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Secreted bacterial proteins

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Abstract

Predicted extracellular proteins of *Bacillus subtilis* have been compared with the experimental identification of the extracellular proteome by two-dimensional gel electrophoresis, peptide sequencing and mass spectrometry.

Significance and context

Bacteria depend on the secretion of extracellular degradative enzymes and other protein products for their survival. Protein secretion from the bacterial cell occurs after cleavage of the amino-terminal signal peptide. Four different signal peptidases (SPases) are responsible for processing the exported products: five type I SPases (SipS, T, U, V and W), LspA (type II SPase), ComC (prepilin-specific SPase) and ABC transporters. The specificity of these enzymes has been characterized experimentally. Knowledge of the signal-peptide cleavage sites enabled development of an *in silico* analysis of the whole proteome to estimate the size of the secretome - the full set of secreted proteins - of *Bacillus subtilis* by computational analysis. The next logical step was to compare the predictions with the real secretome. Antelmann *et al.* used two-dimensional electrophoresis, peptide sequencing and mass spectrometry to identify secreted proteins.

Key results

The authors identified 82 extracellular proteins out of approximately 200 visualized on two-dimensional gels. The 82 included several groups of degradative enzymes, as well as detoxification products, lipoproteins, proteins involved in cell-wall biogenesis and flagella formation and others. When analyzed further, 50 of these proteins had a known function, and 32 were apparently hitherto unknown. Of the novel proteins, 19 could be assigned a possible function by similarity searches and 13 were of unknown function. A comparison of the experimental results with the predicted protein composition of the secretome (assembled using the programs SignalP, TopPred and TMHMM) revealed that only 48 out of the 82 secreted proteins had been predicted to have a signal peptide. An SPase I recognition site was identified in most of the proteins. Antelmann *et al*. suggest that proteins missing from predictions were not identified because of a lack of an apparent signal peptide or because of identifiable transmembrane domains.

To determine how the secretome changes in response to mutations in SPase I, the authors used a panel of mutant strains as single and multiple mutants. This analysis revealed that various mutants of the type-I SPase had very limited effects on the secretome, with only one apparently differentially regulated protein - YfnI - which seems to require both SipT and V for processing. In contrast, the SPase II *lspA* mutation had a dramatic effect on the secretome, reducing it to 25% of that of the parent strain, with effects on a number of non-lipoproteins (for example, alpha-amylase AMYE and YolA). The effects of this mutation are puzzling, in that some of the lipoproteins are unaffected (for example, OppA and YclQ) while others increase in the secreted fraction (for example, MntA and YxeB). Additional aspects of lipoprotein processing were analyzed using an *lgt* mutant, deficient in lipoprotein modification. The mutant showed significant shedding of lipoproteins into the medium, resulting in increased extracellular levels of proteins such as OppA, MntA, YvcE, LytD and others. Investigation of the transcriptional levels of these proteins indicated that the changes observed at the protein level are not related to the RNA levels for these genes. These changes were attributed to proteolysis of some of the lipoproteins (LytD and YvcE) or to membrane leakage.

Links

The complete sequence of *Bacillus subtilis* is available from Micado and JAFAN: Japan Functional Analysis Network for *Bacillus subtilis*.

Conclusions

Antelmann *et al*. have identified 82 secreted proteins, 50 of which have a typical signal peptide for SPase I. The authors suggest that SipS or SipT SPases are sufficient for proper precursor processing, although no direct evidence was shown to support this claim (a double mutant was not analyzed and not commented on). The presence of lipoproteins in the medium of the *lspA* mutant is explained by the presence of alternative secretory pathways for lipoproteins. The main conclusion of the paper is that only 50% of the secreted proteins were predicted by computational analysis, mainly because of a lack of signal peptides in the sequences.

Reporter's comments

This is a good example of experimental validation of computational predictions from whole-genome analysis. Bioinformatics approaches are often unable to predict protein structure, localization and function correctly. The main strength of the paper is the comparison of the secretory-pathway mutants. The authors did not, however, examine the effects of all possible SPase I mutant combinations. Further

work is required to determine the reasons for the drastic reduction in secreted proteins in the *lspA* mutant. Reasons for the reduction of non-lipoproteins in this mutant are not addressed in the discussion.

Table of links

Genome Research

Micado

JAFAN: Japan Functional Analysis Network for *Bacillus subtilis*

References

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