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(54) MICROPARTICLES FROM  
STREPTOCOCCUS PNEUMONIAE AS  
VACCINE ANTIGENS

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## (57) ABSTRACT

An isolated *Streptococcus pneumoniae* membrane vesicle microparticle (MP), wherein said MP comprises: the protein Ply at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP; and/or the protein LytA at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP; and/or the protein PspC at the level of  $\geq 0.130 \mu\text{g}/\text{mg}$  total protein in the MP; and/or the protein RrgB at the level of  $\geq 0.020 \mu\text{g}/\text{mg}$  total protein in the MP. Compositions comprising such MPs. Uses thereof in particular in immunization, as well as methods of manufacture thereof.

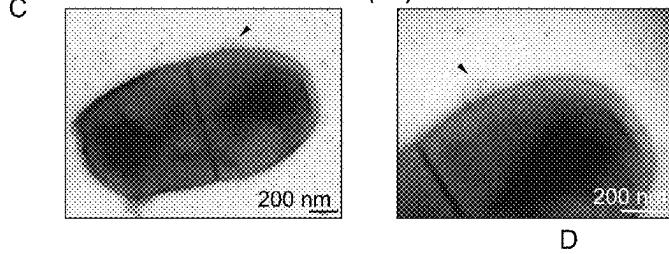
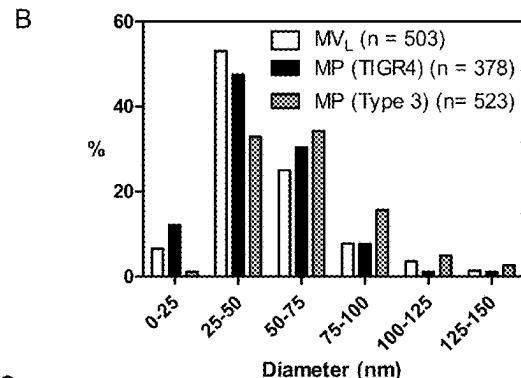
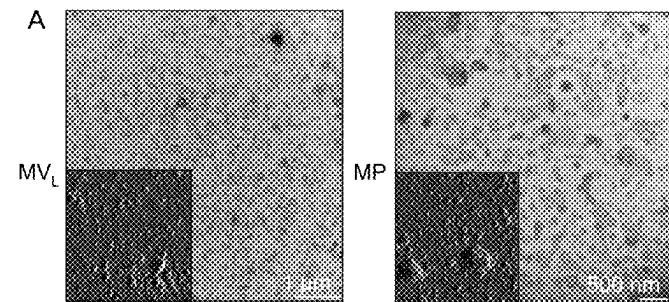


FIG. 1A

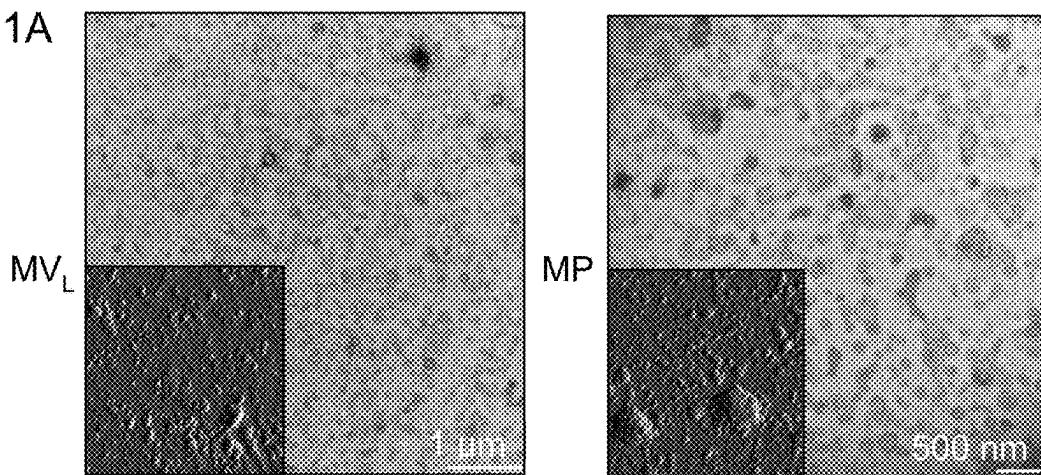


FIG. 1B

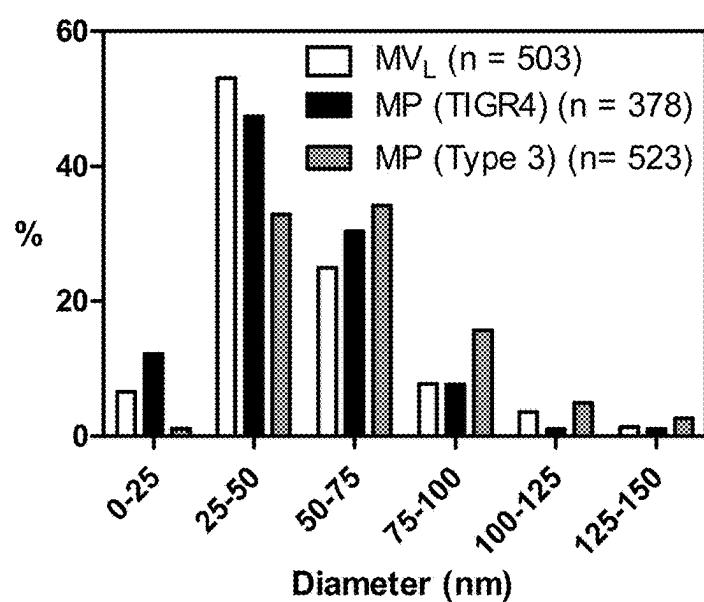


FIG. 1C

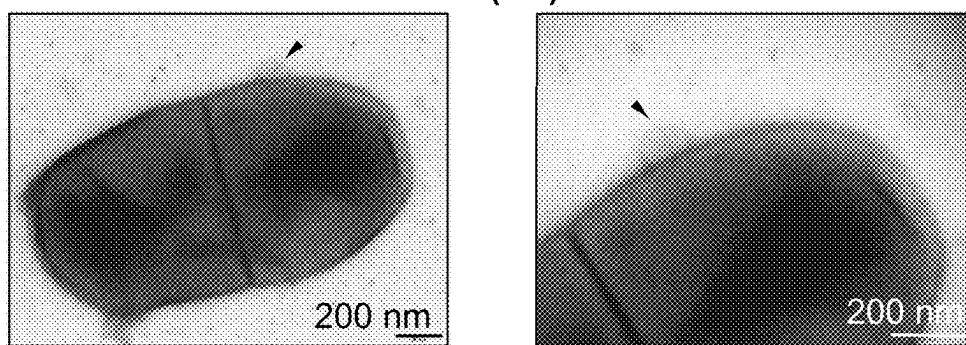
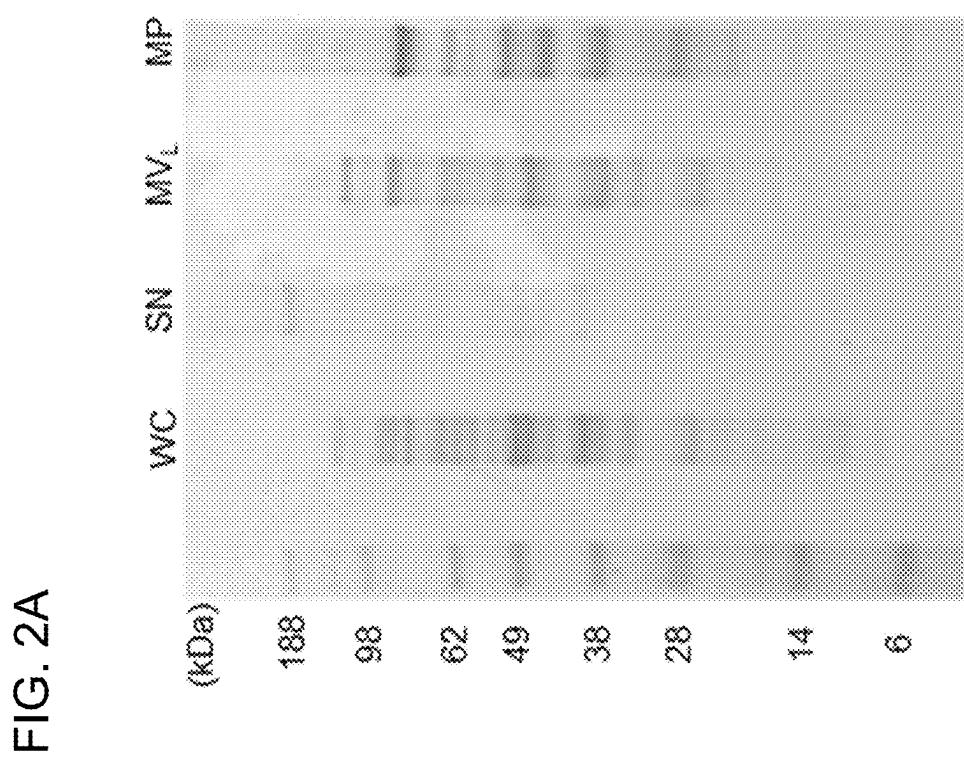
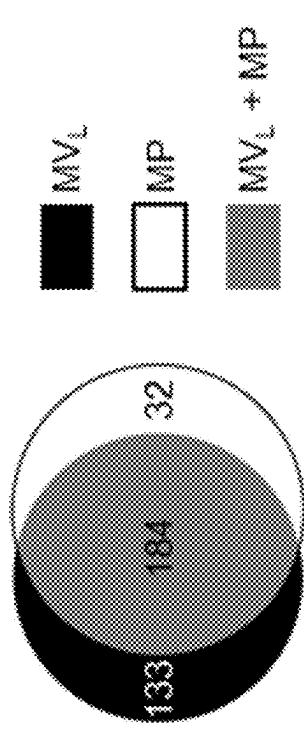


FIG. 1D



**FIG. 2B**



**FIG. 2C**

	T4 MV <sub>L</sub>	T4 MP	Type 3 MV <sub>L</sub>	Type 3 MP
Cytosolic	67.2%	56.0%	77.3%	78.2%
Lipoprotein	8.8%	12.0%	5.6%	6.7%
N-terminally anchored	8.8%	12.0%	6.9%	5.5%
Multi-transmembrane	11.0%	14.8%	8.9%	8.4%
Cell wall	0.9%	0.5%	0.0%	0.0%
Secretory	3.2%	4.6%	1.3%	1.2%

FIG. 2D

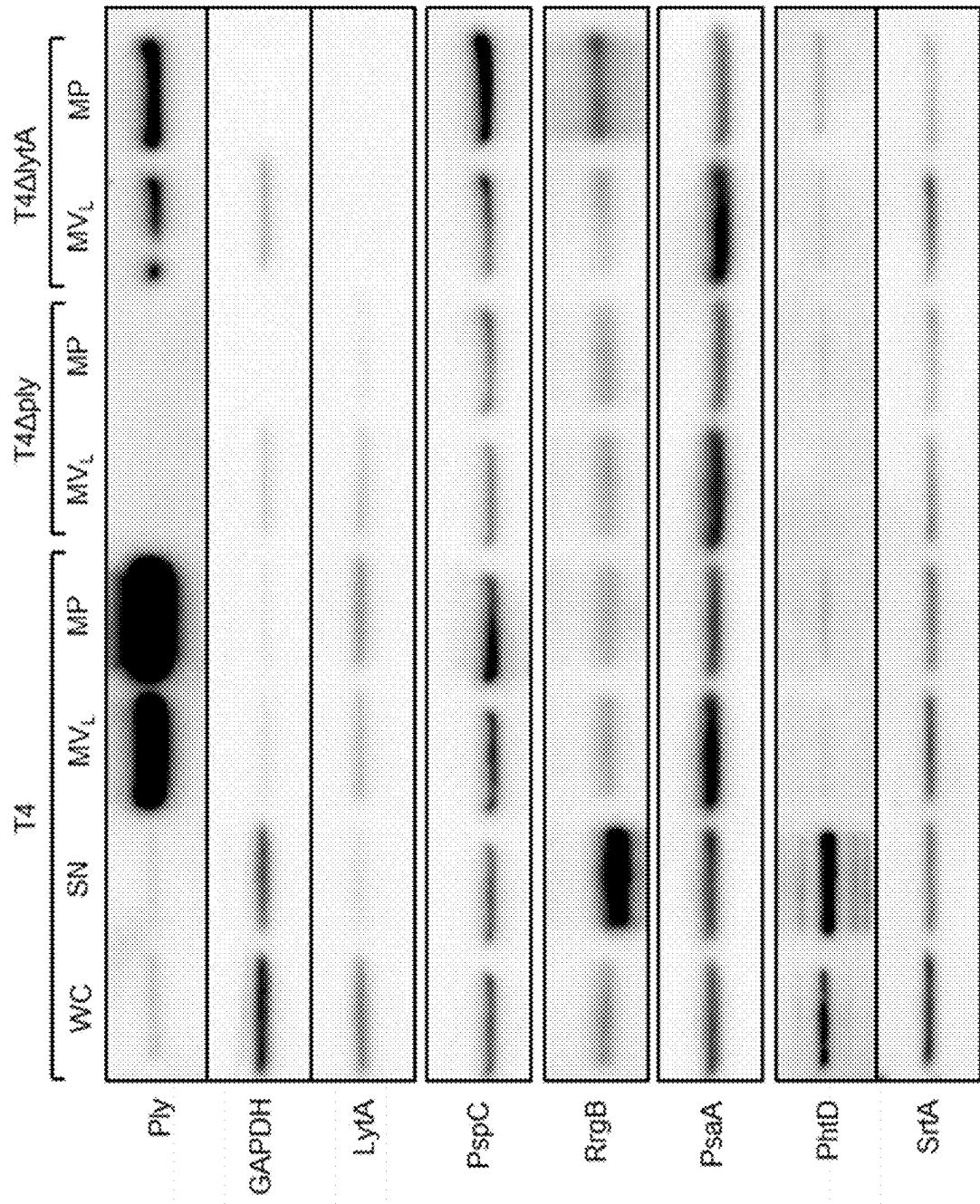


FIG. 3

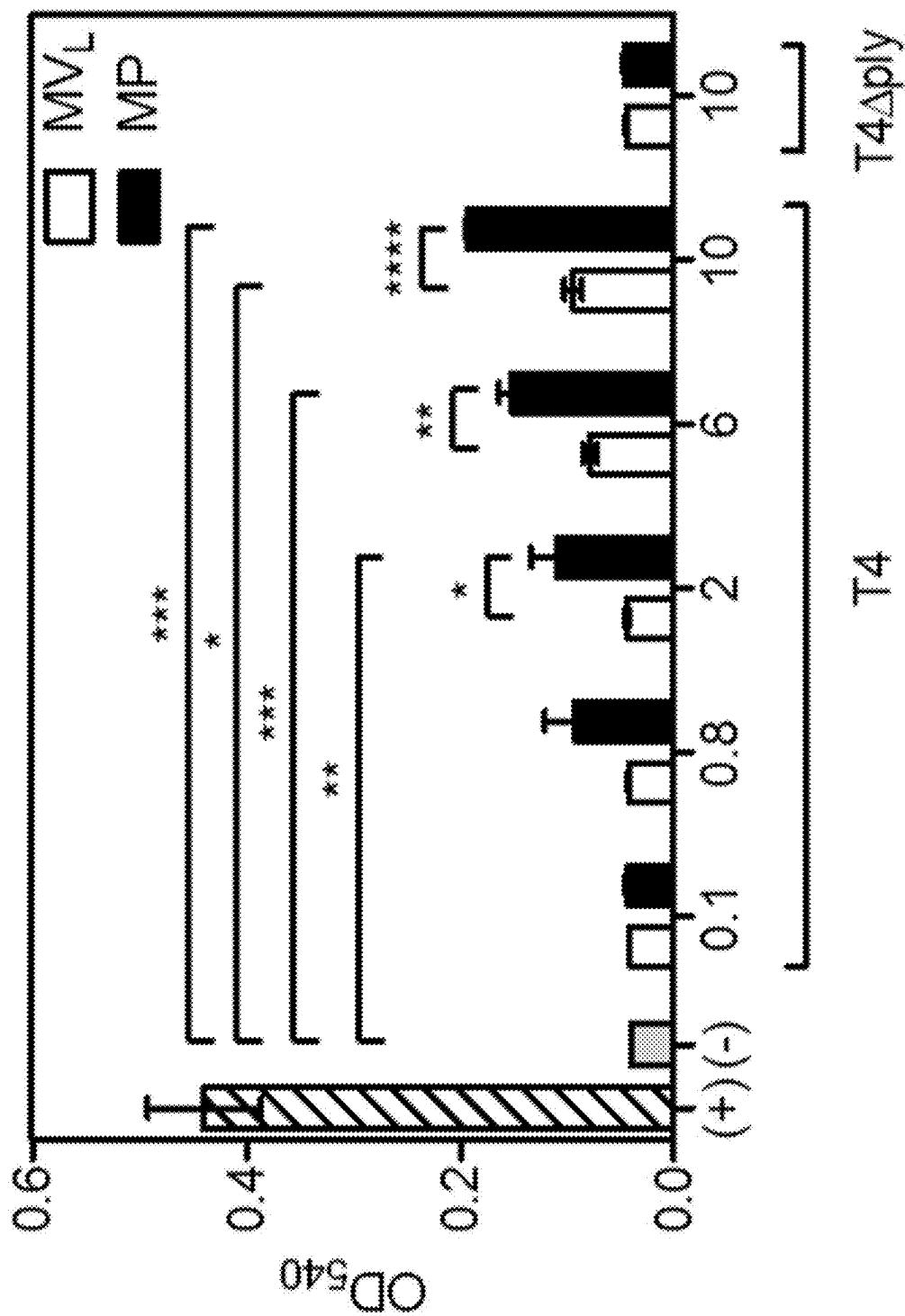


FIG. 4A

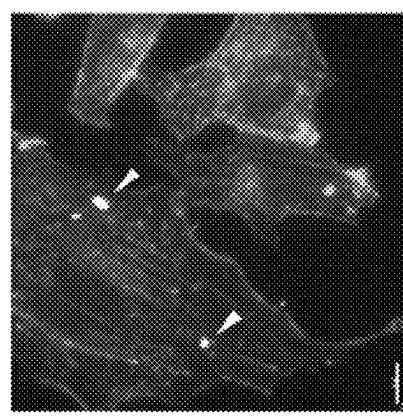


FIG. 4B

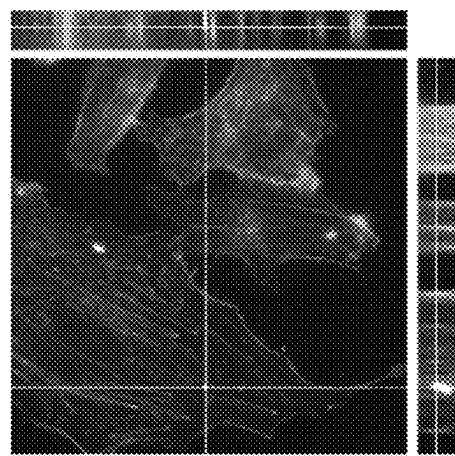


FIG. 4C

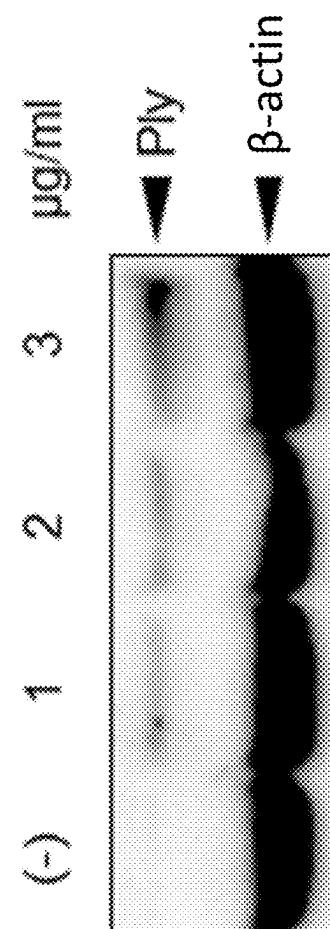
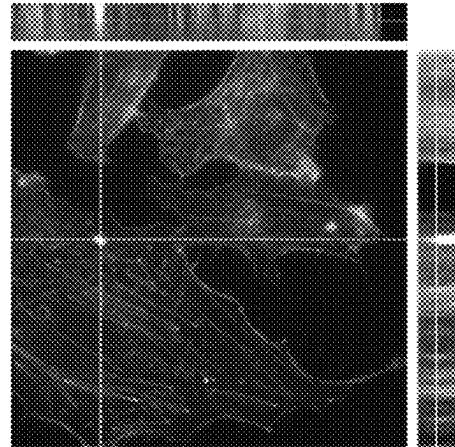


FIG. 4E

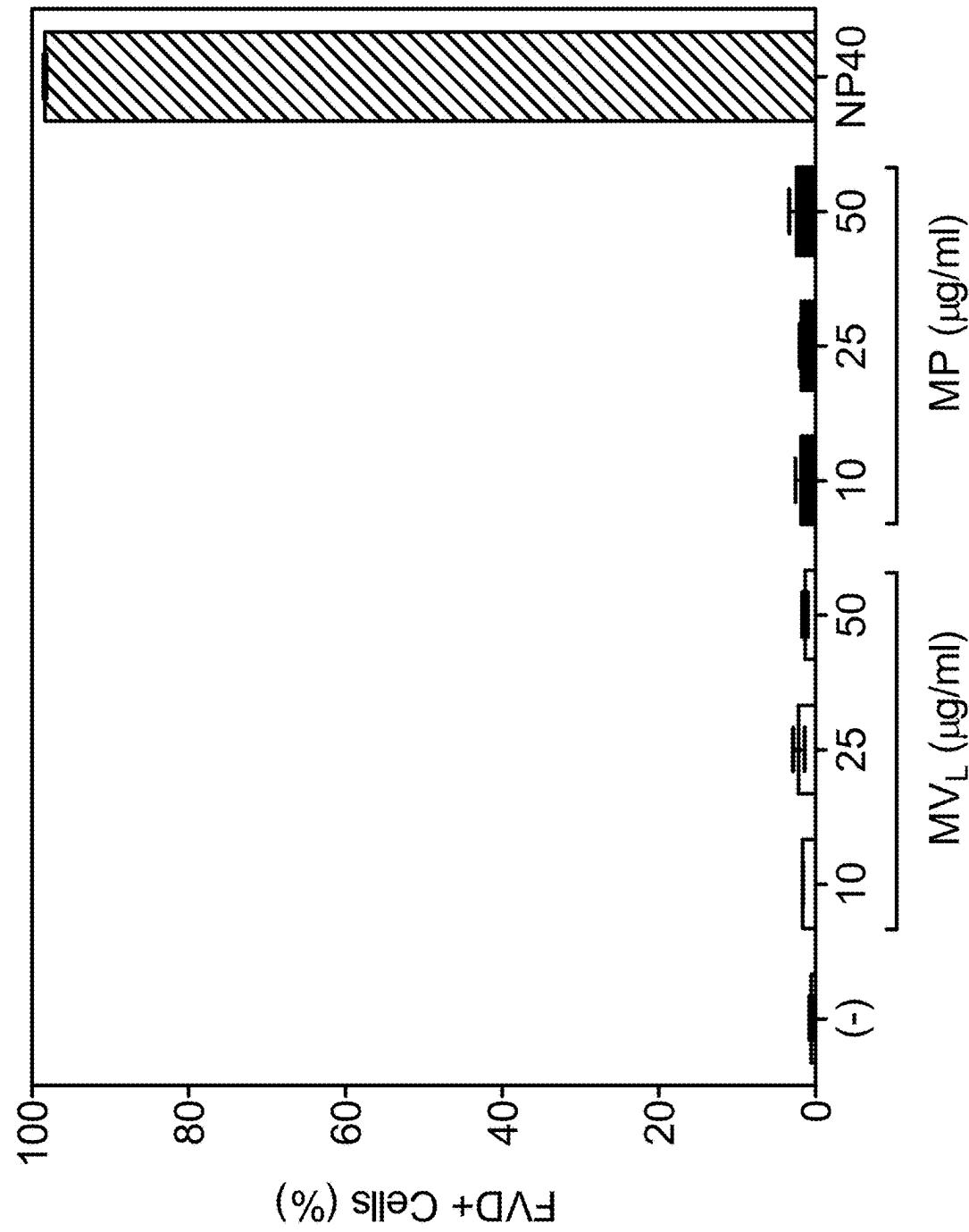


FIG. 5A

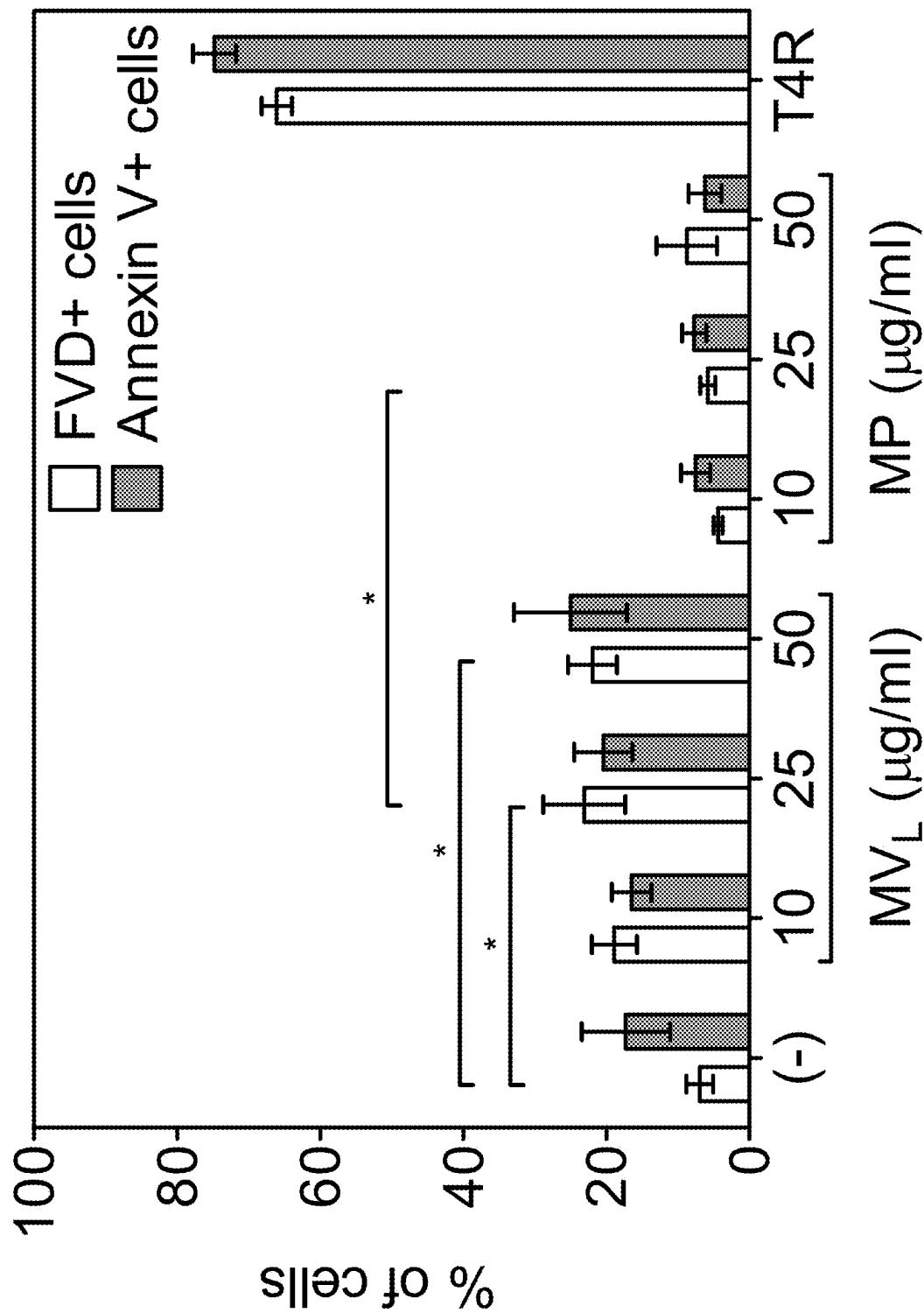


FIG. 5B

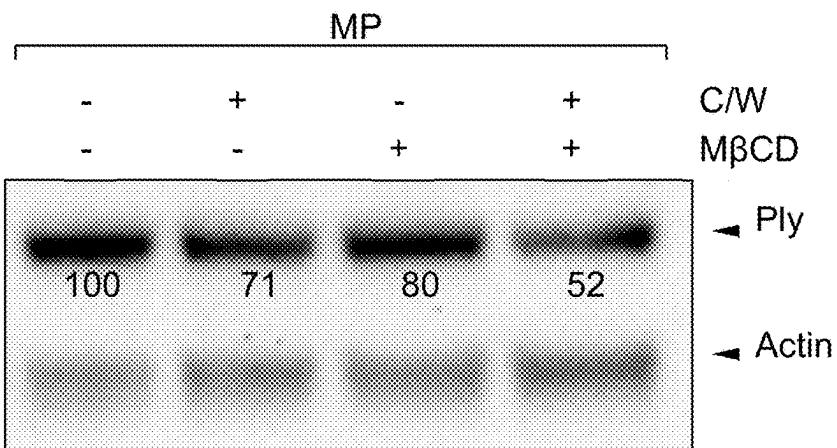


FIG. 5C

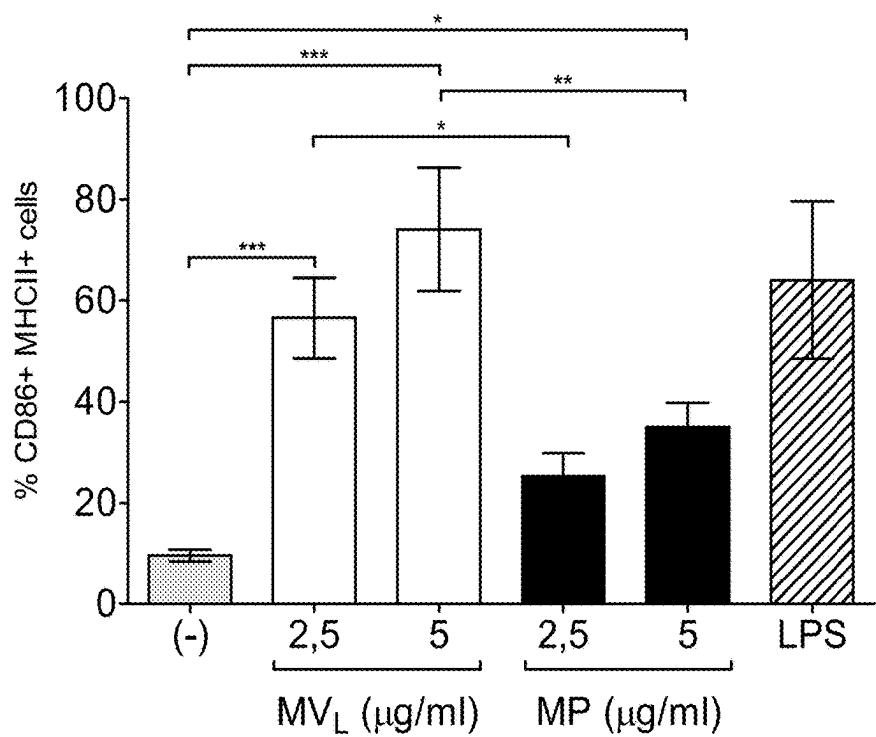


FIG. 6A

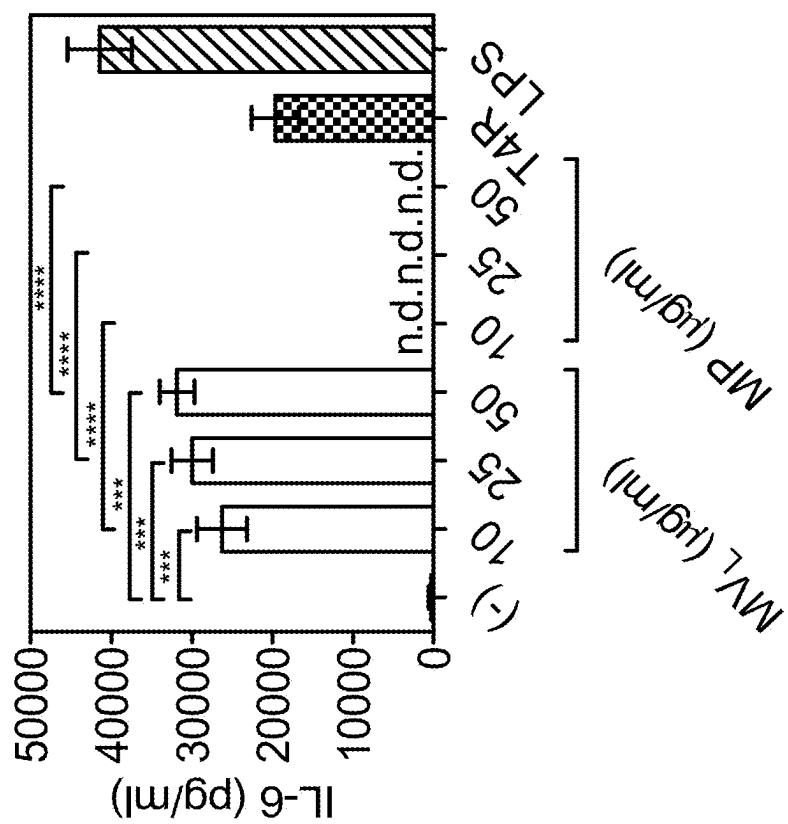


FIG. 6B

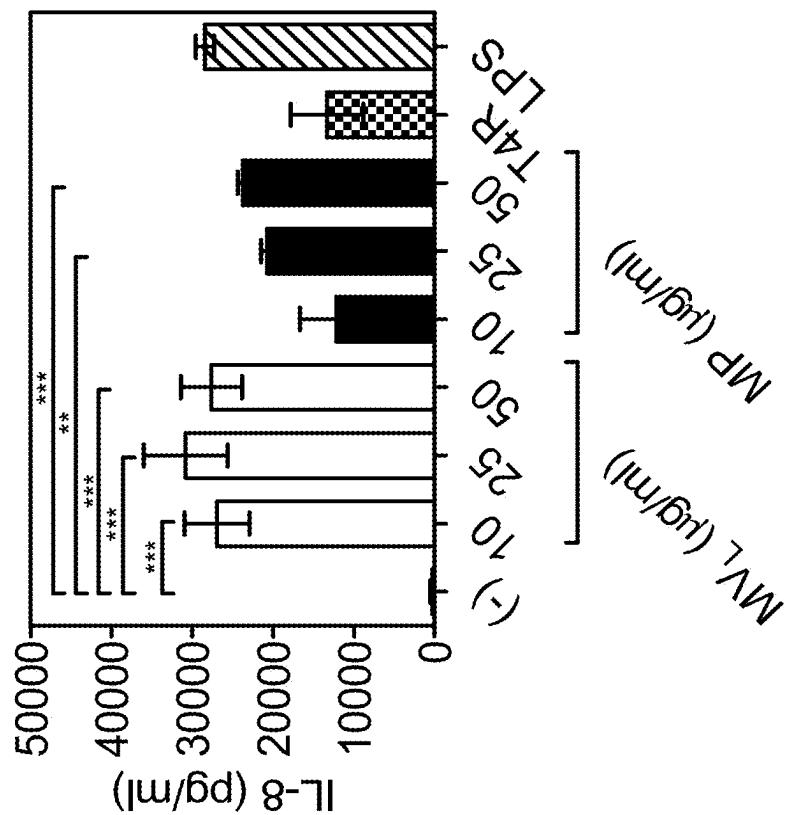


FIG. 6C  
FIG. 6D

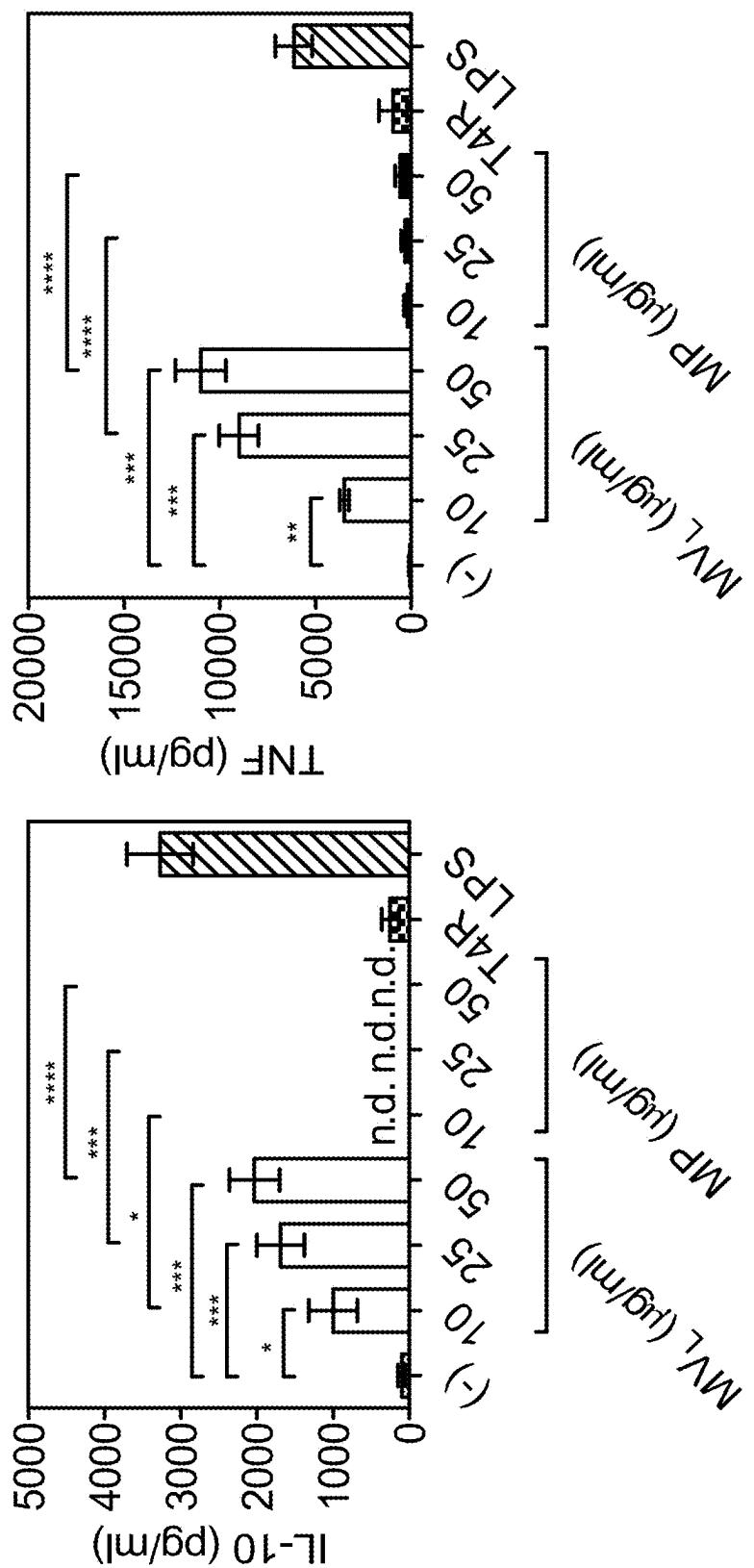


FIG. 7A

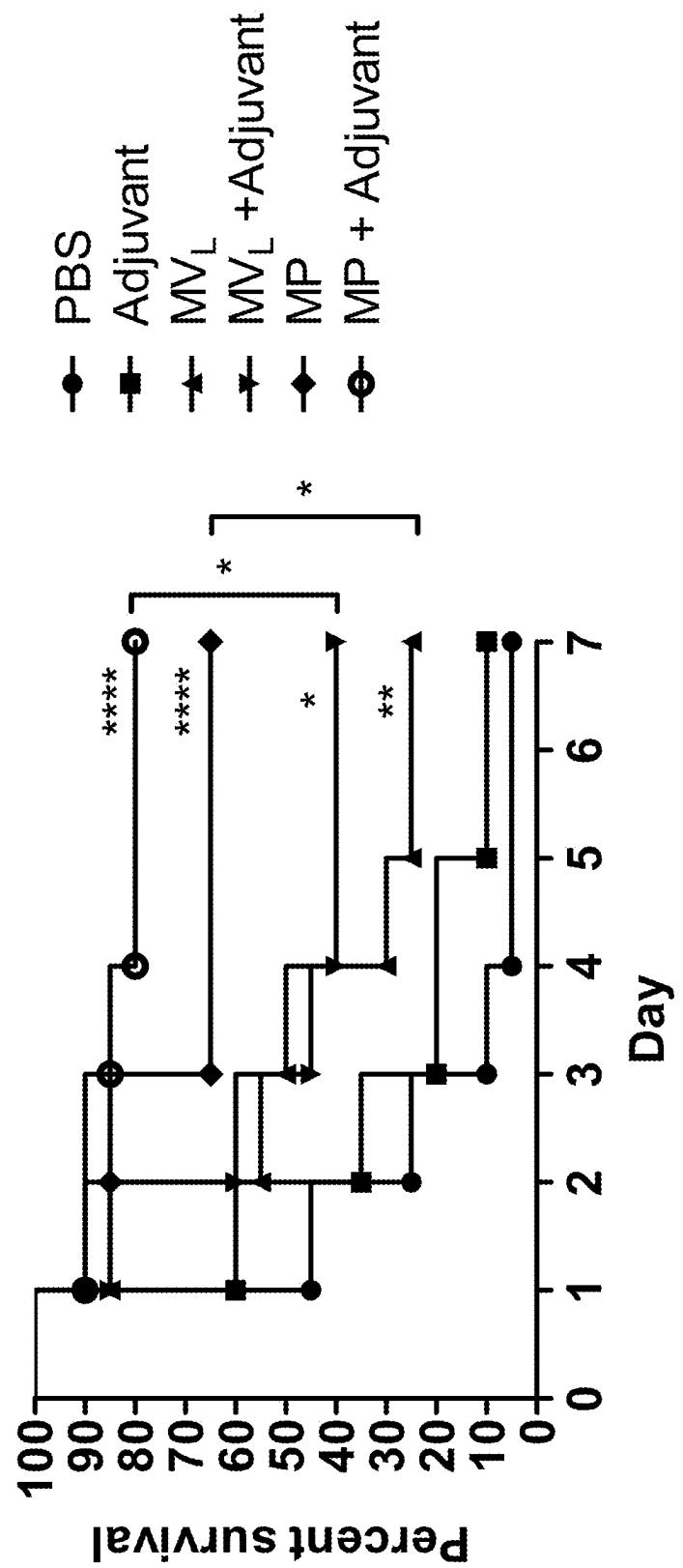
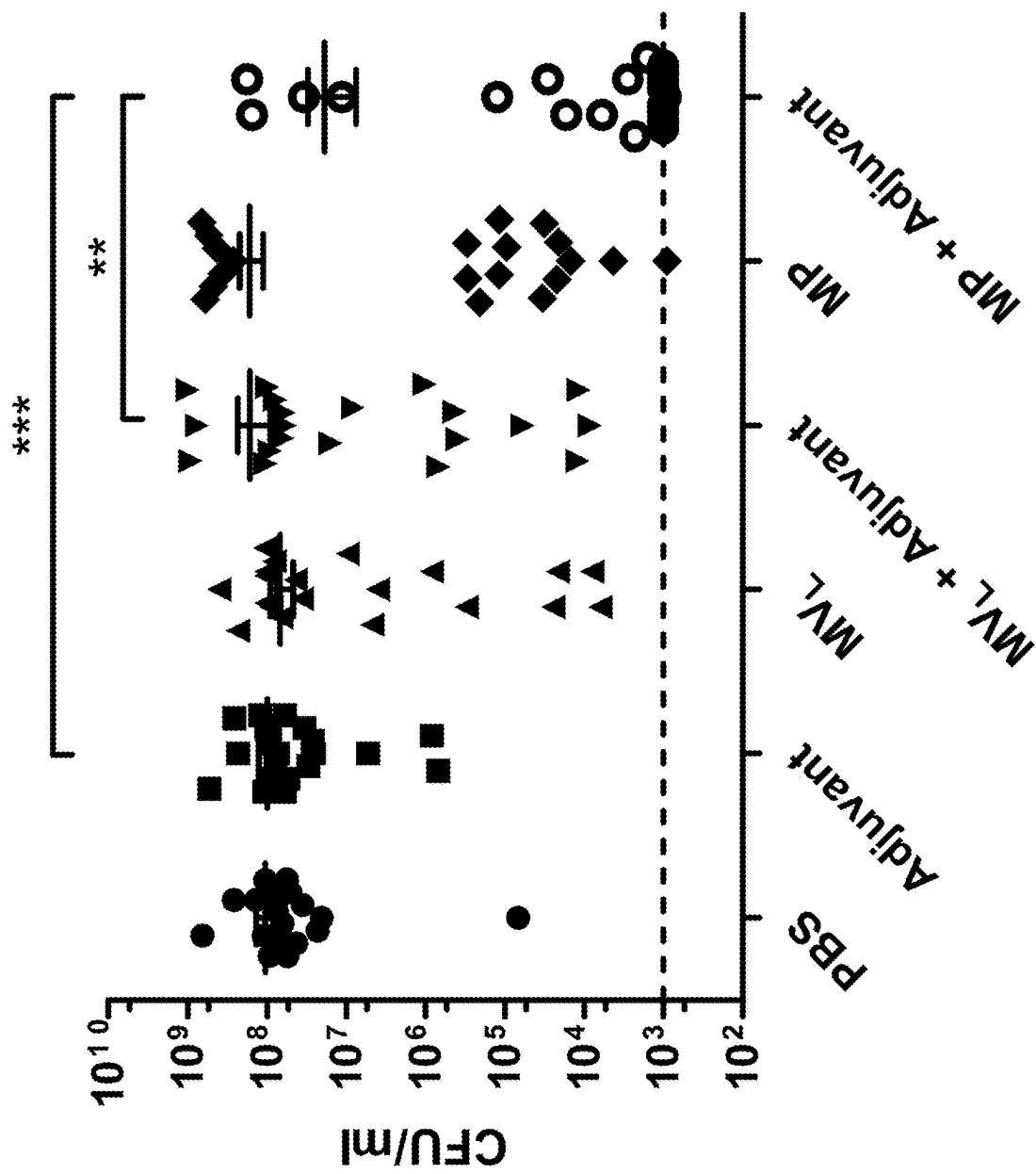


FIG. 7B



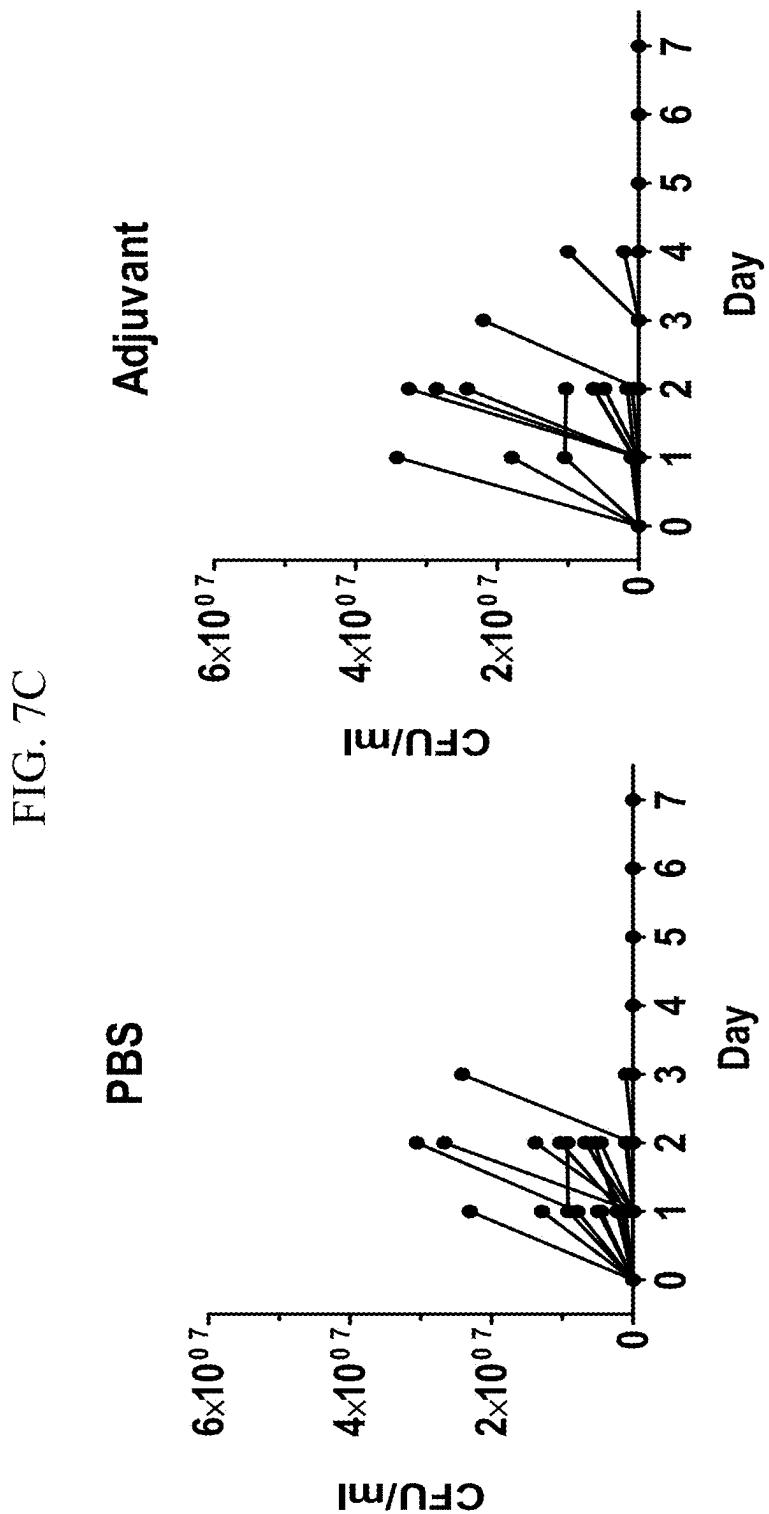


FIG. 7D

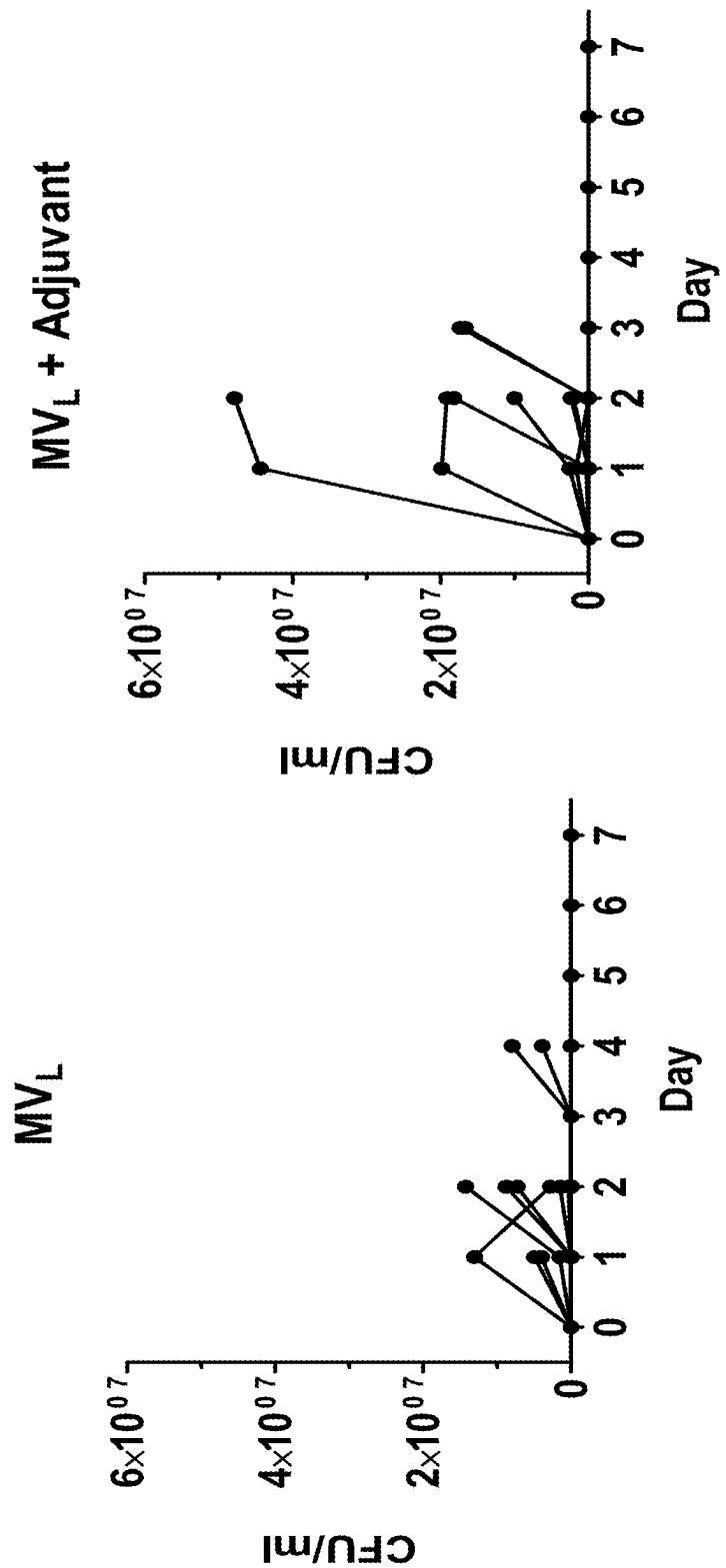


FIG. 7E

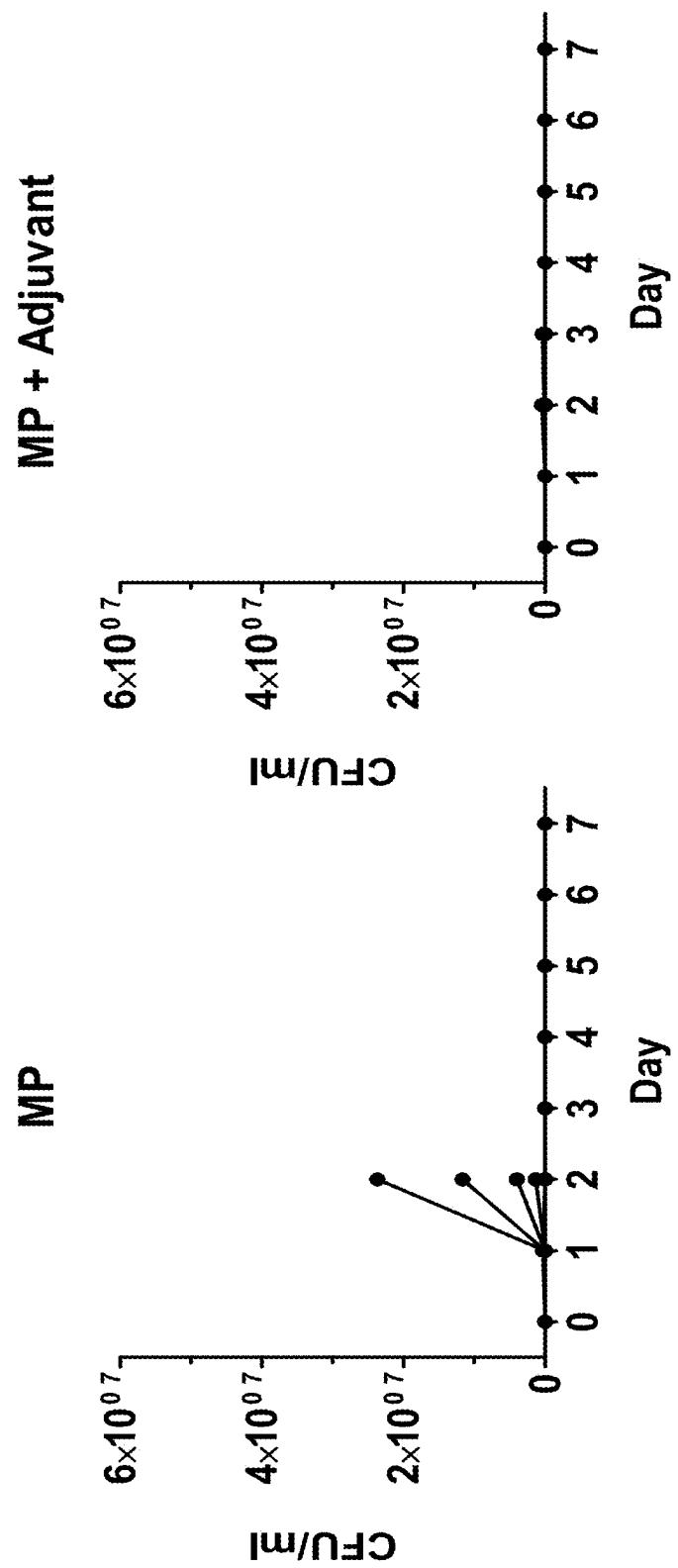


FIG. 8A

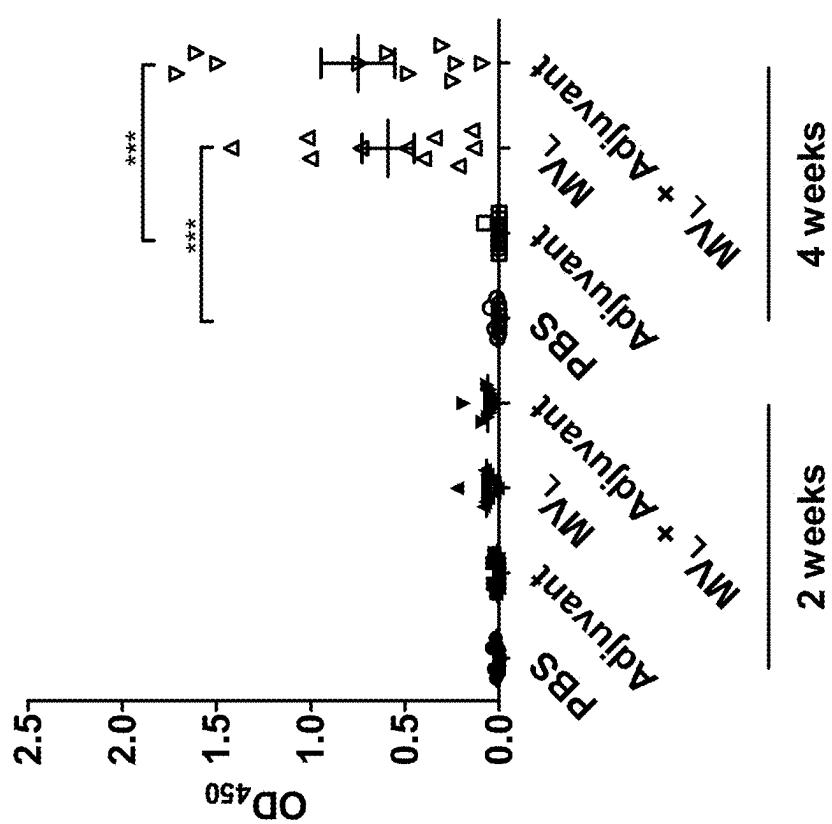


FIG. 8B

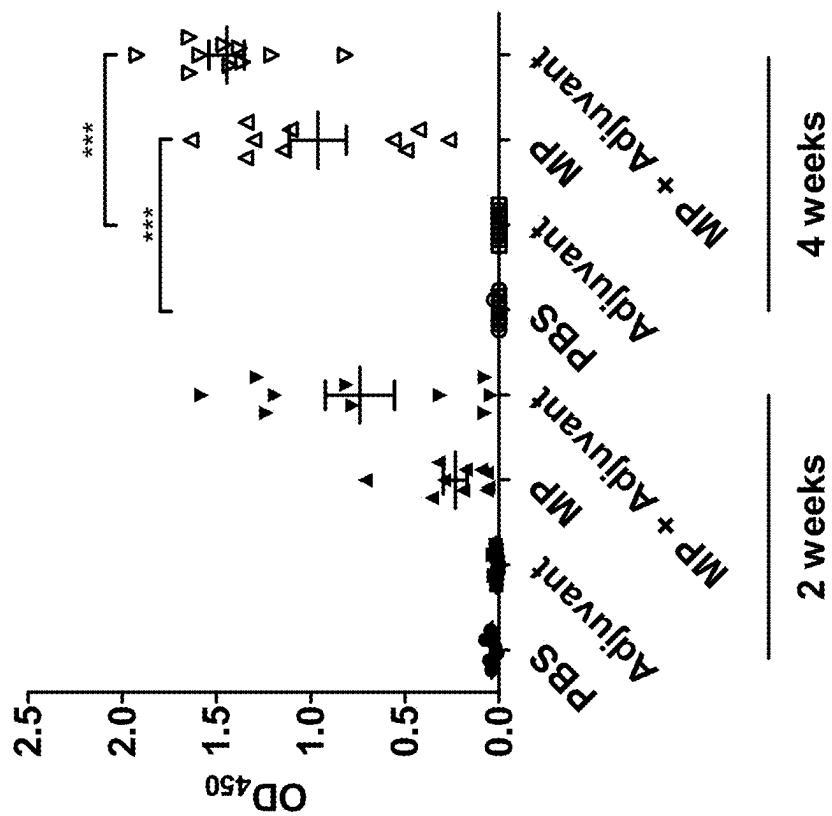


FIG. 8C  
FIG. 8D

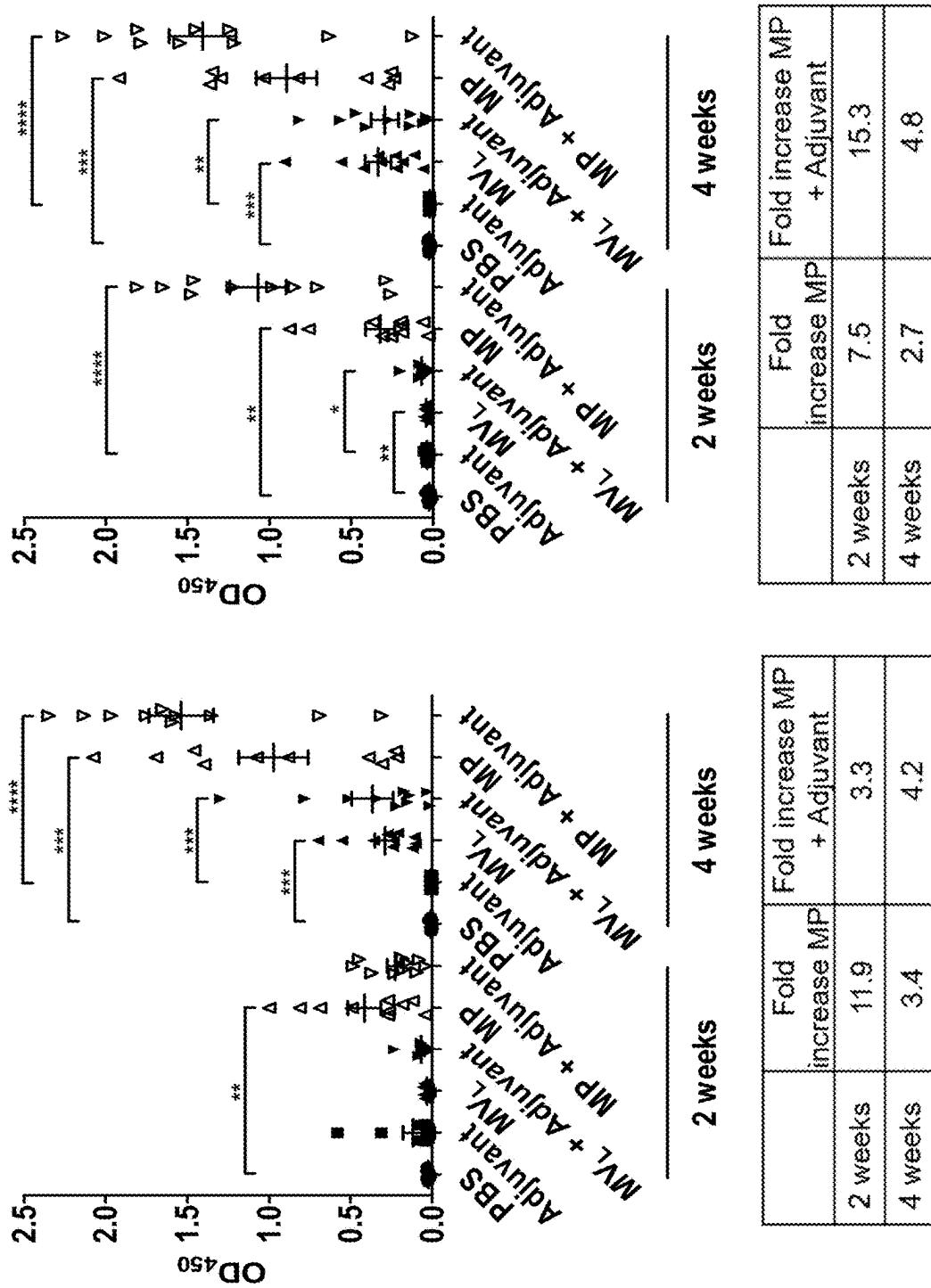


FIG. 9A

*S. pneumoniae* serotypes

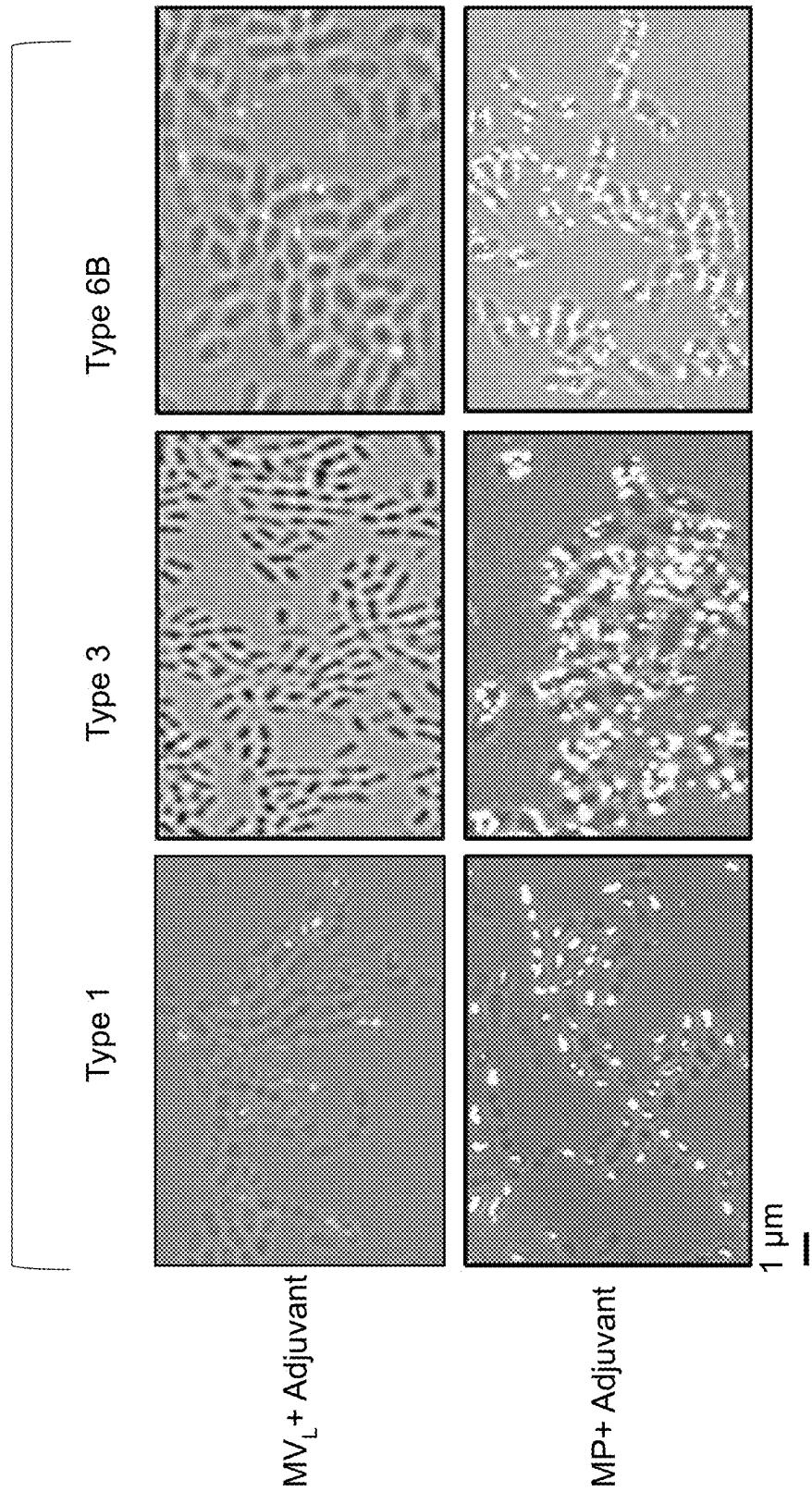
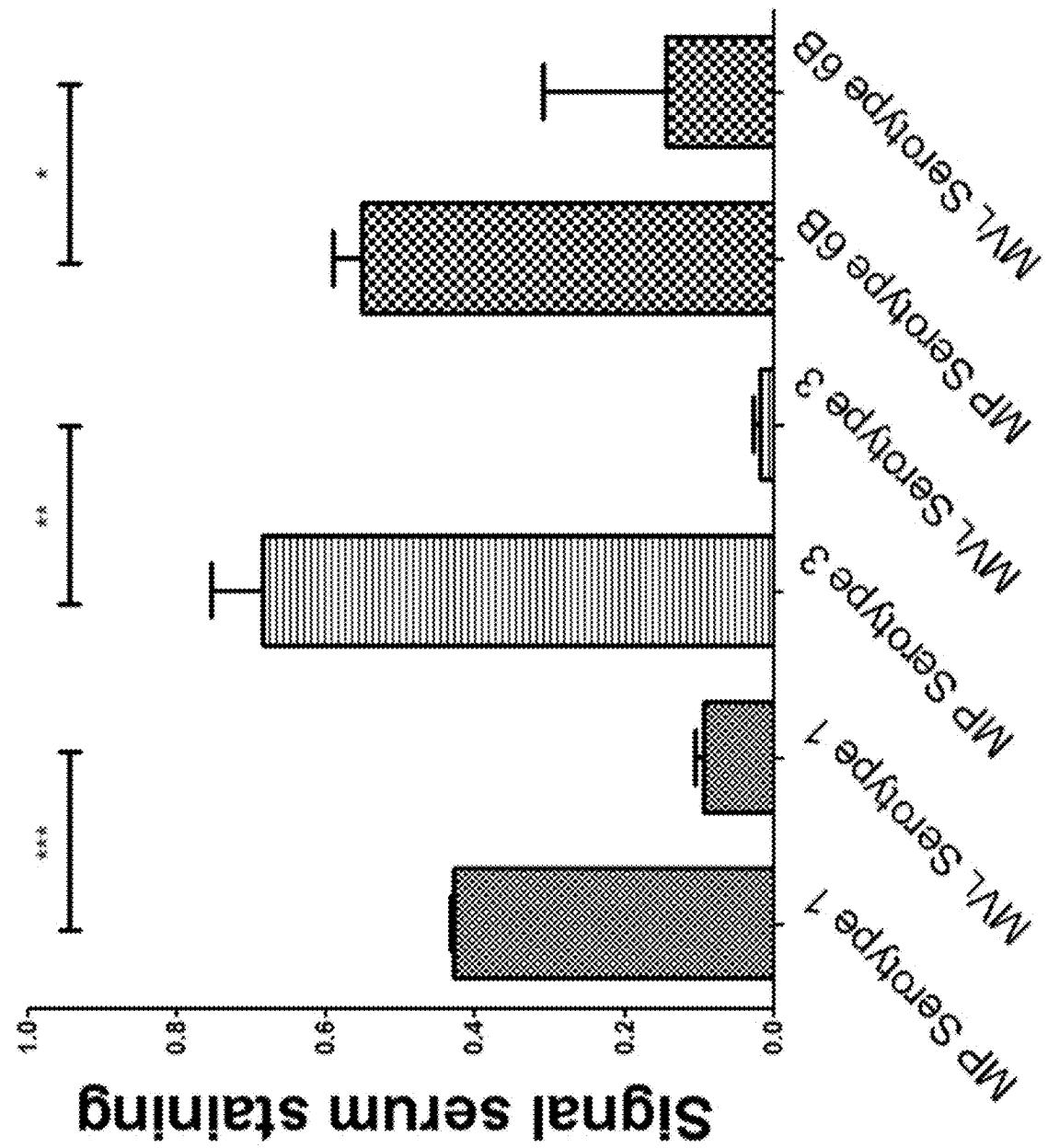


FIG. 9B



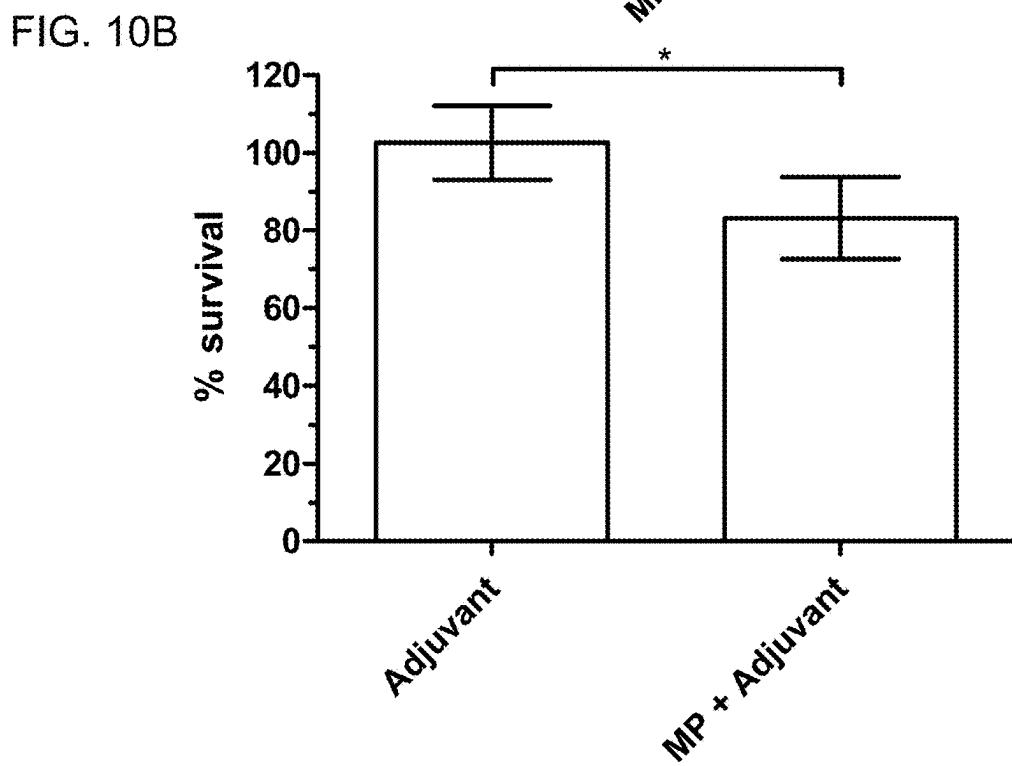
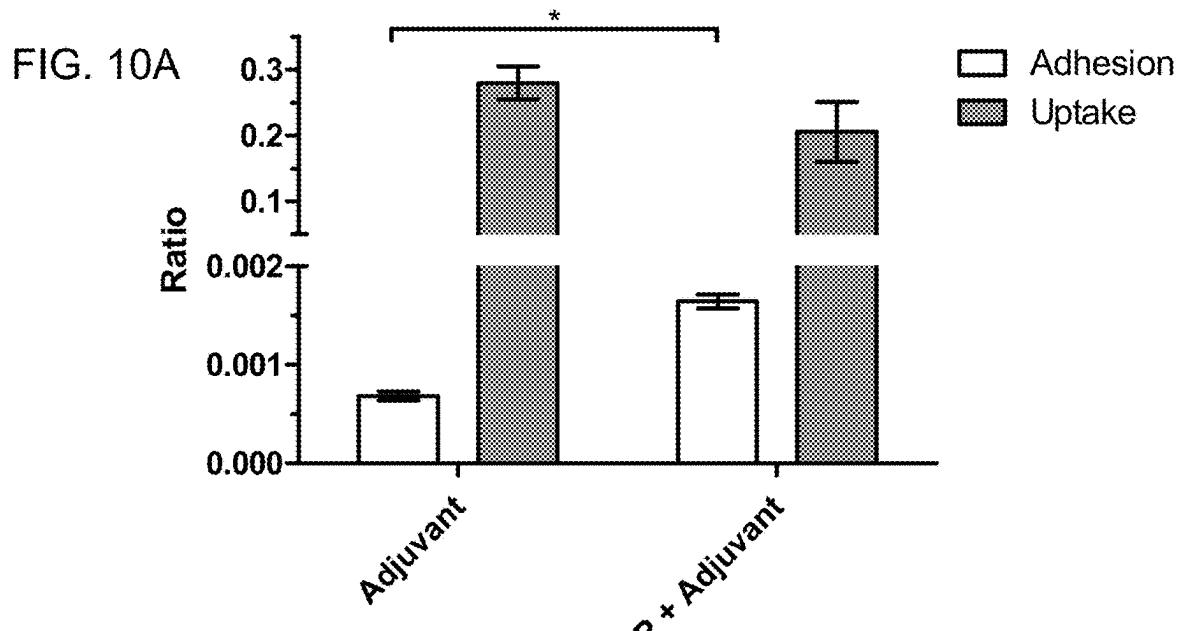


FIG. 11A

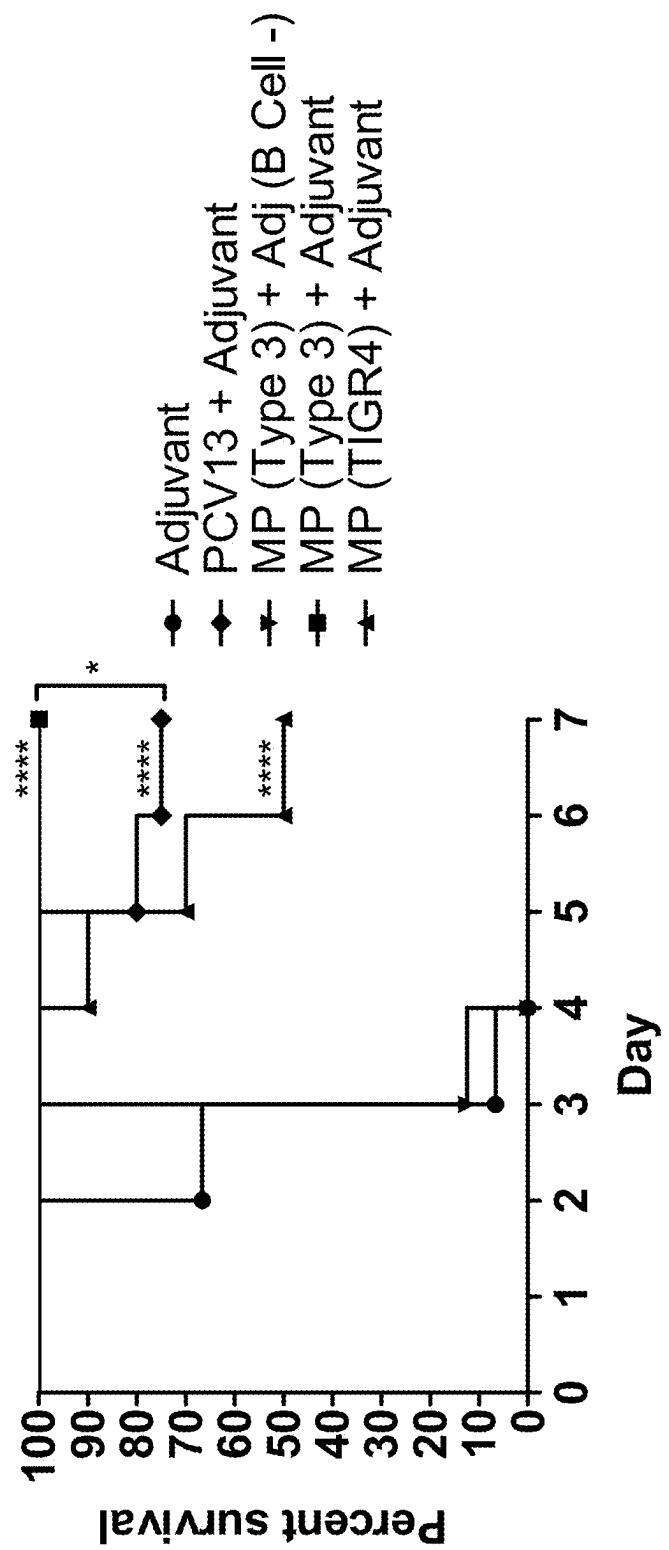
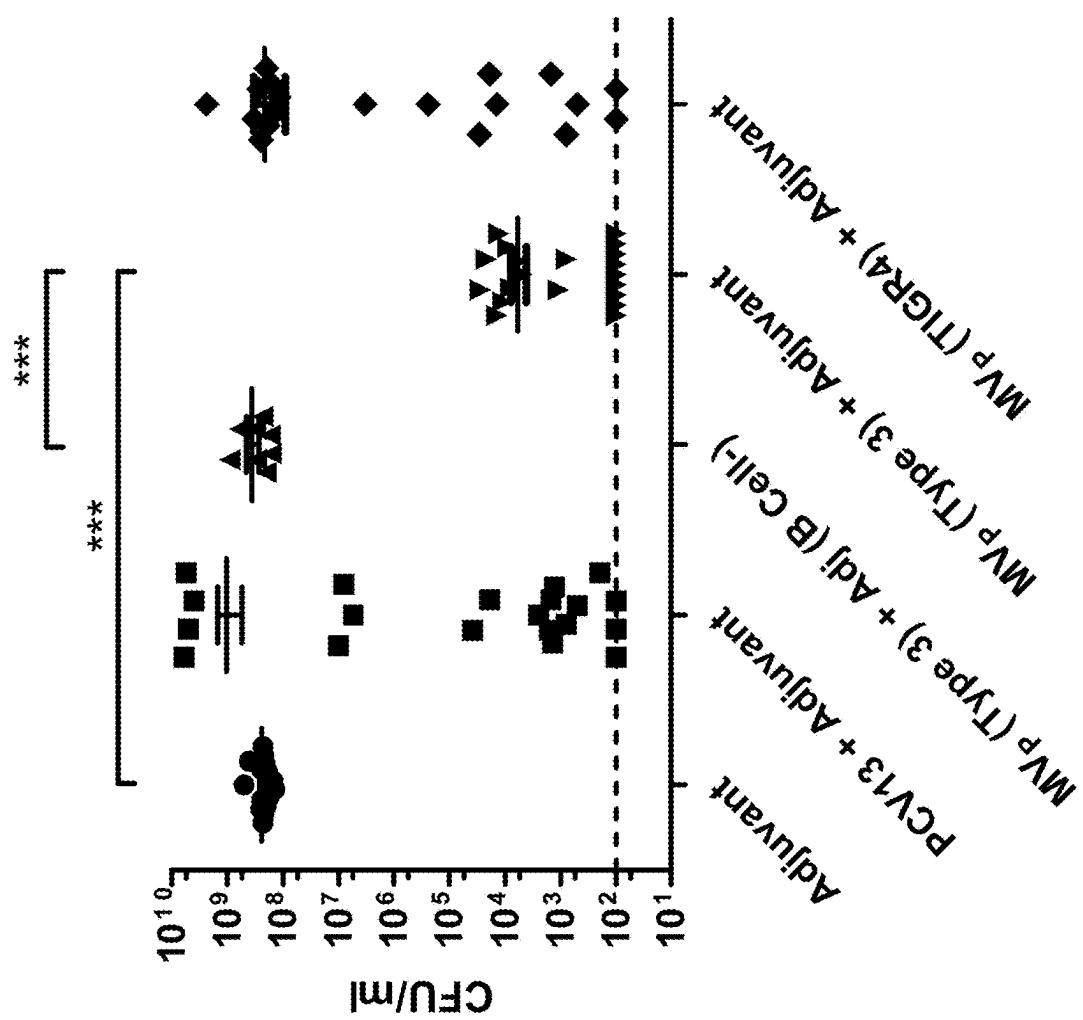


FIG. 11B



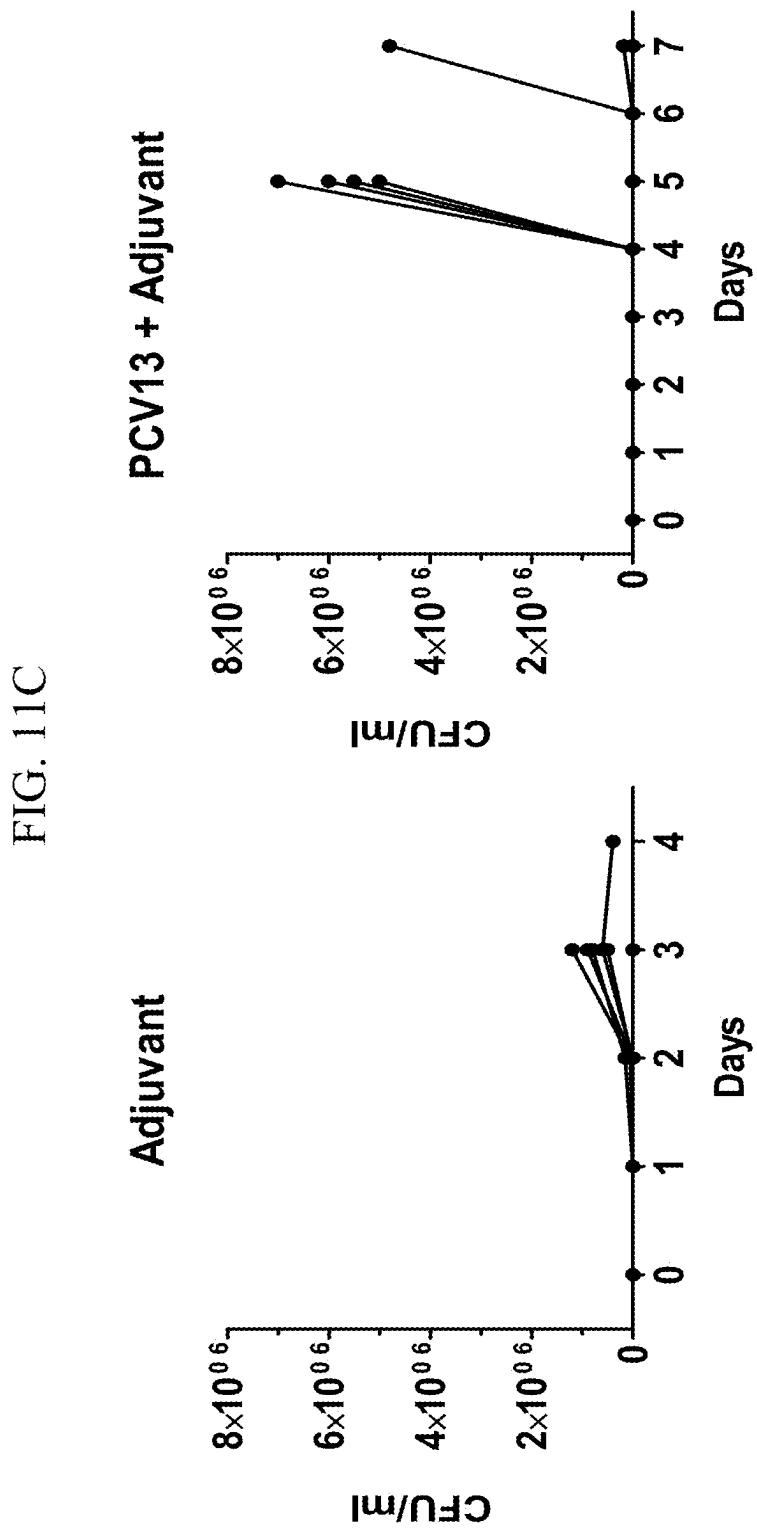


FIG. 11D

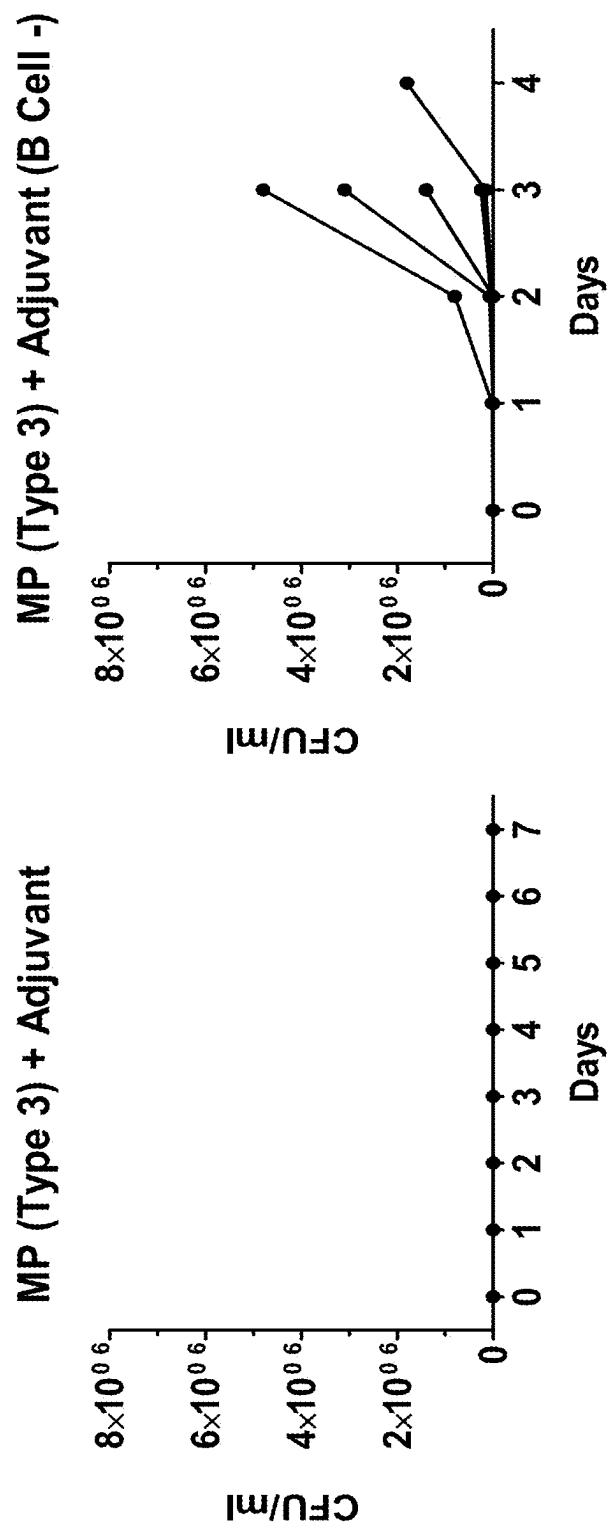
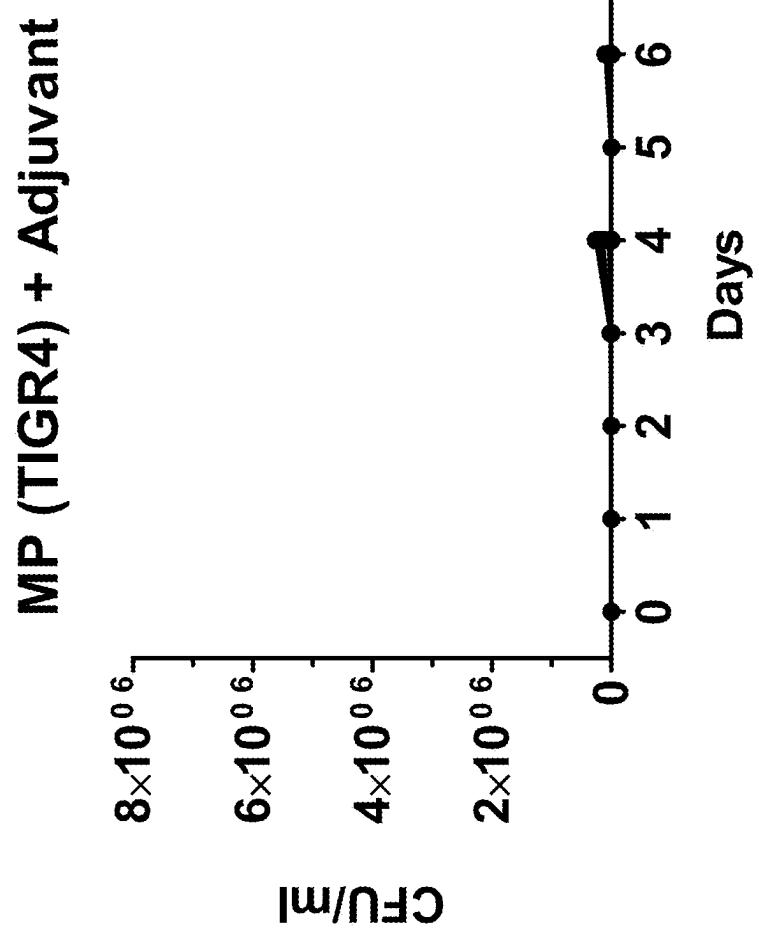


FIG. 11E



## MICROPARTICLES FROM STREPTOCOCCUS PNEUMONIAE AS VACCINE ANTIGENS

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 18/212,857, filed on Jun. 22, 2023, which is a continuation of U.S. application Ser. No. 16/474,940, filed on Jun. 28, 2019, which is a National Stage application under 35 U.S.C. § 371 of International Application No. PCT/SE2017/051323, having an International Filing Date of Dec. 21, 2017, which claims the benefit of SE Application No. 1651746-8 filed Dec. 28, 2016. The contents of the aforementioned applications are hereby incorporated by reference in their entirety.

### TECHNICAL FIELD

[0002] The present invention relates to the field of vaccines against *Streptococcus pneumoniae*.

### BACKGROUND TO THE INVENTION

[0003] *Streptococcus pneumoniae* is a Gram-positive bacterium that is a major contributor to morbidity and mortality worldwide causing about 2 million deaths annually, i.e. the same range as for tuberculosis. Pneumococci are the major cause of common infections such as sinusitis, otitis, and community-acquired pneumonia (CAP), but also a common cause of severe invasive diseases (IPD) such as sepsis and meningitis. Young children and the elderly are the most susceptible age groups to acquire pneumococcal infections, but other factors such as underlying diseases, splenectomy, immunosuppression (including HIV), diabetes and a prior influenza-virus infection also sensitize for a pneumococcal infection. Pneumococcal infections such as CAP also predispose for cardiovascular diseases such as cardiac infarction, and meningitis patients may get neurological sequelae such as hearing loss and cognitive impairments.

[0004] Pneumococci have the human upper airways as their normal ecological niche, and up to 60% of preschool children may be colonized at any given time without symptoms. It is believed that most transmission events to the elderly come from young colonized children. *S. pneumoniae* is a highly genetically diverse species due to an efficient DNA exchange system resulting in a multitude of clonal lineages that may differ from one another in the presence and absence of genes and gene clusters, as well as in variations in individual genes.

[0005] Variations in the capsular locus, the major virulence factor and vaccine target of pneumococci, have resulted in at least 97 known capsular serotypes with different potential of causing disease.

[0006] Pneumococcal conjugate vaccines (PCVs), targeting a limited number (7, 10 or 13 in PCV7, PCV10 and PCV13 respectively) of the known 97 capsular serotypes, have been introduced in the childhood vaccination program in many countries. Vaccine introduction has led to a decrease in IPD caused by vaccine-types in vaccinated children, however, non-vaccine type pneumococci have rapidly expanded and replaced vaccine-types in childhood carriage and in IPD, but also in non-vaccinated population such as the elderly. Furthermore, protection against vaccine types differ, and for serotype 3, a serotype included in PCV13 and with a high mortality rate, there is more or less no evidence for protection against IPD so far. Data from Sweden, where both PCV10 and PCV 13 are used, show that serotype 3 was

a dominating serotype among IPD cases during 2016, also in counties using only PCV13. Hence, novel vaccine approaches are needed.

[0007] In Sweden the total number of patients with IPD has remained almost the same after vaccine introduction. Moreover, in a recent study we found that pneumococcal carriage rates remained around 30% in the youngest children 8 years after vaccine introduction in Stockholm, and non-vaccine types were found in over 90% of the isolates. Antibiotic resistance is emerging among clinical pneumococcal isolates and according to reports from the European Centre for Disease prevention and Control (ECDC), and the European Antimicrobial Resistance Surveillance Network (EARS-Net), reduced susceptibility to penicillin has increased during the last years in Sweden and now exceeds 5% among IPD cases. In addition, the need for multiple antigens covering various capsular serotypes lead to high production costs for the known vaccines.

[0008] Opsonophagocytosis is the primary mechanism for clearance of pneumococci from the host, and the measurement of opsonophagocytic antibodies appears to correlate with vaccine-induced protection. However, it has proven difficult to design vaccines that elicit an efficient response in form of opsonophagocytic antibodies.

[0009] Taken together, the above issues stress the need for novel vaccine approaches. Thus, an object of the present invention is the provision of improved pneumococcal antigens able to confer serotype-independent immunological responses and/or improved response in terms of opsonophagocytic antibodies and protection in experimental models.

### Background for Pneumococcal Microparticles

[0010] Spherical membranous blebs, so called outer membrane vesicles (OMVs), are produced by Gram-negative bacteria. OMVs range in size from 10 to 300 nm and they have been shown to be formed by budding from the bacterial outer membrane, and to have many functions, such as influencing virulence by different mechanisms. In Gram-negative bacteria OMVs have been suggested to act as vehicle to deliver virulence factors to host cells. Only recently, membrane vesicles (MV<sub>L</sub>) were discovered also in Gram-positive bacteria and their formation and function is poorly understood compared to OMVs. Gram-positive bacteria do not contain an outer membrane and the cell wall differs from Gram-negative bacteria.

[0011] For *Streptococcus pneumoniae* MV<sub>L</sub> (membrane vesicle) were isolated from pneumococci belonging to different serotypes (2, 6B, 8 and 23F) grown in liquid culture medium (1). MV<sub>L</sub> from serotype 8 were shown to be protective against pneumococcal challenge with the same serotype in mice. Importantly, no cross protection was shown to pneumococci of other serotypes.

### Definitions

[0012] The pneumococcal capsule. The pneumococcal capsule, consisting of polysaccharide, exists in at least 97 different variants, so called capsular serotypes. The pneumococcal capsule is a major virulence determinant of pneumococci, affecting phagocytosis of the bacteria by host cells, and influence bacterial interactions with the innate immune response.

[0013] Pneumolysin (termed Ply herein) is a 53 kDa cholesterol dependent cytolysin released by *Streptococcus pneumoniae* upon lysis. It is one of the major virulence factors of this bacterium. It forms pores in all eukaryotic

cells that have cholesterol in their membranes. The formation of pores by Ply frequently results in host cell death as membrane integrity is destroyed. Ply plays a central role in protecting the pneumococcus from complement attack and aiding its spread to other tissues/organs. Ply is able to activate the classical complement pathway, even in the absence of Ply specific antibody (2). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 1.

[0014] LytA is the major autolysin of *Streptococcus pneumoniae*. Lysis is caused by cleaving the lactyl-amide bond between the stem peptides and the glycan strands of peptidoglycan, resulting in hydrolysis of the cell wall. The contribution of LytA to pneumococcal virulence is still unclear. It is possible that LytA-mediated lysis releases other virulence factors such as pneumolysin. LytA could also be released to lyse neighboring non-competent pneumococcal cells in a fratricidal manner. This would potentially facilitate genetic exchange between naturally competent pneumococcal populations that easily take up and incorporate DNA by homologous recombination. A third possibility is that LytA mediates lysis to release proteins involved in immune evasion or cell wall components that may interfere with the host immune response (3). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 2.

[0015] PspC/CbpA (choline binding protein A) is a protein that binds the phosphocholine present in the teichoic acid and the lipoteichoic acid of the cell membrane and the cell wall. It is a major pneumococcal adhesin. It promotes pneumococcal adherence via a human-specific interaction with the ectodomain of the polymeric Ig receptor. It also prevents activation of C3b and complement-mediated opsonophagocytosis of pneumococci (4). The pspC locus is highly polymorphic and 11 major groups of this protein have been identified. Single PspC proteins are identified by sequential numbers separated from the group number by a dot (5) as follows: PspC1.1, PspC2.1, PspC2.2, PspC3.1, PspC3.4, PspC4.2/PspC10.1, PspC5.1, PspC6.1/PspC9.1, PspC7.1, PspC8.1, PspC11.1 and PspC11.4 (SEQ ID NOS: 3-14, respectively).

[0016] RrgB is the major subunit and stalk protein of the pneumococcal pilus. *S. pneumoniae* pilus 1 is encoded by a genetic islet (PI-1) present in 30 to 50% of the pneumococcal strains and is implicated in adhesion to epithelial cells, lung infection, and virulence. Pilus 1 is composed of the backbone subunit RrgB, the minor pilin subunits RrgA, and RrgC (6). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 15.

[0017] RrgA is a minor pilin subunit of the pneumococcal pilus. RrgA is the tip protein of the pilus and has been shown to mediate adhesion to epithelial cells (6, 7). Recently, RrgA was shown to promote meningitis development in mice (8). A reference sequence is presented in SEQ ID NO: 16.

[0018] PhtD is a polyhistidine triad protein of *S. pneumoniae*. Polyhistidine triad (Pht) proteins are streptococcal surface proteins that contain multiple copies of a characteristic HxxHxH sequence, designed as histidine triads, which were predicted to bind divalent metal cations. Despite the increasing amount of biochemical, structural and physiological data, the functional role of PhtD and the other Pht proteins remains unclear. PhtD could be involved in zinc uptake. Alternatively, Pht proteins could play a role in protecting the pneumococcus from toxic effects of high Zn<sup>2+</sup> concentrations by scavenging, storing or trapping Zn<sup>2+</sup> ions (9). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 17.

[0019] IgA refers to Immunoglobulin A1 protease of *S. pneumoniae* (10). Said IgA1-protease is a highly specific

enzyme that cleaves amino acid sequences of certain proteins. The natural substrate of the IgA protease is immunoglobulin A1, hence its name. The enzyme is in fact capable of cleavage of proteins with the amino acid sequence N—X-Z-Pro-Pro/-Y-Pro-C, where the X in the sequence is preferably a Proline or Serine; the Y=Threonine, Serine or Alanine; and Z preferably is Arginine or Threonine. Thus, the IgA1 protease acts by cleaving the proline-rich hinge region of the heavy chain of IgA1. Release of the IgA1 protease by the pathogen allows adherence to mucous membranes by degrading host immunoglobulin A. A reference sequence from strain TIGR4 is presented in SEQ ID NO: 18.

[0020] The term protective immunity in the present context refers to immunization measures resulting in any degree of reduction in the likelihood of developing the condition for which the protective immunity is relevant, including a minor, substantial or major reduction in likelihood of developing the condition as well as total prevention. Preferably, the degree of likelihood reduction is at least a minor reduction.

[0021] The term sequence identity expressed in percentage is defined as the value determined by comparing two optimally aligned sequences over a comparison window, wherein a portion of the sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Unless indicated otherwise, the comparison window is the entire length of the sequence being referred to. In this context, optimal alignment is the alignment produced by the BLASTP algorithm as implemented online by the US National Center for Biotechnology Information (see The NCBI Handbook [Internet], Chapter 16), with the following input parameters: Word length=3, Matrix=BLOSUM62, Gap cost=11, Gap extension cost=1.

#### BRIEF DESCRIPTION OF THE FIGURES

[0022] FIGS. 1A through 1D: Membrane-derived vesicles and microparticles produced by *S. pneumoniae* TIGR4 (T4). (FIG. 1A) Electron micrographs and atomic force micrographs (insets) of membrane vesicles and microparticles after isolation and density gradient purification from liquid culture (MV<sub>L</sub>) and microparticles from plate-grown (MP) bacteria. (FIG. 1B) Size distribution of MV<sub>L</sub> MPs from TIGR4 and MPs from serotype 3. (FIG. 1C) Electron micrograph of a membrane budding from *S. pneumoniae* T4R and (FIG. 1D) the same electron micrograph in larger magnification. Released particle is indicated by arrow.

[0023] FIGS. 2A through 2D: Identification of proteins present in pneumococcal MV<sub>L</sub> and MP. (FIG. 1A) Preparations of MV<sub>L</sub> and MP were separated by SDS-PAGE and proteins visualized by coomassie staining. In comparison, proteins present in a whole cell lysate (WC) and supernatant (SN) of a liquid bacterial culture were visualized. (FIG. 1B) Venn diagram displaying the number of proteins identified by mass spectrometry (repeated twice) exclusively found in MV<sub>L</sub> and MP preparations and common to both. (FIG. 1C) Mass spectrometry identification of proteins from MV<sub>L</sub> and MP. Numbers indicate percentages of proteins based on their subcellular localization, defined as cytosolic proteins, membrane-associated proteins (lipoproteins), proteins with one

transmembrane domain (N-terminally anchored), transmembrane proteins, cell wall associated proteins and secreted proteins. (FIG. 1D) Immunoblot detection of pneumococcal proteins and virulence factors present in MV<sub>L</sub> and MP isolated from *S. pneumoniae* T4 WT, pneumolysin-(T4Δply) and LytA-deficient (T4ΔlytA) strains.

[0024] FIG. 3: Pneumolysin associated with pneumococcal MV<sub>L</sub> and MP is biologically active. Quantification of in vitro hemolytic activity of purified MV<sub>L</sub> and MP isolated from *S. pneumoniae* T4 and T4Δply. Erythrocytes from buffy coat blood were incubated for 60 minutes with different concentrations (0.1, 0.8, 2, 6 and 10 µg protein) of MV<sub>L</sub> and MP. As control treatments, erythrocytes were incubated with PBS (-) or 1% Triton X-100 (+). Data are represented as means+/-SEM of three independent experiments. \*=P<0.05; \*\*=P<0.01; \*\*\*=P<0.001.

[0025] FIGS. 4A through 4E: Microparticles (MP) are internalized by A549 epithelial cells.

[0026] (FIGS. 4A, 4B and 4C) Immunofluorescence pictures of A549 cells treated with MP (10 µg/ml) for 24 hours. Cells were stained for F-actin, MP are detected with anti-pneumolysin and LytA antibodies. (FIG. 4A) Z-stack image (nr. stacks=21) of MP-treated cells. Internalized MP are indicated by arrows. (FIGS. 4B and 4C) Orthogonal views of the same picture, taken at stack nr. 11, for the two indicated MP, further showing their intracellular localization.

[0027] (FIG. 4D) Immunoblot detection of anti-Ply, and anti-β-actin as loading control, in A549 cell lysates after incubation with increasing concentrations (1, 2, 3 µg/ml) of MP. As control treatment, A549 cells were incubated with PBS (-). (FIG. 4E) Viability of A549 cells examined by flow cytometry of fixable viability dye (FVD) positive cells after 24 hours of incubation with MV<sub>L</sub> or MP. As control treatment, A549 cells were incubated with PBS (-) or 0.02% NP40 in PBS. Data are represented as means+/-SEM of three independent experiments.

[0028] FIGS. 5A through 5C: MV<sub>L</sub> and microparticles (MP) activate human monocyte-derived dendritic cells (DCs). (FIG. 5A) Percentages of FVD and Annexin V-positive cells analyzed by flow cytometry. Cells were incubated for 24 hours with different concentrations (10, 25, 50 µg/ml) of MV<sub>L</sub>, MP, PBS (-) or the unencapsulated mutant of TIGR4 (T4R). Data are represented as means+/-SEM of three independent experiments. \*=P<0.05. (FIG. 5B) Immunoblot detection of anti-Ply, and anti-β-actin as loading control, in cell lysates after incubation of DCs with MP (4 µg/ml) in presence or absence of the inhibitors Cytochalasin D and Wortmannin (C/W), to block active phagocytosis, or methyl-β-cyclodextrin (MBCD), to block lipid rafts and membrane fusion. Numbers represent the relative intensity of bands corresponding to Ply, adjusted to the loading control bands, in three independent experiments. (FIG. 5C) DC activation measured by flow cytometry of MHCII and CD86-positive cells after 24 hours incubation with different concentrations (2.5, 5 µg/ml) of MV<sub>L</sub>, MP, PBS (-) or LPS (1 µg/ml). Data are represented as means+/-SEM of three independent experiments. \*=P<0.05; \*\*\*=P<0.001.

[0029] FIGS. 6A through 6D: Cytokine release by human monocyte-derived dendritic cells upon incubation with MV<sub>L</sub> or MP. (FIG. 6A) IL-6, (FIG. 6B) IL-8, (FIG. 6C) IL-10 and (FIG. 6D) TNF released by dendritic cells after 24 hours of incubation with different concentrations (10, 25, 50 µg/ml) of MV<sub>L</sub> or MP. Control treatments include PBS (-), the unencapsulated mutant of TIGR4 (T4R) and LPS (1 µg/ml). Not detectable (n.d.) amounts of cytokines are indicated.

Data are represented as means+/-SEM of three independent experiments. \*=P<0.05; \*\*=P<0.01; \*\*\*=P<0.001; \*\*\*\*=P<0.0001.

[0030] FIGS. 7A through 7E: Intranasal immunization of C57BL/6 mice with MV<sub>L</sub> or MP of *S. pneumoniae* TIGR4 increase survival against intranasal infection with serotype 1.

[0031] (FIG. 7A) Percentage of mice survived after challenge. 20 mice per group. \*=P<0.05; \*\*=P<0.01; \*\*\*\*=P<0.0001. (FIG. 7B) CFUs in lungs of mice after sacrifice. Each dot represents one mouse. \*\*=P<0.01; \*\*\*=P<0.001. (C-E) CFUs in blood of mice. Each line represents one mouse.

[0032] FIGS. 8A through 8D: Intranasal immunization of mice with MV or MPs stimulate the production of pneumococcal-specific IgG. Detection of MV-reactive (FIG. 8A), MP-reactive (FIG. 8B), T4-reactive (FIG. 8C) and T4R-reactive (FIG. 8D) mouse IgG in immunized mice sera using ELISA assay. Each dot represents one mouse serum. \*=P<0.05; \*\*=P<0.01; \*\*\*=P<0.001; \*\*\*\*=P<0.0001.

[0033] FIGS. 9A and 9B: Staining of serotypes 1, 3 and 6B using antibodies raised against MP from a serotype 4 strain. (FIG. 9A) Immunofluorescence pictures of pneumococcal strains belonging to serotypes 1, 3 and 6B stained using sera, as primary antibodies, from mice immunized either with MV<sub>L</sub>+adjuvant or MP+adjuvant. The white spots on the bacterial surface indicates the presence of specific antibodies against pneumococci in the sera of the immunized mice. (FIG. 9B) Quantification of the signal (white spots) detected on the bacteria; the signal ratio was calculated by dividing the area of the signal detected on the bacteria (white spots) by the total area detected occupied by the bacteria. The area covered by the bacteria and the area covered by the signal detected on the bacteria after staining with sera were selected, defined and measured using the functions Image>Adjust>Threshold and Analyze>Measure of ImageJ. \*=P<0.05; \*\*=P<0.01; \*\*\*=P<0.001.

[0034] FIGS. 10A and 10B: Opsonophagocytic activity of antibodies in mice sera. (FIG. 10A) RAW mouse macrophages were incubated with *Streptococcus pneumoniae* type 1, pre-incubated with mouse sera from the adjuvant or MP+adjuvant groups. Indicated are the ratios of adherence of bacteria to cells, or uptake of bacteria inside cells. Data are represented as means+/-SEM of three independent experiments. \*=P<0.05. (FIG. 10B) RAW mouse macrophages have been incubated with *Streptococcus pneumoniae* type 1, pre-incubated with mouse sera from the adjuvant or MP+adjuvant groups. Indicated is the percentage of taken up bacteria surviving inside the cells after 1 hour. Data are represented as means+/-SEM of three independent experiments. \*=P<0.05.

[0035] FIGS. 11A through 11E: Intranasal immunization of C57BL/6 mice with MP of *S. pneumoniae* increases survival against intranasal infection with serotype 3. In particular, immunization with MPs of serotype 3 confers 100% protection against serotype 3 infection in comparison to PCV13 immunization, which confers 75% protection. B-cell deficient mice immunized with MPs of serotype 3 are not protected against intranasal infection with serotype 3, strongly suggesting that protection provided by MPs is antibody-dependent.

[0036] (FIG. 11A) Percentage of mice that survived after pneumococcal challenge. 10 mice in the group of "B Cell-deficient mice", 10 mice in the group "Adjuvant", 20 mice per all other groups. \*=P<0.05; \*\*\*\*=P<0.0001. (FIG. 11B) CFUs in lungs of mice after sacrifice. Each dot represents one mouse. \*\*\*=P<0.001. (FIGS. 11C-11E) CFUs in blood of mice. Each line represents one mouse.

## SUMMARY OF THE INVENTION

- [0037] The present invention relates to the following items. The subject matter disclosed in the items below should be regarded disclosed in the same manner as if the subject matter were disclosed in patent claims.
- [0038] 1. An isolated *Streptococcus pneumoniae* membrane vesicle microparticle (MP), wherein said MP comprises:
- [0039] i. the protein Ply at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP;
  - [0040] ii. the protein LytA at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP;
  - [0041] iii. the protein PspC at the level of  $\geq 0.130 \mu\text{g}/\text{mg}$  total protein in the MP; or
  - [0042] iv. the protein RrgB at the level of  $\geq 0.020 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0043] 2. The microparticle according to item 1, further comprising a capsular polysaccharide of a capsular serotype of *Streptococcus pneumoniae*, preferably at a level of  $\geq 0.001$ , more preferably  $\geq 0.01$ , most preferably  $\geq 0.1 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0044] 3. The microparticle according to any of the preceding items, comprising the protein Ply at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0045] 4. The microparticle according to any of the preceding items, comprising the protein Ply at the level of  $\geq 0.15$ , preferably  $\geq 0.2$ , more preferably  $\geq 0.3$ , most preferably  $\geq 0.35 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0046] 5. The microparticle according to any of the preceding items, comprising the protein LytA at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0047] 6. The microparticle according to any of the preceding items, comprising the protein LytA at the level of  $\geq 0.08$ , preferably  $\geq 0.09$ , yet more preferably  $\geq 0.10$ , most preferably  $\geq 0.20 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0048] 7. The microparticle according to any of the preceding items, comprising the protein PspC at the level of  $\geq 0.130 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0049] 8. The microparticle according to any of the preceding items, comprising the protein PspC at the level of  $\geq 0.15$ , preferably  $\geq 0.18$ , more preferably  $\geq 0.20$ , most preferably  $\geq 0.3 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0050] 9. The microparticle according to any of the preceding items, comprising the protein RrgB at the level of  $\geq 0.020 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0051] 10. The microparticle according to any of the preceding items, comprising the protein RrgB at the level of  $\geq 0.022$ , preferably  $\geq 0.025$ , most preferably  $\geq 0.028 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0052] 11. The microparticle according to any of the preceding items, further comprising the protein PhtD.
- [0053] 12. The microparticle according to any of the preceding items, comprising the protein PhtD at a level being at least 2-fold, more preferably at least 2.5-fold compared to *Streptococcus pneumoniae* membrane vesicles obtained in liquid culture in terms of  $\mu\text{g}/\text{mg}$  total protein in the particle or the vesicle, respectively.
- [0054] 13. The microparticle according to any of the preceding items, further comprising the protein RrgA.
- [0055] 14. The microparticle according to any of the preceding items, comprising the protein RrgA at the level of  $\geq 0.02$ , preferably  $\geq 0.05$ , more preferably  $\geq 0.10$ , most preferably  $\geq 0.2 \mu\text{g}/\text{mg}$  total protein in the MP.

- [0056] 15. The microparticle according to any of the preceding items, comprising the protein IgA.
- [0057] 16. The microparticle according to any of the preceding items, comprising the protein IgA at the level of  $>0.02$ , preferably  $\geq 0.05$ , more preferably  $\geq 0.10$ , most preferably  $\geq 0.2 \mu\text{g}/\text{mg}$  total protein in the MP.
- [0058] 17. The microparticle according to any of the preceding items, wherein the protein Ply comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 1.
- [0059] 18. The microparticle according to any of the preceding items, wherein the protein LytA comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 2.
- [0060] 19. The microparticle according to any of the preceding items, wherein the protein PspC comprises a sequence having at least 40%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NOs: 3.
- [0061] 20. The microparticle according to any of the preceding items, wherein the protein PspC comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to any one of SEQ ID NOs: 3-14.
- [0062] 21. The microparticle according to any of the preceding items, wherein the protein RgrB comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 15.
- [0063] 22. The microparticle according to any of the preceding items, wherein the protein PhtD comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 16.
- [0064] 23. The microparticle according to any of the preceding items, comprising the protein RgrA, wherein the protein RgrA comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 17.
- [0065] 24. The microparticle according to any of the preceding items, comprising the protein IgA, wherein the protein IgA comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 18.
- [0066] 25. The microparticle according to any of the preceding items, wherein the MP is 5-300 nm in diameter, preferably 15-175 nm.
- [0067] 26. The microparticle according to any of the preceding items, wherein the MP is 10-125 nm in diameter.
- [0068] 27. The microparticle according to any of the preceding items, wherein the MP is derived from a *Streptococcus pneumoniae* strain selected from any serotype 1 strain, any serotype 3 strain, TIGR4, P1031 and A66, preferably TIGR4.
- [0069] 28. The microparticle according to any of the preceding items, obtainable by:
- [0070] a. Culturing host cells of a *Streptococcus pneumoniae* strain on blood agar plates;
- [0071] b. harvesting the cultured host cells;

- [0072] c. centrifuging the harvested host cells at 17,000 g for 30 minutes at +4°C;
- [0073] d. subjecting the supernatant to filtration through a 0.22 µm filter;
- [0074] e. centrifuging the filtered supernatant at 120,000 g for 2 h at +4°C;
- [0075] f. washing the pellets from e) twice in phosphate-buffered saline with sedimentations at 120,000 g for 2 h at +4°C;
- [0076] g. resuspending the pellet in 1 ml phosphate-buffered saline;
- [0077] h. adjusting the resuspended pellet to 50% (w/v) Optiprep™ density gradient medium in a total volume of 2 ml and overlaying with one fraction of 30% (w/v) Optiprep™ (9 ml) followed by a fraction of 5% (w/v) Optiprep™ (3 ml);
- [0078] i. centrifuging the gradients at 250,000×g for 3 hours at 4° C.;
- [0079] j. collecting the 4 ml top fraction, containing the membrane microparticle; and
- [0080] k. washing the microparticle three times phosphate-buffered saline with sedimentations of 250,000×g for 2 hours at +4° C.
- [0081] 29. A composition comprising a microparticle according to any of items 1-28.
- [0082] 30. The composition according to item 29, wherein the composition is devoid of whole *Streptococcus pneumoniae* cells.
- [0083] 31. The composition according to any of items 29-30, further comprising capsular polysaccharides from *Streptococcus pneumoniae*.
- [0084] 32. The composition according to any of items 29-31, comprising MPs in an amount of 1 µg/ml, preferably 5 µg/ml, more preferably 10 µg/ml, most preferably 100 µg/ml.
- [0085] 33. The composition according to any of items 29-32, further comprising an adjuvant.
- [0086] 34. The composition according to any of items 29-33, further comprising an adjuvant comprising aluminium hydroxide.
- [0087] 35. The composition according to any of items 29-34, being an immunogenic composition.
- [0088] 36. The composition according to any of items 29-35, being an immunogenic composition capable of eliciting opsonophagocytic antibodies against *Streptococcus pneumoniae* when administered to a mammalian host.
- [0089] 37. The composition according to any of items 29-36, being an immunogenic composition capable of eliciting serotype independent antibodies against *Streptococcus pneumoniae* when administered to a mammalian host.
- [0090] 38. The composition according to any of items 29-37, being an immunogenic composition capable of eliciting antibodies against *Streptococcus pneumoniae* serotype 3 when administered to a mammalian host.
- [0091] 39. The composition according to any of items 29-38, further comprising capsular polysaccharides of a capsular serotype of *Streptococcus pneumoniae*.
- [0092] 40. The composition according to any of items 29-39, being a vaccine.
- [0093] 41. The composition according to any of items 29-40, for use in a method for inducing protective immunity against *Streptococcus pneumoniae* in a subject.
- [0094] 42. The composition according to item 41, for use according to item 41, wherein the immunity is protective against a condition selected from pneumococcal sinusitis, pneumococcal otitis, pneumococcal pneumonia and invasive pneumococcal disease including but not limited to pneumococcal sepsis and pneumococcal meningitis, preferably invasive pneumococcal disease.
- [0095] 43. A use of a microparticle according to any of items 1-28 in an immunogenic composition.
- [0096] 44. The use according to item 43, wherein the immunogenic composition is a vaccine.
- [0097] 45. A use of a microparticle according to any of items 1-28 in the manufacture of a vaccine.
- [0098] 46. A method for manufacturing a vaccine, comprising:
- [0099] a. Providing a microparticle according to any of items 1-28;
- [0100] b. Providing an adjuvant;
- [0101] c. Mixing the microparticle and the adjuvant in a suitable vehicle in order to produce a vaccine.
- [0102] 47. A method for producing an isolated *Streptococcus pneumoniae* microparticle according to any of items 1-28, comprising:
- [0103] a. providing a *Streptococcus pneumoniae* host cell;
- [0104] b. culturing said host cell under conditions allowing the production of a microparticle according to any of items 1-28 by the host cell; and
- [0105] c. isolating the microparticle thus produced.
- [0106] 48. The method according to item 47, wherein the host cell is of a strain selected from any serotype 1 strain, any serotype 3 strain, TIGR4, P1031 and A66, preferably TIGR4.
- [0107] 49. The method according to any of items 47-48, wherein the culturing is carried out in solid phase.
- [0108] 50. The method according to any of items 47-49, wherein the culturing is carried out in solid phase on blood agar.
- [0109] 51. The method according to any of items 47-50, wherein isolating the microparticle comprises density gradient centrifugation.

#### DETAILED DESCRIPTION

[0110] The inventors isolated membrane vesicles ( $MV_L$ ) from *S. pneumoniae* of serotype 4 grown in liquid cultures as previously described in the literature (1). They also isolated a novel type of membrane particles termed microparticles (MP) using a method involving growth of the bacteria overnight on blood agar plates. FIG. 1 shows the appearance of the  $MV_L$  and MP using electron microscopy and atomic force microscopy as well as their size distribution. Microparticles from *S. pneumoniae* TIGR4 grown on plates (MP) were smaller in size on average than  $MV_L$  coming from liquid media ( $MV_L$ ). However, MP from *S. pneumoniae* serotype 3 display a different size distribution, containing less of the smallest particles and more of the largest ones.

[0111] Biochemical comparison of  $MV_L$  and MP preparations from TIGR4 revealed different properties for the MP and  $MV_L$ . MP isolated from plates carried certain pneumococcal proteins to a higher extent than liquid grown  $MV_L$ . They were particularly enriched in pneumolysin, the major pore-forming toxin in *S. pneumoniae*, as shown in FIG. 2D.

[0112] The novel MP were characterized using in vitro cell assays, and it was shown that consistent with the quantitated levels, the MP contain more biological pneumolysin activity

than the  $MV_L$  (FIG. 3). It was further shown that the MP are taken up by epithelial cells (FIG. 4A-D) but are not cytotoxic (FIG. 4E). Using human monocyte-derived dendritic cells it was shown that both MP and  $MV_L$  are internalized into DCs, induce maturation of DCs (FIG. 5), and elicit differential cytokine responses (FIG. 6).

[0113] Next, the inventors investigated the potential of MP to protect mice using experimental models of pneumonia and invasive disease (FIGS. 7-11). The results showed that 80% of mice (wild type C57BL/6 mice) immunized with MP from serotype 4 (combined with aluminium hydroxide as adjuvant) survived after an intranasal pneumococcal infec-

method of the present invention is more simple based on bacterial growth combined with high speed-centrifugation. Isolated *Streptococcus pneumoniae* Membrane Vesicles and Microparticles

[0116] As discussed in the Background section, a few types of *Streptococcus pneumoniae* membrane vesicles have been obtained in liquid culture (1). The solid phase culture method used by the inventors resulted in a novel type of particles termed microparticles having different and advantageous properties as discussed above and shown in the Examples. As shown in Table 1, the microparticles differ from the known membrane vesicles,  $MV_L$  in terms of relative protein expression.

TABLE 1

Protein	$MV_L$	MP		Homology in	
		(TIGR4)	MP (Type 3)	P1031*	A66**
Ply	0.055 µg	0.406 µg	0.314 µg	99%	99%
LytA	0.050 µg	0.100 µg	0.266 µg	99%	100%
PspC (PspC3.4)	0.118 µg	0.300 µg	Not determined*	85% (PspC 2.1) 45% (PspC 11.4)	
RrgB	0.016 µg	0.028 µg	Absent	Absent	Absent
PltD	100%	266%	5793%	95%	87%

\*Because of the high genetic diversity of the pspC gene among pneumococcal serotypes it was not possible to use the anti TIGR4 PspC antibody (which was specific for the TIGR4 strain) to detect the serotype 3 PspC

tion caused by serotype 1 (heterologous challenge, cross protection). ELISA analysis showed that mice immunized with MP had a significantly higher MP-specific IgG response in comparison to control mice (treated only with the adjuvant) (FIG. 8). Immunization with serotype 3 and then challenge with serotype 3 led to a 100% protection (FIG. 11). Immunization with serotype 4 MP and then challenge with serotype 3 (a serotype that has been problematic in current vaccines) gave a protection of ca 50% (FIG. 11). Furthermore, immunofluorescence stainings showed that sera from mice immunized with MP from serotype 4 contained antibodies specific against multiple other serotypes, such as serotypes 1, 3 and 6B (FIG. 9).

[0114] In conclusion, it was found that MP protect mice against pneumococcal infection, and, most importantly, such protection is serotype-independent to a significant degree, in contrast to the protection conferred by currently available vaccines (polysaccharide or conjugated vaccines) that are, as mentioned in the background section, strictly serotype-dependent. The MP immunisation furthermore elicited protection against serotype 3 which is a challenge for current vaccines. Indeed, immunization with the currently clinically used vaccine PCV13 provided significantly less protection to mice than MP isolated from serotype 3 (FIG. 11).

[0115] Moreover, for protein based vaccines that are under development today, there is a problem in obtaining opsonophagocytic capability. Importantly, the present data show that using MP as a vaccine affects opsonophagocytosis (Example 3, FIG. 10). Last but not least, available pneumococcal vaccines (PCVs) are extremely important to fight pneumococcal diseases in developing countries. However, the costs to produce current vaccines are very high. An important benefit with the present approach is a drop in production costs since isolation of microparticles does not require high-cost preparations with conjugations, instead the

[0117] Thus, in a first aspect of the present invention, there is provided isolated *Streptococcus pneumoniae* microparticle (MP) (a novel type of pneumococcal membrane vesicle particle), wherein said MP comprise either one, two, three or four of the following proteins at the designated levels:

- [0118] i. the protein Ply at the level of >0.070 µg/mg total protein in the MP;
- [0119] ii. the protein LytA at the level of ≥0.070 µg/mg total protein in the MP;
- [0120] iii. the protein PspC at the level of >0.130 µg/mg total protein in the MP; and/or
- [0121] iv. the protein RrgB at the level of >0.020 µg/mg total protein in the MP.

[0122] The microparticles may further comprise capsular polysaccharides of a capsular serotype of *Streptococcus pneumoniae*, preferably at a level of ≥0.001, more preferably ≥0.01, most preferably ≥0.1 µg/mg total protein in the MP.

[0123] The microparticle may comprise the protein Ply at the level of ≥0.070 µg/mg total protein in the MP. The microparticle may comprise the protein Ply at the level of ≥0.15, preferably ≥0.2, more preferably ≥0.3, most preferably ≥0.35 µg/mg total protein in the MP.

[0124] The microparticle may comprise the protein LytA at the level of ≥0.070 µg/mg total protein in the MP. The microparticle may comprise the protein LytA at the level of ≥0.08, preferably ≥0.09, yet more preferably ≥0.1 µg/mg, most preferably ≥0.2 µg/mg total protein in the MP.

[0125] The microparticle, MP, may comprise the protein PspC at the level of ≥0.130 µg/mg total protein in the MP. The microparticle may comprise comprising the protein PspC at the level of ≥0.15, preferably ≥0.18, more preferably ≥0.2, most preferably ≥0.3 µg/mg total protein in the MP.

[0126] The microparticle may comprise the protein RrgB at the level of ≥0.02 µg/mg total protein in the MP. The

microparticle may comprise the protein RgrB at the level of  $\geq 0.022$ , preferably  $\geq 0.025$ , most preferably  $\geq 0.028 \mu\text{g}/\text{mg}$  total protein in the MP.

[0127] The microparticle may further comprise the protein PhtD. The protein PhtD may be present at a level being at least 2-fold, more preferably at least 2.5-fold compared to *Streptococcus pneumoniae* membrane vesicles obtained in liquid culture in terms of  $\mu\text{g}/\text{mg}$  total protein in the particle or the vesicle, respectively.

[0128] The microparticle may further comprise the protein RrgA. The protein RrgA may be present at the level of  $\geq 0.02$ , preferably  $\geq 0.05$ , more preferably  $\geq 0.10$ , most preferably  $\geq 0.2 \mu\text{g}/\text{mg}$  total protein in the MP.

[0129] The microparticle may further comprise the *Streptococcus pneumoniae* protein IgA. The protein IgA may be present at the level of  $\geq 0.02$ , preferably  $\geq 0.05$ , more preferably  $\geq 0.10$ , most preferably  $\geq 0.2 \mu\text{g}/\text{mg}$  total protein in the MP.

[0130] The protein Ply may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 1.

[0131] The protein LytA may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 2.

[0132] The protein PspC may comprise a sequence having at least 40% or 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NOS: 3.

[0133] The protein PspC may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to any one of SEQ ID NOS: 3-14.

[0134] The protein RgrB may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 15.

[0135] The protein PhtD may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 16.

[0136] The protein RgrA may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 17.

[0137] The protein IgA may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 18.

[0138] Thus, the first aspect encompasses (but is not limited to) embodiments disclosed in Table 2 below.

TABLE 2

Minimum levels of designated proteins in various embodiments of the first aspect. A blank cell indicates that the protein is optional i.e. may be absent or present at any level. Units:  $\mu\text{g}/\text{mg}$  total protein, except for PhtD, where the amount is in comparing to *Streptococcus pneumoniae* membrane vesicles obtained in liquid culture in terms of  $\mu\text{g}/\text{ug}$  total protein in the particle or the vesicle, respectively.

Embodiment#	Ply	LytA	PspC	PhtD	RgrB
1	0.070				
2	0.070	0.070			
3	0.070		0.130		
4	0.070			2-FOLD	

TABLE 2-continued

Minimum levels of designated proteins in various embodiments of the first aspect. A blank cell indicates that the protein is optional i.e. may be absent or present at any level. Units:  $\mu\text{g}/\text{mg}$  total protein, except for PhtD, where the amount is in comparing to *Streptococcus pneumoniae* membrane vesicles obtained in liquid culture in terms of  $\mu\text{g}/\text{ug}$  total protein in the particle or the vesicle, respectively.

Embodiment#	Ply	LytA	PspC	PhtD	RgrB
5	0.070	0.070	0.130		
6	0.070	0.070		2-FOLD	
7	0.070	0.070	0.130	2-FOLD	
8	0.070		0.130	2-FOLD	
9		0.070			
10			0.130		
11				2-FOLD	
12		0.070	0.130		
13			0.130	2-FOLD	
14		0.070	0.130	2-FOLD	
15			0.130	2-FOLD	
16	0.070				0.020
17	0.070	0.070			0.020
18	0.070		0.130		0.020
19	0.070			2-FOLD	0.020
20	0.070	0.070	0.130		0.020
21	0.070	0.070		2-FOLD	0.020
22	0.070	0.070	0.130	2-FOLD	0.020
23	0.070		0.130	2-FOLD	0.020
24		0.070			0.020
25			0.130		0.020
26				2-FOLD	0.020
27		0.070	0.130		0.020
28		0.070		2-FOLD	0.020
29			0.130	2-FOLD	0.020
30			0.130	2-FOLD	0.020
31	0.070				
32	0.070	0.070			
33	0.070		0.130		
34	0.070			2-FOLD	
35	0.070	0.070	0.130		
36	0.070	0.070		2-FOLD	
37	0.070	0.070	0.130	2-FOLD	
38	0.070		0.130	2-FOLD	
39		0.070			
40			0.130		
41				2-FOLD	
42		0.070	0.130		
43		0.070		2-FOLD	
44			0.130	2-FOLD	
45			0.130	2-FOLD	
46	0.070				0.020
47	0.070	0.070			0.020
48	0.070		0.130		0.020
49	0.070			2-FOLD	0.020
50	0.070	0.070	0.130		0.020
51	0.070	0.070		2-FOLD	0.020
52	0.070	0.070	0.130	2-FOLD	0.020
53	0.070		0.130	2-FOLD	0.020
54		0.070			0.020
55			0.130		0.020
56				2-FOLD	0.020
57		0.070	0.130		0.020
58		0.070		2-FOLD	0.020
59			0.130	2-FOLD	0.020
60			0.130	2-FOLD	0.020

[0139] The microparticles may be 5-300 nm in diameter, preferably 10-125 nm in diameter.

[0140] The microparticles may be derived from any *Streptococcus pneumoniae* strain, but is preferably selected from a group consisting of all serotype 3 strains, all serotype 1 strains, TIGR4, P1031 and A66, most preferably TIGR4.

- [0141] The microparticles according to the first aspect may be obtainable by:
- [0142] a. culturing host cells of a *Streptococcus pneumoniae* strain on blood agar plates or other plates;
  - [0143] b. harvesting the cultured host cells;
  - [0144] c. centrifuging the harvested host cells at 17,000 g for 30 minutes at +4° C.;
  - [0145] d. subjecting the supernatant to filtration through a 0.22 µm filter;
  - [0146] e. centrifuging the filtered supernatant at 120,000 g for 2 h at +4° C.;
  - [0147] f. washing the pellets from e) twice in phosphate-buffered saline with sedimentations at 120,000 g for 2 h at +4° C.;
  - [0148] g. resuspending the pellet in 1 ml phosphate-buffered saline;
  - [0149] h. adjusting the resuspended pellet to 50% (w/v) Optiprep™ density gradient medium in a total volume of 2 ml and overlaying with one fraction of 30% (w/v) Optiprep™ (9 ml) followed by a fraction of 5% (w/v) Optiprep™ (3 ml);
  - [0150] i. centrifuging the gradients at 250,000×g for 3 hours at 4° C.;
  - [0151] j. collecting the 4 ml top fraction, containing the membrane microparticles;
  - [0152] k. washing the microparticles three times phosphate-buffered saline with sedimentations of 250,000×g for 2 hours at +4° C.

#### Compositions and Uses

[0153] In a second aspect of the present invention, there is provided a composition comprising a microparticle according to the first aspect. The composition may be devoid of whole *Streptococcus pneumoniae* cells. The composition may optionally comprise capsular polysaccharides from *Streptococcus pneumoniae*.

[0154] The composition according to the second aspect may comprise MP in an amount of 1 µg/ml, preferably 5 µg/ml, more preferably 10 µg/ml, most preferably 100 µg/ml (the concentrations refer to the total protein content of MP per ml).

[0155] The composition may further comprise an adjuvant. The adjuvant preferably comprises aluminium hydroxide.

[0156] The composition may be an immunogenic composition. Preferably, the immunogenic composition is capable of eliciting opsonophagocytic antibodies against *Streptococcus pneumoniae* when administered to a mammalian host. Preferably, the immunogenic composition is capable of eliciting serotype independent antibodies against *Streptococcus pneumoniae* when administered to a mammalian host. Preferably, the immunogenic composition is capable of eliciting antibodies against *Streptococcus pneumoniae* serotype 3 when administered to a mammalian host.

[0157] The composition may be formulated as a vaccine.

[0158] In a third aspect of the present invention, there is provided a composition according to the second aspect, for use in a method for inducing protective immunity against *Streptococcus pneumoniae* in a subject. The third aspect also encompasses a method for inducing protective immunity against *Streptococcus pneumoniae* in a subject in need thereof, comprising administering an effective amount of the composition according to the second aspect to the subject. The third aspect also encompasses the use of a composition according to the second aspect in the manufacture of a vaccine for immunization against *Streptococcus pneumoniae*.

[0159] The protective immunity may be an immunity reducing the likelihood of a condition selected from pneumococcal sinusitis, pneumococcal otitis, pneumococcal pneumonia and invasive pneumococcal disease including but not limited to pneumococcal sepsis and pneumococcal meningitis, preferably invasive pneumococcal disease. Preferably, the subject to be immunized is a young child (e.g. less than 7 years of age) or an elderly person (e.g. over 65 years of age), but also other age groups could be targeted.

[0160] For immunization, the composition may be administered to the subject in various manners known in the art, including but not limited to by way of injection (e.g. intramuscular, intracutaneous, subcutaneous, intravenous), buccal, oral and intranasal administration as well as inhalation.

[0161] In a fourth aspect there is provided a use of a microparticle according to the first aspect in an immunogenic composition. The immunogenic composition may be a vaccine.

[0162] In a fifth aspect, there is provided a use of a microparticle according to the first aspect in the manufacture of a vaccine.

[0163] In a sixth aspect, there is provided a method for manufacturing a vaccine, comprising:

[0164] a. Providing a microparticle according to the first aspect

[0165] b. Providing an adjuvant, such as aluminium hydroxide;

[0166] c. Mixing the microparticle and the adjuvant in a suitable vehicle in order to produce a vaccine.

#### Methods for Producing *Streptococcus pneumoniae* Microparticles

[0167] In a sixth aspect of the present invention there is provided a method for producing an isolated *Streptococcus pneumoniae* microparticle according to the first aspect, comprising:

[0168] a. providing a *Streptococcus pneumoniae* bacterial cell;

[0169] b. culturing said bacterial cell under conditions allowing the production of a microparticle according to the first aspect by the bacterial cell; and

[0170] c. isolating the microparticle thus produced.

[0171] The bacterial cell may be from any pneumococcal strain, but is preferably TIGR4, a serotype 1 and/or a serotype 3 strain.

[0172] The culturing may be carried out using plates or liquid culture. Preferably, the culturing is carried out in solid phase, most preferably on blood agar or other plates. Isolating the microparticles may comprise a density gradient centrifugation step.

#### General Aspects Relating to the Present Disclosure

[0173] The term "comprising" is to be interpreted as including, but not being limited to. All references are hereby incorporated by reference. The arrangement of the present disclosure into sections with headings and subheadings is merely to improve legibility and is not to be interpreted limiting in any way, in particular, the division does not in any way preclude or limit combining features under different headings and subheadings with each other.

#### EXAMPLES

[0174] The following examples are not to be regarded as limiting. For further information on the experimental details, the skilled reader is directed to a separate section titled Materials and Methods.

**Example 1: Isolation and In Vitro Characterization of Novel Pneumococcal Microparticles**

*S. pneumoniae* TIGR4 Produces Microparticles that Differ Depending on Bacterial Growth Conditions.

[0175] Membrane vesicles were isolated and purified from *Streptococcus pneumoniae* serotype 4 strain TIGR4 grown in liquid medium (MV<sub>L</sub>). Microparticles (MP) were isolated from pneumococci grown on blood agar plates. Preparations were analyzed by transmission electron microscopy (TEM) and atomic force microscopy, and revealed spherical bodies surrounded by membranous structures (FIG. 1A). The distribution of the size varied between MV<sub>L</sub>, MP from TIGR4 and MP from serotype 3 (FIG. 1B). In total 503 MV<sub>L</sub>, 378 MP from TIGR4 and 523 MP from serotype 3 were analyzed. As a control we also studied an isogenic pneumolysin mutant strain (TIGR4Δply) and found a similar size distribution. To reveal shedding of MV<sub>L</sub> or MP from the surface of bacteria by TEM we then used an isogenic unencapsulated mutant in TIGR4 (T4R) grown in liquid culture. In the absence of the thick bacterial capsule, MV<sub>L</sub> were clearly visible, emanating from the plasma membrane of *S. pneumoniae* into the extracellular milieu (FIG. 1C-D).

Proteomic Analysis Revealed Differences in Protein Content Between MV<sub>L</sub> from Liquid Culture or MP from Plates

[0176] To analyze the protein content of both pneumococcal preparations, purified MV<sub>L</sub> and MP were subjected to SDS-PAGE analysis. Distinct protein patterns in MV<sub>L</sub> and MPs as compared with proteins present in whole cell lysates or bacterial supernatants (FIG. 2A), were identified. Interestingly, the proteomic content in MV<sub>L</sub> and MP fractions differed, suggesting a differential enrichment of proteins in the different types of particles. We further conducted proteomic analysis using tandem mass spectrometry. In total, in TIGR4, we identified 317 proteins in MV<sub>L</sub> (Table 3), and 216 in MP (Table 4), of which 184 proteins were found in both preparations. 133 proteins were unique to MV<sub>L</sub> and 32 to MP (FIG. 2B). In serotype 3 we identified 462 proteins in MV<sub>L</sub> (Table 5) and 344 in MP (Table 6). All detected proteins were further classified according to their subcellular localization based on the combination of LocateP and GO annotation (FIG. 2C) (11). A majority of the proteins identified in MV<sub>L</sub> and MP were cytosolic proteins, followed by multi-transmembrane proteins, lipoproteins, N-terminally anchored proteins, secretory proteins and cell wall-associated proteins. While MV<sub>L</sub> appeared to be more enriched in cytosolic protein and cell wall-associated proteins than MP from TIGR4, the latter harbored a higher percentage of multi-transmembrane proteins, lipoproteins, N-terminally anchored proteins and secretory proteins.

[0177] We further analyzed the mass spectrometry data in order to determine the presence of 30 known pneumococcal virulence factors in the particles. Both types of particles contained approximately half of these virulence factors present in TIGR4. Just two were unique to MV<sub>L</sub> CbpD (12) and BgaA (13), and one to MP, IgA (10).

[0178] Based on the proteomic results, we next performed immunoblot analysis on specific pneumococcal proteins and virulence factors (FIG. 2D). Among cytosolic proteins we investigated the presence of the cytotoxin pneumolysin (Ply), which is a pore-forming toxin and one of the most important virulence factors in pneumococcal pathogenesis (2), GAPDH as a cytosolic marker (14), and LytA, the major autolysin of *Streptococcus pneumoniae* (3). The most striking observation was the high enrichment of Ply especially in MP, which also contained more LytA than MV<sub>L</sub>. Smaller differences were also observed in the enrichment of virulence factors belonging to other subcellular localizations.

While MV<sub>L</sub> displayed a higher amount of Pneumococcal surface antigen A (PsaA) (15) and Sortase A (SrtA) (16) than MP, the latter contained more Pneumococcal surface protein C (PspC) (17) and Polyhistidine triad protein D (PhtD) (9) (FIG. 2D). The major component of the pneumococcal pilus, RrgB (6), was equally found in both particle preparations. [0179] These findings suggest the presence of a still uncharacterized mechanism for the selective secretion and enrichment of specific proteins and virulence factors of *Streptococcus pneumoniae* in these particle preparations.

**A Biologically Active Pneumolysin was Found in Membrane Microparticles**

[0180] The enrichment of Ply in both MV<sub>L</sub> and MP prompted us to determine whether particle-associated Ply was functional and able to lyse erythrocytes in a hemolytic assay. For both types of particles we observed a dose dependent hemolysis (FIG. 3), however, Ply from MP showed higher activity than from MV<sub>L</sub>, which is consistent with the observation that MP contain more Ply. Notably, hemolysis seemed to be entirely caused by Ply, and particles prepared from a TIGR4 mutant lacking Ply (TIGR4Δply) exhibited no hemolytic activity.

MP are Taken Up by A549 Epithelial Cells, but are not Cytotoxic.

[0181] MV in other Gram-positive bacteria have been described to function as vehicle to deliver vesicle-associated proteins to human cells (18) (19). To test whether pneumococcal MP can deliver pneumococcal proteins, we incubated MP from TIGR4 with A549 lung epithelial cells for 24 hours and visualized Ply and LytA by immunofluorescence staining (FIG. 4A-C). By analyzing orthogonal views of A549 cells treated with MP, we showed by immunofluorescence that MP can be taken up, with Ply and LytA detectable inside the cells (FIG. 4B-C).

[0182] To confirm our observation we further analyzed lysates of A549 cells treated with MP by immunoblotting with anti-Ply antibody (FIG. 4D). In order to minimize extracellular associated particles, treated cells were excessively washed before lysates were made. Consistent with our immunofluorescence results, western blotting revealed a dose dependent increase in Ply further suggesting that MP can be internalized.

[0183] Next we asked whether proteins associated with MV<sub>L</sub> or MP, and in particular Ply, may contribute to cytotoxic effects on cultured human cells. To assess toxicity, A549 cells were treated with different concentrations of MV<sub>L</sub> and MP for 24 hours, stained with fixable viability dye and analyzed by flow cytometry (FIG. 4E). The difference in cell death between samples incubated with MV<sub>L</sub> or MPs and the negative control sample was not statistically significant, suggesting that Ply is not cytotoxic to the cells at concentrations present in MV<sub>L</sub> or MP.

MV<sub>L</sub> and MP are Internalized by Human Monocyte-Derived Dendritic Cells (DCs) and Induce their Maturation.

[0184] Since we found that MP can be internalized by epithelial cells, we then studied potential interactions of MV<sub>L</sub> or MP with innate immune cells, focusing on DCs. First we assessed cytotoxicity by incubating DCs with MV<sub>L</sub> or MP, stained with fixable viability dye and Annexin V, and then analyzed using flow cytometry (FIG. 5A). No significant increase in cytotoxicity was detected in samples incubated with MP, while addition of MV<sub>L</sub> displayed cytotoxic effects at the two highest concentrations used. While both preparations were considerably less cytotoxic than live

bacteria, with 70-80% cytotoxic cells after 24 hours of incubation, more studies are needed to assess the mechanism behind the increased cytotoxicity of MV<sub>L</sub> from liquid cultures. Moreover, the percentage of apoptotic cells did not change after incubation with either of the particle preparations.

[0185] Then we investigated uptake of MP by DCs. First we incubated DCs with MP for 1 hour, lysed the cells and performed SDS-PAGE and immunoblotting on cell lysates to check for presence of Ply. Indeed, Ply was found in the cell lysates (FIG. 5B), suggesting that DCs internalize MP. Next, DCs were challenged for 30 minutes with inhibitors of actin polymerization and phosphatidylinositol 3-kinase (PI3K), cytochalasin and wortmannin, to block active phagocytosis, or with an inhibitor of lipid rafts, methyl-β-cyclodextrin, to block fusion of membranes. Subsequently, cells were incubated with MP for 1 hour, lysed, and the same protocol for immunoblotting was followed. Interestingly, blocking active phagocytosis or membrane fusion resulted in less Ply. However, when all inhibitors were used together, Ply still remained present in the lysates, suggesting either that the particles adhered to the cells or were internalized by another mechanism, not investigated in this study.

[0186] Taken together these data suggest that MP can function as a vehicle to deliver bacterial components to host

cells. Since DCs are professional antigen presenting cells, we then assessed whether MV<sub>L</sub> or MP could influence DC maturation. Cells were incubated for 24 hours with MV<sub>L</sub> or MP, and then stained for MHCII and the co-stimulatory molecule CD86, and analyzed by flow cytometry (FIG. 5C). A dose-dependent increase in the presence of both markers on the surface of DCs was detected when cells were incubated with the particles. MV<sub>L</sub> appeared to induce more maturation than MP, and similar levels of maturation were obtained with LPS.

#### MV<sub>L</sub> and MP Induce Pro-Inflammatory Cytokine Responses in DCs

[0187] Since OMVs from Gram-negative have been shown to trigger a potent innate immune response (20) (21), we studied whether also pneumococcal MV<sub>L</sub> or MP could affect pro-inflammatory cytokine responses. DCs were incubated with MV<sub>L</sub> or MP for 24 hours, and ELISA assays were used on supernatants to determine concentrations of IL-6 (FIG. 6A), IL-8 (FIG. 6B), IL-10 (FIG. 6C) and TNF (FIG. 6D). MP induced release of IL-8 and low levels of TNF, while no induction was seen for IL-6 and IL-10. In contrast, MV<sub>L</sub> induced all four cytokines tested to levels higher than live bacteria. None of the particles induced IL-1β or IL-12 (data not shown).

TABLE 3

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97RH0	76.86	47	350	2615.53	Lipoprotein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0845 PE = 4 SV = 1 - [Q97RH0_STRPN]	Lipid anchored
Q2MGF6	82.45	59	490	1620.74	Lysozyme OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lytC PE = 4 SV = 1 - [Q2MGF6_STRPN]	Secretory (released) (with CS)
Q97NK0	73.39	64	883	1364.43	Aldehyde-alcohol dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2026 PE = 3 SV = 1 - [Q97NK0_STRPN]	Intracellular
P18791	72.99	48	659	1307.17	Oligopeptide-binding protein AmiA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiA PE = 1 SV = 3 - [AMIA_STRPN]	Lipid anchored
Q9L7Q2	55.82	91	1906	1166.89	Zinc metalloprotease ZmpB OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = zmpB PE = 3 SV = 2 - [ZMPB_STRPN]	Multi-trans-membrane
Q97T80	58.3	87	1856	1012.57	Zinc metalloprotease ZmpC OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = zmpC PE = 3 SV = 1 - [ZMPC_STRPN]	LP × TG Cell-wall anchored
Q97NQ8	59.59	75	1225	936.07	DNA-directed RNA polymerase subunit beta' OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpoC PE = 3 SV = 1 - [RPOC_STRPN]	Intracellular
P59213	74.47	48	423	838.2	Maltose/maltodextrin-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = malX PE = 1 SV = 1 - [MALX_STRPN]	Lipid anchored

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
P64022	75.61	46	693	803.77	Elongation factor G OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = fusA PE = 3 SV = 1 - [EFGC_STRPN]	Intracellular
POC2J9	92.99	46	471	753.38	Pneumolysin OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ply PE = 3 SV = 1 - [TACY_STRPN]	Intracellular
Q97N55	75	27	392	729.12	Secreted 45 kd protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = usp45 PE = 4 SV = 1 - [Q97N55_STRPN]	Secretory (released) (with CS)
Q97RY6	42.1	73	2140	715.85	Serine protease, subtilase family OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0641 PE = 4 SV = 1 - [Q97RY6_STRPN]	LP × TG Cell-wall anchored
P64030	68.84	32	398	684.61	Elongation factor Tu OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tuf PE = 3 SV = 1 - [EFTU_STRPN]	Intracellular
Q97NQ7	66.42	61	1203	683.01	DNA-directed RNA polymerase subunit beta OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpoB PE = 3 SV = 1 - [RPOB_STRPN]	Intracellular
Q97PE6	77.38	35	442	645.41	Sugar ABC transporter, sugar-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1683 PE = 4 SV = 1 - [Q97PE6_STRPN]	Lipid anchored
Q97PT1	74.59	45	551	641.52	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1518 PE = 4 SV = 1 - [Q97PT1_STRPN]	Intracellular
Q97RQ0	81.35	26	386	599.79	Branched-chain amino acid ABC transporter, amino acid-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = livJ PE = 4 SV = 1 - [Q97RQ0_STRPN]	Lipid anchored
Q97SV2	62.45	19	277	575.15	50 S ribosomal protein L2 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpIB PE = 3 SV = 1 - [RL2_STRPN]	Intracellular
Q97R51	74.12	32	313	574.61	Foldase protein PrsA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = prsA PE = 3 SV = 1 - [PRSA_STRPN]	Lipid anchored
Q97SV5	59.62	17	208	571.92	50 S ribosomal protein L3 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpIC PE = 3 SV = 1 - [RL3_STRPN]	Intracellular
Q04707	64.12	38	719	564.47	Penicillin-binding protein 1 A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ponA PE = 1 SV = 2 - [PBPA_STRPN]	N-terminally anchored (No CS)
Q97NL1	88.36	26	335	546.25	Glyceraldehyde-3-phosphate dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gap PE = 3 SV = 1 - [Q97NL1_STRPN]	Intracellular
Q97PD6	64.87	46	837	501.57	Protein translocase subunit SecA 1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = secA1 PE = 3 SV = 1 - [SECA1_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
P35592	75.61	44	660	500.22	Oligopeptide-binding protein AlmA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = almA PE = 3 SV = 4 - [ALIA_STRPN]	Lipid anchored
POA4G2	72.49	21	309	482.72	Manganese ABC transporter substrate-binding lipoprotein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = psaA PE = 1 SV = 1 - [MITSA_STRPN]	Lipid anchored
Q54970	61.59	38	591	482.11	Pyruvate oxidase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = spxB PE = 3 SV = 2 - [POXB_STRPN]	Intracellular
Q97R09	68.62	21	341	442.02	Iron-compound ABC transporter, iron compound-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1032 PE = 1 SV = 1 - [Q97R09_STRPN]	Lipid anchored
Q97PU3	67.63	31	278	434.28	Amino acid ABC transporter, amino acid-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = aatB PE = 4 SV = 1 - [Q97PU3_STRPN]	Lipid anchored
POA4C3	72.35	20	217	427.97	30 S ribosomal protein S3 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsC PE = 3 SV = 1 - [RS3_STRPN]	Intracellular
Q97T39	36.42	28	744	407.46	Pneumococcal surface protein A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pspA PE = 4 SV = 1 - [Q97T39_STRPN]	N-terminally anchored (with CS)
I6L8V7	41.79	28	627	381.01	Choline binding protein E OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpE PE = 4 SV = 1 - [I6L8V7_STRPN]	Secretory (released) (with CS)
POA475	54.74	11	137	354.71	50 S ribosomal protein L16 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpIP PE = 1 SV = 1 - [RL16_STRPN]	Intracellular
P66112	51.26	9	119	346.49	50 S ribosomal protein L20 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpIT PE = 3 SV = 1 - [RL20_STRPN]	Intracellular
Q97SV4	56.04	14	207	345.01	50 S ribosomal protein L4 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpID PE = 3 SV = 1 - [RL4_STRPN]	Intracellular
Q97T12	78.99	26	276	332.96	ABC transporter, substrate-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0148 PE = 4 SV = 1 - [Q97T12_STRPN]	Lipid anchored
Q97N74	47.47	31	693	320.12	Choline binding protein A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpA PE = 1 SV = 1 - [Q97N74_STRPN]	N-terminally anchored (with CS)
Q97QS2	58.06	22	434	315.73	Enolase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = eno PE = 1 SV = 1 - [ENO_STRPN]	Intracellular
Q97S57	49.58	34	958	315.37	Translation initiation factor IF-2 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = infB PE = 3 SV = 1 - [IF2_STRPN]	Intracellular
Q97T11	75	27	284	313.6	Lipoprotein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC	Lipid anchored

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
P66359	55.12	10	127	299.97	BAA-334/TIGR4) GN = SP_0149 PE = 3 SV = 1 - [Q97T11_STRPN] 30 S ribosomal protein S11 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsK PE = 3 SV = 1 - [RS11_STRPN]	Intracellular
Q97PT6	68.8	24	468	285.18	ATP synthase subunit beta OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpD PE = 3 SV = 1 - [ATPB_STRPN]	Intracellular
Q97SP2	60.24	18	332	279.63	PTS system, mannose-specific IIAB components OS = <i>Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = manL PE = 4 SV = 1 - [Q97SP2_STRPN]	Intracellular
Q97RZ7	60.08	15	238	278.06	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0629 PE = 1 SV = 1 - [Q97RZ7_STRPN]	Lipid anchored
Q97NL3	55.13	34	731	275.05	Penicillin-binding protein 2A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pbp2A PE = 4 SV = 1 - [Q97NL3_STRPN]	N- terminally anchored (No CS)
Q97PE1	62.92	29	445	269.34	ABC transporter, substrate-binding protein OS = <i>Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1690 PE = 4 SV = 1 - [Q97PE1_STRPN]	Lipid anchored
Q97SX2	81.48	19	189	268.65	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0191 PE = 1 SV = 1 - [Q97SX2_STRPN]	Lipid anchored
Q97RN2	67.79	15	267	248.33	Peptidyl-prolyl cis-trans isomerase, cyclophilin-type OS = <i>Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0771 PE = 3 SV = 1 - [Q97RN2_STRPN]	Lipid anchored
P65887	62.62	25	428	243.93	Adenylosuccinate synthetase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = purA PE = 1 SV = 1 - [PUR_A_STRPN]	Intracellular
Q97QH2	33.7	23	721	242.51	Amino acid ABC transporter, amino acid-binding protein/permease protein OS = <i>Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1241 PE = 1 SV = 1 - [Q97QH2_STRPN]	Multi- trans- membrane
O69076	50.46	25	652	240.51	ATP-dependent zinc metalloprotease FtsH OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4) GN = ftsH PE = 3 SV = 3 - [FTSH_STRPN]	Multi- trans- membrane
Q97N37	62.6	22	393	236.55	Serine protease OS = <i>-Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2239 PE = 1 SV = 1 - [Q97N37_STRPN]	N- terminally anchored (No CS)
Q97T63	60.29	26	491	232.33	ABC transporter, substrate-binding protein OS = <i>-Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0092 PE = 4 SV = 1 - [Q97T63_STRPN]	Lipid anchored
Q97NQ0	68.79	19	330	230.07	Aspartate--ammonia ligase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = asnA PE = 3 SV = 1 - [ASNA_STRPN]	Intracellular
Q97SV1	86.67	12	180	229.35	50 S ribosomal protein L5 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplE PE = 3 SV = 1 - [RL5_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97Q37	69.37	18	271	227.84	Amino acid ABC transporter, amino acid-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1394 PE = 4 SV = 1 - [Q97Q37_STRPN]	Lipid anchored
P66907	42.63	9	380	223.83	Queuine tRNA-ribosyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tgt PE = 3 SV = 1 - [TGT_STRPN]	Intracellular
P59205	43.77	20	658	217.64	Putative endo-beta-N-acetylglucosaminidase OS = <i>Streptococcus pneumoniae</i> (strain ATCC BAA-334/TIGR4) GN = lytB PE = 1 SV = 1 - [LYTB_STRPN]	Secretory (released) (with CS)
Q97SC6	49.35	27	774	216.35	Formate acetyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfl PE = 4 SV = 1 - [Q97SC6_STRPN]	Intracellular
Q97NB5	42.19	22	621	215.44	Choline binding protein PcpA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pcpA PE = 1 SV = 1 - [Q97NB5_STRPN]	Secretory (released) (with CS)
POA2U8	49.58	19	355	215.01	Oligopeptide transport ATP-binding protein AmiE OS = -- <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiE PE = 3 SV = 1 - [AMIE_STRPN]	Intracellular
Q97PA9	39	21	659	212.01	Serine/threonine-protein kinase StkP OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = stkP PE = 1 SV = 1 - [STKP2_STRPN]	Intracellular/TMH start AFTER 60
Q97RF9	56.25	22	400	204.01	Ribosomal protein S1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsA PE = 4 SV = 1 - [Q97RF9_STRPN]	Intracellular
Q97SP4	43.89	12	303	202.88	PTS system, mannose-specific IID component OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0282 PE = 4 SV = 1 - [Q97SP4_STRPN]	Multi-trans-membrane
POA4A7	58.39	11	137	202.86	30 S ribosomal protein S12 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsL PE = 3 SV = 1 - [RS12_STRPN]	Intracellular
Q97Q31	55.82	13	292	202.43	Phosphate-binding protein PstS 1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pstS1 PE = 1 SV = 1 - [PSTS1_STRPN]	Lipid anchored
Q97N56	69.11	20	259	201.44	30 S ribosomal protein S2 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsB PE = 3 SV = 1 - [RS2_STRPN]	Intracellular
Q97RC6	48.66	14	335	201.21	ATP-dependent 6-phosphofructokinase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfkA PE = 3 SV = 1 - [PFKA_STRPN]	Intracellular
Q97QX5	68.02	15	344	200.45	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1069 PE = 1 SV = 1 - [Q97QX5_STRPN]	Secretory (released) (with CS)
Q97NM6	60.64	17	404	197.5	Aminotransferase, class I OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1994 PE = 4 SV = 1 - [Q97NM6_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97N69	65.37	19	335	194.36	Putative ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2197 PE = 4 SV = 1 - [Q97N69_STRPN]	Lipid anchored
Q97NS5	60.95	16	338	193.02	Putative transcriptional regulator OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1942 PE = 4 SV = 1 - [Q97NS5_STRPN]	Secretory (released) (with CS)
P0A4M7	38.35	20	498	189.98	Oligopeptide transport system permease protein AmiC OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiC PE = 3 SV = 1 - [AMIC_STRPN]	Multi-trans-membrane
Q97QD2	56.41	20	523	188.15	Signal recognition particle protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ffh PE = 1 SV = 1 - [Q97QD2_STRPN]	Intracellular
P14677	49.87	26	750	185.33	Penicillin-binding protein 2 × OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pbpX PE = 1 SV = 2 - [PBPX_STRPN]	N-terminally anchored
Q97NQ3	51.88	14	345	183.26	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1967 PE = 4 SV = 1 - [Q97NQ3_STRPN]	N-terminally anchored (No CS)
Q97RW9	59.34	16	332	182.68	Putative pneumococcal surface protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0667 PE = 1 SV = 1 - [Q97RW9_STRPN]	Lipid anchored
Q97RL9	66.17	16	399	180.86	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0785 PE = 4 SV = 1 - [Q97RL9_STRPN]	N-terminally anchored (No CS)
P0A3M9	47.26	14	328	180.34	L-lactate dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = Idh PE = 3 SV = 2 - [LDH_STRPN]	Intracellular
Q97PT4	39.72	17	501	180.17	ATP synthase subunit alpha OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpA PE = 3 SV = 1 - [ATPA_STRPN]	Multi-trans-membrane
Q97QH1	67.48	9	246	175.25	Amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1242 PE = 3 SV = 1 - [Q97QH1_STRPN]	Intracellular
Q97NX9	72.9	19	321	174.95	Iron-compound ABC transporter, iron-compound-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1872 PE = 4 SV = 1 - [Q97NX9_STRPN]	Lipid anchored
Q97QC6	66.09	14	115	173.94	50 S ribosomal protein L19 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplS PE = 3 SV = 1 - [RL19_STRPN]	Intracellular
Q97N99	63.14	18	274	172.57	SPFH domain/Band 7 family OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2156 PE = 4 SV = 1 - [Q97N99_STRPN]	Intracellular
Q97PW1	50.97	17	463	169.74	Peptidoglycan N-acetylglucosamine deacetylase A OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgdA PE = 4 SV = 1 - [Q97PW1_STRPN]	N-terminally anchored (No CS)

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97SE1	36.4	14	555	169.45	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0443 PE = 1 SV = 1 - [Q97SE1_STRPN]	Intracellular
Q97PM5	59.04	16	376	168.92	Sugar ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = msmK PE = 4 SV = 1 - [Q97PM5_STRPN]	Intracellular
Q97SN5	85.81	15	148	165.86	50 S ribosomal protein L13 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplM PE = 3 SV = 1 - [Q97SN5_STRPN]	Intracellular
Q97PQ2	28.88	18	914	162.85	Cation-transporting ATPase, E1-E2 family OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1551 PE = 3 SV = 1 - [Q97PQ2_STRPN]	Multi-trans-membrane
Q97NE4	32.16	21	821	161.61	Penicillin-binding protein 1 B OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pbp1B PE = 4 SV = 1 - [Q97NE4_STRPN]	N-terminally anchored (No CS)
Q97Q62	54.48	20	424	161.35	Psr protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1368 PE = 4 SV = 1 - [Q97Q62_STRPN]	Intracellular/TMH start
P18766	50	13	308	160.11	Oligopeptide transport ATP-binding protein AmiF OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiF PE = 3 SV = 2 - [AMIF_STRPN]	AFTER 60 Intracellular
I6L8U0	42.39	16	394	155.34	UDP-N-acetylglucosamine 2-epimerase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4L PE = 3 SV = 1 - [I6L8U0_STRPN]	Intracellular
POCC08	55.21	15	288	154.74	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = accD PE = 1 SV = 1 - [ACCD_STRPN]	Intracellular
Q97RG9	51.27	21	511	154.04	Sugar ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0846 PE = 3 SV = 1 - [Q97RG9_STRPN]	Intracellular
Q97SJ4	50.28	17	360	153.5	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0355 PE = 4 SV = 1 - [Q97SJ4_STRPN]	Intracellular
P72524	33.82	22	822	148.92	DNA gyrase subunit A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gyrA PE = 3 SV = 3 - [GYRA_STRPN]	Intracellular
P66565	56.16	17	203	148.91	30 S ribosomal protein S4 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsD PE = 3 SV = 1 - [RS4_STRPN]	Intracellular
P0A4G0	41.1	20	652	146.87	Oligopeptide-binding protein AliB OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = alib PE = 3 SV = 1 - [ALIB_STRPN]	Lipid anchored
Q97SG0	57.78	18	424	144.79	Serine-tRNA ligase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = serS PE = 3 SV = 1 - [SYS_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97PF9	51.79	20	419	144.46	Cell division protein FtsZ OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsZ PE = 3 SV = 1 - [Q97PF9_STRPN]	Intracellular
Q97Q34	64.04	17	267	144.16	Phosphate import ATP-binding protein PstB 2 OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pstB2 PE = 3 SV = 1 - [PSTB2_STRPN]	Intracellular
I6L8N1	47.25	14	455	141.81	Acetyl-CoA carboxylase, biotin carboxylase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = accC PE = 4 SV = 1 - [I6L8N1_STRPN]	Intracellular
Q97SR1	45.54	19	617	140.84	Proline-tRNA ligase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = proS PE = 3 SV = 1 - [SYP_STRPN]	Intracellular
Q97SI4	40.88	11	340	140.1	Choline binding protein C OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpC PE = 4 SV = 1 - [Q97SI4_STRPN](with CS)	N-terminally anchored
P0A4D7	40.84	17	524	138.63	DEAD-box ATP-dependent RNA helicase CshA OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cshA PE = 3 SV = 1 - [CSHA_STRPN]	Intracellular
Q97RN3	44.44	17	513	136.66	ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0770 PE = 1 SV = 1 - [Q97RN3_STRPN]	Intracellular
Q97PR0	43.62	17	447	136.28	Asparagine-tRNA ligase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = asnS PE = 1 SV = 1 - [SYN_STRPN]	Intracellular
P22976	40.73	18	658	132.04	Probable transketolase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tkt PE = 3 SV = 2 - [TKT_STRPN]	Intracellular
Q97PX1	34.2	16	459	132	NADH oxidase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = nox PE = 4 SV = 1 - [Q97PX1_STRPN]	Intracellular
O05703	50.5	17	501	129.61	Zinc-binding lipoprotein AdcA OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = adcA PE = 3 SV = 4 - [ADCA_STRPN]	Lipid anchored
P66095	52.4	12	229	128.44	50 S ribosomal protein L1 OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplA PE = 3 SV = 1 - [RL1_STRPN]	Intracellular
Q97RP4	55.65	12	230	127.75	Cell division ABC transporter, ATP-binding protein FtsE OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsE PE = 3 SV = 1 - [Q97RP4_STRPN]	Intracellular
Q97RP3	37.66	12	308	126.99	Cell division protein FtsX OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsX PE = 3 SV = 1 - [Q97RP3_STRPN]	Multi-trans-membrane
Q97N72	38.4	23	810	122.4	ATP-dependent Clp protease, ATP-binding subunit OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2194 PE = 3 SV = 1 - [Q97N72_STRPN]	Intracellular
Q97Q67	43.9	14	344	121.11	Conserved domain protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1363 PE = 4 SV = 1 - [Q97Q67_STRPN]	Multi-trans-membrane

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
P95830	50.79	15	378	120.38	Chaperone protein DnaJ OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnaJ PE = 1 SV = 2 - [DNAJ_STRPN]	Intracellular
Q97SJ6	53.04	8	230	119.51	Capsular polysaccharide biosynthesis protein CpsC OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cpsC PE = 3 SV = 1 - [CPSC_STRPN]	Multi-trans-membrane
P63373	71.03	13	252	116.79	Phosphate import ATP-binding protein PstB 1 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pstB1 PE = 3 SV = 1 - [PSTB1_STRPN]	Intracellular
I6L8Q3	32.35	12	340	114.44	Choline binding protein F OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpF PE = 4 SV = 1 - [I6L8Q3_STRPN] (with CS)	N-terminally anchored
Q97RS8	62.96	16	378	110.09	Lactate oxidase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = IctO-2 PE = 4 SV = 1 - [Q97RS8_STRPN]	Intracellular
Q97N53	70.96	11	272	109.3	Cell shape-determining protein MreC OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = mreC PE = 3 SV = 1 - [Q97N53_STRPN]	N-terminally anchored (No CS)
P0A3M5	41.32	18	680	109.25	Penicillin-binding protein 2 B OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = penA PE = 3 SV = 1 - [PBP2_STRPN]	N-terminally anchored (No CS)
P0A4S1	49.15	9	293	109	Fructose-bisphosphate aldolase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = fba PE = 3 SV = 1 - [ALF_STRPN]	Intracellular
Q97RK0	29.57	13	575	107.28	Septation ring formation regulator EzrA OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ezcA PE = 3 SV = 1 - [EZRA_STRPN]	N-terminally anchored (No CS)
Q97SI9	60.55	7	109	107.21	Cell cycle protein GpsB OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpsB PE = 3 SV = 1 - [GPSB_STRPN]	Intracellular
P63413	36.36	12	396	106.75	Acetate kinase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ackA PE = 3 SV = 1 - [ACKA_STRPN]	Intracellular
P0A2Z2	59.76	10	164	105.18	ATP synthase subunit b OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpF PE = 3 SV = 1 - [ATPF_STRPN]	Intracellular
Q97SQ4	39.74	8	156	103.23	30 S ribosomal protein S7 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsG PE = 3 SV = 1 - [RS7_STRPN]	Intracellular
Q97QK5	48.6	19	607	101.3	Elongation factor 4 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lepA PE = 3 SV = 1 - [LEPA_STRPN]	Intracellular
P65239	39.75	11	322	100.54	Ribose-phosphate pyrophosphokinase 1 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = prs1 PE = 3 SV = 1 - [KPRS1_STRPN]	Intracellular
P95829	36.41	14	607	100.51	Chaperone protein Dnak OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnak PE = 3 SV = 2 - [DNAK_STRPN]	Intracellular
I6L8U5	55.47	11	411	100.01	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97PV1	21.8	6	289	93.98	BAA-334/TIGR4) GN = fabF PE = 3 SV = 1 - [I6L8U5_STRPN] Putative glycerol uptake facilitator protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1491 PE = 3 SV = 1 - [Q97PV1_STRPN]	Multi-trans-membrane
Q2MGH6	15.73	18	1767	93.68	Endo-alpha-N-acetylgalactosaminidase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0368 PE = 1 SV = 1 - [GH101_STRPN]	LP × TG Cell-wall anchored
Q97SQ9	36.05	13	602	92.56	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glmS PE = 3 SV = 3 - [GLMS_STRPN]	Intracellular
Q97P40	53.96	9	202	92.31	Putative general stress protein 24 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1804 PE = 4 SV = 1 - [Q97P40_STRPN]	Intracellular
Q97QE4	25.33	18	1058	90.95	Carbamoyl-phosphate synthase large chain OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = carbB PE = 3 SV = 1 - [CARB_STRPN]	Intracellular
I6L8Q9	40.04	11	457	90.76	Cell division protein ftsA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsA PE = 3 SV = 1 - [I6L8Q9_STRPN]	Intracellular
I6L8S9	45.52	10	413	90.26	D-alanyl-D-alanine carboxypeptidase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = dacA PE = 3 SV = 1 - [I6L859_STRPN]	N-terminally anchored (with CS)
Q97Q48	27.52	18	872	89.81	Alanine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = alaS PE = 3 SV = 1 - [SYA_STRPN]	Intracellular
Q97QD5	64.52	12	186	89.81	LemA protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lemA PE = 1 SV = 1 - [Q97QD5_STRPN]	N-terminally anchored (No CS)
Q97NW2	48.69	13	419	88.69	Sugar ABC transporter, sugar-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = raffE PE = 1 SV = 1 - [Q97NW2_STRPN]	Lipid anchored
I6L8N0	43.01	14	365	88.6	UDP-N-acetylglucosamine-2-epimerase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4l PE = 3 SV = 1 - [I6L8N0_STRPN]	Intracellular
Q97QW4	36.47	13	425	87.93	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1083 PE = 4 SV = 1 - [Q97QW4_STRPN]	Intracellular
Q9FB7	58.43	12	255	86.89	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = accA PE = 1 SV = 1 - [ACCA_STRPN]	Intracellular
P67266	42.42	4	99	86.55	Nucleoid-associated protein SP _1102 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1102 PE = 1 SV = 1 - [Y1102_STRPN]	Intracellular
Q97QM4	24.06	13	719	86.48	Ribonucleoside-diphosphate reductase OS = <i>-Streptococcus</i>	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97PF5	44.17	8	283	85.34	<i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = nrdE PE = 3 SV = 1 - [Q97QM4_STRPN] Putative phosphosugar-binding transcriptional regulator OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1674 PE = 4 SV = 1 - [Q97PF5_STRPN]	Intracellular
P0A3Y3	50.87	10	230	84.95	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpmA PE = 3 SV = 1 - [GPMA_STRPN]	Intracellular
Q97NE1	40.78	4	103	84.35	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2102 PE = 4 SV = 1 - [Q97NE1_STRPN]	Intracellular
P67282	35.58	16	534	83.04	Ribonuclease Y OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rny PE = 3 SV = 1 - [RNY_STRPN]	N-terminally anchored (No CS)
Q97QX6	22.61	14	898	82.38	Phosphoenolpyruvate carboxylase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ppc PE = 3 SV = 1 - [CAPP_STRPN]	Intracellular
Q97SD1	32.63	12	521	81.72	Amino acid ABC transporter, amino acid-binding protein/permease protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0453 PE = 4 SV = 1 - [Q97SD1_STRPN]	Multi-transmembrane
P66419	50.56	8	89	78.12	30 S ribosomal protein S14 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsN PE = 3 SV = 1 - [RS14_STRPN]	Intracellular
P61182	61.4	8	114	77.58	50 S ribosomal protein L22 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplV PE = 1 SV = 1 - [RL22_STRPN]	Intracellular
P66278	33.33	4	66	77.34	50 S ribosomal protein L35 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmL PE = 3 SV = 1 - [RL35_STRPN]	Intracellular
Q97R14	34.57	10	324	76.27	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1027 PE = 4 SV = 1 - [Q97R14_STRPN]	Secretory (released) (with CS)
Q97T52	22.4	12	616	70.86	Putative capsular polysaccharide biosynthesis protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0103 PE = 4 SV = 1 - [Q97T52_STRPN]	Multi-transmembrane
Q97SW2	26.53	13	735	70.68	Anaerobic ribonucleoside-triphosphate reductase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = nrdD PE = 4 SV = 1 - [Q97SW2_STRPN]	Intracellular
Q97SE4	32.3	12	514	70.62	Peptide chain release factor 3 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = prfC PE = 3 SV = 1 - [RF3_STRPN]	Intracellular
Q97PC4	27.24	9	492	68.5	ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1715 PE = 4 SV = 1 - [Q97PC4_STRPN]	Multi-transmembrane

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
I6L8V8	47.33	7	243	68.48	3-oxoacyl-[acyl-carrier protein] reductase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = fabG PE = 3 SV = 1 - [I6L8V8_STRPN]	Intracellular
Q97S28	21.17	11	737	68.26	Polyribonucleotide nucleotidyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pnp PE = 3 SV = 1 - [PNP_STRPN]	Intracellular
Q97RC4	41.38	9	290	67.69	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0899 PE = 4 SV = 1 - [Q97RC4_STRPN]	Lipid anchored
Q97S86	21.65	7	448	65.38	Glutamine synthetase, type I OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glnA PE = 3 SV = 1 - [Q97S86_STRPN]	Intracellular
Q97NH4	41.48	9	352	65.13	Alcohol dehydrogenase, zinc-containing OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2055 PE = 3 SV = 1 - [Q97NH4_STRPN]	Intracellular
POA495	65.79	3	38	64.65	50 S ribosomal protein L36 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmJ PE = 3 SV = 1 - [RL36_STRPN]	Intracellular
Q97ND6	25.72	13	587	64.48	Aspartate-tRNA ligase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = aspS PE = 3 SV = 1 - [SYD_STRPN]	Intracellular
P72525	18.1	10	823	63.42	DNA topoisomerase 4 subunit A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = parC PE = 1 SV = 3 - [PARC_STRPN]	Intracellular
Q97PH2	23.11	12	740	63.08	GTP pyrophosphokinase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = relA PE = 3 SV = 1 - [Q97PH2_STRPN]	Intracellular
Q97T46	32.63	4	95	63.06	Putative bacteriocin OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0109 PE = 4 SV = 1 - [Q97T46_STRPN]	Secretory
I6L8V0	42.17	11	351	60.21	Capsular polysaccharide biosynthesis protein Cps4J OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cap4J PE = 4 SV = 1 - [I6L8V0_STRPN]	Intracellular
Q97RQ6	23.84	6	281	60.06	DegV domain-containing protein SP_0742 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0742 PE = 3 SV = 1 - [Y742_STRPN]	Intracellular
Q97SR2	35.08	9	419	60.03	Putative zinc metalloprotease SP_0263 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0263 PE = 3 SV = 1 - [Y263_STRPN]	Multi-trans-membrane
Q97PB8	15.63	8	627	59.73	PTS system IIABC components OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1722 PE = 4 SV = 1 - [Q97PB8_STRPN]	Multi-trans-membrane
Q97QE6	41.47	9	340	58.95	Alcohol dehydrogenase, zinc-containing OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1270 PE = 4 SV = 1 - [Q97QE6_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97P07	34.56	10	408	58.93	Putative capsular polysaccharide biosynthesis protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1837 PE = 3 SV = 1 - [Q97P07_STRPN]	Intracellular
Q97T09	40.23	9	353	57.99	Methionine import ATP-binding protein MetN OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = metN PE = 3 SV = 1 - [METN_STRPN]	Intracellular
Q97NP5	18.83	7	308	57.43	Membrane protein insertase YidC 1 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = yidC1 PE = 3 SV = 1 - [YIDC1_STRPN]	Multi-trans-membrane
POA451	41.49	12	388	57.14	Protein RecA OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = recA PE = 2 SV = 1 - [RECA_STRPN]	Intracellular
Q97SU7	51.12	5	178	56.93	50 S ribosomal protein L6 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplF PE = 3 SV = 1 - [RL6_STRPN]	Intracellular
Q97NE2	18.49	9	687	56.54	Cation-transporting ATPase, E1-E2 family OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2101 PE = 3 SV = 1 - [Q97NE2_STRPN]	Intracellular
Q97SN4	32.31	6	130	56.22	30 S ribosomal protein S9 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsL PE = 3 SV = 1 - [RS9_STRPN]	Intracellular
P66392	51.24	7	121	56.15	30 S ribosomal protein S13 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsM PE = 3 SV = 1 - [RS13_STRPN]	Intracellular
Q97S10	23.51	11	553	55.98	Ribonuclease J OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rnj PE = 3 SV = 1 - [Q97S10_STRPN]	Intracellular
Q97PG0	20.99	5	262	55.94	Cell division protein DivIVA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = divIVA PE = 4 SV = 1 - [Q97PG0_STRPN]	Intracellular
P35597	17.61	9	778	55.84	Probable cation-transporting ATPase exp7 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = exp7 PE = 3 SV = 2 - [EXP7_STRPN]	Multi-trans-membrane
Q97SJ2	43.52	12	409	55.75	Capsular polysaccharide biosynthesis protein Cps4K OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4K PE = 4 SV = 1 - [Q97SJ2_STRPN]	Intracellular
Q97PI4	29.98	12	647	55.52	Threonine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = thrS PE = 1 SV = 1 - [SYT_STRPN]	Intracellular
Q97QW8	25.81	8	434	55.41	GTPase Obg OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = obg PE = 3 SV = 1 - [OBG_STRPN]	Intracellular
P67293	43.9	3	82	55.21	UPF0154 protein SP_1882 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1882 PE = 3 SV = 1 - [Y1882_STRPN]	N-terminally anchored (No CS)

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
POA4L9	20.52	10	648	54.97	DNA gyrase subunit B OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gyrB PE = 3 SV = 1 - [GYRB_STRPN]	Intracellular
Q97SJ8	33.2	11	494	54.78	UPF0371 protein SP_0341 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0341 PE = 3 SV = 1 - [Y341_STRPN]	Intracellular
Q54869	24.16	10	563	54.43	Arginine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = argS PE = 1 SV = 2 - [SYR_STRPN]	Intracellular
P35595	14.88	7	726	53.64	PTS system glucose-specific EIIIBA component OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = exp5 PE = 3 SV = 2 - [PTG3C_STRPN]	Multi-trans-membrane
Q97SI7	22.41	7	464	53.36	Mid-cell-anchored protein Z OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = mapZ PE = 1 SV = 1 - [Q97SI7_STRPN]	Intracellular
POA4B5	55.91	7	93	52.72	30 S ribosomal protein S19 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsS PE = 3 SV = 1 - [RS19_STRPN]	Intracellular
Q97SU3	23.29	4	146	52.09	50 S ribosomal protein L15 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpLO PE = 3 SV = 1 - [RL15_STRPN]	Intracellular
Q97RV5	27.57	11	613	51.74	Elongation factor Tu family protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0681 PE = 4 SV = 1 - [Q97RV5_STRPN]	Intracellular
Q97NW9	8.4	5	655	50.97	Trehalose PTS system, IIABC components OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1884 PE = 4 SV = 1 - [Q97NW9_STRPN]	Multi-trans-membrane
Q97QE7	41.6	9	262	49.97	Choline kinase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pck PE = 4 SV = 1 - [Q97QE7_STRPN]	Intracellular
P67595	31.96	8	341	48.09	Tryptophan-tRNA ligase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = trpS PE = 3 SV = 1 - [SYW_STRPN]	Intracellular
P66581	55.49	6	164	47.77	30 S ribosomal protein S5 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsE PE = 3 SV = 1 - [RS5_STRPN]	Intracellular
Q97R57	16.33	10	784	47.19	Ribonuclease R OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rnr PE = 3 SV = 1 - [Q97R57_STRPN]	Intracellular
Q97RP0	21.03	7	447	46.86	DEAD-box ATP-dependent RNA helicase CshB OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cshB PE = 3 SV = 1 - [Q97RP0_STRPN]	Intracellular
POA3R1	22.65	9	649	46.48	DNA mismatch repair protein HexB OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = hexB PE = 3 SV = 1 - [HEXB_STRPN]	Intracellular
POA3R3	18.48	11	844	45.87	DNA mismatch repair protein HexA OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = hexA PE = 3 SV = 1 - [HEXA_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97SF6	27.97	7	261	45.73	Enoyl-CoA hydratase/isomerase family protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0415 PE = 4 SV = 1 - [Q97SF6_STRPN]	Intracellular
P66708	45.98	9	311	45.6	DNA-directed RNA polymerase subunit alpha OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpoA PE = 3 SV = 1 - [RPOA_STRPN]	Intracellular
I6L858	28.69	9	481	45.33	Capsular polysaccharide biosynthesis protein Cps4A OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4A PE = 4 SV = 1 - [I6L8S8_STRPN]	Multi-trans-membrane
Q97N43	19.51	7	492	45.08	Inosine-5'-monophosphate dehydrogenase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = guaB PE = 3 SV = 1 - [Q97N43_STRPN]	Intracellular
I6L8W8	16.87	6	332	44.57	Choline binding protein J OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpJ PE = 4 SV = 1 - [I6L8W8_STRPN] (with CS)	N-terminally anchored
Q2MGG2	27.62	7	467	44.4	Serine protease, subtilase family OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1954 PE = 4 SV = 1 - [Q2MGG2_STRPN]	Secretory (released) (with CS)
Q97SP0	42.22	7	270	44.36	Cof family protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0286 PE = 4 SV = 1 - [Q97SP0_STRPN]	Intracellular
Q97PG9	38.75	7	240	44.16	Manganese ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = psaB PE = 3 SV = 1 - [Q97PG9_STRPN]	Intracellular
Q97T72	31.67	6	221	44.14	Potassium uptake protein, Trk family OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0079 PE = 4 SV = 1 - [Q97T72_STRPN]	N-terminally anchored (No CS)
Q97RS9	21.98	7	496	44.07	Lysine-ttRNA ligase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lysS PE = 1 SV = 2 - [SYK_STRPN]	Intracellular
Q97PK5	27.22	2	158	43.99	Uncharacterized protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1604 PE = 4 SV = 1 - [Q97PK5_STRPN]	N-terminally anchored (No CS)
Q97RV8	38.89	6	126	43.74	Uncharacterized protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0678 PE = 4 SV = 1 - [Q97RV8_STRPN]	N-terminally anchored (No CS)
P06653	26.1	6	318	43.37	Autolysin OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lytA PE = 1 SV = 2 - [ALYS_STRPN]	Intracellular
P63791	27.8	8	410	42.54	ATP-dependent Clp protease ATP-binding subunit ClpX OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = clpX PE = 3 SV = 1 - [CLPX_STRPN]	Intracellular
Q97Q36	37.33	7	217	42.45	Phosphate-specific transport system accessory protein PhoU OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1395 PE = 3 SV = 1 - [Q97Q36_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97R16	32.06	10	418	42.38	Serine hydroxymethyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glyA PE = 3 SV = 1 - [GLYA_STRPN]	Intracellular
Q97NM1	38.99	9	336	42.33	Catabolite control protein A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ccpA PE = 4 SV = 1 - [Q97NM1_STRPN]	Intracellular
Q97PQ8	42.66	6	143	40.36	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1545 PE = 4 SV = 1 - [Q97PQ8_STRPN]	Intracellular
Q97SU6	59.32	7	118	40.13	50 S ribosomal protein L18 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplR PE = 3 SV = 1 - [RL18_STRPN]	Intracellular
Q97RC9	30.77	3	156	40.01	Arginine repressor OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = argR PE = 3 SV = 1 - [Q97RC9_STRPN]	Intracellular
Q97RC5	25.15	8	501	39.74	Pyruvate kinase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyk PE = 3 SV = 1 - [Q97RC5_STRPN]	Intracellular
Q97PM1	45.04	7	262	38.81	GTP-sensing transcriptional pleiotropic repressor CodY OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = codY PE = 1 SV = 1 - [CODY_STRPN]	Intracellular
I6L8N6	28.91	5	211	38.64	Capsular polysaccharide biosynthesis protein Cps4E OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4E PE = 4 SV = 1 - [I6L8N6_STRPN]	N-terminally anchored (No CS)
Q97R36	38.38	7	185	38.61	Thioredoxin family protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1000 PE = 1 SV = 1 - [Q97R36_STRPN]	Lipid anchored
Q97S93	17.76	7	535	37.74	CTP synthase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyrG PE = 3 SV = 1 - [PYRG_STRPN]	Intracellular
Q97QW1	20.71	9	763	37.7	DNA helicase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pcrA PE = 4 SV = 1 - [Q97QW1_STRPN]	Intracellular
P63544	51.76	6	170	37.59	Adenine phosphoribosyltransferase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = apt PE = 1 SV = 1 - [APT_STRPN]	Intracellular
P65241	18.18	5	319	37.57	Ribose-phosphate pyrophosphokinase 2 OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = prs2 PE = 3 SV = 1 - [KPRS2_STRPN]	Intracellular
Q97RE5	17.08	6	650	37.17	PTS system, fructose specific IIABC components OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0877 PE = 4 SV = 1 - [Q97RE5_STRPN]	Multi-trans-membrane
P35596	21.38	9	608	37.12	Alpha-glycerophosphate oxidase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glpO PE = 3 SV = 2 - [GLPO_STRPN]	N-terminally anchored (No CS)
Q97R12	19.52	7	543	37.11	Uncharacterized RNA methyltransferase SP_1029 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
P64166	12.91	7	767	36.81	GN = SP_1029 PE = 3 SV = 1 - [Y1029_STRPN] DNA translocase Ftsk OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsK PE = 3 SV = 1 - [FTSK_STRPN]	Multi-trans-membrane
P35594	17.15	10	752	36.7	ATP-dependent Clp protease ATP-binding subunit ClpE OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = clpE PE = 3 SV = 2 - [CLPE_STRPN]	Intracellular
P64072	53.33	8	195	36.63	Probable GTP-binding protein EngB OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = engB PE = 3 SV = 1 - [ENG_B_STRPN]	Intracellular
Q97TC4	31.11	5	180	36.56	Hypoxanthine-guanine phosphoribosyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = hpt PE = 3 SV = 1 - [HPRT_STRPN]	Intracellular
Q97PU2	37.8	4	209	36.45	Amino acid ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1501 PE = 3 SV = 1 - [Q97PU2_STRPN]	Intracellular
POA3S3	38.69	6	274	36.43	DNA-entry nuclease OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = endA PE = 1 SV = 1 - [NUCE_STRPN] (No CS)	N-terminally anchored
I6L8N9	25.49	8	506	36.2	Choline transporter OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = proWX PE = 3 SV = 1 - [I6L8N9_STRPN]	Multi-trans-membrane
Q97PQ5	25.99	8	531	36.19	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1548 PE = 4 SV = 1 - [Q97PQ5_STRPN]	Multi-trans-membrane
Q97NI7	28.66	6	328	36.13	Putative jag protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2040 PE = 4 SV = 1 - [Q97NI7_STRPN]	Intracellular
P63742	19.32	7	502	36.12	Glycerol kinase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glpK PE = 3 SV = 1 - [GLPK_STRPN]	Intracellular
Q97NT7	32.91	4	158	36	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1926 PE = 4 SV = 1 - [Q97NT7_STRPN]	Multi-trans-membrane
P64297	23.85	7	520	35.92	GMP synthase [glutamine-hydrolyzing] OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = guaA PE = 3 SV = 1 - [GUAA_STRPN]	Intracellular
Q97R84	28.38	7	444	35.81	Methylenetetrahydrofolate--tRNA-(uracil-5')-methyltransferase TrmFO OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tmFO PE = 3 SV = 2 - [TRMFO_STRPN]	Intracellular
Q97PK2	19.17	5	339	35.39	UDP-glucose 4-epimerase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = galE-1 PE = 3 SV = 1 - [Q97PK2_STRPN]	Intracellular
Q97RP6	41.53	6	236	35.16	Branched-chain amino acid ABC transporter, ATP-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97NM3	30.74	10	462	35.12	(strain ATCC BAA-334/TIGR4) GN = livF PE = 3 SV = 1 - [Q97RP6_STRPN] Cof family protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1997 PE = 4 SV = 1 - [Q97NM3_STRPN]	Intracellular
Q97S34	10.86	7	801	34.77	Phenylalanine-tRNA ligase beta subunit OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pheT PE = 3 SV = 1 - [SYFB_STRPN]	Intracellular
Q97NQ4	31.38	9	427	34.05	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = murA1 PE = 3 SV = 1 - [MURAI_STRPN]	Intracellular
Q97PY0	33.2	6	247	34.04	Amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1460 PE = 3 SV = 1 - [Q97PY0_STRPN]	Intracellular
Q97RQ3	44.5	6	209	33.91	Uracil phosphoribosyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = upp PE = 3 SV = 1 - [UPP_STRPN]	Intracellular
Q97QP0	15.87	7	567	33.86	Dihydrolipoyl dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1161 PE = 4 SV = 1 - [Q97QP0_STRPN]	Intracellular
P64062	24.77	8	436	33.49	GTPase Der OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = der PE = 3 SV = 1 - [DER_STRPN]	Intracellular
Q97N48	22.83	6	276	33.26	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2223 PE = 4 SV = 1 - [Q97N48_STRPN]	Intracellular/ TMH start AFTER 60
Q97SJ5	22.98	7	409	32.72	Capsular polysaccharide biosynthesis protein Cps4F OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4F PE = 4 SV = 1 - [Q97SJ5_STRPN]	Multi-trans-membrane
P65832	36.73	6	275	32.69	Pur operon repressor OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = purR PE = 3 SV = 1 - [PURR_STRPN]	Intracellular
P63384	12.09	9	943	31.63	UvrABC system protein A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = uvrA PE = 3 SV = 1 - [UVRA_STRPN]	Intracellular
I6L8W3	33.88	8	242	31.53	Choline transporter OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = proV PE = 3 SV = 1 - [I6L8W3_STRPN]	Intracellular
Q9AH2D	40.97	8	227	31.44	Tyrosine-protein kinase CpsD OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cpsD PE = 3 SV = 1 - [CPSE_STRPN]	Intracellular
O08397	23.84	8	453	31.17	Chromosomal replication initiator protein DnaA OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnaA PE = 3 SV = 2 - [DNAA_STRPN]	Intracellular
Q97PT5	23.63	7	292	30.59	ATP synthase gamma chain OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpG PE = 3 SV = 1 - [ATPG_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97PJ1	22.09	5	249	30.29	Acyltransferase family protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1624 PE = 4 SV = 1 - [Q97PJ1_STRPN]	Multi-trans-membrane
Q97NF6	14.36	6	564	30.07	ABC transporter, ATP-binding/permease protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2075 PE = 3 SV = 1 - [Q97NF6_STRPN]	Multi-trans-membrane
Q97PW8	18.36	6	414	30.06	Putative oxidoreductase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1472 PE = 4 SV = 1 - [Q97PW8_STRPN]	Intracellular
Q97QT0	15.61	5	474	29.86	Glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gapN PE = 3 SV = 1 - [Q97QT0_STRPN]	Intracellular
Q97PW6	12.24	6	678	29.5	Glycine-tRNA ligase beta subunit OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glyS PE = 3 SV = 1 - [SYGB_STRPN]	Intracellular
Q97T93	25.93	8	432	29.32	Adenylosuccinate lyase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = purB PE = 3 SV = 1 - [Q97T93_STRPN]	Intracellular
Q97RG3	5.68	2	229	29.15	Putative membrane protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0858 PE = 4 SV = 1 - [Q97RG3_STRPN]	Multi-trans-membrane
Q97TA6	12.42	3	330	28.21	Phosphate acyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = plsX PE = 3 SV = 1 - [PLSX_STRPN]	Intracellular
Q97TA8	19.02	6	389	27.91	Aromatic amino acid aminotransferase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = araT PE = 3 SV = 1 - [Q97TA8_STRPN]	Intracellular
P0A3B7	23.99	5	346	27.48	Elongation factor Ts OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tsf PE = 1 SV = 2 - [EFTS_STRPN]	Intracellular
I6L8Q7	20.99	4	324	27.27	Enoyl-(Acyl-carrier-protein) reductase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = fabE PE = 4 SV = 1 - [I6L8Q7_STRPN]	Intracellular
Q97SG5	9.38	5	778	26.77	Endonuclease MutS2 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = mutS2 PE = 3 SV = 1 - [MUTS2_STRPN]	Intracellular
Q97S79	15.4	5	487	26.69	Type I restriction-modification system, M subunit OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = hsdM PE = 4 SV = 1 - [Q97S79_STRPN]	Intracellular
Q97SF8	12.78	4	454	26.54	Aspartokinase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0413 PE = 3 SV = 1 - [Q97SF8_STRPN]	Intracellular
Q97S89	19.1	5	398	26.46	Phosphoglycerate kinase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgk PE = 3 SV = 1 - [PGK_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97QH0	22.14	6	429	26.44	Signal recognition particle receptor FtsY OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsY PE = 1 SV = 1 - [Q97QH0_STRPN]	Intracellular
P0CB59	5.83	3	326	26.31	Phospho-N-acetylmuramoyl-pentapeptide-transferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = mraY PE = 3 SV = 1 - [MRAY_STRPN]	Multi-trans-membrane
Q97P92	23.64	5	368	26.11	GTP-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1749 PE = 4 SV = 1 - [Q97P92_STRPN]	Intracellular
P63733	20.06	6	359	25.98	Carbamoyl-phosphate synthase small chain OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = carA PE = 3 SV = 1 - [CARA_STRPN]	Intracellular
Q97SP1	28.61	6	339	25.89	Alcohol dehydrogenase, zinc-containing OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0285 PE = 3 SV = 1 - [Q97SP1_STRPN]	Intracellular
Q97PP2	19.92	4	256	25.75	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1561 PE = 1 SV = 1 - [Q97PP2_STRPN]	Multi-trans-membrane
P66339	48.04	4	102	25.74	30 S ribosomal protein S10 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsJ PE = 3 SV = 1 - [RS10_STRPN]	Intracellular
POA335	13.52	6	540	25.62	60 kDa chaperonin OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = groL PE = 1 SV = 1 - [CH60_STRPN]	Intracellular
O07344	15.69	3	204	25.56	Signal peptidase I OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lepB PE = 3 SV = 2 - [LEP_STRPN]	N-terminally anchored (No CS)
Q97PP5	41.41	3	128	25.55	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1558 PE = 1 SV = 1 - [Q97PP5_STRPN]	Intracellular
Q97RP7	15.75	2	254	25.33	Branched-chain amino acid ABC transporter, ATP-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = livG PE = 3 SV = 1 - [Q97RP7_STRPN]	Intracellular
Q97Q12	13.37	5	486	24.99	Nicotinate phosphoribosyltransferase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1421 PE = 3 SV = 1 - [Q97Q12_STRPN]	Intracellular
P67506	26.07	5	211	24.79	tRNA (guanine-N(7)-)-methyltransferase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = trmB PE = 1 SV = 1 - [TRMB_STRPN]	Intracellular
P66155	17.74	2	62	24.21	50 S ribosomal protein L28 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmB PE = 3 SV = 1 - [RL28_STRPN]	Intracellular
P66200	71.25	3	80	23.95	50 S ribosomal protein L31 type B OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmE2 PE = 3 SV = 1 - [RL31B_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97NH2	17.69	5	605	23.83	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2057 PE = 4 SV = 1 - [Q97NH2_STRPN]	Multi-trans-membrane
Q97PP4	12.89	4	450	23.67	Phosphoglucosamine mutase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glmM PE = 3 SV = 1 - [GLMM_STRPN]	Intracellular
Q97R44	50.43	4	115	23.65	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0990 PE = 4 SV = 1 - [Q97R44_STRPN]	Intracellular/TMH start AFTER 60
Q97QB4	15.18	4	448	23.57	Glutamate dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gdhA PE = 3 SV = 1 - [Q97QB4_STRPN]	Intracellular
Q97SW6	23.68	2	152	23.55	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0198 PE = 1 SV = 1 - [Q97SW6_STRPN]	Lipid anchored
Q97PW5	16.07	4	305	23.51	Glycine-tRNA ligase alpha subunit OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glyQ PE = 3 SV = 1 - [SYGA_STRPN]	Intracellular
Q97Q69	19.63	5	428	23.47	Homoserine dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = hom PE = 3 SV = 1 - [Q97Q69_STRPN]	Intracellular
Q97QT2	12.27	6	652	23.37	DNA ligase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ligA PE = 3 SV = 1 - [DNLJ_STRPN]	Intracellular
POA4B3	33.72	3	86	23.26	30 S ribosomal protein S17 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsQ PE = 3 SV = 1 - [RS17_STRPN]	Intracellular
Q97NG0	14.92	4	449	23.07	Glucose-6-phosphate isomerase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgi PE = 3 SV = 1 - [G6PI_STRPN]	Intracellular
Q97T58	14.41	3	354	22.73	Conserved domain protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0097 PE = 4 SV = 1 - [Q97T58_STRPN]	Multi-trans-membrane
Q97P32	16.46	4	407	22.04	Tryptophan synthase beta chain OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = trpB PE = 3 SV = 1 - [TRPB_STRPN]	Intracellular
Q97QX3	28.57	5	252	21.94	ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1071 PE = 3 SV = 1 - [Q97QX3_STRPN]	Intracellular
Q97ST6	32.03	2	128	21.84	50 S ribosomal protein L17 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplQ PE = 3 SV = 1 - [RL17_STRPN]	Intracellular
Q97RB6	8.74	5	709	21.38	Putative transcriptional regulator OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0908 PE = 4 SV = 1 - [Q97RB6_STRPN]	Intracellular

TABLE 3-continued

Proteins identified in MV <sub>L</sub> from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97NB9	17.91	4	335	21.02	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2132 PE = 4 SV = 1 - [Q97NB9_STRPN]	Multi-trans-membrane
P66524	37.93	3	58	20.52	30 S ribosomal protein S21 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsU PE = 3 SV = 1 - [RS21_STRPN]	Intracellular
Q97SI6	14.98	5	474	20.22	6-phosphogluconate dehydrogenase, decarboxylating OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gnd PE = 3 SV = 1 - [Q97SI6_STRPN]	Intracellular
Q97R47	30.45	4	266	20.09	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0987 PE = 1 SV = 1 - [Q97R47_STRPN]	N-terminally anchored (No CS)

TABLE 4

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97RH0	76.86	46	350	2085.84	Lipoprotein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0845 PE = 4 SV = 1 - [Q97RH0_STRPN]	Lipid anchored
Q97PE6	90.27	56	442	1745.9	Sugar ABC transporter, sugar-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1683 PE = 4 SV = 1 - [Q97PE6_STRPN]	Lipid anchored
Q97T39	61.29	50	744	1569.47	Pneumococcal surface protein A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pspA PE = 4 SV = 1 - [Q97T39_STRPN]	N-terminally anchored (with CS)
P59213	82.74	62	423	1500.08	Maltose/maltodextrin-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = malX PE = 1 SV = 1 - [MALX_STRPN]	Lipid anchored
Q97N74	53.25	47	693	1294.91	Choline binding protein A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpA PE = 1 SV = 1 - [Q97N74_STRPN]	N-terminally anchored (with CS)
Q97R51	87.86	45	313	1201.93	Foldase protein PrsA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = prsA PE = 3 SV = 1 - [PRSA_STRPN]	Lipid anchored
Q54970	70.05	54	591	1000.77	Pyruvate oxidase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = spxB PE = 3 SV = 2 - [POXB_STRPN]	Intracellular
Q2MGF6	75.51	50	490	957.09	Lysozyme OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lytC PE = 4 SV = 1 - [Q2MGF6_STRPN]	Secretory (released) (with CS)
Q97PT1	77.13	52	551	947.11	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1518 PE = 4 SV = 1 - [Q97PT1_STRPN]	Intracellular/ TMH start AFTER 60
Q97QS2	75.35	31	434	783.92	Enolase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC	Intracellular

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
P64022	76.77	48	693	749.91	BAA-334/TIGR4) GN = eno PE = 1 SV = 1 - [ENO_STRPN] Elongation factor G OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = fusA PE = 3 SV = 1 - [EFG_STRPN]	Intracellular
Q04707	72.6	47	719	715.79	Penicillin-binding protein 1A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ponA PE = 1 SV = 2 - [PBPA_STRPN]	N-terminally anchored (No CS)
POC2J9	87.47	49	471	703.79	Pneumolysin OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ply PE = 3 SV = 1 - [TACY_STRPN]	Intracellular
Q97PE1	73.93	35	445	655.97	ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1690 PE = 4 SV = 1 - [Q97PE1_STRPN]	Lipid anchored
Q97NQ8	61.8	68	1225	650.84	DNA-directed RNA polymerase subunit beta' OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpoC PE = 3 SV = 1 - [RPOC_STRPN]	Intracellular
Q97NL1	79.1	29	335	611.32	Glyceraldehyde-3-phosphate dehydrogenase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gap PE = 3 SV = 1 - [Q97NL1_STRPN]	Intracellular
Q97T12	82.97	33	276	589.52	ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0148 PE = 4 SV = 1 - [Q97T12_STRPN]	Lipid anchored
Q97T63	83.3	36	491	576.45	ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0092 PE = 4 SV = 1 - [Q97T63_STRPN]	Lipid anchored
I6L8V7	44.02	33	627	457.19	Choline binding protein E OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpE PE = 4 SV = 1 - [I6L8V7_STRPN]	Secretory (released) (with CS)
Q97NQ7	55.61	50	1203	449.03	DNA-directed RNA polymerase subunit beta OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpoB PE = 3 SV = 1 - [RPOB_STRPN]	Intracellular
Q97SV2	64.62	18	277	395.51	50S ribosomal protein L2 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplB PE = 3 SV = 1 - [RL2_STRPN]	Intracellular
P59205	60.33	29	658	388.39	Putative endo-beta-N-acetylglucosaminidase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lytB PE = 1 SV = 1 - [LYTB_STRPN]	Secretory (released) (with CS)
O69076	55.98	31	652	385.64	ATP-dependent zinc metalloprotease FtsH OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsH PE = 3 SV = 3 - [FTSH_STRPN]	Multi-transmembrane
Q97SP2	67.47	25	332	369.57	PTS system, mannose-specific IIAB components OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = manL PE = 4 SV = 1 - [Q97SP2_STRPN]	Intracellular
Q97T11	66.55	23	284	361.41	Lipoprotein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0149 PE = 3 SV = 1 - [Q97T11_STRPN]	Lipid anchored
I6L8Q3	52.65	17	340	354.19	Choline binding protein F OS = <i>Streptococcus pneumoniae</i> serotype 4	N-terminally

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97NX9	80.69	25	321	336.18	(strain ATCC BAA-334/TIGR4) GN = cbpF PE = 4 SV = 1 - [I6L8Q3_STRPN] Iron-compound ABC transporter, iron-compound-binding protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1872 PE = 4 SV = 1 - [Q97NX9_STRPN]	anchored (with CS) Lipid anchored
P64030	57.29	25	398	336.06	Elongation factor Tu OS = - <i>Streptococcus pneumoniaiae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tuf PE = 3 SV = 1 - [EFTU_STRPN]	Intracellular
Q97NW2	64.44	25	419	331.55	Sugar ABC transporter, sugar-binding protein OS = - <i>Streptococcus pneumoniaiae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rafE PE = 1 SV = 1 - [Q97NW2_STRPN]	Lipid anchored
Q97QH2	42.86	29	721	330.61	Amino acid ABC transporter, amino acid-binding protein/permease protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1241 PE = 1 SV = 1 - [Q97QH2_STRPN]	Multi-transmembrane
Q97RS8	78.31	29	378	328.02	Lactate oxidase OS = - <i>Streptococcus pneumoniaiae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lctO-2 PE = 4 SV = 1 - [Q97RS8_STRPN]	Intracellular
Q97PW1	63.71	25	463	316.66	Peptidoglycan N-acetylglucosamine deacetylace A OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgdA PE = 4 SV = 1 - [Q97PW1_STRPN]	N-terminally anchored (No CS)
Q97PT6	75.85	26	468	315.76	ATP synthase subunit beta OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpD PE = 3 SV = 1 - [ATPB_STRPN]	Intracellular
Q97NQ3	58.55	22	345	310	Uncharacterized protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1967 PE = 4 SV = 1 - [Q97NQ3_STRPN]	N-terminally anchored (No CS)
Q97R09	58.94	17	341	309.62	Iron-compound ABC transporter, iron compound-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1032 PE = 1 SV = 1 - [Q97R09_STRPN]	Lipid anchored
P18791	69.8	28	659	298.67	Oligopeptide-binding protein AmiA OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiA PE = 1 SV = 3 - [AMIA_STRPN]	Lipid anchored
Q97RZ7	63.03	15	238	283.45	Uncharacterized protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0629 PE = 1 SV = 1 - [Q97RZ7_STRPN]	Lipid anchored
P0A4G2	65.05	18	309	281.58	Manganese ABC transporter substrate-binding lipoprotein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = psaA PE = 1 SV = 1 - [MTSA_STRPN]	Lipid anchored
P66112	60.5	10	119	280.56	50S ribosomal protein L20 OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplT PE = 3 SV = 1 - [RL20_STRPN]	Intracellular
Q97PM5	63.3	23	376	278.8	Sugar ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = msmK PE = 4 SV = 1 - [Q97PM5_STRPN]	Intracellular
P0A4M7	41.57	23	498	271.04	Oligopeptide transport system permease protein AmiC OS =	Multi-transmembrane

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97SP4	48.18	12	303	262.56	<i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amIC PE = 3 SV = 1 - [AMIC_STRPN] PTS system, mannose-specific IID component OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0282 PE = 4 SV = 1 - [Q97SP4_STRPN]	Multi-transmembrane
Q97NL3	60.74	34	731	258.29	Penicillin-binding protein 2A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pbp2A PE = 4 SV = 1 - [Q97NL3_STRPN]	N-terminally anchored (No CS)
P14677	61.33	32	750	257.72	Penicillin-binding protein 2x OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pbpX PE = 1 SV = 2 - [PBPX_STRPN]	N-terminally anchored (No CS)
Q97PT4	38.92	21	501	249.37	ATP synthase subunit alpha OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpA PE = 3 SV = 1 - [ATPA_STRPN]	Multi-transmembrane
Q97NB5	52.66	30	621	236.54	Choline binding protein PepA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pepA PE = 1 SV = 1 - [Q97NB5_STRPN]	Secretory (released) (with CS)
Q97PQ2	33.26	21	914	236.23	Cation-transporting ATPase, E1-E2 family OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1551 PE = 3 SV = 1 - [Q97PQ2_STRPN]	Multi-transmembrane
POA475	50.36	8	137	229.6	SOS ribosomal protein L16 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplP PE = 1 SV = 1 - [RL16_STRPN]	Intracellular
Q97PU3	64.03	23	278	227.7	Amino acid ABC transporter, amino acid-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = aatB PE = 4 SV = 1 - [Q97PU3_STRPN]	Lipid anchored
Q97RC5	50.3	20	501	223.13	Pyruvate kinase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyk PE = 3 SV = 1 - [Q97RC5_STRPN]	Intracellular
Q97NS5	65.68	19	338	221.3	Putative transcriptional regulator OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1942 PE = 4 SV = 1 - [Q97NS5_STRPN]	Secretory (released) (with CS)
Q97RP4	75.22	14	230	213.93	Cell division ABC transporter, ATP-binding protein FtsE OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsE PE = 3 SV = 1 - [Q97RP4_STRPN]	Intracellular
Q97RN2	66.67	13	267	207.19	Peptidyl-prolyl cis-trans isomerase, cyclophilin-type OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0771 PE = 3 SV = 1 - [Q97RN2_STRPN]	Lipid anchored
P66565	61.58	21	203	205.82	SOS ribosomal protein S4 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsD PE = 3 SV = 1 - [RS4_STRPN]	Intracellular
Q97QH1	61.79	10	246	202.96	Amino acid ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1242 PE = 3 SV = 1 - [Q97QH1_STRPN]	Intracellular
I6L8N9	40.91	18	506	201.9	Choline transporter OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = proWX PE = 3 SV = 1 - [I6L8N9_STRPN]	Multi-transmembrane

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97N69	55.22	15	335	201.71	Putative ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2197 PE = 4 SV = 1 - [Q97N69_STRPN]	Lipid anchored
P65887	53.97	23	428	199.01	Adenylosuccinate synthetase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = purA PE = 1 SV = 1 - [PURA_STRPN]	Intracellular
P18766	60.71	13	308	198.48	Oligopeptide transport ATP-binding protein Amf OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiF PE = 3 SV = 2 - [AMIF_STRPN]	Intracellular
Q97QB4	61.83	23	448	197.42	Glutamate dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gdhA PE = 3 SV = 1 - [Q97QB4_STRPN]	Intracellular
Q97SP5	55.18	19	444	196.27	Aminopeptidase C OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pepC PE = 4 SV = 1 - [Q97SP5_STRPN]	Intracellular
Q97RP3	40.58	17	308	193.01	Cell division protein FtsX OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsX PE = 3 SV = 1 - [Q97RP3_STRPN]	Multi-transmembrane
Q97T46	36.84	5	95	189.78	Putative bacteriocin OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0109 PE = 4 SV = 1 - [Q97T46_STRPN]	Secretory (released) (no CS)
Q97PD6	51.85	31	837	188.69	Protein translocase subunit SecA 1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = secA1 PE = 3 SV = 1 - [SECA1_STRPN]	Intracellular
POA4A7	60.58	13	137	187.87	30S ribosomal protein S12 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsL PE = 3 SV = 1 - [RS12_STRPN]	Intracellular
POA2U8	52.96	20	355	185.88	Oligopeptide transport ATP-binding protein AmiE OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiE PE = 3 SV = 1 - [AMIE_STRPN]	Intracellular
Q97RQ0	70.21	18	386	182.7	Branched-chain amino acid ABC transporter, amino acid-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = livJ PE = 4 SV = 1 - [Q97RQ0_STRPN]	Lipid anchored
POA3M9	67.68	16	328	176.61	L-lactate dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ldh PE = 3 SV = 2 - [LDH_STRPN]	Intracellular
Q97RG9	49.51	20	511	174.4	Sugar ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0846 PE = 3 SV = 1 - [Q97RG9_STRPN]	Intracellular
POA3M5	43.38	22	680	166.84	Penicillin-binding protein 2B OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = penA PE = 3 SV = 1 - [PBP2_STRPN]	N-terminally anchored (No CS)
Q97N53	63.24	12	272	163.14	Cell shape-determining protein MreC OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = mreC PE = 3 SV = 1 - [Q97N53_STRPN]	N-terminally anchored (No CS)
P95829	49.26	20	607	161.66	Chaperone protein Dnak OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnaK PE = 3 SV = 2 - [DNAK_STRPN]	Intracellular

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97S19	67.89	7	109	156.35	Cell cycle protein GpsB OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpsB PE = 3 SV = 1 - [GPSB_STRPN]	Intracellular
Q97NE4	36.3	26	821	153.09	Penicillin-binding protein 1B OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pbp1B PE = 4 SV = 1 - [Q97NE4_STRPN]	N-terminally anchored (No CS)
I6L8W3	71.49	15	242	152.01	Choline transporter OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = proV PE = 3 SV = 1 - [I6L8W3_STRPN]	Intracellular
POA335	52.04	21	540	151.44	60 kDa chaperonin OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = groL PE = 1 SV = 1 - [CH60_STRPN]	Intracellular
Q97NG0	44.77	17	449	145.04	Glucose-6-phosphate isomerase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgI PE = 3 SV = 1 - [G6PI_STRPN]	Intracellular
Q97Q31	57.53	12	292	143.85	Phosphate-binding protein PstS 1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pstS1 PE = 1 SV = 1 - [PSTS1_STRPN]	Lipid anchored
Q97RW9	51.81	16	332	139.95	Putative pneumococcal surface protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0667 PE = 1 SV = 1 - [Q97RW9_STRPN]	Lipid anchored
P22976	44.98	18	658	139.63	Probable transketolase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tkt PE = 3 SV = 2 - [TKT_STRPN]	Intracellular
Q97SQ9	42.52	17	602	138.65	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glmS PE = 3 SV = 3 - [GLMS_STRPN]	Intracellular
Q97PA9	33.84	18	659	132.79	Serine/threonine-protein kinase StkP OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = stkp PE = 1 SV = 1 - [STKP2_STRPN]	Intracellular/TMH start AFTER 60
Q97Q34	70.41	18	267	131.91	Phosphate import ATP-binding protein PstB 2 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pstB2 PE = 3 SV = 1 - [PSTB2_STRPN]	Intracellular
Q97QX5	55.81	14	344	130.66	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1069 PE = 1 SV = 1 - [Q97QX5_STRPN]	Secretory(released)(with CS)
I6L8V0	55.84	18	351	130.62	Capsular polysaccharide biosynthesis protein Cps4J OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cap4J PE = 4 SV = 1 - [I6L8V0_STRPN]	Intracellular
Q97SD1	43.38	17	521	119.96	Amino acid ABC transporter, amino acid-binding protein/permease protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0453 PE = 4 SV = 1 - [Q97SD1_STRPN]	Multi-transmembrane
Q97SR1	50.73	21	617	119.02	Proline-tRNA ligase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = proS PE = 3 SV = 1 - [SYP_STRPN]	Intracellular
POA4D7	36.07	14	524	117.93	DEAD-box ATP-dependent RNA helicase CshA OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC	Intracellular

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97P40	68.81	12	202	117.92	BAA-334/TIGR4) GN = cshA PE = 3 SV = 1 - [CSHA_STRPN] Putative general stress protein 24 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1804 PE = 4 SV = 1 - [Q97P40_STRPN]	Intracellular
Q97NK0	41	21	883	117.4	Aldehyde-alcohol dehydrogenase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2026 PE = 3 SV = 1 - [Q97NK0_STRPN]	Intracellular
Q97N99	51.82	14	274	115.56	SPFH domain/Band 7 family OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2156 PE = 4 SV = 1 - [Q97N99_STRPN]	Intracellular
Q9L7Q2	17.63	23	190 6	114.8	Zinc metalloprotease ZmpB OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = zmpB PE = 3 SV = 2 - [ZMPB_STRPN]	Multi-transmembrane
P67293	43.9	4	82	114.56	UPF0154 protein SP_1882 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1882 PE = 3 SV = 1 - [Y1882_STRPN]	N-terminally anchored (No CS)
Q97N37	61.83	14	393	114.16	Serine protease OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2239 PE = 1 SV = 1 - [Q97N37_STRPN]	N-terminally anchored (No CS)
P66359	55.12	8	127	112.68	30S ribosomal protein S11 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsK PE = 3 SV = 1 - [RS11_STRPN]	Intracellular
Q97PX1	30.72	12	459	110.91	NADH oxidase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = nox PE = 4 SV = 1 - [Q97PX1_STRPN]	Intracellular
P35592	43.64	21	660	109.44	Oligopeptide-binding protein AliA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = aliA PE = 3 SV = 4 - [ALIA_STRPN]	Lipid anchored
Q97PC4	28.66	11	492	108.41	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1715 PE = 4 SV = 1 - [Q97PC4_STRPN]	Multi-transmembrane
Q97P68	69.23	8	104	104.87	Thioredoxin OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = trx PE = 3 SV = 1 - [Q97P68_STRPN]	Intracellular
Q97PL8	41.55	12	438	101	Oxidoreductase, pyridine nucleotide-disulfide, class I OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1588 PE = 3 SV = 1 - [Q97PL8_STRPN]	Intracellular
Q97SX2	68.78	17	189	97.17	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0191 PE = 1 SV = 1 - [Q97SX2_STRPN]	Lipid anchored
P63413	43.94	14	396	96.43	Acetate kinase OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ackA PE = 3 SV = 1 - [ACKA_STRPN]	Intracellular
Q97SJ6	52.61	11	230	96.34	Capsular polysaccharide biosynthesis protein CpsC OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cpsC PE = 3 SV = 1 - [CPSC_STRPN]	Multi-transmembrane
Q97Q67	38.08	11	344	95.87	Conserved domain protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	Multi-transmembrane

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
P0A4S1	49.15	10	293	93.48	GN = SP_1363 PE = 4 SV = 1 - [Q97Q67_STRPN] Fructose-bisphosphate aldolase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = fba PE = 3 SV = 1 - [ALF_STRPN]	Intracellular
Q97SR2	37.71	11	419	93.31	Putative zinc metalloprotease SP_0263 OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0263 PE = 3 SV = 1 - [Y263_STRPN]	Multi-transmembrane
Q97NP5	25.65	9	308	92.53	Membrane protein insertase YidC 1 OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = yidC1 PE = 3 SV = 1 - [YIDC1_STRPN]	Multi-transmembrane
Q97SJ4	44.44	15	360	91.77	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0355 PE = 4 SV = 1 - [Q97SJ4_STRPN]	Intracellular
Q97RK0	19.83	10	575	91.07	Septation ring formation regulator EzrA OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ezcA PE = 3 SV = 1 - [EZRA_STRPN]	N-terminally anchored (No CS)
P63373	71.43	11	252	91.01	Phosphate import ATP-binding protein PstB 1 OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pstB1 PE = 3 SV = 1 - [PSTB1_STRPN]	Intracellular
Q97S89	51.01	13	398	89.47	Phosphoglycerate kinase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgk PE = 3 SV = 1 - [PGK_STRPN]	Intracellular
Q97PT5	47.26	12	292	85.91	ATP synthase gamma chain OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpG PE = 3 SV = 1 - [ATPG_STRPN]	Intracellular
Q97514	32.65	9	340	85.66	Choline binding protein C OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpC PE = 4 SV = 1 - [Q97SI4_STRPN]	N-terminally anchored (with CS)
I6L858	39.71	14	481	85.62	Capsular polysaccharide biosynthesis protein Cps4A OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4A PE = 4 SV = 1 - [I6L858_STRPN]	Multi-transmembrane
Q97PY0	65.59	13	247	85.07	Amino acid ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1460 PE = 3 SV = 1 - [Q97PY0_STRPN]	Intracellular
Q97SQ4	46.79	9	156	83.39	30S ribosomal protein S7 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsG PE = 3 SV = 1 - [RS7_STRPN]	Intracellular
P0A3Y3	60	10	230	82.54	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpmA PE = 3 SV = 1 - [GPMA_STRPN]	Intracellular
P0A4C3	59.91	11	217	82.05	30S ribosomal protein S3 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsC PE = 3 SV = 1 - [RS3_STRPN]	Intracellular
P65607	38.46	11	338	79.74	Ornithine carbamoyltransferase, catabolic OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = arcB PE = 3 SV = 1 - [OTCC_STRPN]	Intracellular
O05703	39.92	12	501	79.68	Zinc-binding lipoprotein AdcA OS = <i>Streptococcus pneumoniae</i> serotype 4	Lipid anchored

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
I6L8U0	27.16	11	394	79.39	(strain ATCC BAA-334/TIGR4) GN = adcA PE = 3 SV = 4 - [ADCA_STRPN] UDP-N-acetylglucosamine 2-epimerase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4) GN = cps4L PE = 3 SV = 1 - [I6L8U0_STRPN]	Intracellular
Q97SI7	28.23	8	464	78.19	Mid-cell-anchored protein Z OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = mapZ PE = 1 SV = 1 - [Q97SI7_STRPN]	Intracellular
Q97N56	58.3	15	259	78.0	30S ribosomal protein S2 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsB PE = 3 SV = 1 - [RS2_STRPN]	Intracellular
Q97SI6	35.44	13	474	77.15	6-phosphogluconate dehydrogenase, decarboxylating OS = - <i>Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gnd PE = 3 SV = 1 - [Q97SI6_STRPN]	Intracellular
Q97PG0	27.1	7	262	77.07	Cell division protein DivIVA OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = divIVA PE = 4 SV = 1 - [Q97PG0_STRPN]	Intracellular
Q97RF9	32	11	400	76.46	Ribosomal protein S1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsA PE = 4 SV = 1 - [Q97RF9_STRPN]	Intracellular
Q97T80	15.68	17	1856	76.28	Zinc metalloprotease ZmpC OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = zmpC PE = 3 SV = 1 - [ZMPC_STRPN]	LPXTG Cell-wall anchored
P0A2Z2	62.8	9	164	75.56	ATP synthase subunit b OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpF PE = 3 SV = 1 - [ATPF_STRPN]	Intracellular
I6L859	47.7	10	413	73.22	D-alanyl-D-alanine carboxypeptidase OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4) GN = dacA PE = 3 SV = 1 - [I6L859_STRPN]	N- terminally anchored (with CS)
Q97PU4	80	7	115	71.8	Bacteriocin transport accessory protein OS = - <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4) GN = bta PE = 4 SV = 1 - [Q97PU4_STRPN]	Intracellular
Q97SU6	70.34	7	118	71.64	50S ribosomal protein L18 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplR PE = 3 SV = 1 - [RL18_STRPN]	Intracellular
P63742	35.46	11	502	71.21	Glycerol kinase OS = - <i>Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = glpK PE = 3 SV = 1 - [GLPK_STRPN]	Intracellular
Q97SN4	32.31	6	130	68.89	30S ribosomal protein S9 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsI PE = 3 SV = 1 - [RS9_STRPN]	Intracellular
P66907	31.84	6	380	68.13	Queuine tRNA-ribosyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tgt PE = 3 SV = 1 - [TGT_STRPN]	Intracellular
Q97SF8	32.16	12	454	65.06	Aspartokinase OS = - <i>Streptococcus</i> <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0413 PE = 3 SV = 1 - [Q97SF8_STRPN]	Intracellular
P0A471	82.79	9	122	62.07	50S ribosomal protein L7/L12 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplL PE = 1 SV = 2 - [RL7_STRPN]	Intracellular

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
I6L8W8	21.08	8	332	61.96	Choline binding protein J OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cbpJ PE = 4 SV = 1 - [I6L8W8_STRPN]	N-terminally anchored (with CS)
P0A4J6	40.8	5	201	61.71	Superoxide dismutase [Mn] OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = sodA PE = 1 SV = 2 - [SODM_STRPN]	Intracellular
Q97T52	30.03	14	616	60.5	Putative capsular polysaccharide biosynthesis protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0103 PE = 4 SV = 1 - [Q97T52_STRPN]	Multi-transmembrane
Q97RE5	17.08	6	650	60.23	PTS system, fructose specific IIABC components OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0877 PE = 4 SV = 1 - [Q97RE5_STRPN]	Multi-transmembrane
Q97SV4	54.11	9	207	60.13	SOS ribosomal protein L4 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplD PE = 3 SV = 1 - [RL4_STRPN]	Intracellular
Q97RK7	19.46	12	848	59.83	Aminopeptidase N OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pepN PE = 4 SV = 1 - [Q97RK7_STRPN]	Intracellular
Q97Q62	34.67	11	424	59.21	Psr protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1368 PE = 4 SV = 1 - [Q97Q62_STRPN]	Intracellular/TMH start AFTER 60
O07344	26.96	5	204	58.64	Signal peptidase I OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lepB PE = 3 SV = 2 - [LEP_STRPN]	N-terminally anchored (No CS)
P0A3S3	34.67	6	274	57.63	DNA-entry nuclelease OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = endA PE = 1 SV = 1 - [NUCE_STRPN]	N-terminally anchored (No CS)
Q97PU5	29.55	10	572	57.38	Phosphoglucomutase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgm PE = 3 SV = 1 - [Q97PU5_STRPN]	Intracellular
Q97RC6	31.34	8	335	57.3	ATP-dependent 6-phosphofructokinase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfkA PE = 3 SV = 1 - [PFKA_STRPN]	Intracellular
Q97SV5	37.5	6	208	56.96	SOS ribosomal protein L3 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplC PE = 3 SV = 1 - [RL3_STRPN]	Intracellular
Q97RL9	25.56	6	399	56.21	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0785 PE = 4 SV = 1 - [Q97RL9_STRPN]	N-terminally anchored (No CS)
P66278	18.18	2	66	55.84	SOS ribosomal protein L35 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmI PE = 3 SV = 1 - [RL35_STRPN]	Intracellular
I6L8N0	40.82	12	365	55.32	UDP-N-acetylglucosamine-2-epimerase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4I PE = 3 SV = 1 - [I6L8N0_STRPN]	Intracellular
Q97PQ8	57.34	6	143	54.74	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1545 PE = 4 SV = 1 - [Q97PQ8_STRPN]	Intracellular
Q97TA6	33.64	10	330	52.71	Phosphate acyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4	Intracellular

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97PK5	27.22	2	158	52.32	(strain ATCC BAA-334/TIGR4) GN = plsX PE = 3 SV = 1 - [PLSX_STRPN] Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1604 PE = 4 SV = 1 - [Q97PK5_STRPN]	N-terminally anchored (No CS)
Q97SN5	67.57	8	148	51.12	50S ribosomal protein L13 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplM PE = 3 SV = 1 - [Q97SN5_STRPN]	Intracellular
Q97RV8	60.32	8	126	49.28	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0678 PE = 4 SV = 1 - [Q97RV8_STRPN]	N-terminally anchored (No CS)
Q97R14	30.25	7	324	48.6	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1027 PE = 4 SV = 1 - [Q97R14_STRPN]	Secretory (released) (with CS)
P35597	15.04	7	778	48.34	Probable cation-transporting ATPase exp7 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = exp7 PE = 3 SV = 2 - [EXP7_STRPN]	Multi-transmembrane
P65239	41.61	9	322	48.08	Ribose-phosphate pyrophosphokinase 1 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = prs1 PE = 3 SV = 1 - [KPRS1_STRPN]	Intracellular
Q97Q37	46.13	7	271	47.67	Amino acid ABC transporter, amino acid-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1394 PE = 4 SV = 1 - [Q97Q37_STRPN]	Lipid anchored
Q97R36	52.43	8	185	47.65	Thioredoxin family protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1000 PE = 1 SV = 1 - [Q97R36_STRPN]	Lipid anchored
Q97N48	26.81	5	276	45.87	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2223 PE = 4 SV = 1 - [Q97N48_STRPN]	Intracellular/TMH start AFTER 60
I6L8R1	22.79	4	215	45.06	ABC transporter, ATP-binding protein Vexp2 OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = vex2 PE = 3 SV = 1 - [I6L8R1_STRPN]	Intracellular
Q97SC6	20.93	10	774	44.93	Formate acetyltransferase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfl PE = 4 SV = 1 - [Q97SC6_STRPN]	Intracellular
Q97QD5	51.08	8	186	44.75	LemA protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lemA PE = 1 SV = 1 - [Q97QD5_STRPN]	N-terminally anchored (No CS)
Q97PF9	37.71	9	419	44.71	Cell division protein FtsZ OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsZ PE = 3 SV = 1 - [Q97PF9_STRPN]	Intracellular
POA3B7	36.71	7	346	44.56	Elongation factor Ts OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tsf PE = 1 SV = 2 - [EFTS_STRPN]	Intracellular
P72524	20.56	10	822	44.46	DNA gyrase subunit A OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gyrA PE = 3 SV = 3 - [GYRA_STRPN]	Intracellular

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
P67282	26.22	11	534	43.28	Ribonuclease Y OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rny PE = 3 SV = 1 - [RNY_STRPN]	N-terminally anchored (No CS)
P66419	38.2	6	89	42.98	30S ribosomal protein S14 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsN PE = 3 SV = 1 - [RS14_STRPN]	Intracellular
Q97SV1	48.89	6	180	42.64	50S ribosomal protein L5 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplE PE = 3 SV = 1 - [RL5_STRPN]	Intracellular
Q97NW9	7.48	5	655	42.08	Trehalose PTS system, IIABC components OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1884 PE = 4 SV = 1 - [Q97NW9_STRPN]	Multi-transmembrane
Q97Q18	91.06	6	123	40.86	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1218 PE = 4 SV = 1 - [Q97Q18_STRPN]	Intracellular
Q97T04	11.92	2	193	39.59	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0157 PE = 4 SV = 1 - [Q97T04_STRPN]	Multi-transmembrane
Q97PB8	9.09	4	627	39.49	PTS system IIABC components OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1722 PE = 4 SV = 1 - [Q97PB8_STRPN]	Multi-transmembrane
Q97P19	57.3	5	89	39.48	30S ribosomal protein S15 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsO PE = 3 SV = 1 - [RS15_STRPN]	Intracellular
P61182	40.35	4	114	39.35	50S ribosomal protein L22 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplV PE = 1 SV = 1 - [RL22_STRPN]	Intracellular
Q97SU7	51.69	6	178	38.14	50S ribosomal protein L6 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplF PE = 3 SV = 1 - [RL6_STRPN]	Intracellular
Q97NB9	39.1	8	335	38.04	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2132 PE = 4 SV = 1 - [Q97NB9_STRPN]	Multi-transmembrane
Q97QP7	5.34	8	2004	37.84	Immunoglobulin A1 protease OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = iga PE = 3 SV = 1 - [IGA1A_STRPN]	Multi-transmembrane
Q97N55	23.72	6	392	37.16	Secreted 45 kd protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = usp45 PE = 4 SV = 1 - [Q97N55_STRPN]	Secretory (released) (with CS)
P66942	18.25	4	252	36.63	Triosephosphate isomerase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = tpiA PE = 3 SV = 1 - [TPIS_STRPN]	Intracellular
Q97QC6	66.09	7	115	35.81	50S ribosomal protein L19 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplS PE = 3 SV = 1 - [RL19_STRPN]	Intracellular
I6L8S7	14.6	5	459	35.54	Transmembrane protein Vexp3 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = vex3 PE = 4 SV = 1 - [I6L8S7_STRPN]	Multi-transmembrane
P35595	12.4	6	726	35.44	PTS system glucose-specific EIICBA component OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC	Multi-transmembrane

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97PV1	14.88	3	289	34.89	BAA-334/TIGR4) GN = exp5 PE = 3 SV = 2 - [PTG3C_STRPN] Putative glycerol uptake facilitator protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1491 PE = 3 SV = 1 - [Q97PV1_STRPN]	Multi-transmembrane
Q97NM6	21.04	6	404	33.79	Aminotransferase, class I OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1994 PE = 4 SV = 1 - [Q97NM6_STRPN]	Intracellular
Q97SD2	45.93	5	246	33.44	Amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0452 PE = 3 SV = 1 - [Q97SD2_STRPN]	Intracellular
Q97NN3	36.15	6	213	33.1	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1987 PE = 1 SV = 1 - [Q97NN3_STRPN]	Intracellular
Q97RJ0	23.77	5	244	33.09	Amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0824 PE = 3 SV = 1 - [Q97RJ0_STRPN]	Intracellular
Q97NJ7	27.27	3	99	32.76	Preprotein translocase, YajC subunit OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = yajC-2 PE = 4 SV = 1 - [Q97NJ7_STRPN]	N-terminally anchored (No CS)
P66095	34.5	8	229	32.4	50S ribosomal protein L1 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplA PE = 3 SV = 1 - [RL1_STRPN]	Intracellular
Q97NT7	38.61	6	158	32.3	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1926 PE = 4 SV = 1 - [Q97NT7_STRPN]	Multi-transmembrane
P63667	29.5	4	139	31.7	ATP synthase epsilon chain OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpC PE = 3 SV = 1 - [ATPE_STRPN]	Intracellular
Q97T58	18.08	4	354	31.6	Conserved domain protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0097 PE = 4 SV = 1 - [Q97T58_STRPN]	Multi-transmembrane
Q97R06	36.74	6	264	31.17	Iron-compound ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1035 PE = 3 SV = 1 - [Q97R06_STRPN]	Intracellular
Q97PQ5	20.34	6	531	30.81	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1548 PE = 4 SV = 1 - [Q97PQ5_STRPN]	Multi-transmembrane
Q97R44	33.91	2	115	30.62	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0990 PE = 4 SV = 1 - [Q97R44_STRPN]	Intracellular/TMH start AFTER 60
Q97SW3	47.73	3	88	29.98	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0201 PE = 4 SV = 1 - [Q97SW3_STRPN]	Intracellular

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
I6L8N6	27.01	4	211	29.5	Capsular polysaccharide biosynthesis protein Cps4E OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4E PE = 4 SV = 1 - [I6L8N6_STRPN]	N-terminally anchored (No CS)
Q97SU3	34.25	5	146	28.53	50S ribosomal protein L15 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplO PE = 3 SV = 1 - [RL15_STRPN]	Intracellular
Q97QS6	25.86	6	379	28.15	Glycogen biosynthesis protein GlgD OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = gldG PE = 4 SV = 1 - [Q97QS6_STRPN]	Intracellular
Q97P39	52.24	2	67	27.23	UPF0337 protein SP_1805 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1805 PE = 3 SV = 1 - [Y1805_STRPN]	Intracellular
P06653	20.44	6	318	25.81	Autolysin OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = lytA PE = 1 SV = 2 - [ALYS_STRPN]	Intracellular
Q97PX9	12.78	5	266	25.41	Amino acid ABC transporter, permease protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1461 PE = 3 SV = 1 - [Q97PX9_STRPN]	Multi-transmembrane
Q97QW4	17.65	5	425	24.84	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1083 PE = 4 SV = 1 - [Q97QW4_STRPN]	Intracellular
Q97S93	13.27	7	535	23.79	CTP synthase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyrG PE = 3 SV = 1 - [PYRG_STRPN]	Intracellular
Q97SX1	26.14	2	88	22	UPF0297 protein SP_0192 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0192 PE = 1 SV = 1 - [Y192_STRPN]	Intracellular
Q97RH7	50	4	78	21.34	30S ribosomal protein S20 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsT PE = 3 SV = 1 - [RS20_STRPN]	Intracellular
P66050	45.78	5	166	20.98	50S ribosomal protein L10 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplJ PE = 3 SV = 2 - [RL10_STRPN]	Intracellular
Q97RW6	32.14	3	56	20.85	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0670 PE = 4 SV = 1 - [Q97RW6_STRPN]	Secretory (released) (with CS)
Q9FB7	29.41	4	255	20.65	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = accA PE = 1 SV = 1 - [ACCA_STRPN]	Intracellular
I6L8V8	30.45	4	243	20.58	3-oxoacyl-[acyl-carrier protein] reductase OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = fabG PE = 3 SV = 1 - [I6L8V8_STRPN]	Intracellular
P66581	41.46	4	164	20.46	30S ribosomal protein S5 OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsE PE = 3 SV = 1 - [RS5_STRPN]	Intracellular
Q97T45	11.82	5	694	20.39	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	Multi-transmembrane

TABLE 4-continued

Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
Q97P18	18.31	4	355	20.06	GN = SP_0110 PE = 4 SV = 1 - [Q97T45_STRPN] ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1826 PE = 4 SV = 1 - [Q97P18_STRPN]	Lipid anchored

TABLE 5

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQV2	91.09	50	404	5251.94	Elongation factor Tu OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tuf PE = 3 SV = 1 - [B2IQV2_STRPS]	Intracellular
B2IPX8	95.62	40	434	2291.84	Endonuclease OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = eno PE = 3 SV = 1 - [ENO_STRPS]	Intracellular
B2INW2	70.86	42	374	2221.7	Lipoprotein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0787 PE = 4 SV = 1 - [B2INW2_STRPS]	Lipid anchored
B2ISJ9	85.28	67	693	1851.66	Elongation factor G OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fusA PE = 3 SV = 1 - [EFG_STRPS]	Intracellular
B2IMI7	73.82	33	359	1675.28	Glyceraldehyde-3-phosphate dehydrogenase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gapA PE = 3 SV = 1 - [B2IMI7_STRPS]	Intracellular
B2IM39	82.94	101	1225	1348.46	DNA-directed RNA polymerase subunit beta OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rpoC PE = 3 SV = 1 - [RPOC_STRPS]	Intracellular
B2IMJ9	86.85	68	890	1321.16	Aldehyde-alcohol dehydrogenase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = adhE PE = 3 SV = 1 - [B2IMJ9_STRPS]	Intracellular
B2IPD4	73.16	38	313	1171.98	Foldase protein PrsA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prsA PE = 3 SV = 1 - [PRSA_STRPS]	Lipid anchored
B2INY1	91	53	400	1141.84	30S ribosomal protein S1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsA PE = 4 SV = 1 - [B2INY1_STRPS]	Intracellular
B2IPS3	87.46	27	335	830.14	ATP-dependent 6-phosphofructokinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pfkA PE = 3 SV = 1 - [PFKA_STRPS]	Intracellular
B2IQX0	90.6	38	468	772.66	ATP synthase subunit beta OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpD PE = 3 SV = 1 - [ATPB_STRPS]	Intracellular
B2IRV7	72.76	62	837	760.12	Protein translocase subunit SecA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = secA PE = 3 SV = 1 - [B2IRV7_STRPS]	Intracellular
B2IN25	78.22	51	450	751.01	Maltose/maltodextrin ABC transporter, maltose/maltodextrin-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2072 PE = 4 SV = 1 - [B2IN25_STRPS]	Lipid anchored

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2INB2	72.76	41	591	749.62	Pyruvate oxidase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = spxB PE = 3 SV = 1 - [B2INB2_STRPS]	Intracellular
B2ISZ0	69.04	47	659	746.61	Oligopeptide ABC transporter, oligopeptide-binding protein AmiA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = amiA PE = 4 SV = 1 - [B2ISZ0_STRPS]	Lipid anchored
B2ISK8	56.44	22	303	734.11	PTS system, mannose-specific IID component OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = manN PE = 4 SV = 1 - [B2ISK8_STRPS]	Multi-transmembrane
B2INN2	85.33	30	259	682.23	30S ribosomal protein S2 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsB PE = 3 SV = 2 - [RS2_STRPS]	Intracellular
B2IP54	76.05	39	501	676.55	Pyruvate kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0873 PE = 3 SV = 1 - [B2IP54_STRPS]	Intracellular
B2IR47	69.48	50	652	673.91	Oligopeptide ABC transporter, oligopeptide-binding protein AliB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = alib PE = 4 SV = 1 - [B2IR47_STRPS]	Lipid anchored
B2IR00	66.26	44	652	636.95	ATP-dependent zinc metalloprotease FtsH OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsH PE = 3 SV = 1 - [B2IR00_STRPS]	Multi-transmembrane
B2IM40	77.67	50	824	633.52	DNA-directed RNA polymerase subunit beta OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rpoB PE = 3 SV = 1 - [B2IM40_STRPS]	Intracellular
B2IS43	70.76	23	277	575.93	50S ribosomal protein L2 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplB PE = 3 SV = 1 - [RL2_STRPS]	Intracellular
B2IS46	79.26	27	217	569.56	30S ribosomal protein S3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsC PE = 3 SV = 1 - [RS3_STRPS]	Intracellular
B2IME4	64.95	51	930	553.42	Translation initiation factor IF-2 OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = infB PE = 3 SV = 1 - [IF2_STRPS]	Intracellular
B2IQY1	73.14	43	551	550.86	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1503 PE = 4 SV = 1 - [B2IQY1_STRPS]	Intracellular/TMH start AFTER 60
B2INT1	58.9	34	584	531.45	Septation ring formation regulator Ezra OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ezaA PE = 3 SV = 1 - [B2INT1_STRPS]	N-terminally anchored (No CS)
B2IPY8	67.93	32	474	511.28	Glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gapN PE = 3 SV = 1 - [B2IPY8_STRPS]	Intracellular
B2IRQ6	79.23	20	313	461.56	Manganese ABC transporter, manganese-binding adhesion liprotein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1623 PE = 3 SV = 1 - [B2IRQ6_STRPS]	Lipid anchored
B2IPU8	63.14	36	719	457.32	Ribonucleoside-diphosphate reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = nrdE PE = 3 SV = 1 - [B2IPU8_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IM49	72.42	27	330	449.87	Aspartate--ammonia ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = asnA PE = 3 SV = 1 - [ASNA_STRPS]	Intracellular
B2IQX2	68.86	31	501	447.54	ATP synthase subunit alpha OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpA PE = 3 SV = 1 - [ATPA_STRPS]	Intracellular
B2IMK3	70.36	32	658	435.4	Transketolase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tktA PE = 3 SV = 1 - [B2IMK3_STRPS]	Intracellular
B2IRH9	64.45	36	616	434.01	Capsular polysaccharide biosynthesis protein, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0098 PE = 4 SV = 1 - [B2IRH9_STRPS]	Multi-transmembrane
B2IN95	82.06	37	496	432.16	Lysine--tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lysS PE = 3 SV = 1 - [SYK_STRPS]	Intracellular
B2IS90	71.32	40	537	413.57	Ribonuclease Y OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rny PE = 3 SV = 1 - [B2IS90_STRPS]	Intracellular
B2IRT9	66.06	32	442	412.96	Sugar ABC transporter, sugar-binding protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1656 PE = 4 SV = 1 - [B2IRT9_STRPS]	Lipid anchored
B2IN10	73.37	15	338	401	Glycerol-3-phosphate dehydrogenase [NAD(P)+] OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gpsA PE = 3 SV = 1 - [GPDA_STRPS]	Intracellular
B2IP07	38.46	23	650	398.03	PTS system, fructose specific IIABC component OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fruA PE = 4 SV = 1 - [B2IP07_STRPS]	Multi-transmembrane
B2IPB5	52.94	10	119	388.03	50S ribosomal protein L20 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplT PE = 3 SV = 1 - [RL20_STRPS]	Intracellular
B2IS19	66.29	35	617	385.71	Proline--tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = proS PE = 3 SV = 1 - [SYP_STRPS]	Intracellular
B2INH5	83.95	31	299	370.13	SPFH domain/Band 7 family OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2124 PE = 4 SV = 1 - [B2INH5_STRPS]	N-terminally anchored (No CS)
B2IQ40	64.14	46	898	367.23	Phosphoenolpyruvate carboxylase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ppc PE = 3 SV = 1 - [B2IQ40_STRPS]	Intracellular
B2IML8	76.35	25	406	358.33	Acetate kinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ackA PE = 3 SV = 1 - [B2IML8_STRPS]	Intracellular
B2IRS1	53.94	28	419	341.4	Cell division protein FtsZ OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS1_STRPS]	Intracellular
B2IMN0	96.59	22	352	339.61	Alcohol dehydrogenase, zinc-containing OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = adh PE = 3 SV = 1 - [B2IMNO_STRPS]	Intracellular
B2ISJ7	51.09	13	137	337.74	30S ribosomal protein S12 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsL PE = 3 SV = 1 - [RS12_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IS41	63.29	16	207	337.71	50S ribosomal protein L4 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplD PE = 3 SV = 1 - [RL4_STRPS]	Intracellular
B2ILW7	80.68	18	414	332.02	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fabF PE = 3 SV = 1 - [B2ILW7_STRPS]	Intracellular
B2IRM9	70.29	22	276	330.56	ABC transporter, substrate-binding protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0152 PE = 4 SV = 1 - [B2IRM9_STRPS]	Lipid anchored
B2ISY9	47.99	21	498	319.21	Oligopeptide ABC transporter, permease protein Amic OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = amiC PE = 3 SV = 1 - [B2ISY9_STRPS]	Multi-transmembrane
B2IQT6	62.75	22	459	313.52	NADH oxidase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nox PE = 4 SV = 1 - [B2IQT6_STRPS]	Intracellular
B2IPP2	47.02	32	721	312	Amino acid ABC transporter, amino acid-binding protein/permease protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1062 PE = 3 SV = 1 - [B2IPP2_STRPS]	Multi-transmembrane
B2IS47	63.5	13	137	300.82	50S ribosomal protein L16 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplP PE = 3 SV = 1 - [RL16_STRPS]	Intracellular
B2IRC1	66.67	19	339	299.62	UDP-glucose 4-epimerase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gale PE = 4 SV = 1 - [B2IRC1_STRPS]	Intracellular
B2IQW3	71.22	25	278	296.15	Amino acid ABC transporter, amino acid-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1485 PE = 4 SV = 1 - [B2IQW3_STRPS]	Lipid anchored
B2IRR1	67.83	19	230	295.06	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gpmA PE = 3 SV = 1 - [GPMA_STRPS]	Intracellular
B2IS34	61.87	39	737	290.83	Anaerobic ribonucleoside triphosphate reductase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0212 PE = 4 SV = 1 - [B2IS34_STRPS]	Intracellular
B2IS52	87.78	18	180	288.42	50S ribosomal protein L5 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplE PE = 3 SV = 1 - [RL5_STRPS]	Intracellular
B2IQU1	65.63	33	678	288.36	Glycine-tRNA ligase beta subunit OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glyS PE = 3 SV = 1 - [SYGB_STRPS]	Intracellular
B2ILY5	60.36	21	555	284.08	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0438 PE = 4 SV = 1 - [B2ILY5_STRPS]	Intracellular
B2IS40	59.62	14	208	283.69	50S ribosomal protein L3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplC PE = 3 SV = 1 - [RL3_STRPS]	Intracellular
B21M73	32.44	18	521	281.46	Amino acid ABC transporter, amino acid-binding protein/permease protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14)	Multi-transmembrane

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IR19	52.65	17	302	275.76	GN = gInH PE = 3 SV = 1 - [B2IM73_STRPS] Amino acid ABC transporter, periplasmic amino acid-binding protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0108 PE = 4 SV = 1 - [B2IR19_STRPS]	Lipid anchored
B2IRN0	57.75	22	284	275.2	Lipoprotein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0153 PE = 3 SV = 1 - [B2IRN0_STRPS]	Lipid anchored
B2IM81	70.93	36	774	270.81	Formate acetyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pfl PE = 4 SV = 1 - [B2IM81_STRPS]	Intracellular
B2IRN7	65.61	30	660	269.02	Threonine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = thrS PE = 3 SV = 1 - [B2IRN7_STRPS]	Intracellular
B2IMN6	63.36	22	494	268.42	Threonine synthase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = thrC PE = 4 SV = 1 - [B2IMN6_STRPS]	Intracellular
B2IR98	69.68	20	376	261.66	Sugar ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1565 PE = 3 SV = 1 - [B2IR98_STRPS]	Intracellular
B2IMG8	58.42	23	404	253.09	Aspartate aminotransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aspC PE = 4 SV = 1 - [B2IMG8_STRPS]	Intracellular
B2ILW6	65.43	13	243	252.82	3-ketoacyl-(Acyl-carrier-protein) reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = fabG PE = 4 SV = 1 - [B2ILW6_STRPS]	Intracellular
B2INF2	70.18	25	513	248.48	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0719 PE = 4 SV = 1 - [B2INF2_STRPS]	Intracellular
B2IRS2	78.12	20	457	247.37	Cell division protein FtsA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS2_STRPS]	Multi-transmembrane
B2IM41	70.82	22	377	246.54	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1926 PE = 3 SV = 1 - [B2IM41_STRPS]	Intracellular
B2IQ88	66.09	13	115	240.71	50S ribosomal protein L19 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplS PE = 3 SV = 1 - [RL19_STRPS]	Intracellular
B2IQ10	56.1	22	344	240.32	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1352 PE = 4 SV = 1 - [B2IQ10_STRPS]	Multi-transmembrane
B2IND2	63.73	18	386	236.96	Branched-chain amino acid ABC transporter, amino acid-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0699 PE = 4 SV = 1 - [B2IND2_STRPS]	Lipid anchored
B2INP5	71.34	24	492	236.94	Inosine-5'-monophosphate dehydrogenase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = imdH PE = 3 SV = 1 - [B2INP5_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ISL2	67.26	18	339	235.53	Alcohol dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = adhP PE = 3 SV = 1 - [B2ISL2_STRPS]	Intracellular
B2ISM4	59.23	9	130	228.51	30S ribosomal protein S9 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsI PE = 3 SV = 1 - [RS9_STRPS]	Intracellular
B2IR71	40.26	23	914	227.01	Cation-transporting ATPase, E1-E2 family OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pacL PE = 3 SV = 1 - [B2IR71_STRPS]	Multi-transmembrane
B2IPP1	85.77	17	246	225.41	Amino acid ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1061 PE = 4 SV = 1 - [B2IPP1_STRPS]	Intracellular
B2ISY7	58.31	18	355	221.22	Oligopeptide ABC transporter, ATP-binding protein AmiE OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = amiE PE = 3 SV = 1 - [B2ISY7_STRPS]	Intracellular
B2IQL1	56.83	16	271	219.73	Amino acid ABC transporter, amino acid-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1383 PE = 4 SV = 1 - [B2IQL1_STRPS]	Lipid anchored
B2ISG3	77.23	19	202	219.45	General stress protein 24, putative OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1781 PE = 4 SV = 1 - [B2ISG3_STRPS]	Intracellular
B2IMA2	70.1	20	398	216.35	Phosphoglycerate kinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pgk PE = 3 SV = 1 - [PGK_STRPS]	Intracellular
B2IN71	49.03	25	620	212.01	Elongation factor Tu family protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0638 PE = 4 SV = 1 - [B2IN71_STRPS]	Intracellular
B2IPR1	75.6	16	332	210.14	L-lactate dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = Idh PE = 3 SV = 1 - [B2IPR1_STRPS]	Intracellular
B2IQK0	51.83	30	872	209.16	Alanine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = alaS PE = 3 SV = 1 - [SYA_STRPS]	Intracellular
B2IS44	61.29	12	93	208.67	30S ribosomal protein S19 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsS PE = 3 SV = 1 - [RS19_STRPS]	Intracellular
B2IMN9	48.47	17	425	208.24	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2034 PE = 4 SV = 1 - [B2IMN9_STRPS]	Intracellular
B2IR17	72.27	16	339	208.13	Ribose-phosphate pyrophosphokinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prsA PE = 3 SV = 1 - [B2IR17_STRPS]	Intracellular
B2IQR1	62.69	25	520	208	GMP synthase [glutamine-hydrolyzing] OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = guaA PE = 3 SV = 1 - [GUAA_STRPS]	Intracellular
B2INL3	50.25	23	810	202.66	ATP-dependent Clp protease, ATP-binding subunit OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2162 PE = 3 SV = 1 - [B2INL3_STRPS]	Intracellular
B2IN11	58.53	17	299	197.31	UTP-glucose-1-phosphate uridylyltransferase OS = <i>Streptococcus pneumoniae</i> (strain	Secretory (released) (with CS)

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQ5	44.38	17	480	197.17	CGSP14) GN = galU PE = 3 SV = 1 - [B2IN11_STRPS] Peptidoglycan N-acetylglucosamine deacetylase A OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pgdA PE = 4 SV = 1 - [B2IQ5_STRPS]	N-terminally anchored (with CS)
B2INE4	57.49	19	447	196.45	DEAD-box ATP-dependent RNA helicase CshB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rheB PE = 3 SV = 1 - [B2INE4_STRPS]	Intracellular
B2IMU6	65.97	15	238	196.17	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0590 PE = 4 SV = 1 - [B2IMU6_STRPS]	Lipid anchored
B2INT7	54.92	27	752	195.47	ATP-dependent Clp protease, ATP-binding subunit ClpE OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = clpE PE = 3 SV = 1 - [B2INT7_STRPS]	Intracellular
B2IM28	44.59	17	388	194.98	Protein RecA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = recA PE = 3 SV = 1 - [RECA_STRPS]	Intracellular
B2IQL4	79.78	18	267	192.86	Phosphate import ATP-binding protein PstB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL4_STRPS]	Intracellular
B2ISL0	43.67	13	332	190.43	PTS system, mannose-specific IIAB components OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = manL PE = 4 SV = 1 - [B2ISL0_STRPS]	Intracellular
B2INW3	54.6	19	511	188.38	Sugar ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0788 PE = 4 SV = 1 - [B2INW3_STRPS]	Intracellular
B2INT0	40.9	22	648	185.21	DNA gyrase subunit B OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gyrB PE = 3 SV = 1 - [B2INT0_STRPS]	Intracellular
B2IP17	57.18	13	341	184.52	Iron-compound ABC transporter, iron compound-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1009 PE = 4 SV = 1 - [B2IP17_STRPS]	Lipid anchored
B2IPT0	48.09	22	628	183.98	Elongation factor 4 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lepA PE = 3 SV = 1 - [B2IPT0_STRPS]	Intracellular
B2IN23	39.89	17	752	181.97	Alpha-1,4 glucan phosphorylase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = malP PE = 3 SV = 1 - [B2IN23_STRPS]	Intracellular
B2IQZ2	64.71	20	374	180.03	Ribosome-binding ATPase YchF OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ychF PE = 3 SV = 1 - [B2IQZ2_STRPS]	Intracellular
B2IMU8	66.81	17	229	179.85	50S ribosomal protein L1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplA PE = 3 SV = 1 - [RL1_STRPS]	Intracellular
B2ISY6	51.06	14	331	178.98	Oligopeptide ABC transporter, ATP-binding protein AmfF OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = amfF PE = 3 SV = 1 - [B2ISY6_STRPS]	Intracellular
B2IR87	54.15	18	410	170.5	ATP-dependent Clp protease ATP-binding subunit ClpX OS = <i>Streptococcus pneumoniae</i> (strain	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IR07	58.6	24	442	169.98	CGSP14) GN = clpX PE = 3 SV = 1 - [CLPX_STRPS] Adenylosuccinate synthetase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = purA PE = 3 SV = 1 - [B2IR07_STRPS]	Intracellular
B2ILV9	61.23	20	454	169.93	Aspartokinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lysC PE = 3 SV = 1 - [B2ILV9_STRPS]	Intracellular
B2IMZ6	42.12	25	857	169.84	DNA mismatch repair protein MutS OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hexA PE = 3 SV = 1 - [B2IMZ6_STRPS]	Intracellular
B2IS57	84.76	17	164	169.34	30S ribosomal protein S5 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsE PE = 3 SV = 1 - [B2IS57_STRPS]	Intracellular
B2ILR1	29.62	20	719	166.63	Penicillin-binding protein 1A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pbp1A PE = 4 SV = 1 - [B2ILR1_STRPS]	N-terminally anchored (with CS)
B2IQ78	53.73	22	523	165.23	Signal recognition particle protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ffh PE = 3 SV = 1 - [B2IQ78_STRPS]	Intracellular
B2ISM3	78.38	17	148	163.82	50S ribosomal protein L13 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplM PE = 3 SV = 1 - [B2ISM3_STRPS]	Intracellular
B2INJ2	70.02	21	427	163.61	DltD protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = dltD PE = 4 SV = 1 - [B2INJ2_STRPS]	N-terminally anchored (with CS)
B2ISJ1	48.84	19	602	159.21	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glmS PE = 3 SV = 1 - [B2ISJ1_STRPS]	Intracellular
B2IPM7	52.69	18	577	159.04	Phosphoenolpyruvate-protein phosphotransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1121 PE = 3 SV = 1 - [B2IPM7_STRPS]	Intracellular
B2IQ29	61.01	17	436	157.98	GTPase Obg OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = obg PE = 3 SV = 1 - [OBG_STRPS]	Intracellular
B2IS66	74.8	9	127	157.55	30S ribosomal protein S11 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsK PE = 3 SV = 1 - [RS11_STRPS]	Intracellular
B2IQ67	33.36	24	1058	157.35	Carbamoyl-phosphate synthase large chain OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = carb PE = 3 SV = 1 - [CARB_STRPS]	Intracellular
B2IQ25	62.29	16	419	154.75	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murZ PE = 3 SV = 1 - [B2IQ25_STRPS]	Intracellular
B2IQX4	82.93	14	164	154.18	ATP synthase subunit b OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpF PE = 3 SV = 1 - [ATPF_STRPS]	N-terminally anchored (with CS)
B2IM72	69.51	10	246	154.11	Amino acid ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glnQ PE = 4 SV = 1 - [B2IM72_STRPS]	Intracellular
B2INR2	35.79	19	679	153.3	Methionine--tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = metG PE = 3 SV = 1 - [B2INR2_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IS42	81.63	6	98	152.99	50S ribosomal protein L23 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplW PE = 3 SV = 1 - [RL23_STRPS]	Intracellular
B2IPIO	44.02	18	418	152.53	Serine hydroxymethyltransferase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glyA PE = 3 SV = 1 - [GLYA_STRPS]	Intracellular
B2IRG4	73.89	21	203	151.28	30S ribosomal protein S4 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsD PE = 3 SV = 1 - [RS4_STRPS]	Intracellular
B2ILV7	59.29	21	452	150.33	Serine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = serS PE = 3 SV = 1 - [B2ILV7_STRPS]	Intracellular
B2INC8	60.65	13	216	146.88	Uracil phosphoribosyltransferase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = upp PE = 3 SV = 1 - [B2INC8_STRPS]	Intracellular
B2IQL3	70.63	16	252	145.61	Phosphate import ATP-binding protein PstB OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL3_STRPS]	Intracellular
B2IMZ5	31.91	19	564	144.21	ABC transporter, ATP-binding/permease protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2042 PE = 4 SV = 1 - [B2IMZ5_STRPS]	Multi-transmembrane
B2IS39	59.8	8	102	143.18	30S ribosomal protein S10 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsJ PE = 3 SV = 1 - [RS10_STRPS]	Intracellular
B2IP78	53.87	13	375	141.59	Carboxynorspermidine decarboxylase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nspC PE = 4 SV = 1 - [B2IP78_STRPS]	Intracellular
B2IM60	54.81	13	416	139.41	Diaminopimelate decarboxylase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lysA PE = 3 SV = 1 - [B2IM60_STRPS]	Intracellular
B2IMH3	65.03	14	346	139.16	Catabolite control protein A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ccpA PE = 4 SV = 1 - [B2IMH3_STRPS]	Intracellular
B2IS68	57.03	8	128	139.07	50S ribosomal protein L17 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplQ PE = 3 SV = 1 - [RL17_STRPS]	Intracellular
B2INE0	34.95	13	329	136.77	Cell division protein FtsX OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsX PE = 3 SV = 1 - [B2INE0_STRPS]	Multi-transmembrane
B2ILV2	41.65	20	778	134.89	Endonuclease MutS2 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mutS2 PE = 3 SV = 1 - [MUTS2_STRPS]	Intracellular
B2IQU4	63.93	15	280	133.96	Oxidoreductase, aldo/keto reductase family OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1466 PE = 4 SV = 1 - [B2IQU4_STRPS]	Intracellular
B2IS55	71.35	14	178	129.78	50S ribosomal protein L6 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplF PE = 3 SV = 1 - [RL6_STRPS]	Intracellular
B2ISJ8	67.95	15	156	129.16	30S ribosomal protein S7 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsG PE = 3 SV = 1 - [RS7_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IRR6	24.81	8	266	128.96	Cell division protein DivIVA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1633 PE = 4 SV = 1 - [B2IRR6_STRPS]	Intracellular
B2IS18	43.68	14	419	128.49	Zinc metalloprotease OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = eep PE = 3 SV = 1 - [B2IS18_STRPS]	Multi-transmembrane
B2INF3	47.94	9	267	128.19	Peptidyl-prolyl cis-trans isomerase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ppiA PE = 3 SV = 1 - [B2INF3_STRPS]	Lipid anchored
B2IR96	96.59	13	176	127.15	Adenine phosphoribosyltransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = apt PE = 3 SV = 1 - [B2IR96_STRPS]	Intracellular
B2IQX1	63.36	12	292	124.75	ATP synthase gamma chain OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpG PE = 3 SV = 1 - [ATPG_STRPS]	Intracellular
B2IQL7	40.41	12	292	124.49	Phosphate ABC transporter, phosphate-binding protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1389 PE = 4 SV = 1 - [B2IQL7_STRPS]	Lipid anchored
B2IS49	69.77	10	86	124.47	30S ribosomal protein S17 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsQ PE = 3 SV = 1 - [RS17_STRPS]	Intracellular
B2IQV3	35.99	8	289	123.64	Glycerol uptake facilitator protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glpF PE = 3 SV = 1 - [B2IQV3_STRPS]	Multi-transmembrane
B2IM98	46.54	14	535	123.63	CTP synthase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pyrG PE = 3 SV = 1 - [B2IM98_STRPS]	Intracellular
B2IQ39	47.38	9	344	123.25	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1211 PE = 4 SV = 1 - [B2IQ39_STRPS]	Secretory (released) (with CS)
B2IP00	65.99	14	397	123.01	Serine protease OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = sphra PE = 4 SV = 1 - [B2IP00_STRPS]	N-terminally anchored (No CS)
B2IN41	35.2	16	429	122.13	Histidine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hisS PE = 3 SV = 1 - [SYH_STRPS]	Intracellular
B2IRW4	40.6	15	436	120.41	GTPase Der OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = der PE = 3 SV = 1 - [DER_STRPS]	Intracellular
B2IR68	35.97	13	531	119.86	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1535 PE = 4 SV = 1 - [B2IR68_STRPS]	Multi-transmembrane
B2IPR2	27.94	18	841	118.97	DNA gyrase subunit A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gyra PE = 3 SV = 1 - [B2IPR2_STRPS]	Intracellular
B2IRA1	44.08	17	524	118.46	DEAD-box ATP-dependent RNA helicase CshA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = cshA PE = 3 SV = 1 - [B2IRA1_STRPS]	Intracellular
B2IM46	55.94	11	345	117.68	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1931 PE = 4 SV = 1 - [B2IM46_STRPS]	N-terminally anchored (with CS)
B2ILR7	48.44	16	481	117.48	6-phosphogluconate dehydrogenase, decarboxylating	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IR57	62.02	19	466	116.97	OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gnd PE = 3 SV = 1 - [B2ILR7_STRPS] Cof family protein/peptidyl-prolyl cis-trans isomerase, cyclophilin type OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1524 PE = 4 SV = 1 - [B2IR57_STRPS]	Intracellular
B2IND9	76.09	12	230	114.4	Cell division ABC transporter, ATP-binding protein FtsE OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsE PE = 4 SV = 1 - [B2IND9_STRPS]	Intracellular
B2IPU7	45	13	320	113.45	Ribonucleoside-diphosphate reductase subunit beta OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nrdF PE = 3 SV = 1 - [B2IPU7_STRPS]	Intracellular
B2IMS9	43.58	20	553	112.79	Ribonuclease J OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rnj PE = 3 SV = 1 - [B2IMS9_STRPS]	Intracellular
B2ISQ9	52.02	15	494	111.9	UPF0371 protein SPCG_0344 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0344 PE = 3 SV = 1 - [Y344_STRPS]	Intracellular
B2IPF4	53.38	14	311	110.86	Adhesion lipoprotein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = Imb PE = 3 SV = 1 - [B2IPF4_STRPS]	Lipid anchored
B2IM45	50.82	12	427	110.79	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murA PE = 3 SV = 1 - [B2IM45_STRPS]	Intracellular
B2INN5	58.46	10	272	109.88	Cell shape-determining protein MrcC OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mreC PE = 3 SV = 1 - [B2INN5_STRPS]	N-terminally anchored (with CS)
B2IM15	46.84	10	158	109.73	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1901 PE = 4 SV = 1 - [B2IM15_STRPS]	Multi-transmembrane
B2IRP9	25.68	14	740	109.17	GTP pyrophosphokinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = reIA PE = 3 SV = 1 - [B2IRP9_STRPS]	Intracellular
B2INE1	34.71	11	726	108.73	PTS system, IIABC components OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ptsG PE = 4 SV = 1 - [B2INE1_STRPS]	Multi-transmembrane
B2IST7	48.44	12	417	108.26	Capsular polysaccharide biosynthesis protein, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1812 PE = 3 SV = 1 - [B2IST7_STRPS]	Intracellular
B2IP76	71.33	14	286	106.74	Polyamine aminopropyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = speE PE = 3 SV = 1 - [B2IP76_STRPS]	Intracellular
B2IS23	34.49	22	954	105.34	UvrABC system protein A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = uvra PE = 3 SV = 1 - [B2IS23_STRPS]	Intracellular
B2IS53	52.81	7	89	105.15	30S ribosomal protein S14 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsN PE = 3 SV = 1 - [RS14_STRPS]	Intracellular
B2IQN9	49.79	20	486	105.03	Nicotinate phosphoribosyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1408 PE = 3 SV = 1 - [B2IQN9_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ISZ6	49.88	13	419	104.34	Sugar ABC transporter, sugar-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1871 PE = 4 SV = 1 - [B2ISZ6_STRPS]	Lipid anchored
B2IMA9	47.02	17	487	101.63	Type I restriction-modification system, M subunit OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hsdM PE = 4 SV = 1 - [B2IMA9_STRPS]	Intracellular
B2IQH2	72.19	13	187	100.73	50S ribosomal protein L10 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplI PE = 3 SV = 1 - [B2IQH2_STRPS]	Intracellular
B2IR61	39.82	14	447	100.57	Asparagine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = asnS PE = 3 SV = 1 - [B2IR61_STRPS]	Intracellular
B2IM30	50	15	338	100.22	Transcriptional regulator, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lytR PE = 4 SV = 1 - [B2IM30_STRPS]	Secretory (released) (with CS)
B2ISV9	32.29	10	511	99.16	Choline transporter OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1834 PE = 3 SV = 1 - [B2ISV9_STRPS]	Multi-transmembrane
B2IS51	78.22	12	101	98.15	50S ribosomal protein L24 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplX PE = 3 SV = 1 - [RL24_STRPS]	Intracellular
B2IRX0	76.67	11	240	97.84	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1687 PE = 4 SV = 1 - [B2IRX0_STRPS]	Intracellular
B2ISX8	46.73	11	336	97.53	Non-canonical purine NTP pyrophosphatase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1853 PE = 3 SV = 1 - [B2ISX8_STRPS]	Intracellular
B2IN24	45.35	14	505	97.46	4-alpha-glucanotransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = malm PE = 3 SV = 1 - [B2IN24_STRPS]	Intracellular
B2IM61	54.55	12	275	97.4	Purine operon repressor OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = purR PE = 4 SV = 1 - [B2IM61_STRPS]	Intracellular
B2ILX0	55.38	16	455	97.1	Acetyl-CoA carboxylase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = accC PE = 4 SV = 1 - [B2ILX0_STRPS]	Intracellular
B2IQ19	35.91	15	763	96.38	DNA helicase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pcrA PE = 3 SV = 1 - [B2IQ19_STRPS]	Intracellular
B2IPZ6	69.23	10	91	95.95	DNA-binding protein HU OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hlpA PE = 3 SV = 1 - [B2IPZ6_STRPS]	Intracellular
B2INE5	31.57	12	396	95.45	S-adenosylmethionine synthase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = metK PE = 3 SV = 1 - [METK_STRPS]	Intracellular
B2IS82	28.53	15	659	94.22	Serine/threonine protein kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pkn2 PE = 3 SV = 1 - [B2IS82_STRPS]	Intracellular/TMH start AFTER 60
B2IS65	58.68	10	121	93.19	30S ribosomal protein S13 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsM PE = 3 SV = 1 - [RS13_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B21MI4	35.02	18	731	92.99	Penicillin-binding protein 2A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pbp2A PE = 4 SV = 1 - [B21MI4_STRPS]	N-terminally anchored (with CS)
B2IRK4	37.05	17	637	92.87	tRNA uridine 5'-carboxymethylaminomethyl modification enzyme MnmG OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mnmG PE = 3 SV = 1 - [B2IRK4_STRPS]	Intracellular
B2ISW0	69.01	11	242	92.71	Choline transporter OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1835 PE = 4 SV = 1 - [B2ISW0_STRPS]	Intracellular
B2INP6	39.59	10	341	92.59	Tryptophan-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = trpS PE = 3 SV = 1 - [B2INP6_STRPS]	Intracellular
B2INC5	39.5	11	281	92.15	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0692 PE = 4 SV = 1 - [B2INC5_STRPS]	Intracellular
B2IP66	36.64	17	715	91.51	Transcriptional regulator, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0885 PE = 4 SV = 1 - [B2IP66_STRPS]	Intracellular
B2ISY1	46.58	14	541	91.27	Dextran glucosidase DexS, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dexs PE = 4 SV = 1 - [B2ISY1_STRPS]	Intracellular
B2ISK7	38.51	11	444	91.26	Aminopeptidase C OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pepC PE = 4 SV = 1 - [B2ISK7_STRPS]	Intracellular
B2INL6	30.75	8	335	91.2	ABC transporter, substrate-binding protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2165 PE = 4 SV = 1 - [B2INL6_STRPS]	Lipid anchored
B2IQY9	25.83	11	453	90.37	Chromosomal replication initiator protein DnaA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaA PE = 3 SV = 1 - [DNAA_STRPS]	Intracellular
B2IRS8	22.34	13	685	90.12	Penicillin-binding protein 2B OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pbp2B PE = 4 SV = 1 - [B2IRS8_STRPS]	N-terminally anchored (with CS)
B2IS56	53.44	8	131	88.46	50S ribosomal protein L18 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplR PE = 3 SV = 1 - [B2IS56_STRPS]	Intracellular
B2IQ12	40.13	9	319	88.41	Ribose-phosphate pyrophosphokinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prs PE = 3 SV = 1 - [B2IQ12_STRPS]	Intracellular
B2IS67	52.73	9	311	88.17	DNA-directed RNA polymerase subunit alpha OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rpoA PE = 3 SV = 1 - [B2IS67_STRPS]	Intracellular
B2IS59	64.38	9	146	88.05	50S ribosomal protein L15 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplO PE = 3 SV = 1 - [RL15_STRPS]	Intracellular
B2IQ61	41.52	9	289	87.7	Choline kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pck PE = 4 SV = 1 - [B2IQ61_STRPS]	Intracellular
B2ISY2	24.4	10	705	87.27	Trehalose PTS system, IIABC components OS = <i>-Streptococcus pneumoniae</i> strain CGSP14) GN = treP PE = 4 SV = 1 - [B2ISY2_STRPS]	Multi-transmembrane

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IMQ3	25.97	12	801	86.8	Phenylalanine-tRNA ligase beta subunit OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pheT PE = 3 SV = 1 - [B2IMQ3_STRPS]	Intracellular
B2IQ02	60.82	9	97	86.49	50S ribosomal protein L27 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmA PE = 3 SV = 1 - [RL27_STRPS]	Intracellular
B2IN58	40.3	11	335	86.4	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2101 PE = 4 SV = 1 - [B2IN58_STRPS]	Multi-transmembrane
B2IQW4	62.2	10	209	84.4	Amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1486 PE = 4 SV = 1 - [B2IQW4_STRPS]	Intracellular
B2IP74	35.23	13	491	84.24	Lysine decarboxylase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = cad PE = 4 SV = 1 - [B2IP74_STRPS]	Intracellular
B2IS70	42.49	11	433	83.11	UPF0210 protein SPCG_0246 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0246 PE = 3 SV = 1 - [B2IS70_STRPS]	Intracellular
B2IQ66	64.29	13	280	83.05	LicD2 protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = licD2 PE = 4 SV = 1 - [B2IQ66_STRPS]	Intracellular
B2IRK5	34.17	12	559	81.76	Ribonuclease J OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rnj PE = 3 SV = 1 - [B2IRK5_STRPS]	Intracellular
B2IR84	68.24	12	296	81.42	Nucleotide-binding protein SPCG_1551 OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1551 PE = 3 SV = 1 - [Y1551_STRPS]	Intracellular
B2IMB5	34.93	15	607	81.24	Chaperone protein Dnak OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaK PE = 3 SV = 1 - [DNAK_STRPS]	Intracellular
B2IQ62	58.09	12	346	80.31	Alcohol dehydrogenase, zinc-containing OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1234 PE = 4 SV = 1 - [B2IQ62_STRPS]	Intracellular
B2IMB9	37.86	11	243	79.94	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0494 PE = 4 SV = 1 - [B2IMB9_STRPS]	Intracellular
B2IRD4	56.18	5	89	79.7	30S ribosomal protein S15 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsO PE = 3 SV = 1 - [RS15_STRPS]	Intracellular
B2IN68	50.79	8	126	79.14	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0635 PE = 4 SV = 1 - [B2IN68_STRPS]	N-terminally anchored (with CS)
BEIQN0	44.09	3	127	78.99	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1399 PE = 4 SV = 1 - [B2IQN0_STRPS]	N-terminally anchored (with CS)
B2ILY0	36.58	14	514	78.96	Peptide chain release factor 3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prfC PE = 3 SV = 1 - [RF3_STRPS]	Intracellular
B2IS54	57.58	8	132	78.42	30S ribosomal protein S8 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsH PE = 3 SV = 1 - [RS8_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IMB6	35.19	13	378	78.27	Chaperone protein DnaJ OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaJ PE = 3 SV = 1 - [B2IMB6_STRPS]	Intracellular
B2IQS5	42.11	12	247	78.16	Amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1447 PE = 4 SV = 1 - [B2IQS5_STRPS]	Intracellular
B2IRH0	33.01	12	512	76.84	ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0089 PE = 4 SV = 1 - [B2IRH0_STRPS]	Lipid anchored
B2IPW9	50.88	11	283	76.72	Ribosome biogenesis GTPase A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1141 PE = 3 SV = 1 - [B2IPW9_STRPS]	Intracellular
B2IN18	48.09	11	418	76.44	Tyrosine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tyrS PE = 3 SV = 1 - [SYY_STRPS]	Intracellular
B2IQV9	33.39	13	572	75.14	Phosphoglucomutase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pgm PE = 3 SV = 1 - [B2IQV9_STRPS]	Intracellular
B2IP77	38.9	9	419	74.94	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0896 PE = 4 SV = 1 - [B2IP77_STRPS]	Intracellular
B2IQ63	53.19	8	235	74.22	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ispD PE = 3 SV = 1 - [ISP_D_STRPS]	Intracellular
B2IRQ4	65.34	11	251	74.2	Manganese ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1621 PE = 4 SV = 1 - [B2IRQ4_STRPS]	Intracellular
B2IQX3	62.36	8	178	73.32	ATP synthase subunit delta OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpH PE = 3 SV = 1 - [ATPD_STRPS]	Intracellular
B2INX4	27.48	12	826	73.21	DNA topoisomerase 4 subunit A OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = parC PE = 3 SV = 1 - [B2INX4_STRPS]	Intracellular
B2IN76	40.67	12	450	72.88	UDP-N-acetylmuramoylalanine--D-glutamate ligase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = murD PE = 3 SV = 1 - [MURD_STRPS]	Intracellular
B2IPC3	41.47	10	299	72.75	GTPase Era OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = era PE = 3 SV = 1 - [ERA_STRPS]	Intracellular
B2IRX7	40.79	13	353	72.1	Methionine import ATP-binding protein MetN OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = metN PE = 3 SV = 1 - [B2IRX7_STRPS]	Intracellular
B2INU1	50.65	9	306	70.6	Bifunctional protein F0ID OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = foID PE = 3 SV = 1 - [B2INU1_STRPS]	Lipid anchored
B2ILW4	36.11	6	324	69.62	Enoyl-(Acyl-carrier-protein) reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = fabK PE = 4 SV = 1 - [B2ILW4_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IS45	40.35	3	114	69.56	50S ribosomal protein L22 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplV PE = 3 SV = 1 - [RL22_STRPS]	Intracellular
B2IPZ1	30.21	13	652	67.59	DNA ligase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ligA PE = 3 SV = 1 - [DNLJ_STRPS]	Intracellular
B2IMZ3	34.66	12	603	66.96	ABC transporter, ATP-binding/permease protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2040 PE = 4 SV = 1 - [B2IMZ3_STRPS]	Multi-transmembrane
B2IS63	54.72	6	212	66.76	Adenylate kinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = adk PE = 3 SV = 1 - [KAD_STRPS]	Intracellular
B2INV5	53.01	6	83	65.46	30S ribosomal protein S20 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsT PE = 3 SV = 1 - [B2INV5_STRPS]	Intracellular
B2IRK2	38.07	10	373	65.45	tRNA-specific 2-thiouridylase MnM <sub>a</sub> OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mnmA PE = 3 SV = 2 - [MNMA_STRPS]	Intracellular
B2IRB7	27.22	2	158	65.21	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1584 PE = 4 SV = 1 - [B2IRB7_STRPS]	N-terminally anchored (with CS)
B2INE6	56.27	10	311	64.68	Dihydroorotate dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pyrd PE = 3 SV = 1 - [B2INE6_STRPS]	Intracellular
B2IMB0	27.41	15	777	64.13	Type I restriction-modification system, R subunit OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hsdR PE = 4 SV = 1 - [B2IMB0_STRPS]	Intracellular
B2ISQ4	20.27	11	750	63.95	Penicillin-binding protein 2X OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pbp2X PE = 4 SV = 1 - [B2ISQ4_STRPS]	N-terminally anchored (with CS)
B2IPQ3	27.88	12	556	63.73	Formate-tetrahydrofolate ligase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fhs PE = 3 SV = 1 - [FTHS_STRPS]	Intracellular
B2IMU7	41.84	5	141	63.38	50S ribosomal protein L11 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplK PE = 3 SV = 1 - [RL11_STRPS]	Intracellular
B2ISY0	38	4	100	62.82	UPF0154 protein SPCG_1855 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1855 PE = 3 SV = 1 - [B2ISY0_STRPS]	N-terminally anchored (with CS)
B2IQP4	29.49	9	434	62.33	Peptidase, U32 family OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1416 PE = 4 SV = 1 - [B2IQP4_STRPS]	Intracellular
B2IR79	57.14	11	259	62.31	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1546 PE = 4 SV = 1 - [B2IR79_STRPS]	N-terminally anchored (with CS)
B2ILW2	21.6	8	324	62.19	3-oxoacyl-[acyl-carrier-protein] synthase 3 OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fabH PE = 3 SV = 1 - [FABH_STRPS]	Intracellular
B2IPZ9	43.28	9	305	61.07	Riboflavin biosynthesis protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mreA PE = 3 SV = 1 - [B2IPZ9_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IN32	29.98	13	587	60.72	Aspartate-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aspS PE = 3 SV = 1 - [SYD_STRPS]	Intracellular
B2IRR9	42.46	7	179	60.72	Cell division protein SepF OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = sepF PE = 3 SV = 1 - [SEPF_STRPS]	Intracellular
B2IN19	28.12	13	690	60.07	Cation-transporting ATPase, E1-E2 family OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ctpC PE = 3 SV = 1 - [B2IN19_STRPS]	Intracellular
B2IQY8	36.71	9	444	59.95	UDP-N-acetylmuramate-L-alanine ligase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murC PE = 3 SV = 1 - [MURC_STRPS]	Intracellular
B2IS26	33.86	8	189	59.81	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0204 PE = 4 SV = 1 [B2IS26_STRPS]	Lipid anchored
B2INY7	45.25	8	263	59.27	ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0812 PE = 4 SV = 1 - [B2INY7_STRPS]	Intracellular
B2ILR6	14.66	6	464	58.78	Mid-cell-anchored protein Z OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mapZ PE = 3 SV = 1 - [B2ILR6_STRPS]	N-terminally anchored (with CS)
B2ILX8	36.68	10	488	58.59	Glutamyl-tRNA(Gln) amidotransferase subunit A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gata PE = 3 SV = 1 - [GATA_STRPS]	Intracellular
B2IMN3	35.26	7	380	58.5	Queuine tRNA-ribosyltransferase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tgt PE = 3 SV = 1 - [TGT_STRPS]	Intracellular
B2IN17	21.09	12	825	58.4	Penicillin-binding protein 1B OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pbp1B PE = 4 SV = 1 - [B2IN17_STRPS]	Intracellular/TMH start AFTER 60
B2IR27	45.45	8	330	57.61	Phosphate acyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = plsX PE = 3 SV = 1 - [PLSX_STRPS]	Intracellular
B2INF7	73.21	6	112	56.77	ATP cone domain-containing protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0728 PE = 4 SV = 1 - [B2INF7_STRPS]	Intracellular
B2IMZ8	20.78	9	563	56.13	Arginine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = argS PE = 3 SV = 1 - [SYR_STRPS]	Intracellular
B2ILU5	35.6	10	427	55.96	Trigger factor OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tig PE = 3 SV = 1 - [TIG_STRPS]	Intracellular
B2INK8	56.55	9	336	55.86	tRNA-dihydrouridine synthase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2157 PE = 3 SV = 1 [B2INK8_STRPS]	Intracellular
B2IM90	37.14	11	560	55.51	ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0465 PE = 4 SV = 1 - [B2IM90_STRPS]	Intracellular
B2IQA4	37.72	12	448	54.94	Glutamate dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gdhA PE = 3 SV = 1 - [B2IQA4_STRPS]	Intracellular
B2IPPO	30.1	8	495	54.8	Glucose-6-phosphate 1-dehydrogenase OS = - <i>Streptococcus</i>	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IPZ5	24.33	13	633	54.29	pneumoniae (strain CGSP14) GN = zwf PE = 3 SV = 1 - [B2IPPO_STRPS] ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1167 PE = 4 SV = 1 - [B2IPZ5_STRPS]	Intracellular
B2IS10	23.73	8	649	54.27	DNA mismatch repair protein MutL OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = mutL PE = 3 SV = 1 - [MUTL_STRPS]	Intracellular
B2IN96	47.62	10	378	54.14	Lactate oxidase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = IctO PE = 4 SV = 1 - [B2IN96_STRPS]	Intracellular
B2IND6	51.69	6	236	53.89	Branched-chain amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0703 PE = 4 SV = 1 - [B2IND6_STRPS]	Intracellular
B2IS98	58.85	9	209	53.7	Probable nicotinate-nucleotide adenyllyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nadD PE = 3 SV = 1 - [B2IS98_STRPS]	Intracellular
B2IPN8	43.01	8	272	53.21	Cof family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1058 PE = 4 SV = 1 - [B2IPN8_STRPS]	Intracellular
B2INN8	51.27	10	275	52.83	Energy-coupling factor transporter ATP-binding protein EcfA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ecfA PE = 3 SV = 1 - [B2INN8_STRPS]	N-terminally anchored (with CS)
B2ISR0	23.55	8	535	52.43	Glucan 1,6-alpha-glucosidase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = dexB PE = 4 SV = 1 - [B2ISR0_STRPS]	Intracellular
B2IRF6	52.19	8	228	52.22	Potassium uptake protein, Trk family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = trkA PE = 4 SV = 1 - [B2IRF6_STRPS]	N-terminally anchored (with CS)
B2IM43	17.16	7	443	51.63	CBS domain protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1928 PE = 4 SV = 1 - [B2IM43_STRPS]	Multi-transmembrane
B2ILX2	40	6	255	51.58	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = accA PE = 3 SV = 1 - [ACCA_STRPS]	Intracellular
B2ISA0	34.78	10	368	51.32	GTP-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1723 PE = 4 SV = 1 - [B2ISA0_STRPS]	Intracellular
B2IPB9	23.19	10	677	50.7	Endo-beta-N-acetylglucosaminidase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lytB PE = 4 SV = 1 - [B2IPB9_STRPS]	Secretory (released) (with CS)
B2IQW9	61.87	6	139	50.69	ATP synthase epsilon chain OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpC PE = 3 SV = 1 - [ATPE_STRPS]	Intracellular
B2IPN9	24.94	7	429	50.34	Signal recognition particle receptor FtsY OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsY PE = 3 SV = 1 - [B2IPN9_STRPS]	Intracellular
B2IRI7	17.95	9	713	49.57	Uncharacterized protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0106 PE = 4 SV = 1 - [B2IRI7_STRPS]	Multi-transmembrane
B2IQH3	24.39	7	488	49.39	Chlorohydrolase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14)	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IRD2	21.59	7	264	48.68	GN = trzA PE = 4 SV = 1 - [B2IQH3_STRPS] Acyltransferase family protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1599 PE = 4 SV = 1 - [B2IRD2_STRPS]	Multi-transmembrane
B2IR63	31.14	7	395	48.62	Aspartate aminotransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aspB PE = 3 SV = 1 - [B2IR63_STRPS]	Intracellular
B2INK4	23.36	10	608	48.46	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2153 PE = 4 SV = 1 - [B2INK4_STRPS]	N-terminally anchored (with CS)
B2IN18	28.14	7	501	48.06	Zinc ABC transporter, zinc-binding lipoprotein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2137 PE = 3 SV = 1 - [B2IN18_STRPS]	Lipid anchored
B2IPU4	40.71	7	253	47.82	Lactose phosphotransferase system repressor OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lacR PE = 4 SV = 1 - [B2IPU4_STRPS]	Intracellular
B2INN7	41.94	8	279	47.56	Energy-coupling factor transporter ATP-binding protein EcfA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ecfA PE = 3 SV = 1 - [B2INN7_STRPS]	N-terminally anchored (with CS)
B2IS50	52.46	7	122	47.46	50S ribosomal protein L14 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplN PE = 3 SV = 1 - [RL14_STRPS]	Intracellular
B2IRI6	32.63	3	95	47.46	Bacteriocin putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0105 PE = 4 SV = 1 - [B2IRI6_STRPS]	N-terminally anchored (with CS)
B2IPJ1	50	9	264	47.22	Iron-compound ABC transporter, ATP-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1013 PE = 4 SV = 1 - [B2IPJ1_STRPS]	Intracellular
B2IPR3	40.89	7	247	47.21	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1083 PE = 4 SV = 1 - [B2IPR3_STRPS]	N-terminally anchored (with CS)
B2IR24	28.28	9	389	47.15	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0036 PE = 3 SV = 1 - [B2IR24_STRPS]	Intracellular
B2ISX7	60.12	8	173	47.03	Phosphoesterase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1852 PE = 3 SV = 1 - [B2ISX7_STRPS]	Intracellular
B2IM86	29.62	8	449	46.75	Potassium uptake protein, Trk family OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = trkA PE = 4 SV = 1 - [B2IM86_STRPS]	N-terminally anchored (with CS)
B2IPT8	33.13	9	326	46.7	Tagatose 1,6-diphosphate aldolase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lacD PE = 3 SV = 1 - [B2IPT8_STRPS]	Intracellular
B2ILZ5	39.81	10	540	46.59	60 kDa chaperonin OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = grol PE = 3 SV = 1 - [CH60_STRPS]	Intracellular
B2ILX7	34.58	8	480	46.43	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gatB PE = 3 SV = 1 - [GATB_STRPS]	Intracellular
B2IMQ9	16.96	8	737	45.59	Polyribonucleotide nucleotidyltransferase OS =	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQN3	45.45	5	66	44.91	<i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pnp PE = 3 SV = 2 - [PNP_STRPS]	Intracellular
B2IR90	38.37	2	172	44.68	30S ribosomal protein S21 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsU PE = 3 SV = 1 - [B2IQN3_STRPS]	Intracellular
B2IMS2	42.66	7	293	44.59	Non-heme iron-containing ferritin OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1557 PE = 3 SV = 1 - [B2IR90_STRPS]	Intracellular
B2IRX2	10.53	5	399	44.06	Fructose-bisphosphate aldolase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fba PE = 3 SV = 1 - [B2IMS2_STRPS]	Intracellular
B2ISH7	20.98	10	815	43.34	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1689 PE = 4 SV = 1 - [B2IRX2_STRPS]	Multi-transmembrane
B2ISG1	26.84	7	190	43.06	Formate acetyltransferase, putative OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pIF PE = 4 SV = 1 - [B2ISH7_STRPS]	Intracellular
B2IMY2	73.21	7	56	42.86	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0626 PE = 4 SV = 1 - [B2ISG1_STRPS]	Secretory (released) (with CS)
B2IM36	15.5	8	671	42.81	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1921 PE = 4 SV = 1 [B2IM36_STRPS]	Multi-transmembrane
B2IM12	26.96	8	471	42.77	Pneumolysin OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ply PE = 4 SV = 1 - [B2IM12_STRPS]	Intracellular
B2INM4	34.62	6	182	42.71	Ribosomal subunit interface protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2173 PE = 4 SV = 1 [B2INM4_STRPS]	Intracellular
B2IPW4	27.34	9	567	42.61	Dihydrolipoyl dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = acol PE = 4 SV = 1 - [B2IPW4_STRPS]	Intracellular
B2IQZ9	38.89	6	180	42.56	Hypoxanthine-guanine phosphoribosyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hgt PE = 4 SV = 1 - [B2IQZ9_STRPS]	Intracellular
B2IS21	19.23	6	234	42.44	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0199 PE = 4 SV = 1 - [B2IS21_STRPS]	Multi-transmembrane
B2IPB4	18.18	3	66	42.39	50S ribosomal protein L35 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmL PE = 3 SV = 1 - [RL35_STRPS]	Intracellular
B2IM29	23.68	5	418	42.24	Putative competence-damage inducible protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = cinA PE = 3 SV = 1 - [CINA_STRPS]	Intracellular
B2IR86	66.5	11	197	41.89	Probable GTP-binding protein EngB OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = engB PE = 3 SV = 1 - [ENG_B_STRPS]	Intracellular
B2IPS2	46.7	4	212	41.29	Uridine kinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = udk PE = 3 SV = 1 - [URK_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2INP0	22.46	5	276	41.12	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2189 PE = 4 SV = 1 - [B2INP0_STRPS]	Intracellular/TMH start AFTER 60
B2ILX1	42.71	7	288	41.03	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = accD PE = 3 SV = 1 - [B2ILX1_STRPS]	Intracellular
B2IMY9	31.48	10	486	40.7	Glutamate-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gltX PE = 3 SV = 1 - [SYE_STRPS]	Intracellular
B2ISL4	51.11	9	270	40.64	Cof family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0299 PE = 4 SV = 1 - [B2ISL4_STRPS]	Intracellular
B2IQH7	42.21	5	289	40.55	Homoserine kinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = thrB PE = 3 SV = 1 - [KHNE_STRPS]	Intracellular
B2IS89	36.54	7	208	40.54	Guanylate kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gmk PE = 3 SV = 1 - [B2IS89_STRPS]	Intracellular
B2IRQ3	18.92	7	650	40.16	Endopeptidase O OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pepO PE = 4 SV = 1 - [B2IRQ3_STRPS]	Intracellular
B2IR80	26.03	6	292	39.76	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1547 PE = 4 SV = 1 - [B2IR80_STRPS]	Multi-transmembrane
B2INZ2	20.82	6	413	39.4	D-alanyl-D-alanine carboxypeptidase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dacA PE = 3 SV = 1 - [B2INZ2_STRPS]	N-terminally anchored (with CS)
B2IR93	45.95	6	259	39.26	Triosephosphate isomerase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tpiA PE = 3 SV = 1 - [B2IR93_STRPS]	Intracellular
B2IP56	34.48	8	290	39.23	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0875 PE = 4 SV = 1 - [B2IP56_STRPS]	Lipid anchored
B2IPZ7	46.59	6	279	39.17	DegV family protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1169 PE = 4 SV = 1 - [B2IPZ7_STRPS]	Intracellular
B2INK5	19.52	8	502	39.03	Glycerol kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glpK PE = 3 SV = 1 - [GLPK_STRPS]	Intracellular
B2INM2	32.67	7	150	38.84	50S ribosomal protein L9 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplI PE = 3 SV = 1 - [RL9_STRPS]	Intracellular
B2IPH6	21.17	4	359	38.78	Peptide chain release factor 1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prfA PE = 3 SV = 1 - [RF1_STRPS]	Intracellular
B2ING0	44.21	3	95	38.52	30S ribosomal protein S16 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsP PE = 3 SV = 1 - [RS16_STRPS]	Intracellular
B2IQZ0	33.07	7	378	38.34	DNA polymerase III subunit beta OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaN PE = 4 SV = 1 - [B2IQZ0_STRPS]	Intracellular
B2IR11	29.29	8	420	37.79	DNA repair protein radA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = radA PE = 3 SV = 1 - [B2IR11_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ISKO	7.86	8	1463	37.3	DNA polymerase III PolC-type OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = polC PE = 3 SV = 1 - [B2ISKO_STRPS]	Intracellular
B2IMD8	40.15	8	264	37.04	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0513 PE = 4 SV = 1 - [B2IMD8_STRPS]	Intracellular
B2IP06	33.66	7	303	36.91	Tagatose-6-phosphate kinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fruB PE = 3 SV = 1 - [B2IP06_STRPS]	Intracellular
B2IPT6	46.2	5	171	36.86	Galactose-6-phosphate isomerase subunit LacB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lacB PE = 3 SV = 1 - [LACB_STRPS]	Intracellular
B2IMT1	28.33	7	406	36.74	Beta-lactam resistance factor OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murM PE = 4 SV = 1 - [B2IMT1_STRPS]	Intracellular
B2IN52	24.87	8	567	36.72	Dihydroxy-acid dehydratase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ilvD PE = 3 SV = 1 - [ILVD_STRPS]	Intracellular
B2IRH4	23.13	5	320	36.59	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0093 PE = 4 SV = 1 - [B2IRH4_STRPS]	N-terminally anchored (No CS)
B2IME1	27.51	7	378	36.3	Transcription termination/antitermination protein NusA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = nusA PE = 3 SV = 1 - [B2IME1_STRPS]	Intracellular
B2ISX2	51.67	9	240	36.23	Pseudouridine synthase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rluB PE = 3 SV = 1 - [B2ISX2_STRPS]	Intracellular
B2IN61	77.55	6	49	35.89	50S ribosomal protein L33 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmG PE = 3 SV = 1 - [RL33_STRPS]	Intracellular
B2IQ98	60.64	4	94	35.66	50S ribosomal protein L31 type B OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmE PE = 3 SV = 1 - [B2IQ98_STRPS]	Intracellular
B2IPY5	32.19	7	379	35.53	Glycogen biosynthesis protein GlgD OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glgD PE = 4 SV = 1 - [B2IPY5_STRPS]	Intracellular
B2IRA3	21.23	4	438	35.35	Pyridine nucleotide-disulfide oxidoreductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = merA PE = 3 SV = 1 - [B2IRA3_STRPS]	Intracellular
B2IR65	48.8	6	209	35.25	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1532 PE = 4 SV = 1 - [B2IR65_STRPS]	Intracellular
B2INN1	36.99	7	346	34.94	Elongation factor Ts OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tsf PE = 3 SV = 1 - [EFTS_STRPS]	Intracellular
B2IRV5	19.83	3	343	34.85	Phospho-2-dehydro-3-deoxyheptonate aldolase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aroF PE = 3 SV = 1 - [B2IRV5_STRPS]	Intracellular
B2IN60	53.33	4	60	34.75	50S ribosomal protein L32 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmF PE = 3 SV = 1 - [RL32_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQ13	32.08	6	371	34.73	Aminotransferase, class-V OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1185 PE = 3 SV = 1 - [B2IQ13_STRPS]	Intracellular
B2INQ1	36.11	5	180	34.11	Transcriptional regulator, TetR family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2200 PE = 4 SV = 1 - [B2INQ1_STRPS]	Intracellular
B2IP08	15.91	7	767	33.57	SpoE family protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsK PE = 3 SV = 1 - [B2IP08_STRPS]	Multi-transmembrane
B2IRW8	41.24	6	177	33.56	Transcriptional repressor NrdR OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nrdR PE = 3 SV = 1 - [B2IRW8_STRPS]	Intracellular
B2ISL9	57.61	6	184	33.49	GTP cyclohydrolase 1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = foIE PE = 3 SV = 1 - [GCH1_STRPS]	Intracellular
B2IQL2	25.35	4	217	32.6	Phosphate-specific transport system accessory protein PhoU OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1384 PE = 3 SV = 1 - [B2IQL2_STRPS]	Intracellular
B2ILW1	39.58	4	144	32.47	Transcriptional regulator, MarR family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0414 PE = 4 SV = 1 - [B2ILW1_STRPS]	Intracellular
B2IS33	15.25	5	518	32.44	Competence-induced protein Ccs4 OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0211 PE = 4 SV = 1 - [B2IS33_STRPS]	Multi-transmembrane
B2INX7	16.53	4	236	32.43	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0802 PE = 4 SV = 1 - [B2INX7_STRPS]	Multi-transmembrane
B2INM3	14.16	7	657	32.29	DHH subfamily 1 protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2172 PE = 4 SV = 1 - [B2INM3_STRPS]	Multi-transmembrane
B2INY8	22.62	6	420	32.18	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0813 PE = 4 SV = 1 - [B2INY8_STRPS]	Intracellular
B2IN02	20.77	5	443	32.03	Sensor histidine kinase PnpS OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2049 PE = 4 SV = 1 - [B2IN02_STRPS]	Multi-transmembrane
B2IMR8	13.73	5	459	31.87	Transmembrane protein Vexp3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0562 PE = 3 SV = 1 - [B2IMR8_STRPS]	Multi-transmembrane
B2IP11	34.65	7	404	31.83	Probable tRNA sulfurtransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = thiI PE = 3 SV = 1 - [THII_STRPS]	Intracellular
B2INZ1	26.38	8	470	31.82	FeS assembly protein SuffB OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0816 PE = 4 SV = 1 - [B2INZ1_STRPS]	Intracellular
B2IQ37	41.27	5	252	31.78	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1209 PE = 4 SV = 1 - [B2IQ37_STRPS]	Intracellular
B2IN78	31.83	6	399	31.49	Cell division protein DivIB OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = divIB PE = 3 SV = 1 - [B2IN78_STRPS]	Intracellular/TMH start AFTER 60
B2IS71	21.69	5	249	31.41	Phosphoglycerate mutase family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14)	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQJ2	23.71	5	388	31.39	GN = gpmB PE = 4 SV = 1 - [B2IS71_STRPS] Chorismate synthase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aroC PE = 3 SV = 1 - [AROC_STRPS]	Intracellular
B2IST9	13.89	7	583	31.29	ABC transporter, ATP-binding/permease protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1814 PE = 4 SV = 1 - [B2IST9_STRPS]	Multi-transmembrane
B2IPQ2	32.05	6	234	30.92	Uncharacterized protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1072 PE = 4 SV = 1 - [B2IPQ2_STRPS]	Intracellular
B2ISM0	18.89	3	270	30.69	7,8-dihydronopterin aldolase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = sulD PE = 3 SV = 1 - [B2ISMO_STRPS]	Intracellular
B2IMH1	16.74	6	472	30.67	Cof family protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1962 PE = 4 SV = 1 - [B2IMH1_STRPS]	Intracellular
B2ISV2	17.09	5	392	30.34	Galactokinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gaIK PE = 3 SV = 1 - [B2ISV2_STRPS]	Intracellular
B2IR20	12.6	8	889	30.19	DNA polymerase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = poIA PE = 3 SV = 1 - [B2IR20_STRPS]	Intracellular
B2IR50	26.4	6	481	30	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-L-lysine ligase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murE PE = 3 SV = 1 - [B2IR50_STRPS]	Intracellular
B2ISI6	20.93	6	258	29.94	Isoprenyl transferase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = upps PE = 3 SV = 1 - [B2ISI6_STRPS]	Intracellular
B2IMQ1	31.47	7	375	29.85	Phenylalanine-tRNA ligase alpha subunit OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pheS PE = 3 SV = 1 - [B2IMQ1_STRPS]	Intracellular
B2ISS1	20.39	6	407	29.77	Tryptophan synthase beta chain OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = trpB PE = 3 SV = 1 - [TRPB_STRPS]	Intracellular
B2IMZ0	22.05	5	449	29.65	Glucose-6-phosphate isomerase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pgI PE = 3 SV = 1 - [G6PI_STRPS]	Intracellular
B2IRD1	13.75	4	778	29.59	Cation-transporting ATPase, E1-E2 family OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ctpE PE = 3 SV = 1 - [B2IRD1_STRPS]	Multi-transmembrane
B2IQ17	51.09	5	229	29.49	Glutamine amidotransferase, class I OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1189 PE = 4 SV = 1 - [B2IQ17_STRPS]	Intracellular
B2IPV5	30.5	5	200	29.44	Uncharacterized protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1127 PE = 4 SV = 1 - [B2IPV5_STRPS]	Intracellular
B2ILW5	27.78	6	306	29.34	Malonyl CoA-acyl carrier protein transacylase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fabD PE = 3 SV = 1 - [B2ILW5_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQ16	29.48	6	424	29.26	Psr protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1358 PE = 4 SV = 1 - [B2IQ16_STRPS]	Intracellular/ TMH start AFTER 60
B2IPB3	40.51	5	195	28.99	Translation initiation factor IF-3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = infC PE = 3 SV = 1 - [B2IPB3_STRPS]	Intracellular
B2IM63	18.52	7	432	28.92	Competence-induced protein Ccs50 OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1944 PE = 4 SV = 1 - [B2IM63_STRPS]	N-terminally anchored (No CS)
B2IP99	44.53	6	247	28.66	Uridylate kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pyrH PE = 3 SV = 1 - [B2IP99_STRPS]	Intracellular
B2IPF5	10.79	5	834	28.65	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0977 PE = 4 SV = 1 - [B2IPF5_STRPS]	N-terminally anchored (No CS)
B2IM26	26.1	6	318	28.61	Autolysin OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lytA PE = 4 SV = 1 - [B2IM26_STRPS]	Intracellular
B2INF6	43.1	5	239	28.52	tRNA (guanine-N(1))-methyltransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = trnD PE = 3 SV = 1 - [TRMD_STRPS]	Intracellular
B2ISI0	15.25	6	833	28.41	Leucine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = leuS PE = 3 SV = 1 - [SYL_STRPS]	Intracellular
B2IQH8	20.5	6	439	28.08	Homoserine dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hom PE = 3 SV = 1 - [B2IQH8_STRPS]	Intracellular
B2INT8	57.89	3	76	27.91	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0763 PE = 4 SV = 1 - [B2INT8_STRPS]	Intracellular
B2IMD9	15.64	2	211	27.64	tRNA (guanine-N(7))-methyltransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = trnB PE = 3 SV = 1 - [TRMB_STRPS]	Intracellular
B2IQS2	26.88	4	253	27.63	SpoU rRNA methylase family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = trmB PE = 3 SV = 1 - [B2IQS2_STRPS]	Intracellular
B2IR73	16.69	5	623	27.4	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1540 PE = 4 SV = 1 - [B2IR73_STRPS]	Intracellular
B2IPP4	10.12	6	662	27.29	UvrABC system protein B OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = uvrB PE = 3 SV = 1 - [UVRB_STRPS]	Intracellular
B2IQK6	26.91	4	301	27.27	UDP-N-acetylglucosamine reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = murB PE = 3 SV = 1 - [MURB_STRPS]	Intracellular
B2IP98	25.45	6	444	27.26	Methylenetetrahydrofolate--tRNA-(uracil-5-)methyltransferase TrmFO OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = trmFO PE = 3 SV = 2 - [TRMFO_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQK5	19.48	5	385	27.06	Spermidine/putrescine import ATP-binding protein Pota OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pota PE = 3 SV = 1 - [B2IQK5_STRPS]	Intracellular
B2INN3	30.61	7	392	26.86	Secreted 45 kd protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2182 PE = 4 SV = 1 - [B2INN3_STRPS] (released)	Secretory (released) (with CS)
B2IN20	40.78	4	103	26.71	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2067 PE = 4 SV = 1 - [B2IN20_STRPS]	Intracellular
B2IPN6	8.14	6	1179	26.7	Chromosome partition protein Smc OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = smc PE = 3 SV = 1 - [B2IPN6_STRPS]	Intracellular
B2IRW9	52.07	5	121	26.67	Transcriptional regulator, GntR family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1686 PE = 4 SV = 1 - [B2IRW9_STRPS]	Intracellular
B2IS84	13.47	2	438	26.55	Ribosomal RNA small subunit methyltransferase B OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = sunl PE = 3 SV = 1 - [B2IS84_STRPS]	Intracellular
B2IMX9	19.88	7	332	26.49	Pneumococcal surface protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0623 PE = 4 SV = 1 - [B2IMX9_STRPS]	Lipid anchored
B2IP15	8.69	4	541	26.36	RNA methyltransferase, TrmA family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1007 PE = 3 SV = 1 - [B2IP15_STRPS]	Intracellular
B2IPS8	15.14	7	555	25.77	DNA repair protein RecN OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = recn PE = 3 SV = 1 - [B2IPS8_STRPS]	Intracellular
B2INY5	17.06	6	551	25.65	DNA polymerase III subunits gamma and tau OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaX PE = 4 SV = 1 - [B2INY5_STRPS]	Intracellular
B2ISU0	10.1	4	594	25.61	ABC transporter, ATP-binding/permease protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1815 PE = 4 SV = 1 - [B2ISU0_STRPS]	Multi-transmembrane
B2INU6	24.53	6	424	25.32	Phosphopentomutase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = decb PE = 3 SV = 1 - [B2INU6_STRPS]	Intracellular
B2IM77	17.44	2	281	25.26	Undecaprenyl-diphosphatase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = uppP PE = 3 SV = 1 - [UPPP_STRPS]	Intracellular
B2IS76	21.11	5	398	25.26	Hydroxymethylglutaryl-CoA synthase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = mvaS PE = 4 SV = 1 - [B2IS76_STRPS]	Intracellular
B2IPD9	20.3	4	266	24.91	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0961 PE = 4 SV = 1 - [B2IPD9_STRPS]	N-terminally anchored (No CS)
B2IP88	14.52	6	420	24.84	Gamma-glutamyl phosphate reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = proA PE = 3 SV = 1 - [PROA_STRPS]	Intracellular
B2IQU2	18.36	4	305	24.77	Glycine-tRNA ligase alpha subunit OS = <i>-Streptococcus pneumoniae</i> (strain	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2INW0	25	5	220	24.67	CGSP14) GN = glyQ PE = 3 SV = 1 - [SYGA_STRPS] Deoxyribose-phosphate aldolase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = decC PE = 3 SV = 1 - [DEOC_STRPS]	Intracellular
B2IRB6	28.25	6	223	24.63	Cytidylate kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = cmk PE = 3 SV = 1 - [KCY_STRPS]	Intracellular
B2IP93	24.91	5	289	24.4	Ribosomal RNA small subunit methyltransferase I OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rsml PE = 3 SV = 1 - [B2IP93_STRPS]	Intracellular
B2IPN4	29.88	5	328	24.28	GMP reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = guaC PE = 3 SV = 1 - [GUAC_STRPS]	Intracellular
B2IN17	24.9	5	257	24.1	Fucose operon repressor, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = fcsR PE = 4 SV = 1 - [B2IN17_STRPS]	Intracellular
B2IMP1	31.07	5	206	24.09	Hydrolase, haloacid dehalogenase-like family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2031 PE = 4 SV = 1 - [B2IMP1_STRPS]	Intracellular
B2IRV6	17.78	3	343	23.78	Phospho-2-dehydro-3-deoxyheptone aldolase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aroG PE = 3 SV = 1 - [B2IRV6_STRPS]	Intracellular
B2IQ68	27.02	5	359	23.7	Carbamoyl-phosphate synthase small chain OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = carA PE = 3 SV = 1 - [B2IQ68_STRPS]	Intracellular
B2IMY1	17.2	3	279	23.45	Thymidylate synthase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = thyA PE = 3 SV = 1 - [B2IMY1_STRPS]	Intracellular
B2IM44	17.88	3	274	23.39	DNA-entry nuclease OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = endA PE = 4 SV = 1 - [B2IM44_STRPS]	N-terminally anchored (No CS)
B2IR18	22.54	3	213	23.34	Amino acid ABC transporter, ATP-binding protein, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0107 PE = 4 SV = 1 - [B2IR18_STRPS]	Intracellular
B2IRQ1	5.51	2	653	23.17	PTS system IIABC components OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = scra PE = 4 SV = 1 - [B2IRQ1_STRPS]	Multi-transmembrane
B2IQR5	19.92	4	246	23.16	CppA protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = cppA PE = 4 SV = 1 - [B2IQR5_STRPS]	Intracellular
B2ILR4	22.22	2	117	23.07	Cell cycle protein GpsB OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gpsb PE = 3 SV = 1 - [B2ILR4_STRPS]	Intracellular
B2IQN2	20.25	4	316	22.86	HPr kinase/phosphorylase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hprk PE = 3 SV = 1 - [B2IQN2_STRPS]	Intracellular
B2IMA5	25	6	448	22.78	Glutamine synthetase, type I OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glnA PE = 3 SV = 1 - [B2IMA5_STRPS]	Intracellular

TABLE 5-continued

Proteins identified in MV <sub>L</sub> from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQ75	33.87	4	186	22.38	LemA protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lemA PE = 4 SV = 1 - [B2IQ75_STRPS]	N-terminally anchored (No CS)
B2INX5	19.41	5	340	21.91	Branched-chain-amino-acid aminotransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ilvE PE = 3 SV = 1 - [B2INX5_STRPS]	Intracellular
B2IMY7	43.48	4	207	21.8	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0630 PE = 4 SV = 1 - [B2IMY7_STRPS]	Intracellular
B2IPY6	14.21	4	380	21.63	Glucose-1-phosphate adenylyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glgC PE = 3 SV = 1 - [GLGC_STRPS]	Intracellular
B2IM69	20.91	4	416	21.42	L-threonine dehydratase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ilVA PE = 3 SV = 1 - [B2IM69_STRPS]	Intracellular
B2INA8	35.36	5	263	21.29	Phosphomethylpyrimidine kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = thiD PE = 4 SV = 1 - [B2INA8_STRPS]	Intracellular
B2ISK9	13.48	3	267	21.26	PTS system, mannose-specific IIC component OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = manM PE = 4 SV = 1 - [B2ISK9_STRPS]	Multi-transmembrane(Lipid modified N-termini)
B2IQ32	12.93	3	441	21.17	Glycosyl transferase, group 1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1204 PE = 4 SV = 1 - [B2IQ32_STRPS]	Intracellular
B21M58	8.12	3	308	20.91	Membrane protein insertase YidC OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = yidC PE = 3 SV = 1 - [B21M58_STRPS]	Multi-transmembrane
B2INR5	26.54	4	309	20.79	Oxidoreductase, aldo/keto reductase family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0740 PE = 4 SV = 1 - [B2INR5_STRPS]	Intracellular
B2INC9	36.22	3	196	20.35	ATP-dependent Clp protease proteolytic subunit OS = CGSP14) GN = clpP PE = 3 SV = 1 - [CLPP_STRPS]	Intracellular
B2IQ35	25.75	4	369	20.25	RNA polymerase sigma factor SigA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rpoD PE = 3 SV = 1 - [B2IQ35_STRPS]	Intracellular
B21MR7	20	3	215	20.15	ABC transporter, ATP-binding protein Vexp2 OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0561 PE = 4 SV = 1 - [B21MR7_STRPS]	Intracellular

TABLE 6

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQV2	90.84	45	404	2064.28	Elongation factor Tu OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tuf PE = 3 SV = 1 - [B2IQV2_STRPS]	Intracellular
B2IRT9	69.68	49	442	1423.4	Sugar ABC transporter, sugar-binding protein OS = <i>-Streptococcus</i>	Lipid anchored

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2INY1	82.25	60	400	1396.37	pneumoniae (strain CGSP14) GN = SPCG_1656 PE = 4 SV = 1 - [B2IRT9_STRPS]	Intracellular
B2ISJ9	86.15	57	693	1352.29	30S ribosomal protein S1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsA PE = 4 SV = 1 - [B2INY1_STRPS]	Intracellular
B2IPX8	86.64	41	434	1214.93	Elongation factor G OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = eno PE = 3 SV = 1 - [ENO_STRPS]	Intracellular
B2INB2	75.63	49	591	1080.64	Pyruvate oxidase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = spxB PE = 3 SV = 1 - [B2INB2_STRPS]	Intracellular
B2IN25	76.44	47	450	1068.79	Maltose/maltodextrin ABC transporter, maltose/maltodextrin-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2072 PE = 4 SV = 1 - [B2IN25_STRPS]	Lipid anchored
B2INK4	76.15	51	608	829.85	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2153 PE = 4 SV = 1 - [B2INK4_STRPS]	N-terminally anchored (No CS)
B2ISK8	52.48	17	303	715.49	PTS system, mannose-specific IID component OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = manN PE = 4 SV = 1 - [B2ISK8_STRPS]	Multi-transmembrane
B2IR98	86.7	33	376	633.37	Sugar ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1565 PE = 3 SV = 1 - [B2IR98_STRPS]	Intracellular
B2IMI7	75.21	29	359	616.73	Glyceraldehyde-3-phosphate dehydrogenase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gapA PE = 3 SV = 1 - [B2IMI7_STRPS]	Intracellular
B2ISL0	55.12	20	332	608.4	PTS system, mannose-specific IIAB components OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = manL PE = 4 SV = 1 - [B2ISL0_STRPS]	Intracellular
B2IRH0	78.91	39	512	573.11	ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0089 PE = 4 SV = 1 - [B2IRH0_STRPS]	Lipid anchored
B2IS43	74.01	23	277	570.24	50S ribosomal protein L2 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIB PE = 3 SV = 1 - [RL2_STRPS]	Intracellular
B2INK5	70.12	34	502	534.8	Glycerol kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glpK PE = 3 SV = 1 - [GLPK_STRPS]	Intracellular
B2IRV7	73.6	55	837	530.96	Protein translocase subunit SecA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = secA PE = 3 SV = 1 - [B2IRV7_STRPS]	Intracellular
B2INW2	65.51	28	374	519.89	Lipoprotein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0787 PE = 4 SV = 1 - [B2INW2_STRPS]	Lipid anchored

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IPD4	58.15	28	313	480.11	Foldase protein PrsA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prsA PE = 3 SV = 1 - [PRSA_STRPS]	Lipid anchored
B2IR00	59.97	38	652	462.58	ATP-dependent zinc metalloprotease FtsH OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsH PE = 3 SV = 1 - [B2IR00_STRPS]	Multi-transmembrane
B2IM39	54.12	57	1225	460.95	DNA-directed RNA polymerase subunit beta' OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpoC PE = 3 SV = 1 - [RPOC_STRPS]	Intracellular
B2IM81	63.18	37	774	432.81	Formate acetyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pfl PE = 4 SV = 1 - [B2IM81_STRPS]	Intracellular
B2IM49	72.73	25	330	394.5	Aspartate--ammonia ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = asnA PE = 3 SV = 1 - [ASNA_STRPS]	Intracellular
B2IN96	69.05	25	378	394.18	Lactate oxidase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lctO PE = 4 SV = 1 - [B2IN96_STRPS]	Intracellular
B2IPT8	93.56	27	326	382.46	Tagatose 1,6-diphosphate aldolase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lacD PE = 3 SV = 1 - [B2IPT8_STRPS]	Intracellular
B2INN2	73.36	26	259	380.63	30S ribosomal protein S2 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsB PE = 3 SV = 2 - [RS2_STRPS]	Intracellular
B2IQX0	86.32	28	468	375.02	ATP synthase subunit beta OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpD PE = 3 SV = 1 - [ATPB_STRPS]	Intracellular
B2IMA2	85.18	25	398	374.17	Phosphoglycerate kinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pgk PE = 3 SV = 1 - [PGK_STRPS]	Intracellular
B2IS46	81.11	26	217	368.3	30S ribosomal protein S3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsC PE = 3 SV = 1 - [RS3_STRPS]	Intracellular
B2ISZ0	64.49	31	659	336.33	Oligopeptide ABC transporter, oligopeptide-binding protein AmiA OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = amiA PE = 4 SV = 1 - [B2ISZ0_STRPS]	Lipid anchored
B2ISG3	77.23	21	202	332.38	General stress protein 24, putative OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1781 PE = 4 SV = 1 - [B2ISG3_STRPS]	Intracellular
B2IML8	69.46	25	406	332.33	Acetate kinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ackA PE = 3 SV = 1 - [B2IML8_STRPS]	Intracellular
B2IP54	71.66	27	501	328.6	Pyruvate kinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0873 PE = 3 SV = 1 - [B2IP54_STRPS]	Intracellular
B2IN10	57.69	12	338	324.09	Glycerol-3-phosphate dehydrogenase [NAD(P)+] OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gpsA PE = 3 SV = 1 - [GPDA_STRPS]	Intracellular
B2IRR1	66.09	12	230	318.07	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14)	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IRI6	36.84	5	95	310.42	GN = gpmA PE = 3 SV = 1 - [GPMA_STRPS] Bacteriocin, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0105 PE = 4 SV = 1 - [B2IRI6_STRPS]	N- terminally anchored (with CS)
B2IQY1	57.35	26	551	302.6	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1503 PE = 4 SV = start 1 - [B2IQY1_STRPS]	Intracellular/TMH
B2ISJ7	67.88	14	137	301.47	30S ribosomal protein S12 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsL PE = 3 SV = 1 - [RS12_STRPS]	AFTER 60 Intracellular
B2IQX2	38.92	21	501	285.3	ATP synthase subunit alpha OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpA PE = 3 SV = 1 - [ATPA_STRPS]	Intracellular
B2IP53	50.15	16	335	269.91	ATP-dependent 6-phosphofructokinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pfkA PE = 3 SV = 1 - [PFKA_STRPS]	Intracellular
B2IR47	51.69	27	652	263.77	Oligopeptide ABC transporter, oligopeptide-binding protein AliB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = aliB PE = 4 SV = 1 - [B2IR47_STRPS]	Lipid anchored
B2ISZ6	50.36	17	419	260.6	Sugar ABC transporter, sugar-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1871 PE = 4 SV = 1 - [B2ISZ6_STRPS]	Lipid anchored
B2IMB5	52.72	24	607	254.75	Chaperone protein Dnak OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaK PE = 3 SV = 1 - [DNAK_STRPS]	Intracellular
B2INT1	42.12	26	584	253.89	Septation ring formation regulator EzrA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ezrA PE = 3 SV = 1 - [B2INT1_STRPS]	N- terminally anchored (No CS)
B2IM12	66.03	26	471	237.78	Pneumolysin OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ply PE = 4 SV = 1 - [B2IM12_STRPS]	Intracellular
B2IS47	55.47	11	137	228.66	50S ribosomal protein L16 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIP PE = 3 SV = 1 - [RL16_STRPS]	Intracellular
B2IRM9	68.48	18	276	218.21	ABC transporter, substrate-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0152 PE = 4 SV = 1 - [B2IRM9_STRPS]	Lipid anchored
B2IRG4	64.53	23	203	217.88	30S ribosomal protein S4 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsD PE = 3 SV = 1 - [RS4_STRPS]	Intracellular
B2IS57	86.59	19	164	213.33	30S ribosomal protein S5 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsE PE = 3 SV = 1 - [B2IS57_STRPS]	Intracellular
B2IM40	44.9	29.14	824	208.41	DNA-directed RNA polymerase subunit beta OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rpoB PE = 3 SV = 1 - [B2IM40_STRPS]	Intracellular
B2ISM3	87.16	20	148	207.33	50S ribosomal protein L13 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIM PE = 3 SV = 1 - [B2ISM3_STRPS]	Intracellular
B2IQ88	76.52	13	115	207.04	50S ribosomal protein L19 OS = <i>Streptococcus pneumoniae</i> (strain	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ISD1	80.77	12	104	201.22	CGSP14) GN = rpIS PE = 3 SV = 1 - [RL19_STRPS] Thioredoxin OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = trxA PE = 3 SV = 1 - [B2ISD1_STRPS]	Intracellular
B2IS40	62.98	15	208	197.25	50S ribosomal protein L3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIC PE = 3 SV = 1 - [RL3_STRPS]	Intracellular
B2IMJ9	41.57	28	890	195.17	Aldehyde-alcohol dehydrogenase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = adhE PE = 3 SV = 1 - [B2IMJ9_STRPS]	Intracellular
B2IPW4	45.86	24	567	191.95	Dihydrolipoil dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = acol PE = 4 SV = 1 - [B2IPW4_STRPS]	Intracellular
B2IP07	28.31	14	650	190.79	PTS system, fructose specific IIABC components OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fnaA PE = 4 SV = 1 - [B2IP07_STRPS]	Multi-transmembrane
B2IS55	84.27	13	178	180.96	50S ribosomal protein L6 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIF PE = 3 SV = 1 - [RL6_STRPS]	Intracellular
B2IME4	33.44	20	930	180.14	Translation initiation factor IF-2 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = infB PE = 3 SV = 1 - [IF2_STRPS]	Intracellular
B2ISW0	53.72	12	242	178.66	Choline transporter OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1835 PE = 4 SV = 1 - [B2ISW0_STRPS]	Intracellular
B2IS66	43.31	8	127	176.98	30S ribosomal protein S11 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsK PE = 3 SV = 1 - [RS11_STRPS]	Intracellular
B2IPR1	43.98	13	332	173.61	L-lactate dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = Idh PE = 3 SV = 1 - [B2IPR1_STRPS]	Intracellular
B2IRS1	44.15	15	419	169.63	Cell division protein FtsZ OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS1_STRPS]	Intracellular
B2IPP2	36.89	22	721	166.14	Amino acid ABC transporter, amino acid-binding protein/permease protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1062 PE = 3 SV = 1 - [B2IPP2_STRPS]	Multi-transmembrane
B2ILZ5	43.89	21	540	165.77	60 kDa chaperonin OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = grol PE = 3 SV = 1 - [CH60_STRPS]	Intracellular
B2IS41	65.22	16	207	162.59	50S ribosomal protein L4 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpID PE = 3 SV = 1 - [RL4_STRPS]	Intracellular
B2ILW6	75.31	15	243	162.56	3-ketoacyl-(Acyl-carrier-protein) reductase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = fabG PE = 4 SV = 1 - [B2ILW6_STRPS]	Intracellular
B2ISY9	34.54	14	498	161.94	Oligopeptide ABC transporter, permease protein Amic OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = amiC PE = 3 SV = 1 - [B2ISY9_STRPS]	Multi-transmembrane

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ISY6	58.31	18	331	161.56	Oligopeptide ABC transporter, ATP-binding protein Amf OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = amf PE = 3 SV = 1 - [B2ISY6_STRPS]	Intracellular
B2IS52	77.22	13	180	159.07	50S ribosomal protein L5 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplE PE = 3 SV = 1 - [RL5_STRPS]	Intracellular
B2ILW7	50.24	10	414	153.03	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = fabF PE = 3 SV = 1 - [B2ILW7_STRPS]	Intracellular
B2ISY7	46.76	16	355	151.21	Oligopeptide ABC transporter, ATP-binding protein AmiE OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = amiE PE = 3 SV = 1 - [B2ISY7_STRPS]	Intracellular
B2ILU5	34.89	13	427	150.71	Trigger factor OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = tig PE = 3 SV = 1 - [TIG_STRPS]	Intracellular
B2IMU8	55.46	15	229	148.33	50S ribosomal protein L1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIA PE = 3 SV = 1 - [RL1_STRPS]	Intracellular
B2IS44	56.99	10	93	148.01	30S ribosomal protein S19 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsS PE = 3 SV = 1 - [RS19_STRPS]	Intracellular
B2IQT6	47.49	18	459	146.83	NADH oxidase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = nox PE = 4 SV = 1 - [B2IQT6_STRPS]	Intracellular
B2INP5	40.65	15	492	145.57	Inosine-5'-monophosphate dehydrogenase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = imdH PE = 3 SV = 1 - [B2INP5_STRPS]	Intracellular
B2INF2	51.27	20	513	140.36	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0719 PE = 4 SV = 1 - [B2INF2_STRPS]	Intracellular
B2IRS2	50.33	12	457	140.22	Cell division protein FtsA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS2_STRPS]	Intracellular
B2IPY5	51.98	15	379	138.63	Glycogen biosynthesis protein GlgD OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glgD PE = 4 SV = 1 - [B2IPY5_STRPS]	Intracellular
B2IQH2	47.59	12	187	137.62	50S ribosomal protein L10 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpII PE = 3 SV = 1 - [B2IQH2_STRPS]	Intracellular
B2IPM7	29.64	14	577	137.31	Phosphoenolpyruvate-protein phosphotransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1121 PE = 3 SV = 1 - [B2IPM7_STRPS]	Intracellular
B2ISJ8	57.69	11	156	133.5	30S ribosomal protein S7 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsG PE = 3 SV = 1 - [RS7_STRPS]	Intracellular
B2IS54	74.24	10	132	133.28	30S ribosomal protein S8 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsH PE = 3 SV = 1 - [RS8_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2INZ1	46.38	17	470	132.61	FeS assembly protein SufB OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0816 PE = 4 SV = 1 - [B2INZ1_STRPS]	Intracellular
B2IS53	62.92	10	89	131.91	30S ribosomal protein S14 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsn PE = 3 SV = 1 - [RS14_STRPS]	Intracellular
B2IS59	63.7	9	146	131.45	50S ribosomal protein L15 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIO PE = 3 SV = 1 - [RL15_STRPS]	Intracellular
B2INH5	50.5	17	299	127.85	SPFH domain/Band 7 family OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2124 PE = 4 SV = 1 - [B2INH5_STRPS]	N-terminally anchored (No CS)
B2IRE3	73.42	12	158	127.54	PTS system, IIB component OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0062 PE = 4 SV = 1 - [B2IRE3_STRPS]	Intracellular
B2IN11	64.88	14	299	125.53	UTP-glucose-1-phosphate uridylyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = galU PE = 3 SV = 1 - [B2IN11_STRPS]	Secretory (released) (with CS)
B2IS19	43.76	23	617	125.33	Proline-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pros PE = 3 SV = 1 - [SYP_STRPS]	Intracellular
B2IPB5	60.5	12	119	125.17	50S ribosomal protein L20 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIT PE = 3 SV = 1 - [RL20_STRPS]	Intracellular
B2IMN9	40.47	11	425	125.01	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2034 PE = 4 SV = 1 - [B2IMN9_STRPS]	Intracellular
B2IS90	47.86	20	537	123.3	Ribonuclease Y OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rny PE = 3 SV = 1 - [B2IS90_STRPS]	Intracellular
B2IPB4	36.36	6	66	122.81	50S ribosomal protein L35 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmI PE = 3 SV = 1 - [RL35_STRPS]	Intracellular
B2IQW2	62.61	5	115	121.34	Bacteriocin transport accessory protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = bta PE = 4 SV = 1 - [B2IQW2_STRPS]	Intracellular
B2INS1	30.9	21	848	120	Aminopeptidase N OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pepN PE = 4 SV = 1 - [B2INS1_STRPS]	Intracellular
B2IPP1	53.66	9	246	116.21	Amino acid ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1061 PE = 4 SV = 1 - [B2IPP1_STRPS]	Intracellular
B2IMN0	34.94	8	352	116.08	Alcohol dehydrogenase, zinc-containing OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = adh PE = 3 SV = 1 - [B2IMN0_STRPS]	Intracellular
B2IRN0	53.52	14	284	114.19	Lipoprotein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0153 PE = 3 SV = 1 - [B2IRN0_STRPS]	Lipid anchored
B2IQZ2	48.66	14	374	110.45	Ribosome-binding ATPase YchF OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ychF PE = 3 SV = 1 - [B2IQZ2_STRPS]	Intracellular
B2IQ10	41.57	13	344	105.33	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain	Multi-transmembrane

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IS49	60.47	7	86	105.08	CGSP14) GN = SPCG_1352 PE = 4 SV = 1 - [B2IQ10_STRPS] 30S ribosomal protein S17 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsQ PE = 3 SV = 1 - [RS17_STRPS]	Intracellular
B2IR07	35.97	15	442	104.04	Adenylosuccinate synthetase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = purA PE = 3 SV = 1 - [B2IR07_STRPS]	Intracellular
B2IMU7	42.55	6	141	103.8	50S ribosomal protein L11 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIK PE = 3 SV = 1 - [RL11_STRPS]	Intracellular
B2ISV9	21.53	9	511	103.52	Choline transporter OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1834 PE = 3 SV = 1 - [B2ISV9_STRPS]	Multi-transmembrane
B2IQV9	40.56	16	572	103.11	Phosphoglucomutase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pgm PE = 3 SV = 1 - [B2IQV9_STRPS]	Intracellular
B2IR17	52.51	12	339	101.9	Ribose-phosphate pyrophosphokinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prsA PE = 3 SV = 1 - [B2IR17_STRPS]	Intracellular
B2IRQ3	37.38	18	650	101.35	Endopeptidase O OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pepO PE = 4 SV = 1 - [B2IRQ3_STRPS]	Intracellular
B2IS63	54.72	6	212	100	Adenylate kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = adk PE = 3 SV = 1 - [KAD_STRPS]	Intracellular
B2IMK3	38.15	13	658	99.61	Transketolase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = tktA PE = 3 SV = 1 - [B2IMK3_STRPS]	Intracellular
B2IMS2	55.97	12	293	98.56	Fructose-bisphosphate aldolase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = fba PE = 3 SV = 1 - [B2IMS2_STRPS]	Intracellular
B2IMH3	55.78	12	346	96.72	Catabolite control protein A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ccpA PE = 4 SV = 1 - [B2IMH3_STRPS]	Intracellular
B2IPY8	48.31	15	474	96.35	Glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gapN PE = 3 SV = 1 - [B2IPY8_STRPS]	Intracellular
B2ILY5	30.81	10	555	96.12	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0438 PE = 4 SV = 1 - [B2ILY5_STRPS]	Intracellular
B2IS42	95.92	11	98	95.91	50S ribosomal protein L23 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIw PE = 3 SV = 1 - [RL23_STRPS]	Intracellular
B2IM28	43.56	13	388	95.69	Protein RecA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = recA PE = 3 SV = 1 - [RECA_STRPS]	Intracellular
B2IRR6	27.44	10	266	95.52	Cell division protein DivIVA OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1633 PE = 4 SV = 1 - [B2IRR6_STRPS]	Intracellular
B2IS68	64.06	8	128	93.87	50S ribosomal protein L17 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIQ PE = 3 SV = 1 - [RL17_STRPS]	Intracellular
B2IPR2	22.47	13	841	93.61	DNA gyrase subunit A OS = <i>Streptococcus pneumoniae</i> (strain	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IM73	29.94	12	521	93.46	CGSP14) GN = gyra PE = 3 SV = 1 - [B2IPR2_STRPS] Amino acid ABC transporter, amino acid-binding protein/permease protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gInH PE = 3 SV = 1 - [B2IM73_STRPS]	Multi-transmembrane
B2INV5	59.04	7	83	93.23	30S ribosomal protein S20 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsT PE = 3 SV = 1 - [B2INV5_STRPS]	Intracellular
B2IRH9	39.61	21	616	93.09	Capsular polysaccharide biosynthesis protein, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0098 PE = 4 SV = 1 - [B2IRH9_STRPS]	Multi-transmembrane
B2INY7	55.13	9	263	92.69	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0812 PE = 4 SV = 1 - [B2INY7_STRPS]	Intracellular
B2INW3	45.21	14	511	92.64	Sugar ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0788 PE = 4 SV = 1 - [B2INW3_STRPS]	Intracellular
B2IQU4	57.5	9	280	89.47	Oxidoreductase, aldo/keto reductase family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1466 PE = 4 SV = 1 - [B2IQU4_STRPS]	Intracellular
B2ILR7	36.8	13	481	87.34	6-phosphogluconate dehydrogenase, decarboxylating OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gnd PE = 3 SV = 1 - [B2ILR7_STRPS]	Intracellular
B2IRW4	23.85	14	436	87.08	GTPase Der OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = der PE = 3 SV = 1 - [DER_STRPS]	Intracellular
B2IQW3	46.76	10	278	86.59	Amino acid ABC transporter, amino acid-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1485 PE = 4 SV = 1 - [B2IQW3_STRPS]	Lipid anchored
B2IPI7	33.14	8	341	85.78	Iron-compound ABC transporter, iron compound-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1009 PE = 4 SV = 1 - [B2IPI7_STRPS]	Lipid anchored
B2IS65	58.68	12	121	85.63	30S ribosomal protein S13 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsM PE = 3 SV = 1 - [RS13_STRPS]	Intracellular
B2ISM4	59.23	8	130	85.44	30S ribosomal protein S9 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsI PE = 3 SV = 1 - [RS9_STRPS]	Intracellular
B2INT7	37.9	17	752	85.35	ATP-dependent Clp protease, ATP-binding subunit ClpE OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = clpE PE = 3 SV = 1 - [B2INT7_STRPS]	Intracellular
B2IMU6	41.18	8	238	85.34	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0590 PE = 4 SV = 1 - [B2IMU6_STRPS]	Lipid anchored
B2IQR1	39.42	15	520	84.59	GMP synthase [glutamine-hydrolyzing] OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = guaA PE = 3 SV = 1 - [GUAA_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IPT6	68.42	8	171	84.01	Galactose-6-phosphate isomerase subunit LacB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lacB PE = 3 SV = 1 - [LACB_STRPS]	Intracellular
B2INL3	30.25	17	810	83.74	ATP-dependent Clp protease, ATP-binding subunit OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2162 PE = 3 SV = 1 - [B2INL3_STRPS]	Intracellular
B2IS51	78.22	10	101	83.43	50S ribosomal protein L24 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIX PE = 3 SV = 1 - [RL24_STRPS]	Intracellular
B2INN1	42.77	10	346	83.3	Elongation factor Ts OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tsf PE = 3 SV = 1 - [EFTS_STRPS]	Intracellular
B2IS56	67.18	10	131	82.77	50S ribosomal protein L18 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIR PE = 3 SV = 1 - [B2IS56_STRPS]	Intracellular
B2ISX8	27.68	8	336	82.74	Non-canonical purine NTP pyrophosphatase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1853 PE = 3 SV = 1 - [B2ISX8_STRPS]	Intracellular
B2INT0	24.07	12	648	81.85	DNA gyrase subunit B OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gyrB PE = 3 SV = 1 - [B2INT0_STRPS]	Intracellular
B2IN71	31.13	15	620	81.8	Elongation factor Tu family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0638 PE = 4 SV = 1 - [B2IN71_STRPS]	Intracellular
B2IPU8	19.19	9	719	79.81	Ribonucleoside-diphosphate reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = nrdE PE = 3 SV = 1 - [B2IPU8_STRPS]	Intracellular
B2INM4	49.45	9	182	79.8	Ribosomal subunit interface protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2173 PE = 4 SV = 1 - [B2INM4_STRPS]	Intracellular
B2ISV2	28.57	7	392	79.49	Galactokinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gaIK PE = 3 SV = 1 - [B2ISV2_STRPS]	Intracellular
B2IMZO	45.21	9	449	77.7	Glucose-6-phosphate isomerase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pgI PE = 3 SV = 1 - [G6PI_STRPS]	Intracellular
B2IRD4	61.8	6	89	77.46	30S ribosomal protein S15 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsO PE = 3 SV = 1 - [RS15_STRPS]	Intracellular
B2IR65	69.38	11	209	76.49	Uncharacterized protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1532 PE = 4 SV = 1 - [B2IR65_STRPS]	Intracellular
B2IR90	43.02	4	172	76.3	Non-heme iron-containing ferritin OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1557 PE = 3 SV = 1 - [B2IR90_STRPS]	Intracellular
B2IPY6	28.16	9	380	75.91	Glucose-1-phosphate adenylyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glgC PE = 3 SV = 1 - [GLGC_STRPS]	Intracellular
B2IN68	38.1	7	126	75.25	Uncharacterized protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0635 PE = 4 SV = (No CS) 1 - [B2IN68_STRPS]	N-terminally anchored

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IR60	97.92	8	96	74.99	30S ribosomal protein S6 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsF PE = 3 SV = 1 - [RS6_STRPS]	Intracellular
B2IQ02	48.45	7	97	74.43	50S ribosomal protein L27 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmA PE = 3 SV = 1 - [RL27_STRPS]	Intracellular
B2IM41	40.05	10	377	74.15	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1926 PE = 3 SV = 1 - [B2IM41_STRPS]	Intracellular
B2IR71	16.41	12	914	73.99	Cation-transporting ATPase, E1-E2 family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pacL PE = 3 SV = 1 - [B2R71_STRPS]	Multi-transmembrane
B2IQV3	35.99	6	289	72.94	Glycerol uptake facilitator protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glpF PE = 3 SV = 1 - [B2IQV3_STRPS]	Multi-transmembrane
B2ILW5	35.95	9	306	72.85	Malonyl CoA-acyl carrier protein transacylase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = fabD PE = 3 SV = 1 - [B2ILW5_STRPS]	Intracellular
B2IRQ6	42.81	10	313	71.49	Manganese ABC transporter, manganese-binding adhesion lipoprotein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1623 PE = 3 SV = 1 - [B2IRQ6_STRPS]	Lipid anchored
B2IQX4	60.98	9	164	71.29	ATP synthase subunit b OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpF PE = 3 SV = 1 - [ATPF_STRPS]	N-terminally anchored (No CS)
B2IPT0	26.59	10	628	71.03	Elongation factor 4 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lepA PE = 3 SV = 1 - [B2IPT0_STRPS]	Intracellular
B2ISJ1	27.74	11	602	69.46	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glmS PE = 3 SV = 1 - [B2ISJ1_STRPS]	Intracellular
B2IQU1	23.75	13	678	68.47	Glycine--tRNA ligase beta subunit OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glyS PE = 3 SV = 1 - [SYGB_STRPS]	Intracellular
B2INH0	41.42	11	338	68.3	Ornithine carbamoyltransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = arcB PE = 3 SV = 1 - [OTC_STRPS]	Intracellular
B2IQL4	61.42	12	267	68.04	Phosphate import ATP-binding protein PstB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL4_STRPS]	Intracellular
B2ING9	37.9	10	409	65.94	Arginine deiminase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = arcA PE = 3 SV = 1 - [ARCA_STRPS]	Intracellular
B2IR87	27.8	9	410	65.69	ATP-dependent Clp protease ATP-binding subunit ClpX OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = clpX PE = 3 SV = 1 - [CLPX_STRPS]	Intracellular
B2IPY4	19.29	7	477	64.75	Glycogen synthase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glgA PE = 3 SV = 1 - [GLGA_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ISY0	36	4	100	63.86	UPF0154 protein SPCG_1855 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1855 PE = 3 SV = 1 - [B2ISY0_STRPS]	N-terminally anchored (No CS)
B2IQL1	28.04	6	271	63.33	Amino acid ABC transporter, amino acid-binding protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1383 PE = 4 SV = 1 - [B2IQL1_STRPS]	Lipid anchored
B2IN60	55	5	60	63.27	50S ribosomal protein L32 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmF PE = 3 SV = 1 - [RL32_STRPS]	Intracellular
B2IQK0	22.48	15	872	63.2	Alanine -- tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = alaS PE = 3 SV = 1 - [SYA_STRPS]	Intracellular
B2INE4	30.43	8	447	62.91	DEAD-box ATP-dependent RNA helicase CshB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rheB PE = 3 SV = 1 - [B2INE4_STRPS]	Intracellular
B2IS50	59.84	8	122	62.57	50S ribosomal protein L14 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplN PE = 3 SV = 1 - [RL14_STRPS]	Intracellular
B2IND2	34.2	8	386	62.51	Branched-chain amino acid ABC transporter, amino acid-binding protein OS = <i>-Streptococcus pneumoniae</i> strain CGSP14) GN = SPCG_0699 PE = 4 SV = 1 - [B2IND2_STRPS]	Lipid anchored
B2IRC1	40.12	8	339	61.9	UDP-glucose 4-epimerase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gale PE = 4 SV = 1 - [B2IRC1_STRPS]	Intracellular
B2IR96	77.27	7	176	61.33	Adenine phosphoribosyltransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = apt PE = 3 SV = 1 - [B2IR96_STRPS]	Intracellular
B2IM46	42.61	9	345	61.11	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1931 PE = 4 SV = 1 - [B2IM46_STRPS]	N-terminally anchored (No CS)
B2IMG8	28.22	8	404	61	Aspartate aminotransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aspC PE = 4 SV = 1 - [B2IMG8_STRPS]	Intracellular
B2IQN0	59.06	5	127	60.7	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1399 PE = 4 SV = 1 - [B2IQN0_STRPS]	N-terminally anchored (No CS)
B2IMQ9	22.52	11	737	60.06	Polyribonucleotide nucleotidyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pnp PE = 3 SV = 2 - [PNP_STRPS]	Intracellular
B2IQ40	24.39	14	898	59.69	Phosphoenolpyruvate carboxylase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ppc PE = 3 SV = 1 - [B2IQ40_STRPS]	Intracellular
B2IND9	60.43	10	230	59.42	Cell division ABC transporter, ATP-binding protein FtsE OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsE PE = 4 SV = 1 - [B2IND9_STRPS]	Intracellular
B2IQU5	27.92	10	480	59.4	Peptidoglycan N-acetylglucosamine deacetylase A OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pgdA PE = 4 SV = 1 - [B2IQU5_STRPS]	N-terminally anchored (No CS)

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IQ23	39.06	8	425	59.39	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1195 PE = 4 SV = 1 - [B2IQ23_STRPS]	Intracellular
B2INH1	22.54	6	315	59	Carbamase kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = arcC PE = 3 SV = 1 - [B2INH1_STRPS]	Secretory (released) (with CS)
B2IRI7	20.76	11	713	58.73	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0106 PE = 4 SV = 1 - [B2IRI7_STRPS]	Multi-transmembrane
B2IMP1	37.86	6	206	56.51	Hydrolase, haloacid dehalogenase-like family OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2031 PE = 4 SV = 1 - [B2IMP1_STRPS]	Intracellular
B2IQH6	45.83	13	312	56.05	Bifunctional methionine sulfoxide reductase A/B protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = msra PE = 3 SV = 1 - [B2IQH6_STRPS]	N-terminally anchored (No CS)
B2IMB6	28.84	8	378	56.02	Chaperone protein DnaJ OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaJ PE = 3 SV = 1 - [B2IMB6_STRPS]	Intracellular
B2IS18	32.46	7	419	55.7	Zinc metalloprotease OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = eep PE = 3 SV = 1 - [B2IS18_STRPS]	Multi-transmembrane
B2IR93	26.64	4	259	55.23	Triosephosphate isomerase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tpiA PE = 3 SV = 1 - [B2IR93_STRPS]	Intracellular
B2ILX0	37.58	11	455	54.82	Acetyl-CoA carboxylase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = accC PE = 4 SV = 1 - [B2ILX0_STRPS]	Intracellular
B2IS39	65.69	6	102	54.76	30S ribosomal protein S10 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsJ PE = 3 SV = 1 - [RS10_STRPS]	Intracellular
B2IQZ9	53.89	9	180	54.16	Hypoxanthine-guanine phosphoribosyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hgt PE = 4 SV = 1 - [B2IQZ9_STRPS]	Intracellular
B2IMZ6	14.35	10	857	54.16	DNA mismatch repair protein MutS OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = hexA PE = 3 SV = 1 - [B2IMZ6_STRPS]	Intracellular
B2IR84	36.49	7	296	53.72	Nucleotide-binding protein SPCG_1551 OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1551 PE = 3 SV = 1 - [Y1551_STRPS]	Intracellular
B2ISX0	31.78	8	321	53.7	Iron-compound ABC transporter, iron-compound-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1845 PE = 4 SV = 1 - [B2ISX0_STRPS]	Lipid anchored
B2ILX6	56.45	6	186	52.99	Elongation factor P OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = efp PE = 3 SV = 1 - [EFP_STRPS]	Intracellular
B2IMZ8	23.45	9	563	52.53	Arginine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = argS PE = 3 SV = 1 - [SYR_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2INY8	20.48	9	420	52.28	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0813 PE = 4 SV = 1 - [B2INY8_STRPS]	Intracellular
B2IRA3	23.52	7	438	52.2	Pyridine nucleotide-disulfide oxidoreductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = merA PE = 3 SV = 1 - [B2IRA3_STRPS]	Intracellular
B2IPW2	16.67	6	330	51.71	Acetoin dehydrogenase, E1 component, beta subunit, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = acob PE = 4 SV = 1 - [B2IPW2_STRPS]	Intracellular
B2IRU1	40.52	9	232	51.05	Putative N-acetylmannosamine-6-phosphate 2-epimerase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nane PE = 3 SV = 1 - [B2IRU1_STRPS]	Intracellular
B2ISY2	5.67	4	705	50.76	Trehalose PTS system, IIABC components OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = treP PE = 4 SV = 1 - [B2ISY2_STRPS]	Multi-transmembrane
B2IRX3	29.29	10	297	50.56	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1690 PE = 4 SV = 1 - [B2IRX3_STRPS]	Intracellular
B2IQY9	24.72	10	453	50.5	Chromosomal replication initiator protein DnaA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dnaA PE = 3 SV = 1 - [DNAA_STRPS]	Intracellular
B2IMI1	36.61	4	183	50.13	Transcription termination/antitermination protein NusG OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = nusG PE = 3 SV = 1 - [B2IMI1_STRPS]	Intracellular
B2ING0	66.32	5	95	49.96	30S ribosomal protein S16 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsP PE = 3 SV = 1 - [RS16_STRPS]	Intracellular
B2INE1	11.85	6	726	48.69	PTS system, IIABC components OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ptsG PE = 4 SV = 1 - [B2INE1_STRPS]	Multi-transmembrane
B2IMN6	33	9	494	48.52	Threonine synthase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = thrC PE = 4 SV = 1 - [B2IMN6_STRPS]	Intracellular
B2ILR1	12.8	8	719	47.81	Penicillin-binding protein 1A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pbp1A PE = 4 SV = 1 - [B2ILR1_STRPS]	N-terminally anchored (No CS)
B2IR57	22.53	8	466	47.14	Cof family protein/peptidyl-prolyl cis-trans isomerase, cyclophilin type OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1524 PE = 4 SV = 1 - [B2IR57_STRPS]	Intracellular
B2IRQ1	10.57	5	653	46.37	PTS system IIABC components OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = scrA PE = 4 SV = 1 - [B2IRQ1_STRPS]	Multi-transmembrane
B2IRE5	23.91	5	276	46.36	PTS system, IID component OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0064 PE = 4 SV = 1 - [B2IRE5_STRPS]	Multi-transmembrane
B2IRX0	47.08	8	240	46.21	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14)	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IR19	40.73	7	302	45.81	GN = SPCG_1687 PE = 4 SV = 1 - [B2IRX0_STRPS] Amino acid ABC transporter, periplasmic amino acid-binding protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0108 PE = 4 SV = 1 - [B2IR19_STRPS]	Lipid anchored
B2IQL3	29.76	6	252	45.8	Phosphate import ATP-binding protein PstB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL3_STRPS]	Intracellular
B2IQ98	60.64	5	94	45.67	50S ribosomal protein L31 type B OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmE PE = 3 SV = 1 - [B2IQ98_STRPS]	Intracellular
B2IRE7	15.8	6	386	44.99	Sugar isomerase domain protein AgaS OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = agaS PE = 4 SV = 1 - [B2IRE7_STRPS]	Intracellular
B2IMZ5	21.99	9	564	44.92	ABC transporter, ATP-binding/permease protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2042 PE = 4 SV = 1 - [B2IMZ5_STRPS]	Multi-transmembrane
B2IRQ7	56.4	7	172	44.31	Probable thiol peroxidase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = tpx PE = 3 SV = 1 - [B2IRQ7_STRPS]	Intracellular
B2ILX8	33.61	8	488	43.22	Glutamyl-tRNA(Gln) amidotransferase subunit A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gata PE = 3 SV = 1 - [GATA_STRPS]	Intracellular
B2IM45	29.04	8	427	43.07	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murA PE = 3 SV = 1 - [B2IM45_STRPS]	Intracellular
B2IM60	25	6	416	43.06	Diaminopimelate decarboxylase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lysA PE = 3 SV = 1 - [B2IM60_STRPS]	Intracellular
B2IRA1	21.95	9	524	42.94	DEAD-box ATP-dependent RNA helicase CshA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = cshA PE = 3 SV = 1 - [B2IRA1_STRPS]	Intracellular
B2IR78	17.33	6	450	42.72	Phosphoglucosamine mutase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glmM PE = 3 SV = 1 - [GLMM_STRPS]	Intracellular
B2IST7	22.06	7	417	42.6	Capsular polysaccharide biosynthesis protein, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1812 PE = 3 SV = 1 - [B2IST7_STRPS]	Intracellular
B2INT8	57.89	2	76	41.48	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0763 PE = 4 SV = 1 - [B2INT8_STRPS]	Intracellular
B2IQ61	25.95	6	289	41.38	Choline kinase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pck PE = 4 SV = 1 - [B2IQ61_STRPS]	Intracellular
B2ILR6	14.66	5	464	41.32	Mid-cell-anchored protein Z OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mapZ PE = 3 SV = 1 - [B2ILR6_STRPS]	Multi-transmembrane

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ILR4	29.91	3	117	40.9	Cell cycle protein GpsB OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gpsB PE = 3 SV = 1 - [B2ILR4_STRPS]	Intracellular
B2IQ25	28.88	8	419	40.5	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murZ PE = 3 SV = 1 - [B2IQ25_STRPS]	Intracellular
B2IQH8	27.11	8	439	40.48	Homoserine dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hom PE = 3 SV = 1 - [B2IQH8_STRPS]	Intracellular
B2IN58	28.96	6	335	40.08	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2101 PE = 4 SV = 1 - [B2IN58_STRPS]	Multi-transmembrane
B2IRK5	20.21	7	559	40.01	Ribonuclease J OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rnj PE = 3 SV = 1 - [B2IRK5_STRPS]	Intracellular
B2INR2	26.51	10	679	39.94	Methionine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = metG PE = 3 SV = 1 - [B2INR2_STRPS]	Intracellular
B2IQ29	30.5	8	436	39.93	GTPase Obg OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = obg PE = 3 SV = 1 - [OBG_STRPS]	Intracellular
B2IMY2	73.21	7	56	39.82	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0626 PE = 4 SV = 1 - [B2IMY2_STRPS]	Secretory (released)
B2IPW3	33.6	8	375	39.59	Dihydrolipoamide acetyltransferase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = acoC PE = 3 SV = 1 - [B2IPW3_STRPS]	Intracellular
B2IR18	38.5	6	213	39.57	Amino acid ABC transporter, ATP-binding protein, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0107 PE = 4 SV = 1 - [B2IR18_STRPS]	Intracellular
B2ISR0	13.08	6	535	39.53	Glucan 1,6-alpha-glucosidase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = dexB PE = 4 SV = 1 - [B2ISR0_STRPS]	Intracellular
B2IM72	56.91	6	246	39.3	Amino acid ABC transporter, ATP-binding protein OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glnQ PE = 4 SV = 1 - [B2IM72_STRPS]	Intracellular
B2IS45	48.25	3	114	39.27	50S ribosomal protein L22 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpIV PE = 3 SV = 1 - [RL22_STRPS]	Intracellular
B2IM26	26.1	5	318	39.16	Autolysin OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lytA PE = 4 SV = 1 - [B2IM26_STRPS]	Intracellular
B2IM71	46.48	4	71	38.4	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0446 PE = 4 SV = 1 - [B2IM71_STRPS]	Intracellular
B2INE0	30.09	8	329	38.01	Cell division protein FtsX OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsX PE = 3 SV = 1 - [B2INE0_STRPS]	Multi-transmembrane
B2IQ19	15.99	8	763	37.52	DNA helicase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = pcrA PE = 3 SV = 1 - [B2IQ19_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IRN7	21.52	6	660	37.25	Threonine-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = thrS PE = 3 SV = 1 - [B2IRN7_STRPS]	Intracellular
B2IS67	24.44	7	311	36.89	DNA-directed RNA polymerase subunit alpha OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpoA PE = 3 SV = 1 - [B2IS67_STRPS]	Intracellular
B2IQA4	21.65	8	448	36.16	Glutamate dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gdhA PE = 3 SV = 1 - [B2IQA4_STRPS]	Intracellular
B2INE5	20.96	6	396	36.08	S-adenosylmethionine synthase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = metK PE = 3 SV = 1 - [METK_STRPS]	Intracellular
B2IQN3	51.52	4	66	35.91	30S ribosomal protein S21 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpsU PE = 3 SV = 1 - [B2IQN3_STRPS]	Intracellular
B2IRK4	18.21	8	637	35.62	tRNA uridine 5'-carboxymethylaminomethyl modification enzyme MnmG OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = mnmG PE = 3 SV = 1 - [B2IRK4_STRPS]	Intracellular
B2IR76	14.89	4	282	35.33	DegV family protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1543 PE = 4 SV = 1 - [B2IR76_STRPS]	Intracellular
B2IRT0	25.85	4	294	35.15	ROK family protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1647 PE = 4 SV = 1 - [B2IRT0_STRPS]	Intracellular
B2IR21	58.62	7	145	35.06	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0033 PE = 4 SV = 1 - [B2IR21_STRPS]	Intracellular
B2IPF4	25.4	8	311	35.02	Adhesion lipoprotein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = Imb PE = 3 SV = 1 - [B2IPF4_STRPS]	Lipid anchored
B2ILV9	26.21	6	454	34.9	Aspartokinase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = lysC PE = 3 SV = 1 - [B2ILV9_STRPS]	Intracellular
B2IPPO	17.37	7	495	34.88	Glucose-6-phosphate 1-dehydrogenase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = zwf PE = 3 SV = 1 - [B2IPPO_STRPS]	Intracellular
B2IRU0	8.43	5	510	34.78	PTS system, IIBC components OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1657 PE = 4 SV = 1 - [B2IRU0_STRPS]	Multi-transmembrane
B2IQX1	34.93	7	292	34.34	ATP synthase gamma chain OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = atpG PE = 3 SV = 1 - [ATPG_STRPS]	Intracellular
B2ISZ2	16.94	5	490	34.05	Sucrose phosphorylase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1867 PE = 4 SV = 1 - [B2ISZ2_STRPS]	Intracellular
B2IP00	28.21	6	397	33.73	Serine protease OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = sphra PE = 4 SV = 1 - [B2IP00_STRPS]	N-terminally anchored (No CS)
B2INW0	31.82	5	220	33.64	Deoxyribose-phosphate aldolase OS = - <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = deoC PE = 3 SV = 1 - [DEOC_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IM98	15.7	6	535	33.16	CTP synthase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pyrG PE = 3 SV = 1 - [B2IM98_STRPS]	Intracellular
B2IMU0	11.8	4	466	33.1	Dipeptidase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = pepV PE = 4 SV = 1 - [B2IMU0_STRPS]	Intracellular
B2IPU4	37.15	5	253	33.02	Lactose phosphotransferase system repressor OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lacR PE = 4 SV = 1 - [B2IPU4_STRPS]	Intracellular
B2IRR9	38.55	5	179	32.57	Cell division protein SepF OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = sepf PE = 3 SV = 1 - [SEPF_STRPS]	Intracellular
B2IQJ5	27.56	5	225	32.43	3-dehydroquinate dehydratase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = aroD PE = 3 SV = 1 - [AROD_STRPS]	Intracellular
B2INC8	38.43	7	216	32.37	Uracil phosphoribosyltransferase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = upp PE = 3 SV = 1 - [B2INC8_STRPS]	Intracellular
B2INU6	33.73	8	424	32.09	Phosphopentomutase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = deob PE = 3 SV = 1 - [B2INU6_STRPS]	Intracellular
B2IME1	24.87	6	378	32.06	Transcription termination/antitermination protein NusA OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = nusA PE = 3 SV = 1 - [B2IME1_STRPS]	Intracellular
B2INN5	22.79	4	272	31.93	Cell shape-determining protein MreC OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = mreC PE = 3 SV = 1 - [B2INN5_STRPS]	N-terminally anchored (No CS)
B2IN76	12.22	6	450	31.55	UDP-N-acetylglucosaminyloylalanine--D-glutamate ligase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = murD PE = 3 SV = 1 - [MURD_STRPS]	Intracellular
B2INF3	16.1	4	267	31.17	Peptidyl-prolyl cis-trans isomerase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ppiA PE = 3 SV = 1 - [B2INF3_STRPS]	Lipid anchored
B2IQ22	31.82	6	286	30.97	Methionine aminopeptidase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = map PE = 1 SV = 1 - [B2IQ22_STRPS]	Intracellular
B2IS48	70.59	5	68	30.8	50S ribosomal protein L29 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmC PE = 3 SV = 1 - [RL29_STRPS]	Intracellular
B2IS10	19.57	6	649	30.36	DNA mismatch repair protein MutL OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = mutL PE = 3 SV = 1 - [MUTL_STRPS]	Intracellular
B2IM44	29.93	6	274	30.16	DNA-entry nuclelease OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = endA PE = 4 SV = 1 - [B2IM44_STRPS]	N-terminally anchored (No CS)
B2INF7	54.46	5	112	29.66	ATP cone domain-containing protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0728 PE = 4 SV = 1 - [B2INF7_STRPS]	Intracellular
B2IS34	13.3	7	737	29.66	Anaerobic ribonucleoside triphosphate reductase OS = <i>Streptococcus pneumoniae</i> (strain	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2IN20	40.78	3	103	29.58	CGSP14) GN = SPCG_0212 PE = 4 SV = 1 - [B2IS34_STRPS] Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2067 PE = 4 SV = 1 - [B2IN20_STRPS]	Intracellular
B2IMB7	38.95	4	95	29.51	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0492 PE = 4 SV = 1 - [B2IMB7_STRPS]	Intracellular
B2IMY9	20.58	6	486	29.3	Glutamate-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = gltX PE = 3 SV = 1 - [SYE_STRPS]	Intracellular
B2IQ75	46.77	5	186	29.27	LemA protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lemA PE = 4 SV = 1 - [B2IQ75_STRPS]	N-terminally anchored (No CS)
B2IPN9	20.75	6	429	29.19	Signal recognition particle receptor FtsY OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ftsY PE = 3 SV = 1 - [B2IPN9_STRPS]	Intracellular
B2INM2	45.33	7	150	28.94	50S ribosomal protein L9 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rplI PE = 3 SV = 1 - [RL9_STRPS]	Intracellular
B2IR80	20.89	5	292	28.84	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1547 PE = 4 SV = 1 - [B2IR80_STRPS]	Multi-transmembrane
B2IPW9	32.16	8	283	28.64	Ribosome biogenesis GTPase A OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1141 PE = 3 SV = 1 - [B2IPW9_STRPS]	Intracellular
B2IN61	75.51	4	49	28.27	50S ribosomal protein L33 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmG PE = 3 SV = 1 - [RL33_STRPS]	Intracellular
B2IM15	25.95	5	158	28.26	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1901 PE = 4 SV = 1 - [B2IM15_STRPS]	Multi-transmembrane
B2IPZ5	13.9	7	633	28.19	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1167 PE = 4 SV = 1 - [B2IPZ5_STRPS]	Intracellular
B2IMA9	20.94	6	487	28.12	Type I restriction-modification system, M subunit OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hsdM PE = 4 SV = 1 - [B2IMA9_STRPS]	Intracellular
B2IQ67	11.63	7	1058	28.04	Carbamoyl-phosphate synthase large chain OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = carb PE = 3 SV = 1 - [CARB_STRPS]	Intracellular
B2IMV8	16.5	4	491	28.03	PTS system, IIC component, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0602 PE = 4 SV = 1 - [B2IMV8_STRPS]	Multi-transmembrane
B2INP6	15.54	4	341	28	Tryptophan-tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = trpS PE = 3 SV = 1 - [B2INP6_STRPS]	Intracellular
B2INU9	26.02	4	269	27.83	Purine nucleoside phosphorylase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = npn PE = 3 SV = 1 - [B2INU9_STRPS]	Intracellular
B2IMS9	20.8	7	553	27.26	Ribonuclease J OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = rnj PE = 3 SV = 1 - [B2IMS9_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ILY3	41.94	4	62	27.17	50S ribosomal protein L28 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = rpmB PE = 3 SV = 1 - [RL28_STRPS]	Intracellular
B2IRW8	34.46	5	177	26.92	Transcriptional repressor NrdR OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nrdR PE = 3 SV = 1 - [B2IRW8_STRPS]	Intracellular
B2IMH1	20.97	7	472	26.92	Cof family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1962 PE = 4 SV = 1 - [B2IMH1_STRPS]	Intracellular
B2IPM 6	13.79	2	87	26.87	Phosphocarrier protein HPr OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1120 PE = 4 SV = 1 - [B2IPM6_STRPS]	Intracellular
B2IRB7	27.22	2	158	26.84	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1584 PE = 4 SV = 1 - [B2IRB7_STRPS] (No CS)	N- terminally anchored
B2ILW4	23.46	5	324	26.69	Enoyl-(Acyl-carrier-protein) reductase OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = fabK PE = 4 SV = 1 - [B2ILW4_STRPS]	Intracellular
B2IN41	16.32	7	429	26.52	Histidine -- tRNA ligase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = hisS PE = 3 SV = 1 - [SYH_STRPS]	Intracellular
B2IQU2	16.07	3	305	26.2	Glycine -- tRNA ligase alpha subunit OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = glyQ PE = 3 SV = 1 - [SYGA_STRPS]	Intracellular
B2IPW1	23.91	4	322	26.19	Acetooin dehydrogenase, E1 component, alpha subunit, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = acoA PE = 4 SV = 1 - [B2IPW1_STRPS]	Intracellular
B2ISG1	25.79	5	190	26.04	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1779 PE = 4 SV = 1 - [B2ISG1_STRPS]	Multi-transmembrane
B2IPZ7	19.35	4	279	25.42	DegV family protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1169 PE = 4 SV = 1 - [B2IPZ7_STRPS]	Intracellular
B2ISK9	13.48	4	267	25.25	PTS system, mannose-specific IIC component OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = manM PE = 4 SV = 1 - [B2ISK9_STRPS] (Lipid modified N-termini)	Multi-transmembrane
B2IS70	20.32	5	433	25.16	UPF0210 protein SPCG_0246 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0246 PE = 3 SV = 1 - [B2IS70_STRPS]	Intracellular
B2INL6	15.22	4	335	24.97	ABC transporter, substrate-binding protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2165 PE = 4 SV = 1 - [B2INL6_STRPS]	Lipid anchored
B2IPF2	46.6	4	191	24.92	Thioredoxin family protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0974 PE = 4 SV = 1 - [B2IPF2_STRPS]	Lipid anchored
B2INJ2	24.59	7	427	24.82	DltD protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dltD PE = 4 SV = 1 - [B2INJ2_STRPS] (No CS)	N- terminally anchored
B2IPH6	13.65	3	359	24.36	Peptide chain release factor 1 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prfA PE = 3 SV = 1 - [RF1_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2ILY0	20.43	4	514	24.35	Peptide chain release factor 3 OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prfC PE = 3 SV = 1 - [RF3_STRPS]	Intracellular
B2ILV5	20.88	4	182	24.04	Alkyl hydroperoxide reductase AhpD OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = mip PE = 3 SV = 1 - [B2ILV5_STRPS]	Intracellular
B2IRX2	21.8	4	399	23.82	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1689 PE = 4 SV = 1 - [B2IRX2_STRPS]	Multi-transmembrane
B2IM58	11.36	4	308	23.79	Membrane protein insertase YidC OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = yidC PE = 3 SV = 1 - [B2IM58_STRPS]	Multi-transmembrane
B2IR27	23.03	4	330	23.79	Phosphate acyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = plsX PE = 3 SV = 1 - [PLSX_STRPS]	Intracellular
B2IQL7	22.95	4	292	23.74	Phosphate ABC transporter, phosphate-binding protein, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1389 PE = 4 SV = 1 - [B2IQL7_STRPS]	Lipid anchored
B2IR86	41.62	7	197	23.54	Probable GTP-binding protein EngB OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = engB PE = 3 SV = 1 - [ENGGB_STRPS]	Intracellular
B2IP77	21	5	419	23.53	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0896 PE = 4 SV = 1 - [B2IP77_STRPS]	Intracellular
B2ISL2	23.6	5	339	23.5	Alcohol dehydrogenase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = adhP PE = 3 SV = 1 - [B2ISL2_STRPS]	Intracellular
B2IPR3	21.05	5	247	23.35	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1083 PE = 4 SV = 1 - [B2IPR3_STRPS]	N-terminally anchored (with CS)
B2IR97	25	6	252	23.12	Methyltransferase, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1564 PE = 4 SV = 1 - [B2IR97_STRPS]	Intracellular
B2IS98	24.4	4	209	22.81	Probable nicotinate-nucleotide adenylyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = nadD PE = 3 SV = 1 - [B2IS98_STRPS]	Intracellular
B2IQS3	22.11	4	303	22.58	Thioredoxin reductase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = trxB PE = 3 SV = 1 - [B2IQS3_STRPS]	Intracellular
B2IPW5	31.61	6	329	22.56	Lipoate-protein ligase, putative OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1137 PE = 4 SV = 1 - [B2IPW5_STRPS]	Intracellular
B2IMB4	30.77	5	182	22.24	Protein GrpE OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = grpE PE = 3 SV = 1 - [B2IMB4_STRPS]	Intracellular
B2ILY4	43.8	3	121	22.23	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = asp23 PE = 4 SV = 1 - [B2ILY4_STRPS]	Intracellular
B2IPH3	18.37	7	479	22.18	tRNA modification GTPase MnmE OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = thdF PE = 3 SV = 1 - [B2IPH3_STRPS]	Intracellular

TABLE 6-continued

Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing.						
Accession #	Coverage	# Peptides	#AAs	Score	Description	Localization
B2INP7	17.59	6	540	22.16	ABC transporter, ATP-binding protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_2196 PE = 4 SV = 1 - [B2INP7_STRPS]	Intracellular
B2IP74	14.66	5	491	21.9	Lysine decarboxylase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = cad PE = 4 SV = 1 - [B2IP74_STRPS]	Intracellular
B2ISG4	52.24	2	67	21.88	Uncharacterized protein OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1782 PE = 3 SV = 1 - [B2ISG4_STRPS]	Intracellular
B2ISY1	17.74	7	541	21.79	Dextran glucosidase DexS, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = dexS PE = 4 SV = 1 - [B2ISY1_STRPS]	Intracellular
B2IMA5	15.4	5	448	21.67	Glutamine synthetase, type I OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = glnA PE = 3 SV = 1 - [B2IMA5_STRPS]	Intracellular
B2IN77	24.72	6	352	21.61	UDP-N-acetylglucosamine -- N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = murG PE = 3 SV = 1 - [MURG_STRPS]	Intracellular
B2IQ12	25.39	5	319	21.48	Ribose-phosphate pyrophosphokinase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = prs PE = 3 SV = 1 - [B2IQ12_STRPS]	Intracellular
B2ISL4	16.3	4	270	21.32	Cof family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_0299 PE = 4 SV = 1 - [B2ISL4_STRPS]	Intracellular
B2IQ78	13.38	5	523	21.2	Signal recognition particle protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = ffh PE = 3 SV = 1 - [B2IQ78_STRPS]	Intracellular
B2IS71	21.69	5	249	21.06	Phosphoglycerate mutase family protein OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = gpmB PE = 4 SV = 1 - [B2IS71_STRPS]	Intracellular
B2IM30	15.38	5	338	20.64	Transcriptional regulator, putative OS = <i>-Streptococcus pneumoniae</i> (strain CGSP14) GN = lytR PE = 4 SV = 1 - [B2IM30_STRPS]	Secretory (released with CS)
B2IQN9	10.91	4	486	20.01	Nicotinate phosphoribosyltransferase OS = <i>Streptococcus pneumoniae</i> (strain CGSP14) GN = SPCG_1408 PE = 3 SV = 1 - [B2IQN9_STRPS]	Intracellular

**Example 2: Microparticles (MPs) Confer Serotype-Independent Protective Immunity**

**[0188]** For each immunization experiment male C57BL/6 wild-type mice ca 5 weeks old were used. Before immunization, mice were anesthetized by inhalation of isofluorane (Abbott) and then intranasally administered with 50 µl/mouse of microparticles (MP) combined with the adjuvant aluminium hydroxide (Sigma Aldrich, 10 mg/ml in PBS) or the adjuvant alone for the control groups. Immunization was repeated again after two weeks from the first immunization following the same conditions described above. After 4 weeks of immunization, mice were infected

by intranasal administration of 50 µl/mouse of  $5 \times 10^6$  CFU, for *S. pneumoniae* type 1 infection experiments (FIG. 7), or  $10^6$  CFU, for *S. pneumoniae* type 3 infection experiments (FIG. 11).

**[0189]** All mice were anesthetized by inhalation of isofluorane prior to challenge with bacteria. After the infection, clinical symptoms of the mice were monitored multiple times per day (in accordance with the ethical permit). Blood samples (5 µl/mouse) were taken every day of infection and level of bacteremia was assessed by plating serial dilutions of blood samples onto blood-agar plates. Mice that reached humane end-points were anesthetized by inhalation of isofluorane and intranasal administered with 100 µl/mouse of

the fluorescent marker Bacterisense 645 (Perkin Elmer) 30 minutes before the sacrifice. Prior to sacrifice, mice were anesthetized again (isoflurane inhalation). After sacrifice, mice were placed in the IVIS Spectrum Imaging System to detect the fluorescent signal of the bacterial infection. After the IVIS imaging, lungs and spleens were collected for further analysis. Bacterial amount in the lungs was assessed by CFU count of bacteria after plating serial dilutions of lung homogenates onto blood-agar plates.

[0190] Intranasal immunization with microparticles (MP) from a serotype 4 *S. pneumoniae* increased survival after a challenge with serotype 1 *S. pneumoniae* (FIG. 7A). The increase in survival was significant also in comparison to immunization with MV<sub>L</sub>. The immunization greatly reduced the bacterial load in the lungs of the mice surviving the challenge with serotype 1 *S. pneumoniae* (FIG. 7B). Furthermore, mice immunized with serotype 3 MP and then challenged with serotype 3 bacteria showed 100% protection in the pneumonia model, higher than immunization with the currently available vaccine PCV13, which only conferred a protection of 75% in the model. Moreover, protection against pneumococcal infection was absent in mice lacking B cells, suggesting the involvement of an adaptive immune response with anti-pneumococcal antibodies (FIG. 11).

**Example 3: Serotype-Independent Binding and Opsonophagocytic Activity of Antibodies Raised Against Pneumococcal Membrane Particles**

[0191] Antibodies raised against the inventive MP from serotype 4 as antigen were reactive against *Streptococcus pneumoniae* of other serotypes, such as 1, 6B and 3 (FIG. 9). In other words, the MP were able to elicit production of serotype-independent antibodies, much more so than MV<sub>L</sub> (FIG. 9B).

[0192] The production of anti-MP antibodies in mice was more efficient (in particular at time point 2 weeks) when the antigen was administered together with an adjuvant. However, satisfactory results were obtainable even without an adjuvant (FIG. 8). Moreover, antibodies in sera were able to bind both the encapsulated T4 (TIGR4) and the unencapsulated isogenic mutant T4R to the same extent, indicating that the antibody response was capsule-independent.

[0193] Importantly, the anti-MP antibodies showed opsonophagocytic activity, increasing adhesion of bacteria and killing of internalized bacteria by RAW murine macrophages (FIG. 10).

#### Materials and Methods

[0194] Bacterial strains and growth conditions. In this study six strains of *Streptococcus pneumoniae* were used: TIGR4 or T4 (serotype 4) (22), its isogenic mutants lacking the cytotoxin pneumolysin (T4Δply) (23), the autolysin LytA (T4ΔlytA) (3), or the capsule (T4R) (24), ATCC 6301 (serotype 1) and 1-33 (serotype 3). Bacteria were grown in C+Y medium pH 7.9-8.0 (for serotype 4) or THY medium (for serotype 1 and 3) at 37° C. A spectrophotometer (Genesys 20, Thermo Spectronic) was used to follow the growth by monitoring the optical density (OD) at 600 nm.

[0195] Isolation and purification of membrane vesicles (MV) and microparticles (MP). For isolation of MV<sub>L</sub> from a liquid culture, pneumococcal strains (TIGR4, T4Δply or T4ΔlytA) were grown at 37° C. in C+Y medium, pH 7.9-8.0 until OD<sub>600 nm</sub>=0.9. The culture was then centrifuged (17,000×g for 30 minutes at 4° C.) to remove bacterial cells from the supernatant. The cell-free supernatant was filtered through a 0.22 μm filter (Sarstedt) and centrifuged (120,

000×g for 2 hours at 4° C.) to sediment the vesicles. Pellets were washed twice in phosphate-buffered saline (PBS) and resuspended in PBS.

[0196] For isolation of microparticles (MP) from bacteria grown on plates, the pneumococcal strains were streaked on blood agar plates and incubated overnight at 37° C. with 5% CO<sub>2</sub>. Bacteria were harvested from plates, resuspended in PBS and MP were pelleted following the same procedure as mentioned above.

[0197] Crude MV<sub>L</sub>/MP preparations were further purified by density gradient centrifugation using Optiprep™ Density Gradient Medium (Sigma). Pelleted particle fractions were adjusted to 50% (w/v) Optiprep™ in a total volume of 2 ml and overlayed with one fraction of 30% (w/v) Optiprep™ (9 ml) followed by a fraction of 5% (w/v) Optiprep™ (3 ml). Gradients were centrifuged at 250,000×g for 3 hours at 4° C. and the first 4 ml on top, containing the particles, were collected. After 3 washes with PBS (250,000×g for 2 hours at 4° C.), pellets were recovered in PBS and stored at -80° C.

[0198] Electron microscopy. To visualize MV<sub>L</sub> and MP on bacteria, *S. pneumoniae* T4R was grown in C+Y medium until OD<sub>600</sub>=0.4 at 37° C. Bacteria were harvested by centrifugation for 10 min at 4,000×g, 4° C. and pellets were suspended in 100 μl PBS. Glow discharged carbon coated grids (Oxford Instruments, UK) were incubated for 1 min with a drop of bacterial solution or purified MV<sub>L</sub>/MP preparation and negatively stained with 2% uranyl acetate in water (7 times for 10 sec). Specimens were examined on a FEI CM120 microscope operated at 80 kV. Images were collected with a side mounted camera MegaView III (Olympus Soft Imaging solutions).

[0199] Atomic force microscopy. 5 μl of MP samples isolated from *S. pneumoniae* were placed onto freshly cleaved mica (Goodfellow Cambridge Ltd., Cambridge, United Kingdom). The specimens on the mica were blot dried and placed into a desiccator for at least 2 h. Imaging was performed on a Nanoscope IIIa (Digital Instruments, Santa Barbara) Atomic Force Microscope using Tapping Mode with standard silicon cantilevers oscillating at resonant frequency (270 to 305 kHz). Images were collected at a scan rate of 0.8-1.5 Hz, depending on sample number and the size of the scan. The final images were fitted in both axes and presented in a surface plot of the height mode.

[0200] SDS-PAGE and western blotting. The total amount of proteins in purified MV<sub>L</sub> or MP, lysates of A549 cells or dendritic cells stimulated with MV<sub>L</sub> or MP were quantified with Pierce™ BCA Protein Assay Kit (Life Technologies). Samples containing equal amounts of total protein were resolved by SDS-PAGE using 4-12% Bis-Tris gels (Life Technologies) and transferred to PVDF membranes. Membranes were then blocked with 5% skim milk in PBS containing 0.1% Tween-20 and incubated with antibodies as indicated. For detection of pneumolysin a mouse monoclonal antibody (Abcam, final dilution 1:500) was used. Polyclonal GAPDH (1:2,000) and LytA (1:2,000) (3) antisera raised in rabbits were used. Rabbit polyclonal antibodies against PspC (1:1,000), RrgB (1:1,000), PsaA (1:25,000), PhtD (1:25,000) and mouse polyclonal antibodies against SrtA (1:500) were kindly provided by Novartis Vaccines and Diagnostics. As loading control for A549 cells and dendritic cell lysates, a mouse monoclonal β-actin antibody (Santa Cruz) was used as primary antibody. Anti-mouse IgG or anti-rabbit IgG conjugated to horseradish peroxidase (GE Healthcare) were used as secondary antibodies (final dilution 1:10,000). Blots were developed with Amersham™ ECL

Plus Western blotting detection system (GE Healthcare Life Sciences), using a ChemiDoc™ XRS+ (Bio-Rad Laboratories).

[0201] Tandem mass spectrometry. Proteins in MV<sub>L</sub> or MP samples were reduced, alkylated and in-solution digested by trypsin according to a standard operating procedure. Thereafter the samples were purified by Pierce C18 Spin Columns (Thermo Scientific), dried and resolved in 0.1% formic acid. The resulting peptides were separated in reversed-phase on a C18-column and electrosprayed on-line to a Q Exactive Plus mass spectrometer (Thermo Finnigan). Tandem mass spectrometry was performed applying HCD.

[0202] Database searches were made using the Sequest algorithm towards a FASTA database including proteins from *Streptococcus pneumoniae* TIGR4. The search criteria for protein identification were set to at least two matching peptides of 95% confidence level per protein. Only proteins with a Sequest score above 20 were considered for analysis, to avoid the possibility of false positives.

[0203] Subcellular localizations of proteins were predicted using the algorithm of website Locate P located at the following URL: [www.cmbi.ru.nl/locatep-db/cgi-bin/locatepdb.py](http://www.cmbi.ru.nl/locatep-db/cgi-bin/locatepdb.py) (11).

[0204] Hemolysis assay. Purified MV<sub>L</sub> or MP were incubated in 96-well plates with blood from buffy coats (diluted 1:50 in PBS containing 1 mM dithiothreitol (DTT)) for 1 hour at 37° C. After 50 minutes 1% Triton X-100 in PBS was added to the positive control wells in order to lyse all the erythrocytes. Plates were then spun at 400×g for 15 minutes at 4° C.; supernatant was transferred to an optical plate and the optical density was measured at 540 nm.

[0205] A549 cell toxicity assay. A549 lung epithelial cells were grown and maintained at 37° C., with 5% CO<sub>2</sub> in RPMI medium (Gibco) supplemented with 10% (v/v) Fetal bovine serum (FBS) (HyClone). To assess MV<sub>L</sub> or MP associated cytotoxic effects, 0.6×10<sup>6</sup> A549 cells were seeded in 6-well plates and incubated overnight at 37° C. Cells were then washed with PBS and incubated for 24 hours with medium containing MV<sub>L</sub> or MP at indicated concentrations. Washed cells were labelled with Fixable Viability Dye eFluor® 780 (1:50,000, eBioscience) for 30 minutes at 4° C. in the dark, in presence or absence of 0.02% NP40 (Sigma) as positive control, and fixed with 4% paraformaldehyde (PFA) for 30 minutes at room temperature. Next, cells were gently scraped into PBS containing 1% FBS and analyzed in a Gallios™ Flow Cytometer (Beckman Coulter).

[0206] A549 cell uptake assay. To assess MV<sub>L</sub> and MP uptake by A549 cells via immunoblotting, 0.6×10<sup>6</sup> A549 cells were seeded in 6-well plates and incubated overnight at 37° C. Cells were then washed with PBS and incubated for 24 hours with medium containing the particles at indicated concentrations. After washing, cells were lysed in RIPA buffer containing 1× protease inhibitor cocktail (Roche). Cell lysates were analyzed by SDS-PAGE and western blotting.

[0207] Immunofluorescence microscopy. A549 cells (6.25×10<sup>4</sup>) were seeded in 24-well plates with coverslips on the bottom of the wells, and incubated at 37° C. overnight. Cells were then washed, fixed with 4% PFA for 30 minutes and permeabilized with 1% Triton X-100 in PBS for 5 minutes. MV<sub>L</sub> or MP were detected with mouse monoclonal anti-Ply (1:200) and polyclonal rabbit antibodies to LytA (1:200). Respective antigens were visualized with Alexa Fluor® 488-conjugated goat-anti mouse IgG antibody (Life Technologies) and Alexa Fluor® 350-conjugated goat-anti rabbit IgG (Life Technologies) (1:1,000).

[0208] Actin cytoskeleton was stained with Alexa Fluor® 594 Phalloidin (Life Technologies, 1:40 dilution) for 1 hour and coverslips were mounted with Vectashield (Vector Laboratories, Inc.) on microscope slides. Images were acquired with a DeltaVision microscope equipped with a 60x-objective. Quick projection images of approximately 20 z-stacks were taken. Orthogonal views were used to visualize the cell monolayer from the x, y, and z axes.

[0209] After growth, pneumococci (serotypes 1, 3 and 6B) have been stained using the sera from the immunized mice as primary antibody (dilution 1:100 in PBS 1% BSA) and Alexa Fluor 488 goat anti mouse (dilution 1:500 in PBS 1% BSA) as secondary antibody.

[0210] Imaging has been performed with high-resolution Delta Vision Elite System using 100×objective (1000× total magnification). FITC Laser intensity 50%, exposure time 0.025 ms (FIGS. 13-15).

Quantification of the Signal Detected on the Bacteria after Immunofluorescence Staining

[0211] Using the functions Image>Adjust>Threshold and Analyze>Measure of ImageJ, the area covered by the bacteria and the area covered by the signal detected on the bacteria after staining with sera were selected, defined and measured. The final signal ratio was calculated by dividing the area of the signal (detected using the sera) by the total area of the bacteria.

[0212] Isolation and differentiation of human monocyte-derived dendritic cells. Dendritic cells were isolated using RosetteSep™ Human Monocyte Enrichment Cocktail (Stemcell Technologies) according to the manufacturer's instructions. In brief, blood from buffy coats from healthy donors was incubated for 20 minutes with RosetteSep™ Human Monocyte Enrichment Cocktail (Stemcell Technologies), layered on top of Ficoll-Paque™ Plus (GE Healthcare) and centrifuged at 1200×g for 20 minutes without acceleration or brake. The monocyte containing layer was recovered, cells washed 7 times with PBS and passed through a 100 µm cell strainer. Monocytes were then differentiated for 6 days in RPMI containing 10% FBS supplemented with 37.5 ng/ml of Granulocyte macrophage colony-stimulating factor (GM-CSF) (Peprotech) and 37.5 ng/ml of Interleukin 4 (IL-4) (Peprotech), changing the medium after 4 days. For experiments, cells were resuspended in RPMI containing 10% FBS.

[0213] Toxicity and apoptosis assay of dendritic cells. 6×10<sup>5</sup> cells were seeded in 96-well plates and incubated with RPMI containing 10% FBS and MV<sub>L</sub> or MP at indicated concentrations, or with T4R in a multiplicity of infection (MOI) of 20, as positive control, for 24 hours. Gentamicin (100 µg/ml, Sigma) was added after 1 hour of incubation to stop bacteria from growing in samples stimulated with T4R. Before staining, cells were washed once in PBS and once in Annexin V Binding Buffer (BD Pharmingen). Staining was performed with Fixable Viability Dye eFluor® 780 (1:50,000, eBioscience) and FITC Annexin V (1:20, BD Pharmingen) for 30 minutes at 4° C., followed by two washes with Annexin buffer. Labelled cells were fixed in 4% PFA for 30 minutes, resuspended in PBS containing 1% FBS and analyzed in a Gallios™ Flow Cytometer (Beckman Coulter).

[0214] Activation assay of dendritic cells. 6×10<sup>5</sup> cells were seeded in 96-well plates and incubated in RPMI containing 10% FBS with MV<sub>L</sub> or MP at indicated concentrations, or with 1 µg/ml lipopolysaccharide (LPS) (Sigma) as positive control, for 24 hours. Cells were stained with Phycoerythrin (PE) Mouse Anti-Human CD86 (BD Pharmingen) and PE-Cy™5 Mouse Anti-Human HLA-DR (BD Pharmingen) for 20 minutes at 4° C., washed twice with

PBS and resuspended in PBS 1% FBS. Labelled cells were analyzed in a Gallios™ Flow Cytometer (Beckman Coulter).

[0215] Uptake assay with dendritic cells.  $10^6$  cells were seeded in 96-well plates and incubated with RPMI containing 10% FBS, in presence or absence of the inhibitors cytochalasin D (0.5 µg/ml, Sigma) and wortmannin (0.5 µg/ml, Sigma) (C/W), or methyl-β-cyclodextrin (MβCD) (10 µM, Sigma), for 30 minutes. Cells were then incubated with particles at indicated concentration for 1 hour and lysed in RIPA buffer containing 1x protease inhibitor cocktail (Roche). Cell lysates were analyzed by SDS-PAGE and western blotting.

[0216] Quantification of cytokines. Different cytokines were assessed (IL-6, IL-8, IL-10 and TNF) in cell-free supernatants of  $10^5$  dendritic cells by Enzyme-Linked Immunosorbent Assay (ELISA), using commercially available BD OptEIA™ kits from BD Biosciences. Cells were incubated with particles at indicated concentrations, or with T4R or LPS (as previously described), for 24 hours.

[0217] Opsonophagocytosis assay with RAW cells. RAW 264.7 murine macrophages were grown and maintained at 37° C., with 5% CO<sub>2</sub> in RPMI medium (Gibco) supplemented with 10% (v/v) Fetal bovine serum (FBS) (HyClone). To assess the opsonophagocytosis activity of antibodies in immunized mice sera  $2 \times 10^5$  RAW 264.7 cells were seeded in 24-well plates and incubated overnight at 37° C. Serotype 1 bacteria were incubated for 30 minutes at 37° C. with 5% CO<sub>2</sub> with 20% serum from mice immunized with MP+adjuvant, or with adjuvant alone as negative control. RAW cells were then washed with PBS and incubated for 1.5 hours with  $2.5 \times 10^7$ /well of pre-treated bacteria. Cells were washed three times with PBS to remove unattached bacteria. To measure total uptake of bacteria, cells were incubated with a 50/50 solution of 2% saponin (Sigma) and trypsin-EDTA (Gibco) for 15 minutes at 37° C., to lyse eukaryotic cells, and total bacteria were plated for enumeration. To evaluate phagocytosis 300 µg/ml of Gentamicin (Sigma) and 0.12 mg/ml of Penicillin G (Sigma) were added to separate wells and incubated 15 minutes at 37° C., to kill extracellular bacteria. Cells were then washed three times with PBS and incubated with a 50/50 solution of 2% saponin and trypsin-EDTA for 15 minutes at 37° C. to lyse eukaryotic cells. To evaluate killing of bacteria inside macrophages separate wells were treated with antibiotics (as for phagocytosis), washed three times with PBS and then incubated for 1 hour at 37° C. with medium. Cells were then washed three times with PBS and incubated with a 50/50 solution of 2% saponin and trypsin-EDTA for 15 minutes at 37° C. to lyse eukaryotic cells.

[0218] Mouse IgG ELISA assay. To detect MV<sub>L</sub>- and MP-specific mouse IgG in sera of immunized mice optical plates (Sarstedt) were coated with 1 µg/ml of MP in 0.1 M Sodium Carbonate buffer pH 9.5, overnight at 4° C. Wells were then washed three times with PBS containing 0.05% Tween-20 and incubated with PBS with 10% FBS, for 1 hours at room temperature. After three washes wells were incubated with mice sera diluted 1:500 in PBS with 10% FBS, for 2 hours at room temperature. Wells were then washed three times and incubated with anti-mouse IgG-HRP (GE Healthcare) diluted 1:500 in PBS 10% FBS, for 2 hours at room temperature. After three washes wells were then incubated with TMB substrate (BD Bioscience) for 10 minutes and the reaction was stopped with 1 M H<sub>3</sub>PO<sub>4</sub>. Absorbance at 450 nm was then measured with a plate reader.

[0219] To detect pneumo-specific mouse IgG in sera of immunized mice, bacteria were grown on blood agar plates overnight at 37° C., resuspended in PBS and heat-inactivated for 2 hours at 60° C. After diluting the bacteria to OD<sub>600</sub> 0.6, optical plates (Sarstedt) were coated with 100 µl of bacteria in 0.1 M Sodium Carbonate buffer pH 9.5, overnight at 4° C. Wells were then washed three times with PBS and incubated with PBS with 2.5% skim milk, for 2 hours at room temperature. After three washes wells were incubated with mice sera diluted 1:500 in PBS, for 1 hour at room temperature. Wells were then washed three times and incubated with anti-mouse IgG-HRP (GE Healthcare) diluted 1:500 in PBS, for 1 hour at room temperature. After three washes well were then incubated with TMB substrate (BD Bioscience) for 10 minutes and the reaction was stopped with 1 M H<sub>3</sub>PO<sub>4</sub>. Absorbance at 450 nm was then measured with a plate reader.

[0220] Statistical analysis. For multiple comparisons the nonparametric ANOVA test was used to assess the presence of the differences between the groups, then the Dunn's test was used to make pairwise comparisons; for two groups comparison the non-parametric two tailed Wilcoxon's rank sum test (also known as Mann-Whitney test) was used. Statistically significant data was defined as \* P<0.05, \*\* P<0.01, \*\*\* P<0.001, \*\*\*\*=P<0.0001.

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- What is claimed is:
1. A method for inducing protective immunity against *Streptococcus pneumoniae* in a subject, comprising administering to the subject a composition comprising a *Streptococcus pneumoniae* membrane vesicle microparticle (MP), wherein said MP comprises:
    - i. the protein pneumolysin (Ply) at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP;
    - ii. the protein autolysin (LytA) at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP;
    - iii. the protein Pneumococcal surface protein C (PspC) at the level of  $\geq 0.130 \mu\text{g}/\text{mg}$  total protein in the MP; or
    - iv. the protein pilus-1 backbone protein (RrgB) at the level of  $\geq 0.020 \mu\text{g}/\text{mg}$  total protein in the MP.
  2. The method according to claim 1, wherein the immunity is protective against a condition selected from pneumococcal sinusitis, pneumococcal otitis, pneumococcal pneumonia, and invasive pneumococcal disease including but not limited to pneumococcal sepsis and pneumococcal meningitis.
  3. The method according to claim 1, wherein the composition is a liquid and comprises MPs in an amount of 1  $\mu\text{g}/\text{ml}$ .
  4. The method according to claim 1, wherein said MP comprises:
    - i. the protein pneumolysin (Ply) at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP;
    - ii. the protein autolysin (LytA) at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP;
    - iii. the protein Pneumococcal surface protein C (PspC) at the level of  $\geq 0.130 \mu\text{g}/\text{mg}$  total protein in the MP; and
    - iv. the protein pilus-1 backbone protein (RrgB) at the level of  $\geq 0.020 \mu\text{g}/\text{mg}$  total protein in the MP.
  5. The method according to claim 1, wherein the MP is 5-300 nm in diameter.
  6. The method according to claim 1, wherein the MP is 10-125 nm in diameter.
  7. The method according to claim 1, wherein the composition elicits antibodies against *Streptococcus pneumoniae* serotype 3 when administered to a mammalian host.
  8. The method according to claim 1, wherein the composition elicits serotype independent antibodies against *Streptococcus pneumoniae* when administered to a mammalian host.
  9. The method according to claim 1, wherein the composition is administered intranasally.
  10. The method according to claim 1, wherein the composition further comprises an adjuvant.
  11. The method according to claim 10, wherein the adjuvant comprises aluminium hydroxide.
  12. The method according to claim 2, wherein the immunity is protective against invasive pneumococcal disease.
  13. The method according to claim 1, wherein the MP comprises a capsular polysaccharide of a capsular serotype of *Streptococcus pneumoniae* at a level of  $\geq 0.001 \mu\text{g}/\text{mg}$  total protein in the MP.
  14. The method according to claim 1, wherein said MP comprises the protein Ply at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP.
  15. The method according to claim 1, wherein said MP comprises the protein LytA at the level of  $\geq 0.070 \mu\text{g}/\text{mg}$  total protein in the MP.
  16. The method according to claim 1, wherein said MP comprises the protein PspC at the level of  $\geq 0.130 \mu\text{g}/\text{mg}$  total protein in the MP.
  17. The method according to claim 1, wherein said MP comprises the protein RrgB at the level of  $\geq 0.020 \mu\text{g}/\text{mg}$  total protein in the MP.

**18.** The method according to claim 1, wherein said MP comprises the protein Ply at the level of  $\geq 0.35 \mu\text{g}/\text{mg}$  total protein in the MP.

**19.** The method according to claim 1, wherein said MP comprises the protein LytA at the level of  $\geq 0.20 \mu\text{g}/\text{mg}$  total protein in the MP.

**20.** The method according to claim 1, wherein said MP comprises the protein PspC at the level of  $>0.3 \mu\text{g}/\text{mg}$  total protein in the MP.

**21.** The method according to claim 1, wherein said MP comprises the protein RrgB at the level of  $\geq 0.028 \mu\text{g}/\text{mg}$  total protein in the MP.

\* \* \* \*