4.2.13

Metabolite-centric approach using genome-scale metabolic network for drug targeting of pathogens

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As microbial pathogens constantly develop resistance to antibiotics, it has become necessary to develop efficient methods for drug targeting and discovery. For this, we paid attention to the role of metabolites in the microbial metabolism, so-called metabolite essentiality. If removal of a certain metabolite leads to cell death, it is considered the essential metabolite. Because its removal is equivalent to simultaneous deletion of outgoing metabolic reactions from that removed metabolite, it is possible to identify various combinations of reactions that suppress cell survival, thereby enabling multi-targeting. This multi-targeting is expected to prevent pathogens' resistance to antibiotics better than single-targeting. We resorted to analysis of genome-scale metabolic network to predict essential metabolites. In particular, we utilized constraints-based flux analysis and chokepoint analysis. The former is an optimization-based simulation technique that calculates metabolic fluxes based on the mass balance of metabolites with assumption of pseudo-steady state. The latter is a network-topology-based method that selects enzymes or metabolites as a target that has a single ingoing and/or outgoing reaction. We combined these methods to generate novel drug targets in several emerging drug-resistant pathogens, including *Escherichia coli*, *Helicobacter pylori*, *Mycobacterium tuberculosis* and *Staphylococcus aureus*. This study reveals the biological and clinical importance of metabolites in metabolism from systems perspectives.

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4.2.14

Metabolic footprinting of *Escherichia coli* grown in fed-batch fermentation during recombinant protein production

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The metabolic activity of a bacterial cell is closely regulated and adapted to the specific growth conditions encountered during biosynthetic processes. The production of foreign proteins in *Escherichia coli* (*E. coli*) poses a severe metabolic burden on the host cell, causing metabolic stress because of the rapid exhaustion of essential metabolic precursors and cellular energy. This stress response is believed to affect negatively the cell physiology during high-level production of recombinant protein in *E. coli* cultures. As a result, the cellular growth is inhibited and toxic compounds are accumulated in the extracellular medium. However, microbial cells can alter their extracellular environment to improve their ability to uptake natural resources by releasing hydrolytic enzymes and metabolites. Secreted metabolites help to maintain the biochemical balance of the cell and assure the optimal operation of the metabolism. The secretion of metabolites can be interpreted as biomarkers of particular intracellular metabolic processes such as cellular stress. Metabolome analysis has been widely applied in the large-scale functional analysis of cellular behaviour. The analysis of the entire set of low molecular weight compounds that are released from organisms into the extracellular medium is known as metabolic footprinting and can yield a large amount of biochemical information, which is a straightforward alternative method for monitoring microbial bioprocesses.

In this work, we determined the changes in extracellular metabolite profiles of *E. coli* during the production of a recombinant protein (AcGFP1, jellyfish green fluorescent protein) in fed-batch fermentations using minimal medium and glucose as the only carbon source. Besides the examination of the main differences in the MS spectra between the pre-induction and post-induction phases, we identified several metabolites in the extracellular medium associated with energy scavenging processes specifically aimed to generate metabolic energy during periods of energy-limitation. C4-dicarboxylic acids, such as fumarate, L-aspartate, malate and succinate, were found in the extracellular medium at high levels. Certain amino acids, like L-glutamate, glycine, L-alanine and L-proline were also found and might be associated with the decoupling of catabolic and anabolic reactions during recombinant protein production. Hence, the analysis of the exometabolome aimed to further inspect the effects of metabolic burden in the physiological state of *E. coli* cells during recombinant bioprocesses.

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4.2.15

Friedreich ataxia: a computational dynamic model of the key proteins involved in the yeast Fe—S cluster biogenesis

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Friedreich ataxia (FRDA) is a human neuro-degenerative and hereditary disease which affects the equilibrium, the coordination, the muscles and the heart mainly. It is the most common autosomal recessive ataxia and it is associated with a pronounced lack of a protein named frataxin. This protein has been associated with iron inside the mitochondria and it seems to play an important role in the assembly/maturation of the mitochondrial iron—sulfur clusters (ISCs). It is supposed a high similarity between the human and the yeast molecular mechanisms that involve frataxin. Moreover, in yeast, it has been demonstrated experimentally that the yeast frataxin (yfh1) interacts with the protein isu1, while this interacts both with the protein isu2 as well as nfs1. These four proteins together might generate the central platform for ISC biogenesis. Our main objective is studying bioinformatically the sequence, the structure and the putative function