Metabolomics as a tool to understand *E. coli* metabolic responses in recombinant processes

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In the last 10 years, studies on *E. coli* metabolism have benefited from the rapid expansion of metabolomics methodologies that allow to capture qualitative as well as quantitative information about the intracellular and extracellular metabolite profiles. The capacity to identify the effect of various known biological factors on the metabolome has been pointed as one of the most promising features of this new 'omics' field [1-4].

Though *E. coli* is certainly one of the best characterized industrial organisms, results from emerging metabolomic studies have revealed important findings about the physiology of these metabolic systems. Indeed, it is well-known that a vast number of metabolic activities in *E. coli* are still poorly characterized and information obtained from such studies can provide us an unprecedented amount of information about their functional roles [5,6]. Since *E. coli* is still used as a model organism, in particular for the elucidation of many metabolic activities, these studies stand as a key source of metabolic information. Lately, the study of metabolic alterations in response to genetic and environmental perturbations has been a central topic in microbial metabolomics [7-9]. Understanding the rapid adjustments required in the metabolic network for cells to cope with these conditions is usually valuable to understand the physiology of *E. coli* cells [10,11] and also to infer novel functional roles in the organism [12].

Our studies on metabolomics analysis of recombinant *E. coli* cells grown under controlled bioprocesses have provided us with detailed information about the metabolic status of cells during the production of heterologous proteins. This is of particular interest, since *E. coli* has become one of the most used microbial systems to produce heterologous proteins. As previously reported, the host cell metabolism undergoes a severe metabolic burden due to the additional production of recombinant protein, resulting in a rapid exhaustion of essential precursors and cellular energy [13]. This ultimately leads to an imbalance in cellular metabolism and low productivities. Furthermore, we have also performed some analyses on the influence of the stringent response, a stress phenomenon caused by the depletion of amino acids, often associated with the metabolic burden caused by the production of recombinant proteins [14]. So far, only few attempts have been made to characterize the impact of the metabolic burden caused by recombinant processes through the unbiased identification and quantification of the entire set of metabolites present in the cell. Similarly, the impact of the stringent response on the cellular metabolism during recombinant processes has been overlooked, remaining unclear which metabolic alterations result from cellular responses to such environmental conditions.

Therefore, the physiological and metabolic changes in *E. coli* cultures during the production of heterologous proteins were investigated by performing GC-MS-based metabolomic analyses. A detailed description of the analytical protocol employed in these studies is given in [15]. 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. According to our results, major alterations in metabolic activities are produced immediately after the induction of recombinant protein production, revealing that the additional drainage of metabolic resources influences the whole metabolic activity of host cells. Furthermore, the triggering of stress responses like the stringent response hampers the protein production and it seems to have a tight control over different metabolic pathways, in particular fatty and amino acids biosynthesis. Finally, it

was also concluded that, though important findings were accomplished, to reach a full understanding of the underlying mechanisms controlling these metabolic imbalances, further inspections on key metabolic activities are required (anaplerotic reactions) and the combination with other experimental strategies (e.g. fluxomics or proteomics) would be also beneficial.

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