Mature Epitope Density – A strategy for target selection based on immunoinformatics and exported prokaryotic proteins

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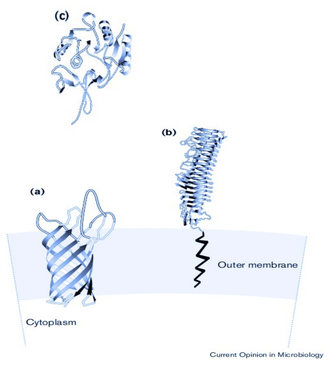
ABSTRACT

Background: Current immunological bioinformatic approaches focus on the prediction of allelespecific epitopes capable of triggering immunogenic activity. The prediction of major histocompatibility complex (MHC) class I epitopes is well studied, and various software solutions exist for this purpose. However, currently available tools do not account for the concentration of epitope products in the mature protein product and its relation to the reliability of target selection.

Results: We developed a computational strategy based on measuring the epitope's concentration in the mature protein, called Mature Epitope Density (MED). Our method, though simple, is capable of identifying promising vaccine targets. Our online software implementation provides a computationally light and reliable analysis of bacterial exoproteins and their potential for vaccines or diagnosis projects against pathogenic organisms. We evaluated our computational approach by using the Mycobacterium tuberculosis (Mtb) H37Rv exoproteome as a gold standard model. A literature search was carried out on 60 out of 553 Mtb's predicted exoproteins, looking for previous experimental evidence concerning their possible antigenicity. Half of the 60 proteins were classified as highest scored by the MED statistic, while the other half were classified as lowest scored. Among the lowest scored proteins, ~13% were confirmed as not related to antigenicity or not contributing to the bacterial pathogenicity, and 70% of the highest scored proteins were confirmed as related. There was no experimental evidence of antigenic or pathogenic contributions for three of the highest MED-scored Mtb proteins. Hence, these three proteins could represent novel putative vaccine and drug targets for Mtb. A web version of MED is publicly available online at http://med.mmci.uni-saarland.de/.



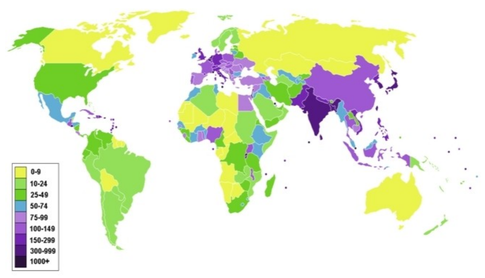
OBJECTIVE

Catalog and characterize bacterial exported proteins according to their possibility to produce cellular immunological responses in hosts.

RESULTS AND DISCUSSIONS

THE MED SCORE CONCEPT IS SIMILAR TO OTHER BROADLY KNEW CONCEPT: THE DEMOGRAPHIC DENSITY ...

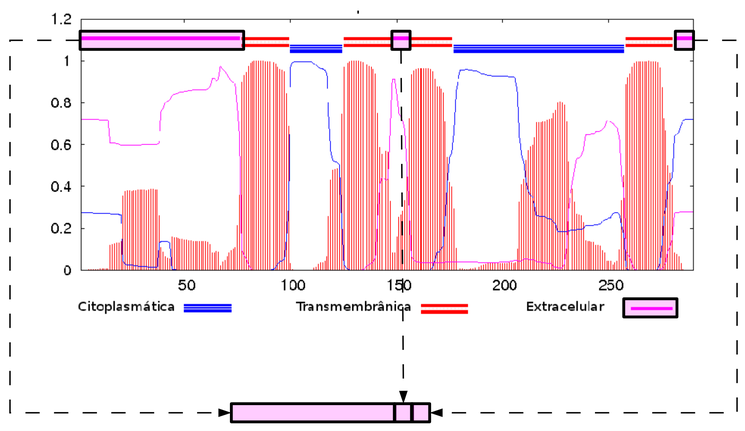
$$Demographic\ Density = \frac{Number\ of\ inhabitants}{Area}$$



Just substitute "inhabitants" for *in silico* predicted epitopes and "Area" for possible 9mer epitopes;

$$MED \simeq \frac{Amount\ of\ predicted\ epitopes * Average\ MHC\ affinity}{Possible\ epitopes\ predicted}$$

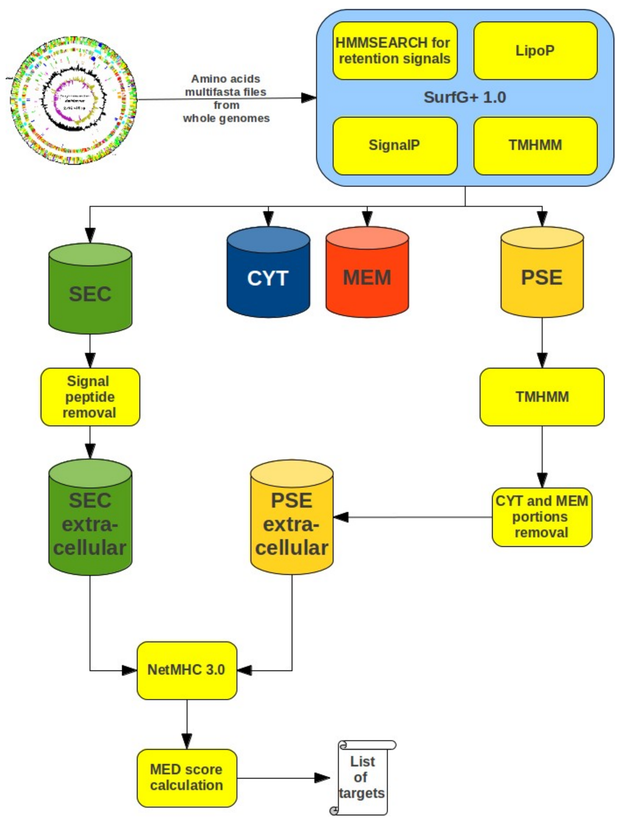
... BUT THE WHOLE AREA DO NOT NEED TO BE CONSIDERED, JUST THE MATURE PORTION OF A PROTEIN.



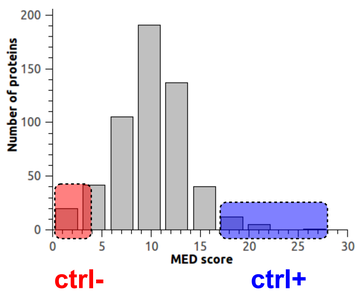
$$MED \simeq \frac{Amount\ of\ predicted\ epitopes * Average\ MHC\ affinity}{Possible\ epitopes\ predicted}$$

The above graph represents the prediction of sub-cellular location from a *C. pseudotuberculosis*' protein portions, strain 1002. For each amino acid a probability of being part of a trans-membrane region is assigned. This graph represents a protein of about 300 amino acids classified as potentially surface exposed (PSE) by the program TMHMM 2.0. Epitopes predicted in the extracellular portion are used to create an artificial protein for the MED score calculations.

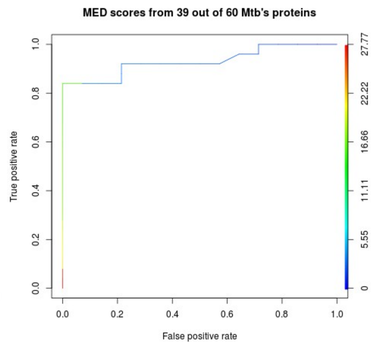
THE MED SCORE CALCULATION WAS AUTOMATED ACCORDING TO THIS MEDPIPE FLOWCHART AND IS AVAILABLE AT THE SITE http://med.mmci.uni-saarland.de/



THE CONCEPT WAS VERIFIED FOR *Mycobacterium tuberculosis* SHOWING A GOOD SENSITIVITY RATE ACCESSED BY LITERATURE SEARCH ...



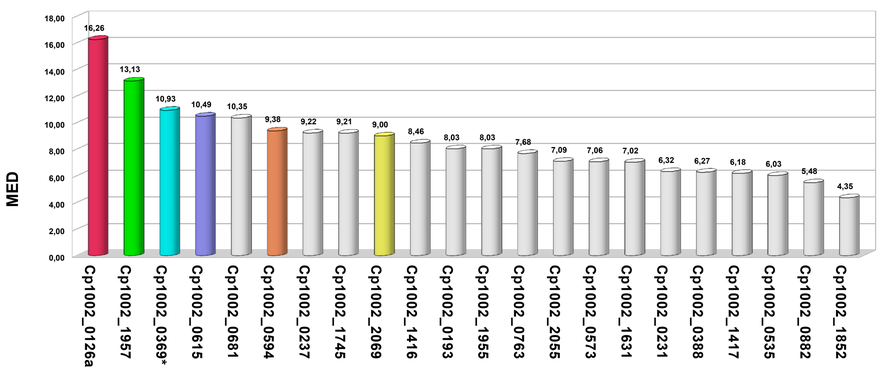
MED scores histogram of 553 *in silico* predicted exoproteins. Sixty proteins were chose for literature search, being half from the left (ctrl-) or lowest scored and other half from right (ctrl+) highest scored.



***ROC* curve for 39 out 60 proteins gathered from the aside histogram. From these 39 proteins, there are 18 and 21 in the ctrl- and ctrl+, respectively, achieving a sensitivity (Tpr) of 84% against a specificity (Fpr) of 7%.**

... AND ALSO FOR *Corynebacterium pseudotuberculosis* WHERE THREE FROM THE TOP FOUR SCORED PROTEINS WERE CONFIRMED AS ANTIGENIC BY DISTINCT EXPERIMENTS.

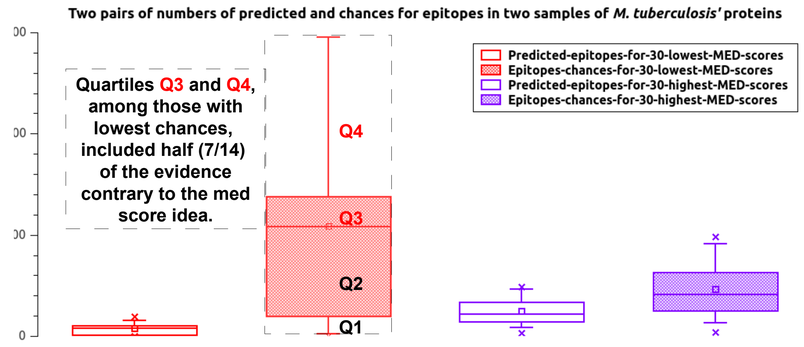
MED scores for the predicted C. pseudotuberculosis pan secretome found simultaneosly in strains 1002 and C231



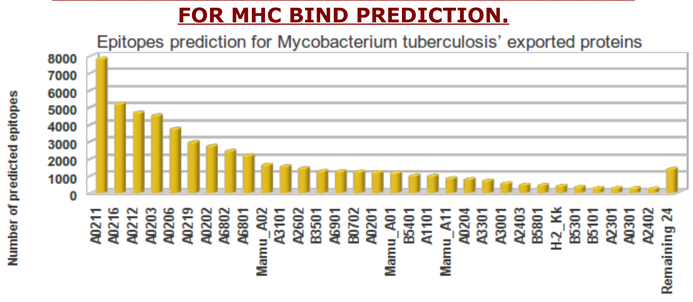
Identifier	*C. pseudotuberculosis* product	MED (nM/mer)	*In silico* and *in vitro* corroborative results
Cp1002_0126a	*Hypothetical protein*	16,26	*Surfg+ ∩ TTP/LC-MS*
Cp1002_1957	*Trehalose corynomycolyl transferase B*	13,13	*Surfg+ ∩ TTP/LC-MS ∩ Antigenicity*
Cp1002_0369*	*Phosphoesterase PA-phosphatase related protein*	10,93	*Surfg+ ∩ TTP/LC-MS ∩ Antigenicity*
Cp1002_0681	*Resuscitation-promoting factor RpfB*	10,35	*Surfg+ ∩ TTP/LC-MS ∩ Immunoproteomic*
Cp1002_0237	*Surface layer protein A*	9,22	*Surfg+ ∩ TTP/LC-MS ∩ Immunoproteomic*
Cp1002_1416	*NlpC/P60 protein*	8,46	*Surfg+ ∩ TTP/LC-MS ∩ Immunoproteomic*

HOWEVER, MED SCORE HAS IT'S LIMITATIONS, FOR INSTANCE, A FOLD GREATHER THAN FOUR BETWEEN PREDICTED EPITOPES AND POSSIBLE 9MER EPITOPES PRODUCES AN UNDER ESTIMATION OF THE MED SCORE.

Two pairs of numbers of predicted and chances for epitopes in two samples of *M. tuberculosis'* proteins



... AND ALSO THE SMALL NUMBER OF MHC CLASS I ALLELES OFFERED BY THE CURRENT COMPUTER PROGRAMS FOR MHC BIND PREDICTION.



MHC alleles in the software NetMHC and the number of predicted strong binders to epitopes from *Mtb* H37Rv exported proteins.

CONCLUSIONS

Conclusions: The software presented here offers a practical and accurate method to identify potential vaccine and diagnosis candidates against pathogenic bacteria by "reading" results from well-established reverse vaccinology software in a novel way, considering the epitope's concentration in the mature portion of the protein.

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Supported by: CNPq