UMEÅ UNIVERSITET

BACTERIAL MYSTERY

Unravelling bacterial metabolic interactions and improving microbial metabolomics

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i byggnad KBC
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Fakultetsopponent: prof. Coral Barbas
The Chemistry and Biochemistry Department, Universidad San Pablo, Spanien.

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Abstract

Metabolomics is a comprehensive analysis of metabolites within a biological system. It offers insight into understanding cellular processes at a molecular level. Bacteria, with their relatively simple structure and rapid growth rates are good candidates for metabolomics studies. The advantages of using metabolomics in bacterial studies are relatively fast bacterial growth, plasticity of bacterial metabolism as well as an easy control over experimental conditions. This allows rapid collection of data and facilitates the interpretation of the results. Bacteria cultures can be maintained under highly controlled *in vitro* conditions. This is crucial for metabolomics, where even minor variations can lead to significant differences in metabolic profiles. However, challenges such as complex data analysis, dynamic metabolic changes, resolving multiple pathways, standardization issues, need for proper controls, and resource requirements can be experienced.

The aim of this study to study effects of bacterial interaction with each other and with chemical compounds from the perspective of their metabolism and to improve the approaches to metabolite analysis in bacteria. Specifically, this thesis includes studies of bacterial metabolism under various experimental conditions, including different growth phases, co-culture of two species, and drug exposure.

Understanding how bacteria respond to drugs at the metabolic level can aid in the development of new therapeutic methods. **Paper I** and **Paper III** studied the lipidome of *Streptococcus pneumoniae* and the metabolome of *Mycobacterium tuberculosis* (*Mtb*) to identify metabolic pathways that are altered in response to drug exposure and, hence, may be responsible for drug resistance.

Bacteria often exist in complex microbial communities. Interspecies interactions may reflect in their metabolism over different growth phases. Metabolomics can extract these interactions by identifying metabolites that differ between single species and consortia. **Paper II** investigates the influence of co-culture and growth phase on metabolite patterns in *Actinobacteria*, showing adaptive strategies for growth, stress survival, and environmental interaction, providing insights into bacterial physiology.

Finally, to improve annotation of bacterial metabolites, a study design was established for a comprehensive database over bacterial metabolites. The design covers ten species of bacteria and the key factors such as growth phase and nutrients Bacterial cultivation, sampling, biomass harvesting, and high-throughput LC-MS/MS metabolomics were done with multiple replicates and in a carefully controlled manner aiming to catalogue and characterize diverse bacterial metabolites (**Paper V)**. **Paper IV** proves advantages of Orthogonal Projections to Latent Structures Effect Projections (OPLS-EP), a paired multivariate analysis that has been used here for the first time to improve model performance of bacterial datasets. Programming was used to develop new code instruments and methodology for metabolomics data treatment and analysis.

In conclusion, this thesis provides insights into metabolism of bacterial systems under various conditions, such as growth stages, co-cultures, and drug exposure as well as evaluation of different tools for metabolomics analyses to study bacterial physiology.

Keywords
metabolomics, bacterial metabolism, metabolite analysis, GC-MS, LC-MS, growth phase, interspecies interactions, drug-resistance.

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