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Review

The lactic acid bacterium as a cell factory for food ingredient production

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Abstract

In this contribution, the homofermentative lactic acid bacterium as an efficient cell factory for different (food) ingredients will be presented. The emphasis will be on some successful examples of metabolic engineering and on the physiology of these bacteria, which makes them so suitable as a cell factory. One interesting conclusion of the metabolism of these bacteria is that they have clearly chosen for speed instead of efficiency, although some evolutionary results can still not be explained on mechanistic level.

Keywords: *Lactococcus lactis*; Metabolic engineering; Systems biology; Homolactic fermentation; Genomics

Contents

1. Introduction

Natural fermentation has already been used for ages to increase the shelf-life of various food materials. This process has resulted in a number of traditional food products such as: the dairy products cheese, butter, buttermilk and yoghurt; fermented meat, plants and fruits such as sausages, silage, sauerkraut, olives and grapes; and finally fermented cereal products such as bread and beer (Caplice & Fitzgerald, 1999). With the exception of beer and wine, both involving alcoholic fermentation by yeast, all these food products result from bacterial acidification, leading to longer shelf-lives (Ross, Morgan, & Hill, 2002). The bacteria that are found in these fermented products are almost always lactic acid bacteria (LAB), named for the organic acid produced during fermentation. In most cases, a specific species of LAB will become dominant in a fermentation as a result of its extremely efficient conversion of the available sugars and the rapid formation of lactic acid that inhibits the growth of most other microorganisms present. Besides producing the (lactic) acid, the acidifying bacteria, also called starter bacteria, contribute to the flavour, the texture and the nutritional value of the fermented foods through production of aroma components, through production or modification of exopolysaccharides and proteins, and through the production of nutritional components such as vitamins.

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The species, strain or variant that becomes dominant during a specific fermentation is determined by the food substrate that is used, the temperature of the process, and other environmental conditions. In Gouda cheese, which does not involve cooking of the curd, the lactic acid bacterium *Lactococcus lactis* prevails, a so-called mesophilic bacterium with a growth optimum at 30 °C and an inability to grow at temperatures above 35–38 °C. In yoghurt and Parmesan cheese, however, where heating to 50 °C is employed, so-called thermophilic LAB (Delcour, Ferain, & Hols, 2000) are found such as *Streptococcus thermophilus* and *Lactobacillus helveticus*.

Nowadays, dairy fermentations are often carried out at such large scale that there is a strong need to control the process. By adding a high dosage of active starter cultures the fermentation is no longer a randomly occurring process, but has become a carefully controlled process. The production of these starter cultures (Hansen, 2002) is now a worldwide business of approximately 500 M€.

In this overview some characteristic features of homofermentative LAB will be described, and if possible, explained. These features are the "choice" of these bacteria for their specific metabolism, the use of these bacteria for metabolic engineering, and the use of these bacteria for delivery of flavour and nutritional components into fermented foods.

2. Homofermentative lactic acid fermentation

Why do (homofermentative) LAB dominate in so many food fermentations? This can be explained by the type of metabolism that is found in these bacteria. Sugars, such as the milk sugar lactose, are rapidly taken up and degraded by these bacteria. Only partial degradation of sugars takes place—to lactic acid—and further conversion to carbon dioxide does not happen since this requires air, which is not present in most fermentations, and since a respiratory metabolism is not found in most LAB.

It turns out that homofermentative LAB, which are focussed on lactic acid production and lack the ability to produce a whole range of other fermentation products, grow substantially faster than other bacteria present in the same ecological niche. This results in very rapid domination of this type of bacteria in a wide range of environments. The higher growth rate of LAB is a result of their simple primary metabolism, but also of adaptation to rich environments. Most LAB have very limited biosynthetic capabilities and depend on their environment for a supply of amino acids, nucleotides and vitamins for growth (Kok, 1990). In most food substrates such as milk, vegetables, fruits and meat, these nutrients are present in high abundance. So, the LAB can focus on the (rapid) conversion of sugars to lactic acid without having to synthesise growth factors. This focus on lactic acid production is strictly maintained under almost all conditions. Over the last 10–15 years numerous attempts have been made to change metabolite production in these bacteria, via metabolic engineering, from lactic acid to production of other organic compounds. The strategy used in most instances was to remove lactate dehydrogenase (LDH) (Aarnikunas, van Weymarn, Ronnholm, Leisola, & Palva, 2003; Bongers et al., 2003; Ferain, Schranck, & Delcour, 1996; Gaspar et al., 2004; Hillman, Chen, & Snoep, 1996; Hols et al., 1999a, b; Korakli, Schwartz, Wolf, & Hammes, 2000; Ladero et al., 2007; Neves et al., 2000; Neves, Ramos, Shearman, Gasson, & Santos, 2002; Platteeuw, Hugenholtz, Starrenburg, van Alen-Boerrigter, & de Vos, 1995; Saha & Nakamura, 2000; Wisselink, Mars, van der Meer, Eggink, & Hugenholtz, 2004a), the enzyme directly responsible for reduction of pyruvate to lactate. In some LAB, this exercise was not possible (Hillman et al., 1996), while in others such as *L. lactis* (Bongers et al., 2003; Gaspar et al., 2004; Hols et al., 1999a, b; Neves et al., 2000, 2002; Platteeuw et al., 1995) and *Lactobacillus plantarum* (Ferain et al., 1996; Ladero et al., 2007) this was remarkably easy to achieve. However, the observed phenotype, a LAB producing no lactic acid, appeared not to be stable. In *L. lactis*, this instability was not a result of reactivation of the inactivated gene, but activation of an alternative lactate dehydrogenase gene via a genetic insertion event (Bongers et al., 2003, Fig. 1). This event was found repeatedly in *L. lactis* under anaerobic conditions, illustrating the competitive advantage of producing lactic acid under these conditions. Another illustration of selection for homolactic fermentation in *L. lactis*, is the unwillingness to utilise alternative catabolic pathways, such as acetic acid formation or complete oxidation via a respiratory pathway (Brooijmans, Schuurmans-Wolters, Poolman, & Hugenholtz, 2007). These alternative pathways are indeed present in *L. lactis*, as shown in the studies mentioned above, using LDH-negative mutants and fermentations under sugar limitation or strong aeration. In these conditions, the LAB produce abundant acetate and acetoin and even only acetate ($+CO_2$) when heme is added during growth (inducing an electron transport chain). Although these alternative pathways would lead to much more energy generation, under "normal" circumstances the lactic acid bacterium still remains homolactic and does not switch to a respiratory life-style by acquiring a heme biosynthesis pathway. There are, nevertheless, ample examples of new traits that have been acquired, by LAB, through horizontal gene transfer (Klaenhammer, Barrangou, Buck, Azcarate-Peril, & Altermann, 2005).

3. Metabolic engineering

The metabolism of LAB is completely geared towards production of a single metabolite—lactic acid. Such a focussed metabolism seems to be a perfect basis for creating cell factories of single metabolites. Over the last ten years, this potential has been demonstrated with several examples of successful metabolic engineering. The development of a very effective gene expression system called NICE—for nisin controlled gene expression—has been