IS1-3

Target Identification for Metabolic Engineering : Incorporation of Metabolome and Transcriptome Strategies to Better Understand Metabolic Fluxes

Nic LINDLEY

Laboratoire Biotechnology-Bioprocédés, UMR CNRS/INRA/INSA, Institut National des Sciences Appliquées, 135 Avenue de Rangueil, 31077 Toulouse cedex 4, France. Email : lindley@insa-tlse.fr

Metabolic engineering is now a well established discipline, used extensively to determine and execute rational strategies of strain development to improve the performance of micro-organisms employed in industrial fermentations. The basic principle of this approach is that performance of the microbial catalyst should be adequately characterised metabolically so as to clearlyidentify the metabolic network constraints, thereby identifying the most probable targets for genetic engineering and the extent to which improvements can be realistically achieved. In order to harness correctly this potential, it is clear that the physiological analysis of each strain studied needs to be undertaken under conditions as close as possible to the physico-chemical environment in which the strain evolves within the full-scale process. Furthermore, this analysis needs to be undertaken throughoutthe entire fermentation so as to take into account the changing environment in an essentially dynamic situation in which metabolic stress is accentuated by the microbial activity itself, leading to increasingly important stress response at a metabolic level. All too often these industrial fermentation constraints are overlooked, leading to identification of targets whose validity within the industrial context is at best limited. Thus the conceptual error is linked to experimental design rather than inadequate methodology.

New tools are becoming available which open up new possibilities in metabolic engineering and the characterisation of complex metabolic networks. Traditionally metabolic analysis was targeted towards pre-identified genes and their corresponding enzymatic activities within pre-selected metabolic pathways. Those pathways not included at the onset were intrinsically removed from the network giving a fundamentally localised vision of pathway functionality. New tools from genome research extend this reductive approach so as to include the global characteristics of a given biological model which can now be seen as an integrated functional unit rather than a specific sub-group of biochemical reactions, thereby facilitating the resolution of complexnetworks whose exact composition cannot be estimated at the onset. This global overview of whole cell physiology enables new targets to be identified which would classically not have been suspected previously. Of course, as with all powerful analytical tools, post-genomic technology must be used carefully so as to avoid expensive errors. This is not always the case and the data obtained need to be examined carefully to avoid embarking on the study of artefacts due to poor

understanding of cell biology.

These basic developments and the underlying concepts will be illustrated with examples from the author's laboratory concerning the industrial production of commodity chemicals using a number of industrially important bacteria. The different levels of possibleinvestigation and the extent to which the data can be extrapolated will be highlighted together with the extent to which realistic yield targets can be attained. Genetic engineering strategies and the performance of the resulting strains will be examined within the context of the prevailing experimental conditions encountered in the industrial fermentor. Examples used will include the production of amino acids, vitamins and polysaccharides. In each case metabolic constraints can be identified and the extent to which performance can be enhanced predicted