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Overview on sugar metabolism and its control in *Lactococcus lactis* – The input from in vivo NMR

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Abstract

The wide application of lactic acid bacteria in the production of fermented foods depends to a great extent on the unique features of sugar metabolism in these organisms. The relative metabolic simplicity and the availability of genetic tools made *Lactococcus lactis* the organism of choice to gain insight into metabolic and regulatory networks. In vivo nuclear magnetic resonance has proven a very useful technique to monitor non-invasively the dynamics of intracellular metabolite and co-factor pools following a glucose pulse. Examples of the application of this methodology to identify metabolic bottlenecks and regulatory sites are presented. The use of this information to direct metabolic engineering strategies is illustrated.

Keywords: LAB; *Lactococcus lactis*; In vivo NMR; Glycolysis regulation; Metabolic engineering

Contents

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1. Introduction

Lactic acid bacteria (LAB) are industrially important microorganisms that are used worldwide in the manufacture of fermented foods and beverages. These microbes produce mainly lactic acid from sugar, providing an effective method of preserving fermented products. The homofermentative and heterofermentative pathways utilized by LAB for the conversion of carbohydrates to lactate are well known and have been described in textbooks [1] and reviews [2,3]. The fact that LAB usually lack functional electron chains, grow under low oxygen tension, and rely mostly on fermentative processes to provide energy, considerably limits their metabolic versatility. Nevertheless, minor products of sugar metabolism have been shown to be highly relevant in dairy fermentations. In addition to preservation, LAB also contribute to other product characteristics, such as flavor, texture and, frequently, nutritional value (for reviews see [4–8]). The well established status of LAB as food organisms together with a relatively simple physiology make them suitable targets for metabolic engineering strategies aimed at the improvement of food quality and human health [9]. Of all LAB, *Lactococcus lactis* is by far the most extensively studied organism. The relative simplicity of *L. lactis* metabolism that converts sugars via the glycolytic (homofermentative) pathway to pyruvate, generating energy mainly through substrate level phosphorylation (Fig. 1), makes it an attractive target for the development of effective cell factories. Moreover, the availability of a large number of genetic tools [10] and the complete genome sequence [11] consolidated its status as a model for LAB and offers the opportunity of adopting global approaches that, it is to be hoped, will provide a comprehensive picture of how cellular components interact to produce a functional organism [12].

A major breakthrough for using *L. lactis* as a cell factory was the development of several genetic tools that made the genetic manipulation of this organism straightforward. In particular, the nisin-controlled overexpression system [13], a toolbox allowing modulation of gene expression at a selected level [14], and the pORI/pVE6007 two-plasmid system to obtain clean food-grade deletions of genes of interest [15] are invaluable tools available for directed genetic manipulation of this bacterium. In the last decade several reports on metabolic re-routing of central carbon metabolism in *L. lactis* were presented and the engineering strategies have been extensively reviewed [16–18]. It is interesting to note that successful cases in metabolic engineering were not accomplished through disruption or overexpression of single genes. Indeed, several examples illustrate this concept [19,20]. Instead, coordinated expression and/or disruption of several genes was required to attain the desired objective. Successful examples were the redirection of pyruvate metabolism to products other than lactate, such as alanine and diacetyl [21,22]. Furthermore, increased production of compounds like exopolysaccharides [23,24] and vitamins [25] has been described. However, engineering such complex biosynthetic pathways still poses a major challenge, and predicting how cell physiology will respond to a genetic modification is not easy. The difficulty originates mainly from the existence of multiple interlocked pathways connected via common metabolites and cofactors through various levels of genetic and metabolic regulation [26,27]. Overall, the consensus is that manipulation of strains leading to the desired metabolic features cannot be achieved without a global understanding of the metabolic network as well as of the interdependent relationships among the different steps.

Regulation of glycolysis in *L. lactis* has been the subject of intensive research. Key glycolytic enzymes, pyruvate kinase (PK) [28–31], phosphofructokinase [32], fructose 1,6-bisphosphate aldolase [33], glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [34] and lactate dehydrogenase (LDH) [35,36] were characterized, and concentrations of glycolytic intermediates, fructose 1,6-bisphosphate (FBP), dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate (3-PGA), 2-phosphoglycerate, phosphoenolpyruvate (PEP), in cell extracts had been obtained already in the eighties (for a review on early studies see [37]). Under certain conditions a metabolic shift from homolactic (lactate production) to mixed acid fermentation (ethanol, acetate and formate production) can occur in *L. lactis* (reviewed in [3,38]). The mechanisms underlying this shift have been the object of considerable controversy and a full explanation has yet to be put forward; this topic will be discussed in more detail later in this paper. More recently, the control of the glycolytic flux has been addressed in a number of elegant experiments (for review see [39]), but an answer to the question of what controls glycolysis in *L. lactis* remains elusive. Despite the wealth of metabolic information collected during years of intensive research and numerous genetic tools available for *L. lactis*, we are still far from achieving a comprehensive understanding of sugar metabolism and regulatory pathways in this model organism. This goal can only be achieved by resorting to quantitative metabolic models, the development of which requires reliable data on intracellular concentrations of intermediates and metabolic fluxes [40].

Powerful analytical methodologies can now be used to characterize the ensemble of low-molecular mass metabolites in the cell [41]. Nuclear magnetic resonance spectroscopy (NMR) is a powerful technique for studying metabolism, making it feasible to probe complex reaction pathways and control points simultaneously and non-invasively in the live cell, obtain invaluable information on the magnitude of intracellular metabo-