P42: Footprinting Microbial Metabolites in Nature and Medicine

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The study of metabolic alterations in response to genetic and environmental perturbations has been a central topic in microbial metabolomics (Fiehn, 2002; Kol *et al*., 2010; Villas-Boas *et al*., 2008). Some of these alterations can be readily detected by changes in their surroundings, normally associated with metabolites that are released by cells as by-products of the metabolism or as extracellular signalling molecules to mediate cross-talk within microbial communities. The analysis of these metabolites, also known as metabolic footprinting, has been widely applied with different purposes: discriminating between metabolic phenotypes in order to classify and identify mutant strains (Villas-Boas et al., 2008); monitoring bioprocesses with the aim to detect specific metabolites that indicate alterations in the culture performance (Carneiro *et al.,* 2011; Sue *et al*., 2011); and identifying quorum-sensing metabolites that indicate potential targets to annihilate pathogens (Birkenstock *et al*., 2012). These metabolic readouts have been also useful to give insights into intracellular metabolic activities and provide a straightforward way to analyse simultaneously multiple metabolic activities, since no extraction procedures are required to analyse the endometabolome (i.e., intracellular metabolites).

Thus, through metabolic footprint analysis we can assess central metabolic activities that characterize the reproduction and survival of organisms.

We have developed a methodology to evaluate the metabolic state of microbial cultures by analysing the footprints of two microbial systems: the bacterium *Escherichia coli* and the human pathogen *Helicobacter pylori* that increases the risk of gastric cancer. Strategies for sampling and sample preparation were developed, as well as the analytical procedures based on gas chromatography with mass spectrometry (GC-MS). A wide variety of metabolites was detected, including fatty, amino and organic acids, which allowed us to address changes in most central metabolic pathways, such as the tricarboxylic acid cycle (TCA cycle), the biosynthesis of amino and fatty acids, as well as other energy generating metabolic reactions.

The analysis of extracellular metabolites of *E. coli* cultures at different growth conditions were first performed to discriminate the physiological state of cultures and to evaluate the metabolic alterations produced at different growth conditions. According to our results in these experiments, metabolic footprints are good indicators of alterations in the intracellular metabolism. Next, the metabolic footprints of *H. pylori* cultures were investigated to get insights on the catabolism of this human pathogen. Overall, fifteen amino acids were detected in extracellular medium; six of them were confirmed as essentials for *H. pylori* growth, four amino acids were identified as non-essentials and can be used as carbon source, whilst five amino acids were identified as non-essentials and non-carbon source. In addition, some organic acids were also identified as carbon sources for *H. pylori*. This metabolic footprint analysis of *H. pylori* cultures allowed us to uncover key metabolic activities, mainly related with amino acids catabolism and to get insight on the metabolic behaviour of this organism.

The characterization of catabolic pathways, as well as of possible metabolic constraints, is of major importance to understand the dynamic basis of the interactions host–microbe in the human gut, and in particular to discover potential 'diagnostic' biomarkers. It is well-known that pathogen's metabolism can influence the host health and may affect drug metabolism, toxicity and the efficacy of therapies (Holmes *et al.,* 2011). However, little is known about their metabolic structure and behaviour. Our methodology allows uncovering part of the metabolic structure of *H. pylori* metabolism and undisclosed catabolic activities.

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