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Lateral Transfers of Serine Hydroxymethyltransferase (*glyA*) and UDP-*N*-Acetylglucosamine Enolpyruvyl Transferase (*murA*) Genes from Free-living Actinobacteria to the Parasitic Chlamydiae

Emma Griffiths, Radhey S. Gupta

Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada L8N 3Z5

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Abstract. The chlamydiae are important human and animal pathogens which form a phylogentically distinct lineage within the Bacteria. There is evidence that some genes in these obligate intracellular parasites have undergone lateral exchange with other free-living organisms. In the present work, we describe two interesting cases of lateral gene transfer between chlamydiae and actinobacteria, which have been identified based on the shared presence of conserved inserts in two important proteins. In the enzyme serine hydroxymethyltransferase (SHMT or GlyA protein), which links amino acid and nucleotide metabolisms by generating the key intermediate for one-carbon transfer reactions, two conserved inserts of 3 and 31 amino acids (aa) are uniquely present in various chlamydiae species as well as in a subset of Actinobacteria and in the *Treponema* species. Similarly, in the enzyme UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA), which is involved in the synthesis of cell wall peptidoglycan, a 16-aa conserved insert is specifically present in various sequenced chlamydiae and a subset of actinobacteria (i.e., *Streptomyces, Actinomyces, Tropheryma, Bifidobacterium, Leifsonia, Arthrobacter*, and *Brevibacterium*). To determine the phylogenetic depths of the GlyA and MurA inserts, the fragments of these genes from two chlamydiae-like species, *Simkania negevensis* and *Waddlia chondrophila*, were PCR amplified and sequenced. The presence of the corresponding inserts in both these species strongly indicates that these inserts are distinctive characteristics of the Chlamydiales order. In phylogenetic trees based on GlyA and MurA protein sequences, the chlamydiae species (and also the *Treponema* species in the case of GlyA) branched with a high affinity with various insert-containing actinobacteria within a clade of other actinobacteria. These results provide strong evidence that the shared presence of these indels in these bacteria is very likely a consequence of ancient lateral gene transfers from actinobacteria to chlamydiae. Pairwise sequence identity and the branching pattern of the GlyA homologues in the phylogenetic tree indicates that the *glyA* gene was initially transferred from an actinobacteria to an ancestor of the *Treponema* genus and from there it was acquired by the common ancestor of the Chlamydiales.

Key words: Chlamydiae — Actinobacteria — Conserved indels — Phylogenetic trees — Rare genomic changes — *Streptomyces* — *Treponema*

Correspondence to: Radhey S. Gupta; *email:* gupta@mcmaster.ca

Introduction

The Chlamydiales and Actinobacteria comprise two main groups within Bacteria, which are quite distinct from each other based on their physiological characteristics and branching pattern in various phylogenetic trees (Woese 1987; Embley and Stackebrandt 1994; Gupta 1998; Ludwig and Klenk 2001; Kalayoglu and Byrne 2001). Chlamydiae are obligate

intracellular parasites of eukaryotic hosts, which are characterized by a unique two-stage division cycle and are responsible for a wide spectrum of diseases in humans and animals. The order Chlamydiales is currently composed of four families, the Chlamydiaceae, which contains all of the traditional chlamydiae species, and three more recently identified families of chlamydiae-related organisms, the Parachlamydiaceae, Waddliaceae, and Simkaniaceae (Everett et al. 1999; Rurangirwa et al. 1999; Kahane et al. 1999; Kalayoglu and Byrne 2001; Corsaro et al. 2003). The complete genome sequences of six chlamydiae species—*Chlamydophila* (*Chlam.*) *pneumoniae*, *Chlam. abortus*, *Chlam. caviae*, *Chlamydia* (*Chl.*) *trachomatis*, *Chl. muridarum*, and the environmental chlamydiae-like organism *Protochlamydia amoebophila* (previously *Parachlamydia* sp. UWE25) (Collingro et al. 2005)—are now known (Stephens et al. 1998; Kalman et al. 1999; Read et al. 2000; Read et al. 2003; Horn et al. 2004; Thomson et al. 2005). Our recent comparative analyses of chlamydiae genomes has identified a large number (>400) of proteins that are distinctive characteristics of either all Chlamydiales or particular subgroups of chlamydiae (e.g., Chlamydiaceae family or *Chlamydia* and *Chlamydophila* genera) but that are not found in any other bacteria. In other work, a large number of conserved indels (i.e., inserts or deletions) in widely distributed proteins that are uniquely present in various chlamydiae species have also been identified, supporting the taxonomic and phylogenetic distinctness of chlamydiae from all other bacteria (Griffiths and Gupta 2002; Griffiths et al. 2005).

In contrast to chlamydiae, which are Gram-negative bacteria, actinobacteria are Gram-positive organisms with a high G + C mol% (>55%) in their genomic DNA (Embley and Stackebrandt 1994; Stackebrandt et al. 1997; Ludwig and Klenk 2001; Gao and Gupta 2005). The Actinobacteria phylum constitutes one of the largest groups within Bacteria and they exhibit enormous diversity in terms of their morphology (rod, coccoid, fragmenting hyphae), metabolism (antibiotic production, pathogen), physiology (anaerobic, aerobic), and lifestyle (endospore-forming, cell cycle) (Stackebrandt et al. 1997). Similar to the chlamydiae, species from the Actinobacteria phylum can also be distinguished from all other bacteria based on a large number of conserved inserts and whole proteins that are uniquely found in these bacteria (Gao and Gupta 2005; Gao et al. 2006). In phylogenetic trees based on 16S rRNA and various proteins, Actinobacteria and chlamydiae branch very distantly and there is no evidence that any specific evolutionary relationship exists between these two groups (Woese 1987; Viale et al. 1994; Embley and Stackebrandt 1994; Gupta 1998; Gupta 2004; Gao and Gupta 2005).

During our extensive work on chlamydiae and actinobacteria, we have come across three conserved indels in two widely distributed proteins that are uniquely shared mainly by the species from these two groups of bacteria. These indels include two conserved inserts of 3 and 31 amino acids (aa) in the enzyme serine hydroxymethyltransferase (SHMT or GlyA protein), which carries out serine-to-glycine conversion (Rao et al. 2000), and a 16-aa insert in the MurA protein, involved in the synthesis of cell wall peptidoglycan (Brown et al. 1995). The indel in MurA has been described in our earlier work (Griffiths and Gupta 2002). However, sequence information for MurA has become available from a large number of other species since then. Hence, it was important to update this information and we have also carried out further work to understand the origin of this insert in different bacteria. The identified inserts in GlyA and MurA proteins were present in all sequenced chlamydiae (limited to members of the Chlamydiaceae family and *P. amoebophila*) species and a small number of actinobacteria. Additionally, the insert in GlyA was also present in the *Treponema* species whose genomes have been sequenced (Fraser et al. 1998; Weinstock et al. 1998). Because the inserts in GlyA and MurA were present in all available chlamydiae, to determine at what stage of chlamydiae evolution these indels were introduced, we have obtained sequence information for these genes from *Simkania negevensis* and *Waddlia chondrophila*, belonging to the remaining two families of the Chlamydiales order for which sequence information is lacking at present. The presence of these inserts in all of the Chlamydiales family members indicates that they were introduced in these genes in a common ancestor of the chlamydiae species. Phylogenetic analyses of GlyA and MurA protein sequences from different groups of bacteria reveal that all of the insert-containing homologues showed a strong affinity for each other and they branched within the actinobacterial species. These results provide evidence that the shared presence of these inserts in chlamydiae, actinobacteria and also *Treponema* is due to ancient lateral gene transfer (LGT) rather than from independent origin.

Materials and Methods

PCR Amplification and Sequencing

DNA from *S. negevensis* (ATCC VR1471) and *W. chondrophila* (ATCC 1470) was kindly provided to us by Dr. Astrid Petrich (St. Joseph's Hospital, Hamilton, Ontario) (Mahony et al. 2000; Griffiths et al. 2005). Oligonucleotide primers, in opposite orientations, were designed for the flanking conserved regions in the sequence alignment for MurA and GlyA based on the sequences from available chlamydiae species. Degeneracy was incorporated into these primers to account for nucleotide variability at different sites

in the alignment. The primers were synthesized at MOBIX, McMaster University and their sequences are given below.

GlyA. The forward and reverse primers used to amplify a 600-bp fragment of the *glyA* gene from *W. chondrophila* are as follows: (forward) 5′-GTNCARCCNCAYWCNGG-3′ and (reverse) 5′-TGRTTRTCNGTNCCNCC-3′. A 750-bp fragment of the *glyA* gene from *S. negevensis* was amplified using the following primers: (forward) 5′-GGNGCSGAYGCSAAYTT-3′ and (reverse) 5′ RAGNGGCAANGGACCACC 3′.

MurA. A 219-bp fragment of the *murA* gene was PCR amplified from *S. negevensis* using the following primers: (forward) 5′-CAYGARACDGTYYAYGARAA-3′ and (reverse) 5′-GC CATNACRTANGCRAANCCNGC-3′. A fragment of the *murA* gene from *W. chondrophila* was cloned and sequenced in our earlier work (Griffiths and Gupta 2002). The unusual letters in the primer sequences represent the following: N = A, T, C, or G; Y = C or T; S = G or C; R = A or G; D = A, T, or G; and W = A or T.

PCR was performed in a Techne Techgene thermocycler under the following conditions. Each reaction had a final volume of 10 μl, and a Mg^{2+} concentration of 4 mM was used during amplification with all primer sets for each DNA strain tested. Also, 2% DMSO (final concentration) was added to each reaction mixture in order to facilitate primer binding and enhance PCR specificity. The PCR amplification using Taq polymerase (Fermentas) was carried out over 30 cycles (15 s at 94°C, 15 s at 45°C, 1 min at 72°C), with an initial 1 min hot start at 94°C and a final extension step (15 s at 94°C, 15 s at 45°C, 7 min at 72°C). DNA fragments of the expected size were purified from 0.8% (w/v) agarose gels (using a GeneClean kit; Qiagen) and subcloned into the plasmid pDRIVE using a TU cloning kit (Invitrogen). *E. coli* JM109 cells were transformed with the recombinant plasmid, and the inserts from a number of positive clones were sequenced through the MOBIX facility. Sequences of all cloned fragments were BLAST searched to ensure that they were from a novel source. The sequence data for various clone obtained in this work have been deposited in GenBank under the following accession numbers: AY845402, AY845409, AY845411, and AAN52535.

Phylogenetic Analysis and Pairwise Comparison of Protein Sequences

The sequences for the MurA and GlyA proteins from different species were retrieved from the NCBI database, and multiple sequence alignments of these were created using the ALIGN PLUS 4 program (Scientific and Educational Software). After this work was completed, at the revision stage of the manuscript, partial sequence information for *S. negevensis* genome became available at the TIGR Microbial Database site (2006). The BLAST searches on this were used to retrieve the sequence information for the remainder portions of the MurA and GlyA protein sequences from this species for phylogenetic purposes. All sequence gaps and poorly conserved regions were removed from the alignments for phylogenetic studies. The edited GlyA and MurA sequence alignments contained 321 and 313 aligned positions, respectively. Phylogenetic analysis of these datasets was carried out both in the presence and in the absence of the large inserts under consideration to determine their effect on the branching patterns. Genetic distances for bootstrapped data sets (100 replicates) were calculated by Kimura's (1983) method and neighbor-joining consensus trees based on these were constructed and a consensus tree was obtained. The trees were rooted using *Bacillus subtilis* sequences. All of the phylogenetic programs that were used to construct the tree are part of the TREECON for Windows software package (Van de Peer and De Wachter 1994). Pairwise amino acid sequence identity for GlyA and MurA homologues was determined using the ALIGN PLUS4 program with the BLOSUM62 scoring matrix and default parameters.

Results

Multiple sequence alignments for different chlamydial proteins were created in our earlier work (Griffiths and Gupta 2002; Griffiths et al. 2005). We have used these alignments to search for conserved inserts and deletions in widely distributed proteins that are unique to the chlamydial group of species and can be used for their identification as well as for taxonomic, genetic, and biochemical studies. These studies have led to the identification of a large number of conserved indels (or signatures) that are specific for the chlamydiae but are not found in any other bacteria (Griffiths and Gupta 2002; Griffiths et al. 2005). However, during this work we have come across some conserved inserts in widely distributed proteins (e.g., GlyA and MurA) that, in addition to various chlamydiae, are also found in the same position in certain subgroups of actinobacteria, as well as, in one case (GlyA), in *Treponema* species. The shared presence of these conserved indels in these phylogenetically distinct groups of bacteria suggested that the genes for these proteins may have been laterally transferred between these groups of bacteria. Although LGT between chlamydiae and some other groups of bacteria has been reported previously (Zomorodipour and Andersson 1999; Wolf et al. 1999; Royo et al. 2000; Brinkman et al. 2002; Ortutay et al. 2003), these are the only examples of LGT between chlamydiae and the free-living actinobacteria (mostly soil bacteria). A brief description of these conserved indels and further work that we have carried out to understand their evolutionary origin is given below.

Description and Phylogenetic Analysis of the Conserved Insert in SHMT (GlyA)

The enzyme SHMT, encoded by the *glyA* gene, carries out the transfer of the hydroxymethyl group of L-serine to 5,6,7,8-tetrahydrofolate, to give rise to glycine and 5,10-methylene tetrahydrofolate, which serves as the source of one-carbon fragments in a variety of biosynthetic reactions (Rao et al. 2000). This widely distributed protein contains two conserved inserts, of 31 and 3 aa, that are uniquely present in GlyA homologues of various sequenced chlamydiae species (e.g., *Chlamydia, Chlamydophila*, and *Protochlamydia amoebophila*) as well as in one of the GlyA homologues from *Streptomyces, Propionibacterium*, and *Nocardioides* (Figs. 1 and 2, respectively). It should be noted that, in contrast to other bacterial species, which contain a single GlyA homologue, three different homologues of GlyA are present in the two *Streptomyces* species, and of these only one of the homologues contained the inserts. Two different homologues of GlyA are also found in

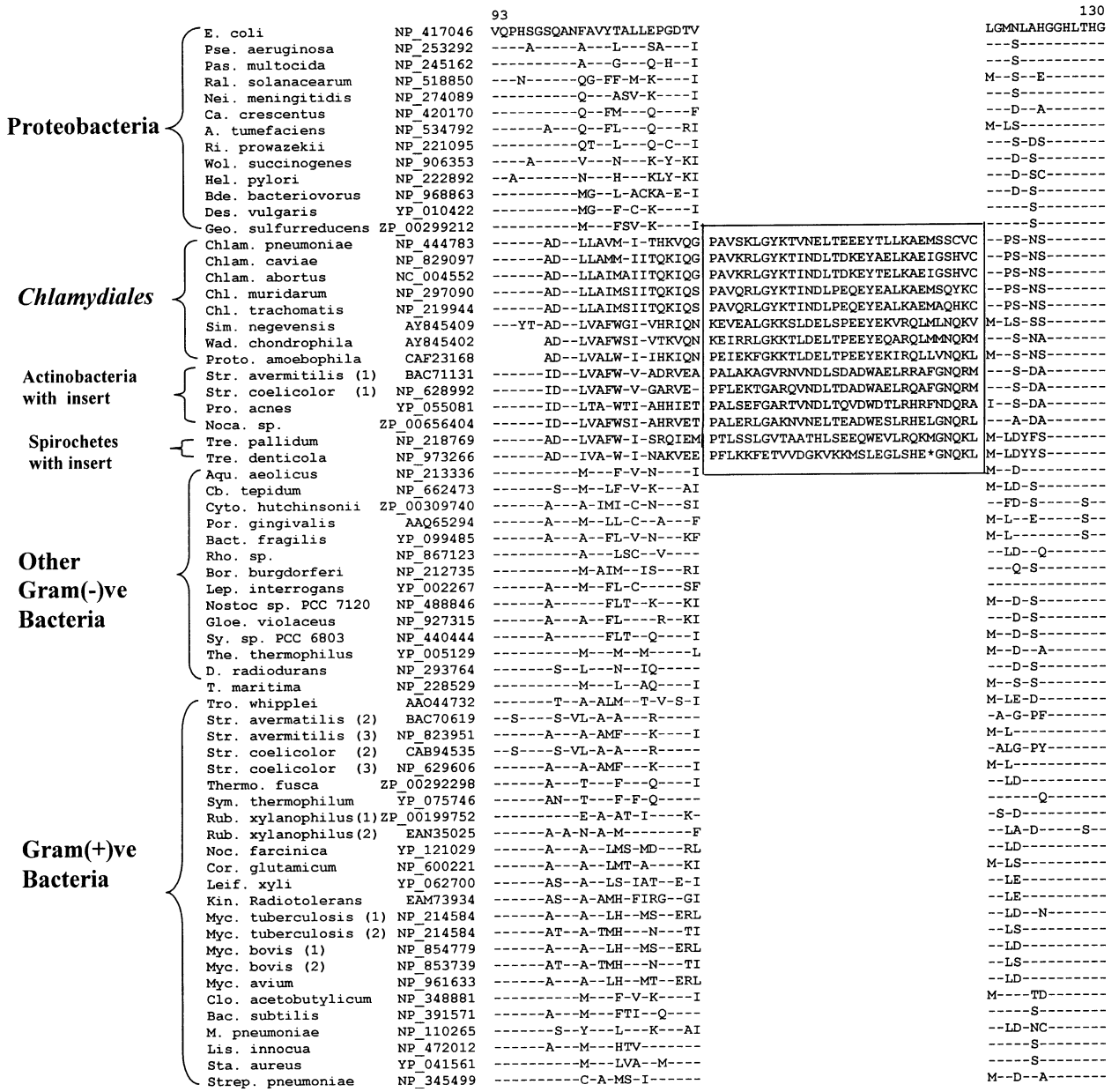

Fig. 1. Partial sequence alignment of GlyA (or SHMT) homologues showing a 31-aa insert which is found only in the Chlamydiales, a subset of Actinobacteria and the *Treponema* species, to the exclusion of all other available bacteria. Dashes in this and all other sequence alignments indicate identity to the amino acid sequence on the top line. The numbers at the top indicate the position of the sequence in the *E. coli* homologue. The insert in *Tre. denticola* contains an additional 9 aa (EWEELRHAL) at the position marked by an asterisk, which are not shown. *A.*, *Agrobacterium*; *Aqui., Aquifex; Bac., Bacillus; Bact., Bacteroides; Bde., Bdellovibrio; Bif., Bifidobacterium; Bor., Borrelia; Brev., Brevibacterium; Bru., Brucella; Ca., Caulobacter; Camp., Campylobacter; Cb., Chlorobium; Chl., Chlamydia; Chlam., Chlamydophila; Clo., Clostridium; Cor., Corynebacterium; Cyto., Cytophaga; D., Deinococcus; Des., Desulfovibrio; E., Escherichia; Geo., Geobacter; Gloe., Gloeobacter; Hel., Helicobacter; Kin., Kineococcus; Leif., Leifsonia; Lep., Leptospira; Lis., Listeria; M., Mycoplasma; Myc., Mycobacterium; Nei., Neisseria; Noc., Nocardia; Noca., Nocardioides; Pas., Pasteurella; Por., Porphyromonas; Pro., Porpionibacterium; Proto., Protochlamydia; Pse., Pseudomonas; Ral., Ralstonia; Rho., Rhodopirellula; Ri., Rickettsia; Rub., Rubrobacter; Sim., Simkania; Sta., Staphylococcus; Str., Streptomyces; Strep., Streptococcus; Sy., Synechocystis; Sym., Symbiobacterium; T., Thermotoga; The., Thermus; Tre., Treponema; Tro., Trophyrema; Thermo., Thermobifida; Wad., Waddlia; Wol., Wolinella.*

the two *Mycobacterium* species, *M. tuberculosis* and *M. bovis*, as well as *Rubrobacter xylanophilus*. Both copies from each of these species lacked the inserts. Interestingly, in this case, in addition to various chlamydiae and a few actinobacteria, conserved inserts of similar lengths were also present in the two sequenced *Treponema* species (*T. denticola* and *T. pallidum*) (Figs. 1 and 2), but they were not present in other spirochetes such as *Borrelia* and *Leptospira*. Since these inserts were present in all sequenced

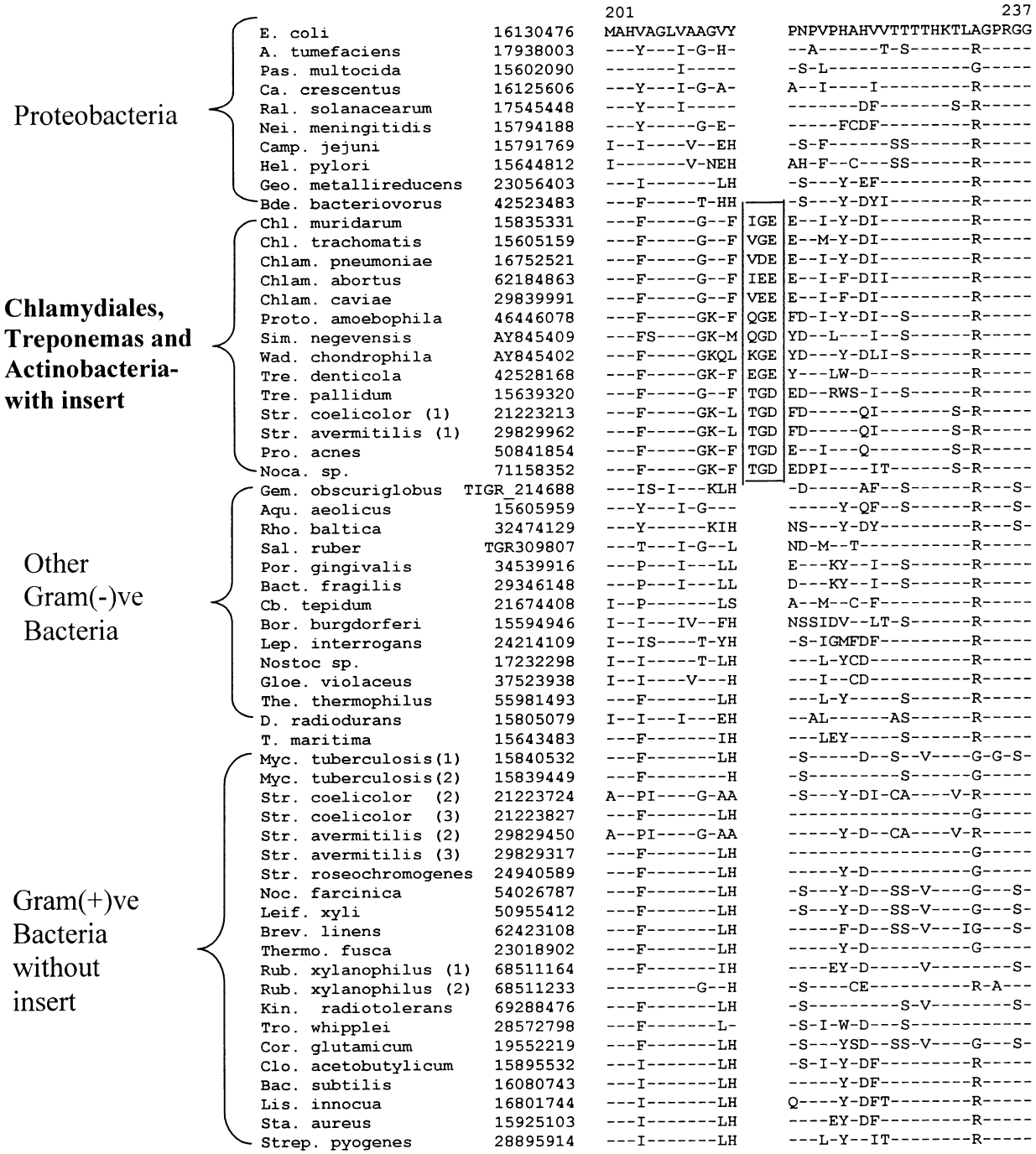

Fig. 2. Excerpts from the GlyA sequence alignment showing an additional 3-aa conserved insert which is uniquely found in various Chlamydiales, some Actinobacteria, and the *Treponema*. *Brev.*, *Brevibacterium; Gem., Gemmata; Sal., Salinibacter*.

chlamydiae genomes, we have sought to determine whether they were also present in species from other Chlamydiales families for which sequence information is lacking at present. To this end, using degenerate primers for highly conserved regions in the *glyA* gene flanking these inserts, we have PCR amplified and sequenced fragments of this gene from *S. negevensis* and *W. chondrophila*. As seen from the partial sequence alignment of GlyA proteins containing these insert regions (Figs. 1 and 2), both these chlamydiae species were found to contain both the inserts, indicating that they are distinctive characteristic of the entire Chlamydiales order.

The three GlyA homologues that are found in *Streptomyces* species are most likely due to multiple gene duplications in this lineage, which occurred prior to the rare genetic event leading to the introduction of the two inserts in one of the *glyA* genes.

Table 1. Amino acid identity of GlyA Homologs between Chlamydial and other Bacteria

Group	Bacterial species	*Chlam. pneumoniae* (497 aa)	*Chl. trachomatis* (497 aa)	*Proto. amoebophila* (491 aa)
Actinobacteria and Spirochetes species with insert	*Str. coelicolor [1]*	46.3% (230)	46.9% (233)	53.8% (264)
	Str. avermatilis [1]	45.7% (227)	46.7% (232)	53.0% (260)
	Pro. acnes	46.1% (229)	47.9% (238)	50.3% (247)
	Tre. pallidum	48.5% (241)	50.1% (249)	56.6% (278)
	Tre. denticola	50.3% (250)	50.3% (250)	57.6% (283)
Actinobacteria without insert	*Str. coelicolor [2]*	40.2% (200)	39.0% (194)	39.1% (192)
	Str. coelicolor [3]	36.2% (180)	36.4% (181)	34.6% (170)
	Str. avermatilis [2]	40.0% (199)	39.4% (196)	40.5% (199)
	Str. avermatilis [3]	36.0% (179)	36.4% (181)	35.0% (172)
	Tro. whipplei	38.0% (189)	38.4% (191)	38.7% (190)
	Brev. linens	41.0% (204)	42.7% (212)	40.9% (201)
	Leif. xylii	40.1% (203)	40.6% (202)	40.1% (197)
	Myc. tuberculosis [1]	38.2% (190)	39.8% (198)	39.3% (193)
	Myc. tuberculosis [2]	39.2% (195)	39.6% (197)	38.5% (189)
	Cor. glutamicum	39.8% (198)	40.8% (203)	38.1% (187)
	Noc. farcinia	39.8% (198)	41.2% (205)	38.5% (189)
Firmicutes	*Bac. subtilis*	39.4% (196)	39.2% (195)	39.5% (194)
	Lis. innocua	39.6% (197)	40.0% (199)	39.3% (193)
Other Gram-negative bacteria	*E. coli*	39.2% (195)	38.4% (191)	37.7% (185)
	Ca. crescentus	40.2% (200)	40.8% (203)	39.5% (194)
	Bde. bacteriovorus	41.2% (205)	40.8% (203)	40.7% (200)
	Camp. jejuni	34.0% (169)	33.8% (168)	35.4% (174)
	Aqu. aeolicus	41.6% (207)	42.4% (211)	41.5% (204)
	Bact. fragilis	36.4% (181)	36.0% (179)	38.1% (187)
	Por. gingivalis	35.6% (177)	35.4% (176)	37.4% (184)
	Cb. tepidum	36.0% (179)	34.8% (173)	37.4% (184)
	Bor. burgdorferi	34.8% (173)	36.8% (183)	35.2% (173)
	Nostoc sp.	41.2% (205)	40.6% (202)	42.0% (206)
	D. radiodurans	37.6% (187)	36.4% (181)	37.4% (184)

Note. Pairwise identities of the chlamydiae GlyA homologues with the corresponding proteins from other bacteria. The numbers of identical positions in different pairwise alignments are given in parentheses. *Streptomyces* species contain three GlyA homologues, which are indicated by [1], [2], and [3].

We have determined the pairwise sequence identity of the chlamydial GlyA homologues to the GlyA homologues from various other species (including multiple homologues from *Streptomyces*) and they were found to exhibit the highest degree of similarity to the GlyA homologues from the two *Treponema* species, followed by the *Streptomyces* homologues, which contained these inserts (Table 1). To understand the evolutionary origin of the GlyA inserts, a neighbor-joining distance tree was constructed from 50 GlyA protein sequences containing representative species from most of the main groups within Bacteria (Fig. 3). In this tree, the chlamydiae or *Treponema* species did not branch among other Gram-negative bacteria, as seen in the phylogenetic trees based on 16S rRNA (Fig. 4) or various protein sequences (Olsen et al. 1994; Viale et al. 1994; Gupta 1998; Gupta 2004). Instead, all insert-containing homologues from divergent bacterial species (*Streptomyces* homologue 1, *Propionibacterium, Nocardioides, Treponema*, the chlamydiae) branched together as a distinct clade (with 100% bootstrap score), which was separated from all other bacterial homologues by a long-branch length. This clade was located within the actinobacterial cluster and the GlyA2 homologues from *Streptomyces* formed its immediate outgroup. Within the clade of insert-containing homologues, the chlamydiae and *Treponema* species were clearly separated from the actinobacterial species. Within the chlamydiae species, the environmental chlamydiae such as Protochlamydia and *Simkania* formed a deeper-branching clade which also included the two *Treponema* species. The distinct branching of these two groups of chlamydiae species (i.e., *Chlamydiaceae* verus the other chlamydiae families) was also noted in our recent studies based on concatenated protein sequences for several proteins (Griffiths et al. 2005). In contrast to the insert-containing *Treponema* species, other spirochete species such as *Borrelia burgdorferi* branched very distantly and with the other Gram-negative phyla of bacteria. Similar branching of the GlyA homologues was observed when the insert region was omitted from the sequence alignment (results not shown). These results strongly

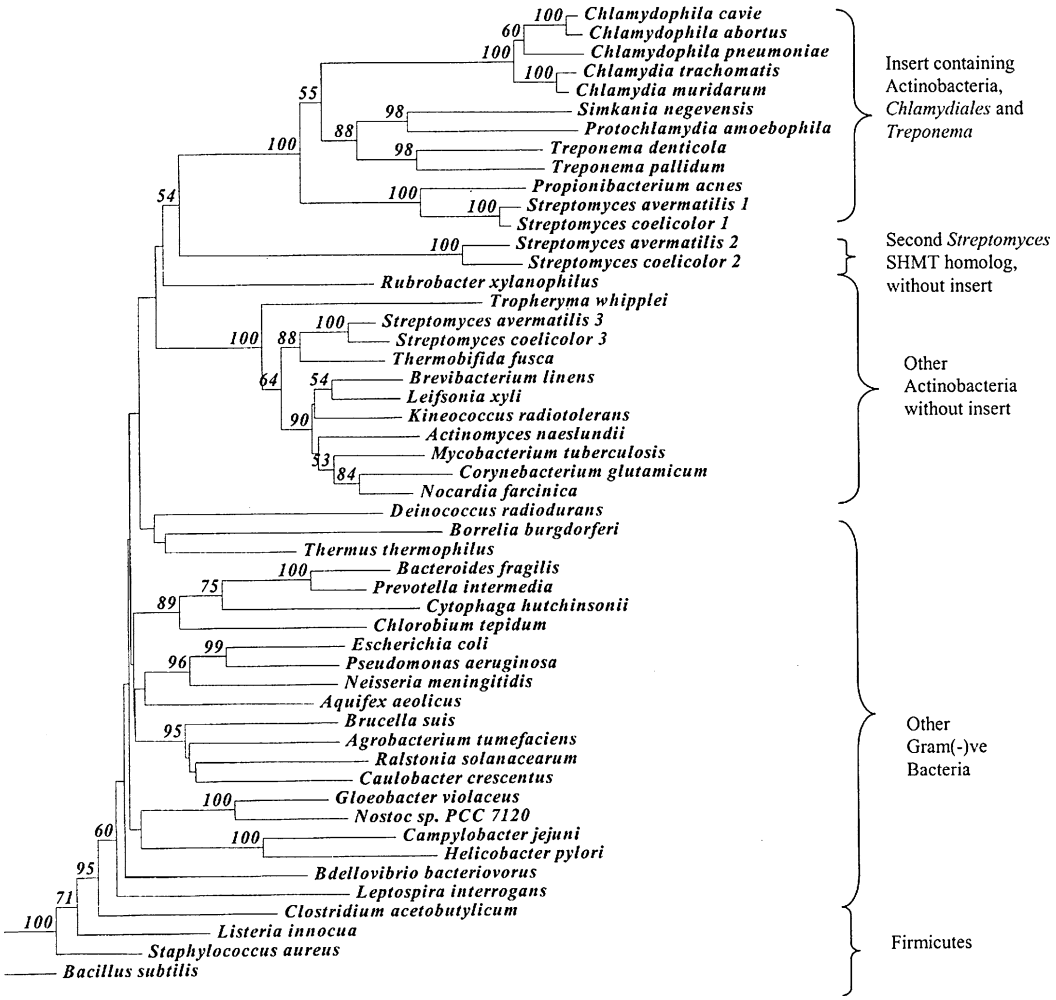

Fig. 3. A bootstrapped neighbor-joining distance tree based on SHMT (GlyA) homologues. Bootstrap scores that were > 50 (of 100) are indicated on various nodes. The chlamydiae and *Treponema* species were found to branch anomalously with the insert-containing SHMT homologues from *Streptomyces* and *Propionibacterium*, indicating lateral gene transfer between these groups.

suggest that the insert-containing *glyA* genes in chlamydiae species as well as in *Treponema* have been acquired via LGT from an insert-containing actinobacteria. In view of our results that the *Treponema* species grouped with the deeper-branching chlamydiae clade and that the chlamydial homologues also exhibited the highest sequence similarity to those from *Treponema*, it is likely that the *glyA* gene was initially transferred from an actinobacterial species to a common ancestor of the *Treponema* genus and from there it was acquired by a common ancestor of the chlamydiae.

Description and Phylogenetic Analysis of the Conserved Insert in the MurA Protein

MurA is an essential enzyme that catalyzes the first committed step in peptidoglycan biosynthesis and it is the cellular target of the antibiotic fosfomycin (Brown et al. 1995; Du et al. 2000). This protein contains a 16-aa insert in a conserved region (Fig. 5), which is uniquely present in all of the sequenced chlamydiae species as well as in some actinobacteria (Fig. 5). All Gram-negative bacteria belonging to different phyla (e.g., Proteobacteria, chlamydiae, Cyanobacteria, *Aquifex*, Spirochetes, *Cytophaga-Flavobacteria-Bacteroidetes-Chlorobi* group, and *Deinococcus-Thermus*) contain only a single homologue of the MurA protein, and of these only the chlamydiae-homologue contained this insert. The insert was also present in the MurA homologues from several actinobacterial species, which included *Streptomyces, Actinomyces, Tropheryma, Bifidobacterium, Leifsonia, Arthrobacter*, and *Brevibacterium*, but it was lacking in other species such as *Corynebacterium, Mycobacterium, Propionibacterium, Nocardia, Rubrobacter, Kineococcus*, and *Thermobifida*. It is important to note that within Actinobacteria, species belonging to the genera *Streptomyces* and *Arthrobacter* were found to contain two different homologues of the MurA protein (referred to as MurA1 and MurA2 in the present work), of which

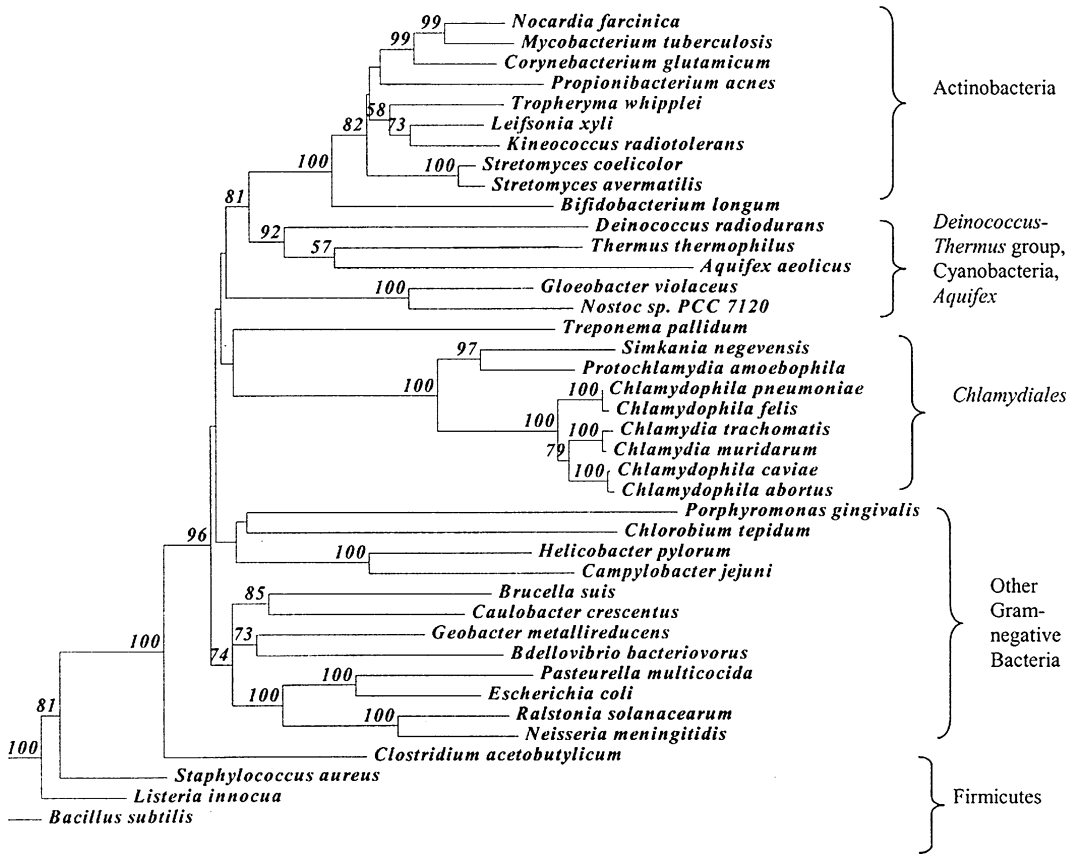

Fig. 4. A bootstrapped neighbor-joining distance tree based on 16S rRNA sequences indicating the distinct branching of the chlamydiae and the *Actinobacteria* phyla.

only MurA1 contained the insert. The other actinobacteria, many of whose genomes have been sequenced, contained only a single MurA homologue. In contrast to the Gram-negative bacteria and actinobacteria, all of the *Firmicutes* species (low G + C Gram-positive bacteria) were found to possess two different MurA homologues (Du et al. 2000), none of which contained the large16 aa insert.

The indel in MurA is present in all of the sequenced chlamydiae species. To determine whether this indel is a distinctive characteristic of the entire Chlamydiales order, a fragment of the *murA* gene was PCR amplified from *S. negevensis* and sequenced. The sequence information for MurA from *W. chondrophila* has been obtained in our earlier work (Griffiths and Gupta 2002). With the cloning of MurA fragment from *S. negevensis*, sequence information for this protein is now available from species belonging to all four families within the Chlamydiales order. As seen from the results presented in Fig. 5, this insert is present in all chlamydiae species, indicating that it is a distinctive characteristic of this group of bacteria, which was likely introduced in a common ancestor of the Chlamydiales.

To understand the origin of this commonly shared insert in chlamydiae and actinobacteria, a phylogenetic tree based on MurA sequences from species representing all major groups within bacteria was constructed (Fig. 6). The overall topology of this tree is again unusual, as the chlamydiae species did not branch with the other Gram-negative bacteria, as seen in the 16S rRNA tree (Fig. 4)(Olsen et al. 1994) or phylogenetic trees based on different protein sequences (Viale et al. 1994; Gupta 1995, 1998, 2004). Instead they were found to branch within the actinobacterial species, and the chlamydiae and actinobacterial homologues which contained the 16-aa insert formed a distinct clade (100% bootstrap score) that was separated from other actinobacterial species by a long-branch length. The observed branching pattern was not dependent on the presence of the insert sequence, as similar results were obtained when phylogenetic analysis was carried out without the insert region (results not shown). As in the GlyA tree (Fig. 3) and in earlier studies (Griffiths et al. 2005), the environmental chlamydiae (i.e., Protochlamydia and *Simkania*) and the traditional chlamydia species formed two distinct clades in this tree (Fig. 6). A close relationship of the chlamydiae homologues to the actinobacterial homologues containing the insert is also evident from a comparison of the pairwise amino acid identity between various MurA homo-

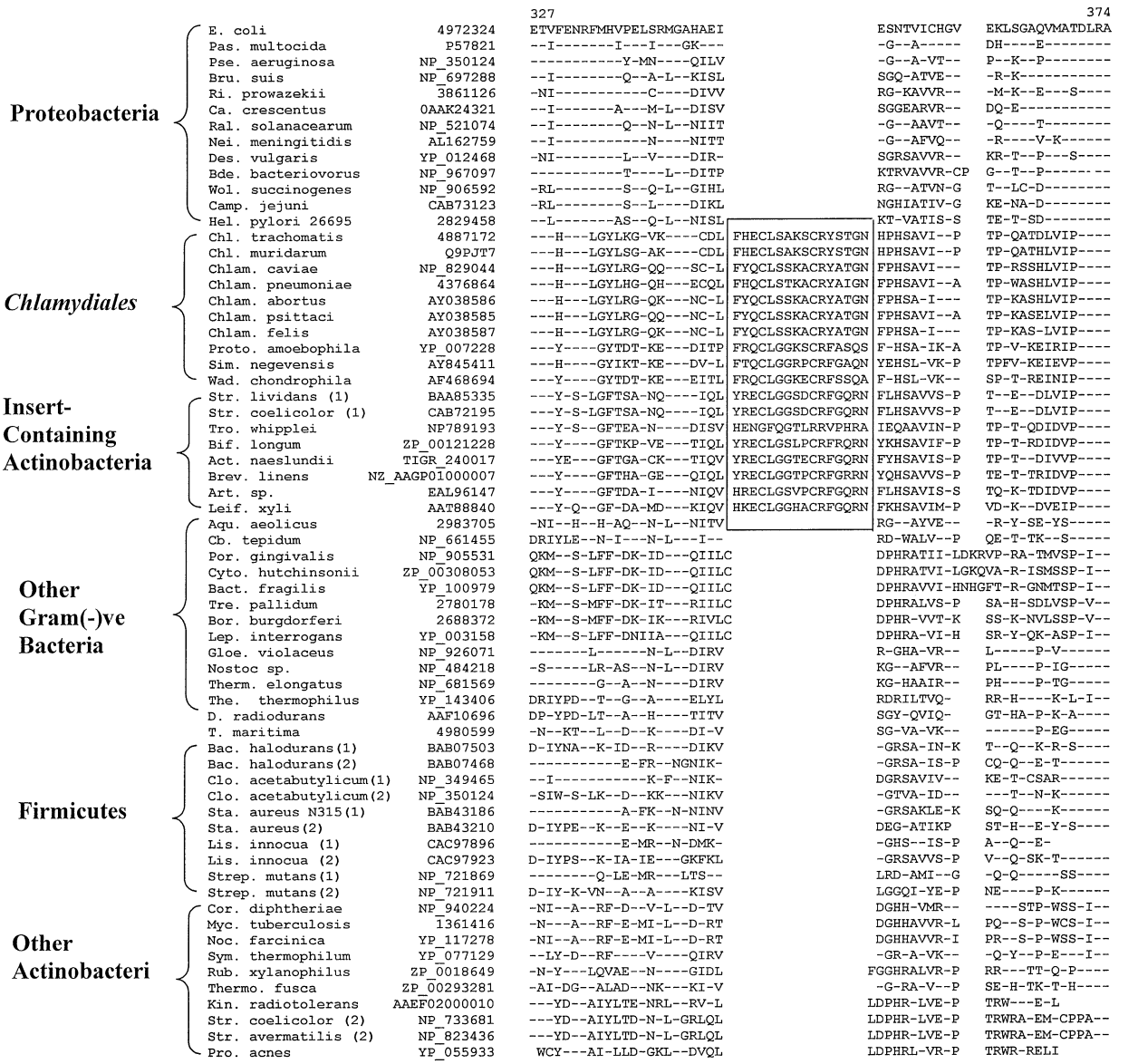

Fig. 5. Partial sequence alignment of MurA proteins from different species showing a 16-aa conserved insert that is commonly shared by various Chlamydiales and a subset of Actinobacteria. *Act., Actinomyces; Art., Arthrobacter; Therm., Thermosynechococcus.*

logues, where the chlamydiae homologues were found to be most similar to the (+) insert homologues from actinobacteria (Table 2). These results provide strong evidence for a common ancestry of the MurA homologues containing the insert. Within this clade of (+) insert homologues, the actinobacteria and chlamydiae species formed two distinct clusters. The immediate outgroup of the insert (+) node was a branch containing the second MurA homologue (MurA2) from *Streptomyces* as well as (−) insert homologues from *Propionibacterium* and *Kineococcus*. This cluster containing the *Streptomyces* MurA2 homologues was separated from the (+) insert clade by a very long-branch length, indicating significant sequence divergence between the MurA1 and the MurA2 homologues. The (−) insert MurA homologues from other actinobacterial species, including *Rubrobacter xylanophilus*, which forms the deepest branch within *Actinobacteria* in the 16S RNA and other phylogenetic trees (Stackebrandt et al. 1997; Gao and Gupta 2005), formed the next adjoining clade in this tree, followed by various Gram-negative bacteria (Fig. 6). The most parsimonious explanation for these results is that the MurA indel was initially lacking in Actinobacteria and that the (−) insert state (also found in various Gram-negative bacteria as well as *Firmicutes*) is the ancestral state of the protein. Subsequently, there was a duplication of the *murA* gene within actinobacteria and the insert was introduced into one of the gene copies. From an actino-

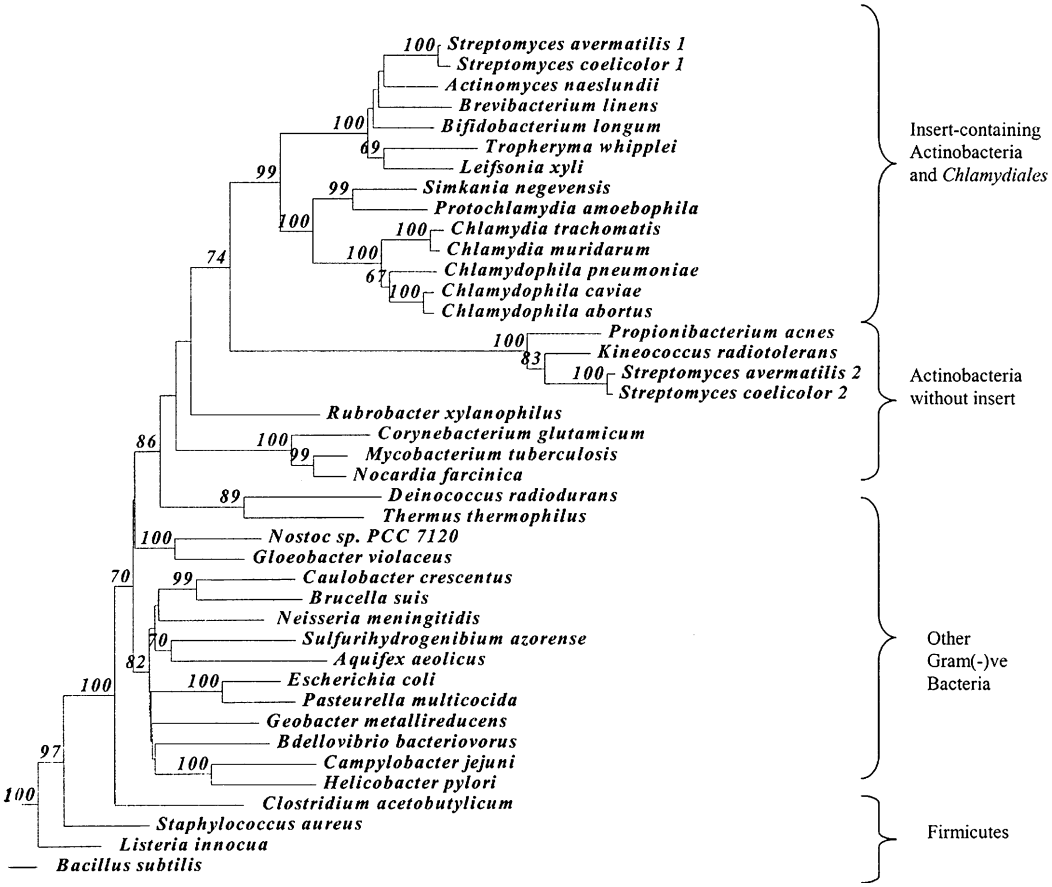

Fig. 6. A neighbor-joining distance tree based on MurA sequences showing the branching of the Chlamydiales with the insert-containing actinobacterial species. Bootstrap scores >50% are shown.

bacterial species which contained the insert, the *murA* gene was laterally transferred to a common ancestor of the Chlamydiales. Since all Gram-negative bacteria contain a (−) insert MurA homologue, it is likely that the ancestral chalmydiae also initially possessed such a homologue, but it was lost after the acquisition of the (+) insert homologue.

Discussion

The chlamydiae species, which are responsible for a broad spectrum of diseases in humans and animals, constitute a distinct phylum within Bacteria (Kalayoglu and Byrne 2001; Bush and Everett 2001; Corsaro et al. 2003). In recent years, complete genomes of several chlamydiae species have been sequenced. The analyses of these genomes by BLASTp searches and phylogenetic methods have indicated that as many as about 4% of the proteins in chlamydiae genomes are probably derived from other bacteria by means of LGT (Wolf et al. 1999). In particular, a significant number of chlamydiae proteins exhibit the highest sequence similarity to the plants or plastids homologues (Royo et al. 2000; Brinkman et al. 2002), leading to the suggestion of an ancestral relationship between cyanobacteria (from which chloroplasts are derived) and the chlamydiae. One well-studied example of LGT involving chlamydiae and some other groups of bacteria is provided by ATP/ADP translocases. These genes are only found in the plant/plastid genomes, the chlamydiae and the rickettsiae (Zomorodipour and Andersson 1999; Wolf et al. 1999; Brinkman et al. 2002; Schmitz-Esser et al. 2004). The ATP/ADP translocases are essential for these intracellular organisms and organelles, as they enable them to import host-derived ATP across their cell membrane, which is otherwise impermeable to nucleotides (Schmitz-Esser et al. 2004). In phylogenetic trees based on ATP/ADP translocases, the rickettsias, chlamydiae, and chloroplasts cluster monophyletically regardless of the treeing method (Brinkman et al. 2002; Schmitz-Esser et al. 2004). The phylogenetic studies on these proteins indicate that the ATP/ADP translocase was originally invented by a chlamydial ancestor and then laterally transferred to an ancestral rickettsiae. Whether the transfer of the ATP/ADP translocase gene(s) occurred directly between chlamydia and plants or to the chloroplast ancestor has not been

Table 2. Amino acid identity of chlamydiae MurA homologues with other bacteria

Group	Bacterial species	Chlam. pneumoniae (458 aa)	Chl. trachomatis (444 aa)	Proto. amoebophila (465 aa)
Actinobacteria with insert	*Str. coelicolor [1]*	43.8% (196)	42.6% (189)	46.5% (216)
	Str. avermatilis [1]	42.7% (199)	43.5% (193)	46.2% (215)
	Bif. longum	43.6% (200)	46.6% (207)	46.7% (217)
	Brev. linens	42.3% (194)	42.1% (187)	46.2% (215)
	Leif. xylii	40.8% (187)	42.3% (188)	44.5% (207)
	Tro. whipplei	39.3% (180)	40.3% (179)	42.2% (196)
Actinobacteria without insert	*Str. coelicolor [2]*	30.3% (139)	29.1% (129)	26.2% (122)
	Str. avermatilis [2]	29.6% (136)	28.8% (128)	26.2% (122)
	Pro. acnes	28.6% (131)	30.4% (135)	27.9% (130)
	Myc. tuberculosis	34.1% (156)	33.6% (149)	31.6% (147)
	Cor. glutamicum	33.4% (153)	36.3% (161)	32.0% (149)
	Noc. farcinia	34.5% (158)	33.8% (150)	32.9% (153)
Firmicutes	*Bac. subtilis [1]*	32.3% (157)	34.5% (153)	34.6% (161)
	Bac. subtilis [2]	32.1% (147)	31.8% (141)	33.8% (157)
	Lis. innocua [1]	34.5% (158)	36.5% (162)	35.3% (164)
	Lis. innocua [2]	32.9% (151)	32.4% (144)	32.7% (152)
Gram-negative bacteria	*E. coli*	32.9% (151)	33.6% (149)	33.1% (154)
	Ca. crescentus	32.3% (148)	33.1% (147)	34.2% (159)
	Bde. bacteriovorus	31.9% (146)	31.3% (139)	32.0% (149)
	Camp. jejuni	31.4% (144)	32.7% (145)	31.8% (148)
	Aqu. aeolicus	30.6% (140)	32.2% (143)	30.3% (141)
	Bact. fragilis	31.0% (142)	30.2% (134)	28.2% (131)
	Por. gingivalis	32.9% (151)	33.1% (147)	29.2% (136)
	Cb. Tepidum	29.7% (136)	31.8% (141)	30.9% (144)
	Bor. burgdorferi	31.2% (143)	32.9% (146)	28.2% (131)
	Tre. pallidum	30.6% (140)	31.1% (138)	27.3% (127)
	Gloe. violaceus	35.6% (163)	35.1% (156)	36.8% (171)
	Nostoc sp.	36.2% (166)	36.5% (162)	34.4% (160)
	D. radiodurans	31.4% (144)	30.4% (135)	31.2% (145)

Note. The percentage identities as well as the actual numbers of identical positions (in parentheses) that are observed in different pairwise sequence alignments are given. *Streptomyces* and *Firmicutes* species contain two MurA homologues, which are indicated as [1] and [2].

resolved. In addition to many examples of LGT between chlamydiae and plant genomes, a few cases of horizontal transfer between the chlamydiae and other groups of prokaryotes (e.g., Archaea, *Thermotoga*, and Spirochetes) have also been reported (Li et al. 1999; Ortutay et al. 2003). However, in these earlier studies based on BLASTp searches, no cases of LGT between chlamydiae and actinobacteria were identified (Zomorodipour and Andersson 1999; Wolf et al. 1999; Li et al. 1999; Dalevi et al. 2002; Brinkman et al. 2002; Ortutay et al. 2003; Schmitz-Esser et al. 2004).

The present study describes two clear examples of LGTs involving a subset of actinobacteria and the chlamydiae group of species. Our attention was drawn to the GlyA and MurA proteins, as they were found to contain prominent indels in identical positions in highly conserved regions of the proteins in very different groups of bacteria. These results were in contrast to a large number of other conserved indels as well as whole proteins that are uniquely present in either all chlamydiae or Actinobacteria and provide strong evidence that these bacteria are very distinct from each other (Griffiths and Gupta 2002; Griffiths et al. 2005; Gao and Gupta 2005; Gao et al. 2006), which is in accordance with their branching pattern in different phylogenetic trees (Olsen et al. 1994; Viale et al. 1994; Gupta 2000, 2004; Ludwig and Klenk 2001). The shared presence of these rare genetic changes (i.e., conserved indels) in these different groups could result from either independent mutational events (due to similar functional or other selective constraints) or by LGTs between these groups. Our finding that the chlamydiae homologues of these proteins (as well as the GlyA homologues of *Treponema*) exhibited the highest sequence similarity to the insert-containing homologues from actinobacteria, and that in phylogenetic trees based on GlyA and MurA protein sequences, they clustered with a high affinity with these homologues within other actinobacterial species, strongly indicates that the shared presence of these conserved indels is due to LGT from an actinobacteria to these other bacteria. LGT is an important mechanism by which a recipient species can acquire exogenous DNA that can confer novel functional capabilities and selective advantages (Syvanen 1994; Koonin et al. 2001; Jain

et al. 2003; Boucher et al. 2003; Gogarten and Townsend 2005). However, what is of great interest in the present context is that the identified inserts in MurA as well as GlyA are present in all Chlamydiales species and they formed a strongly supported subclade within Actinobacteria. These results strongly indicate that the postulated LGT events are very ancient and they likely occurred from an actinobacteria to a common ancestor of the Chlamydiales species. It should be noted that the GC content as well as patterns of codon usage for these genes is very similar to that for the rest of the Chlamydiales genomes, indicating that these LGTs are ancient.

The Chlamydiales species, which are obligate intracellular parasites of eukaryotic hosts, are physiologically very different from Actinobacteria, which are widely distributed in both terrestrial and aquatic ecosystems, especially in soil (Embley and Stackebrandt 1994). Since the laterally transferred *murA* and *glyA* genes are present in all chlamydiae, for genetic exchange to occur between these groups, it is likely that these gene transfers occurred in an ancestral chlamydiae species that was either free-living or more broadly distributed in the environment such as the chlamydiae-like organism *P. amoebophila*, which infects free-living amoebae (Kalayoglu and Byrne 2001; Bush and Everett 2001; Corsaro et al. 2003; Horn et al. 2004). In the case of SHMT or GlyA, the laterally transferred gene is also present in the *Treponema* species, which are anaerobic commensals and parasites of humans and animals (Charon and Goldstein 2002). Our observations that the *Treponema* homologues associated with the deep-branching environmental chlamydiae species (i.e., *Simkania* and Protochlamydia) in the GlyA phylogenetic tree and that chlalmydiae GlyA exhibited highest the sequence similarity to the *Treponema* homologues indicate that the *glyA* gene was initially transferred from actinobacteria to an ancestor of the *Treponema* genus, and from there the gene was acquired by a common ancestor of the Chlamydiales. It is of interest in this regard that in phylogenetic trees based on 16S rRNA and various proteins (Olsen et al. 1994; Viale et al. 1994; Eisen 1995; Ludwig and Klenk 2001; Gupta 2004), as well as in branching pattern deduced based on conserved inserts in protein sequences (Gupta 2001; Gupta and Griffiths 2002), Spirochetes species form the neighboring phylum of the chlamydiae group. It is likely that after the gene transfer, the ancestor of *Treponema*/Chlamydiales initially contained 2 versions of the *murA* and *glyA* genes for some time (i.e., the native [−] insert gene and the newly acquired [+] insert homologue). Subsequently, in the process of streamlining of their genomes during adaptation to parasitism (Zomorodipour and Andersson 1999; Andersson et al. 2002), and possibly due to the selective advantage provided by the (+) insert gene, the native *murA* and *glyA* genes were lost.

It should be mentioned that although based on the branching patterns of the GlyA and MurA homologues in phylogenetic trees, we have favored the possibility that these genes have been laterally transferred from an actinobacteria to a common ancestor of the Chlamydiales, the possibility that these gene transfers have occurred in the other direction cannot be ruled out. Such a possibility is favored by the observation that all of the chlamydiae species posses only one copy of these genes, whereas different actinobacterial species which harbor the insert-containing homologues have two or more copies of these genes. In this scenario, the identified inserts were first introduced in a common ancestor of the Chlamydiales, and from there they were transferred to other soil bacteria.

The possible functional and physiological significance of the observed LGT from actinobacteria to chlamydiae (or vice versa) is presently unclear. Since the location and size of the inserts in MurA and SHMT proteins in the chlamydiae and actinobacteria have been maintained and preserved throughout evolutionary history, it is likely that they confer some novel adaptive advantage to the organisms that possess them. Although the functional significance of these conserved rare genetic changes remains to be determined, it should be noted that the inserts in both MurA and SHMT proteins are proximal to important catalytic residues within these enzymes (Scarsdale et al. 2000; Eschenburg et al. 2005). Further, both chlamydiae and actinobacterial species are characterized by a complex developmental cycle and the proteins with the insert could play some unique but commonly shared function in these distantly related organisms. It is also of much interest that although chlamydiae species lack traditional peptidoglycan, all Chlamydiales genomes contain almost a full complement of genes for the peptidoglycan biosynthetic pathway (Stephens et al. 1998; Rockey et al. 2000). In this context, it should be noted that in addition to the large insert in the MurA protein, we have previously identified another prominent indel (17-aa insert) in a second peptidoglycan biosynthesis pathway enzyme, i.e., UDP-*N*-acetylglucosamine pyrophosphorylase (GlmU), that is commonly shared by various chlamydiae and the archaebacteria (Griffiths and Gupta 2002). In this particular case, an LGT transfer has occurred from an *Archaea* to an common ancestor of the Chlamydiales (Griffiths and Gupta 2002; unpublished results). It is possible that the large inserts in the MurA and GlmU, in addition to modifying the known functions of these proteins, may confer on them some novel functional capabilities enabling

interaction with other cellular proteins and ligands. Hence, functional studies aimed at understanding the functional significance of these rare genetic changes should be of great interest.

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References

Andersson SG, Alsmark C, Canback B, Davids W, Frank C, Karlberg O, Klasson L, Antoine-Legault B, Mira A, Tamas I (2002) Comparative genomics of microbial pathogens and symbionts. Bioinformatics 18(Suppl 2):S17

Boucher Y, Douady CJ, Papke RT, Walsh DA, Boudreau ME, Nesbo CL, Case RJ, Doolittle WF (2003) Lateral gene transfer and the origins of prokaryotic groups. Annu Rev Genet 37:283–328

Brinkman FS, Blanchard JL, Cherkasov A, Av-Gay Y, Brunham RC, Fernandez RC, Finlay BB, Otto SP, Ouellette BF, Keeling PJ, Rose AM, Hancock RE, Jones SJ, Greberg H (2002) Evidence that plant-like genes in Chlamydia species reflect an ancestral relationship between Chlamydiaceae, cyanobacteria, and the chloroplast. Genome Res 12:1159–1167

Brown ED, Vivas EI, Walsh CT, Kolter R (1995) MurA (MurZ), the enzyme that catalyzes the first committed step in peptidoglycan biosynthesis, is essential in Escherichia coli. J Bacteriol 177:4194–4197

Bush RM, Everett KD (2001) Molecular evolution of the Chlamydiaceae. Int J Syst Evol Microbiol 51:203–220

Charon NW, Goldstein SF (2002) Genetics of motility and chemotaxis of a fascinating group of bacteria: the spirochetes. Annu Rev Genet 36:47–73

Collingro A, Toenshoff ER, Taylor MW, Fritsche TR, Wagner M, Horn M (2005) 'Candidatus Protochlamydia amoebophila', an endosymbiont of Acanthamoeba spp. Int J Syst Evol Microbiol 55:1863–1866

Corsaro D, Valassina M, Venditti D (2003) Increasing diversity within Chlamydiae. Crit Rev Microbiol 29:37–78

Dalevi DA, Eriksen N, Eriksson K, Andersson SG (2002) Measuring genome divergence in bacteria: a case study using chlamydian data. J Mol Evol 55:24–36

Du W, Brown JR, Sylvester DR, Huang J, Chalker AF, So CY, Holmes DJ, Payne DJ, Wallis NG (2000) Two active forms of UDP-N-acetylglucosamine enolpyruvyl transferase in gram-positive bacteria. J Bacteriol 182:4146–4152

Eisen JA (1995) The RecA protein as a model molecule for molecular systematic studies of bacteria:comparison of trees of RecAs and 16S rRNAs from the same species. J Mol Evol 41:1105–1123

Embley TM, Stackebrandt E (1994) The molecular phylogeny and systematics of the actinomycetes. Annu Rev Microbiol 48:257–289

Eschenburg S, Priestman M, Schonbrunn E (2005) Evidence that the fosfomycin target Cys115 in UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) is essential for product release. J Biol Chem 280:3757–3763

Everett KD, Bush RM, Andersen AA (1999) Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. Int J Syst Bacteriol 49(Pt 2): 415–440

Fraser CM, Norris SJ, Weinstock CM, White O, Sutton GG, Dodson R, Gwinn M, Hickey EK, Clayton R, Ketchum KA, Sodergren E, Hardham JM, McLeod MP, Salzberg S, Peterson J, Khalak H, Richardson D, Howell JK, Chidambaram M, Utterback T, McDonald L, Artiach P, Bowman C, Cotton MD (1998) Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. Science 281:375–388

Gao B, Gupta RS (2005) Conserved indels in protein sequences that are characteristic of the phylum Actinobacteria. Int J Syst Evol Microbiol 55:2401–2412

Gao B, Parmanathan R, Gupta RS (2006) Signature proteins that are distinctive characteristics of Actinobacteria and their subgroups. Antonie van Leeuwenhoek 3 May 2006, Epub

Gogarten JP, Townsend JP (2005) Horizontal gene transfer, genome innovation and evolution. Nat Rev Microbiol 3:679–687

Griffiths E, Gupta RS (2002) Protein signatures distinctive of chlamydial species:horizontal transfers of cell wall biosynthesis genes glmU from archaea to chlamydiae and murA between chlamydiae and Streptomyces. Microbiology-Sgm 148:2541–2549

Griffiths E, Petrich AK, Gupta RS (2005) Conserved indels in essential proteins that are distinctive characteristics of Chlamydiales and provide novel means for their identification. Microbiology-Sgm 151:2647–2657

Gupta RS (1995) Evolution of the chaperonin families (Hsp60, Hsp10 and Tcp-1) of proteins and the origin of eukaryotic cells. Mol Microbiol 15:1–11

Gupta RS (1998) Protein phylogenies and signature sequences: a reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. Microbiol Mol Biol Rev 62:1435–1491

Gupta RS (2000) The phylogeny of Proteobacteria: relationships to other eubacterial phyla and eukaryotes. FEMS Microbiol Rev 24:367–402

Gupta RS (2001) The branching order and phylogenetic placement of species from completed bacterial genomes, based on conserved indels found in various proteins. Int Microbiol 4:187–202

Gupta RS (2004) The phylogeny and signature sequences characteristics of Fibrobacteres, Chlorobi, and Bacteroidetes. Crit Rev Microbiol 30:123–143

RS, Gupta Griffiths E (2002) Critical issues in bacterial phylogeny. Theor Popul Biol 61:423–434

Horn M, Collingro A, Schmitz-Esser S, Beier CL, Purkhold U, Fartmann B, Brandt P, Nyakatura GJ, Droege M, Frishman D, Rattei T, Mewes HW, Wagner M (2004) Illuminating the evolutionary history of chlamydiae. Science 304:728–730

Jain R, Rivera MC, Moore JE, Lake JA (2003) Horizontal gene transfer accelerates genome innovation and evolution. Mol Biol Evol 20:1598–1602

Kahane S, Everett KD, Kimmel N, Friedman MG (1999) Simkania negevensis strain ZT: growth, antigenic and genome characteristics. Int J Syst Bacteriol 49(Pt 2):815–820

Kalayoglu MV, Byrne GI (2001) Chlamydia. In: Dworkin M (ed) The prokaryotes: an evolving electronic resource for the microbiological community, 3rd ed, release 37 (http://link.springer-ny.com/link/service/books/10125/). Springer-Verlag, New York

Kalman S, Mitchell W, Marathe R, Lammel C, Fan J, Hyman RW, Olinger L, Grimwood J, Davis RW, Stephens RS (1999) Comparative genomes of Chlamydia pneumoniae and C. trachomatis. Nat Genet 21:385–389

Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge

Koonin EV, Makarova KS, Aravind L (2001) Horizontal gene transfer in prokaryotes:quantification and classification. Annu Rev Microbiol 55:709–742

Li T, Graham DE, Stathopoulos C, Haney PJ, Kim HS, Vothknecht U, Kitabatake M, Hong KW, Eggertsson G, Curnow AW, Lin W, Celic I, Whitman W, Soll D (1999) Cysteinyl-tRNA formation: the last puzzle of aminoacyl-tRNA synthesis. FEBS Lett 462:302–306

Ludwig W, Klenk H-P (2001) Overview: a phylogenetic backbone and taxonomic framework for prokaryotic systamatics. In: Boone DR, Castenholz RW (eds) Bergey's manual of systematic bacteriology. Springer-Verlag, Berlin, pp 49–65

Mahony JB, Chong S, Coombes BK, Smieja M, Petrich A (2000) Analytical sensitivity, reproducibility of results, and clinical performance of five PCR assays for detecting Chlamydia pneumoniae DNA in peripheral blood mononuclear cells. J Clin Microbiol 38:2622–2627

Olsen GJ, Woese CR, Overbeek R (1994) The winds of (evolutionary) change:breathing new life into microbiology. J Bacteriol 176:1–6

Ortutay C, Gaspari Z, Toth G, Jager E, Vida G, Orosz L, Vellai T (2003) Speciation in Chlamydia: genomewide phylogenetic analyses identified a reliable set of acquired genes. J Mol Evol 57:672–680

Rao NA, Talwar R, Savithri HS (2000) Molecular organization, catalytic mechanism and function of serine hydroxymethyltransferase—a potential target for cancer chemotherapy. Int J Biochem Cell Biol 32:405–416

Read TD, Brunham RC, Shen C, Gill SR, Heidelberg JF, White O, Hickey EK, Peterson J, Utterback T, Berry K, Bass S, Linher K, Weidman J, Khouri H, Craven B, Bowman C, Dodson R, Gwinn M, Nelson W, DeBoy R, Kolonay J, McClarty G, Salzberg SL, Eisen J, Fraser CM (2000) Genome sequences of Chlamydia trachomatis MoPn and Chlamydia pneumoniae AR39. Nucleic Acids Res 28:1397–1406

Read TD, Myers GS, Brunham RC, Nelson WC, Paulsen IT, Heidelberg J, Holtzapple E, Khouri H, Federova NB, Carty HA, Umayam LA, Haft DH, Peterson J, Beanan MJ, White O, Salzberg SL, Hsia RC, McClarty G, Rank RG, Bavoil PM, Fraser CM (2003) Genome sequence of Chlamydophila caviae (Chlamydia psittaci GPIC): examining the role of niche-specific genes in the evolution of the Chlamydiaceae. Nucleic Acids Res 31:2134–2147

Rockey DD, Lenart J, Stephens RS (2000) Genome sequencing and our understanding of chlamydiae. Infect Immun 68:5473–5479

Royo J, Gimez E, Hueros G (2000) CMP-KDO synthetase: a plant gene borrowed from gram-negative eubacteria. Trends Genet 16:432–433

Rurangirwa FR, Dilbeck PM, Crawford TB, McGuire TC, McElwain TF (1999) Analysis of the 16S rRNA gene of microorganism WSU 86-1044 from an aborted bovine foetus reveals that it is a member of the order Chlamydiales: proposal of Waddliaceae fam. nov., Waddlia chondrophila gen. nov., sp. nov. Int J Syst Bacteriol 49(Pt 2):577–581

Scarsdale JN, Radaev S, Kazanina G, Schirch V, Wright HT (2000) Crystal structure at 24 A resolution of *E. coli* serine hydroxymethyltransferase in complex with glycine substrate and 5-formyl tetrahydrofolate. J Mol Biol 296:155–168

Schmitz-Esser S, Linka N, Collingro A, Beier CL, Neuhaus HE, Wagner M, Horn M (2004) ATP/ADP translocases: a common feature of obligate intracellular amoebal symbionts related to Chlamydiae and Rickettsiae. J Bacteriol 186:683–691

Stackebrandt E, Rainey FA, WardRainey NL (1997) Proposal for a new hierarchic classification system, Actinobacteria classis nov. Int J Syst Bacteriol 47:479–491

Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, Mitchell W, Olinger L, Tatusov RL, Zhao Q, Koonin EV, Davis RW (1998) Genome sequence of an obligate intracellular pathogen of humans: Chlamydia trachomatis. Science 282:754–759

Syvanen M (1994) Horizontal gene transfer:evidence and possible consequences. Annu Rev Genet 28:237–261

Thomson NR, Yeats C, Bell K, Holden MT, Bentley SD, Livingstone M, Cerdeno-Tarraga AM, Harris B, Doggett J, Ormond D, Mungall K, Clarke K, Feltwell T, Hance Z, Sanders M, Quail MA, Price C, Barrell BG, Parkhill J, Longbottom D (2005) The Chlamydophila abortus genome sequence reveals an array of variable proteins that contribute to interspecies variation. Genome Res 15:629–640

TIGR Microbial Database (2006) http:/www.tigr.org/tdb/mdb/mdb.html

Van de Peer Y, De Wachter R (1994) TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Appl Biosci 10:569–570

Viale AM, Arakaki AK, Soncini FC, Ferreyra RG (1994) Evolutionary relationships among eubacterial groups as inferred from GroEL (chaperonin) sequence comparisons. Int J Syst Bacteriol 44:527–533

Weinstock GM, Hardham JM, McLeod MP, Sodergren EJ, Norris SJ (1998) The genome of Treponema pallidum: new light on the agent of syphilis. FEMS Microbiol Rev 22:323–332

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271

Wolf YI, Aravind L, Koonin EV (1999) Rickettsiae and Chlamydiae: evidence of horizontal gene transfer and gene exchange. Trends Genet 15:173–175

Zomorodipour A, Andersson SG (1999) Obligate intracellular parasites: Rickettsia prowazekii and Chlamydia trachomatis. FEBS Lett 452:11–15