Mixed-acid fermentation and polysaccharide production by *Lactobacillus helveticus* in milk cultures

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Abstract

*Lactobacillus helveticus* grown in milk with pH control at 6.2 had a slower growth rate (\(\mu = 0.27\) h\(^{-1}\)) and produced less exopolysaccharide (49 mg l\(^{-1}\)) but increased lactic acid production (425 mM) compared to cultures without pH control (\(\mu = 0.5\) h\(^{-1}\), 380 mg exopolysaccharide l\(^{-1}\), and 210 mM lactate), respectively. Both cultures displayed a mixed-acid fermentation with formation of acetate, which is linked not only to citrate metabolism, but also to alternative pathways from pyruvate.

Introduction

*Lactobacillus helveticus*, an obligate homofermentative lactic acid bacteria (LAB), plays an important role in the dairy industry as starter culture for the manufacture of acid milks, hard-cheeses and Mozzarella-like cheeses. The incorporation of exopolysaccharide (EPS)-producing strains into the starter culture would avoid the use of additives of plant or animal origin which are not allowed in most European Union countries (Gibson & Roberfroid 1995), resulting in the development of novel products with enhanced rheological properties.

The metabolism of citrate in milk to produce acetate and/or diacetyl is also important for the flavour development of many fermented dairy products. However, there are few reports concerning the metabolism of citrate by thermophilic lactobacilli (Hickey *et al.* 1983).

This paper deals with the fermentation pattern of and the EPS production by *L. helveticus* growing in milk as free and pH-controlled fermentation runs.

Materials and methods

**Microorganism and growth conditions**

*Lactobacillus helveticus* ATCC 15807 was grown in sterile (115°C, 20 min), 10% (w/v) reconstituted non-fat skim milk, in a fermenter, working volume of 2000 ml, at 37°C for 60 h. Batch cultures were carried out without aeration, as free- and controlled-pH (adjusted at pH 6.2 with sterile 1 M NH\(_4\)OH) fermentation runs. Temperature (37°C), agitation (100 rev min\(^{-1}\)) and pH were controlled automatically.

Fermentations were allowed to proceed for 60 h; samples were aseptically withdrawn at different intervals from the fermentation vessel and immediately cooled on ice to determine EPS yield, cell viability, end-products, citrate consumption, residual lactose and its hydrolysis products (glucose and galactose). The specific growth rate (\(\mu_{\text{max}}\)) was calculated from the slope of a semi logarithmic plot of c.f.u. ml\(^{-1}\) vs. time. Cell viability was determined by plating in mass appropriated dilutions in MRS agar (De Man *et al.*
Fig. 1. Cell viability (●) and EPS production (○) by Lactobacillus helveticus in milk batch cultures; (a) pH 6.2, (b) free-pH.

Quantification of exopolysaccharide and monomer analysis

Isolation of EPS was carried out in 100 ml samples treated with Pronase E Type XIV (Sigma) to hydrolyse milk proteins. The EPS were isolated by centrifugation, precipitated with ethanol (Mozzi et al. 1996), freeze-dried in 2 ml distilled water, and applied on a Sepharose 4B (Sigma) column to determine its molecular weight (MW). The EPS was eluted with 0.05 M Tris/HCl buffer (pH 7), and the column calibrated with a mixture of dextrans (Sigma) (MW 39 100; 73 000; 515 000 and 2 000 000) at 0.25 mg ml\(^{-1}\) each. The MW of the EPS was determined by using a graphic plot of the MW log of dextrans against the elution volume. The monosaccharide composition of the purified and hydrolysed (3 M trifluoroacetic acid, at 100 °C for 6 h) EPS was determined by HPLC on a Rezex ROA-Organic Acid column (Phenomenex) at 55 °C using water as mobile phase at 0.6 ml min\(^{-1}\). The relative proportion of the peak areas was calculated to estimate the monomer composition. Total EPS (expressed as mg l\(^{-1}\)) was estimated by the phenol sulphuric method (Dubois et al. 1956) using glucose as standard.

Other determinations

The residual lactose, glucose and galactose as well as lactate and acetate were determined by HPLC as described above; citrate was determined by enzymatic methods (Boehringer Mannheim GmbH, Germany). For HPLC analysis, samples were previously clarified with Carrez solution (Boehringer). Results were expressed in mM.

Reproducibility

All results presented in this paper are the average of three assays. The variations among results were less than 10%.

Results and discussion

The growth kinetics of and the lactic acid and EPS production by L. helveticus ATCC 15807 in milk batch cultures with and without pH control was quite different (Figures 1–3). At pH 6.2 (Figure 1a), cultures displayed a low specific growth rate (\(\mu = 0.27\) h\(^{-1}\)), cell viability (3 × 10\(^8\) c.f.u. ml\(^{-1}\)) and EPS formation (49 mg l\(^{-1}\)) but a high lactic acid production (425 mM) (Figure 2a); this fact would indicate that the sugar metabolism was mainly diverted to lactate rather than to the synthesis of EPS. The lactic acid accumulated in the medium at pH 6.2 seemed to be the key factor for the early beginning of the stationary phase and the low cell viability (Figure 1a). In contrast, cultures grown without pH control grew at a higher rate (\(\mu = 0.5\) h\(^{-1}\)) and produced 83% more EPS (360 mg l\(^{-1}\)) (Figure 1b) but 49% less lactate (210 mM) (Figure 2b).

The isolated EPS were polymers of high MW (8.2 × 10\(^5\) to 2 × 10\(^6\)) containing high amounts of