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Influence of polysaccharides on oxygen dependent lactate utilization by an amylolytic *Lactobacillus plantarum* strain

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Abstract

Oxygen-dependent conversion of lactate to acetate by the amylolytic strain *Lactobacillus plantarum* A6 was studied using MRS-grown cells, transferred to a basic medium with lactate. In the presence of oxygen, lactate was stoechiometricaly converted to acetate. When glucose, maltose or cellobiose was added to the basic medium, no utilisation of lactate was observed. However, when starch or glycogen was added, the conversion of lactate to acetate happened. To verify the possible link of this effect with sugar consumption rate, a glucose-fed batch culture was conducted with a lactic acid consuming culture grown on the basic medium with lactate. Even when glucose was fed at the same low rate as the consumption rate observed for polysaccharides, lactic acid was no more consumed. For the amylolytic strain *L. plantarum* A6, the transport and use of oligosaccharides resulting from polysaccharides hydrolysis might affect differently the glycolytic flux, with the putative consequence to suppress the metabolic control of glycolysis by glucose. This fact could play an important role in the fermentation of amylaceous foods.
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Keywords: Amylolytic lactic acid bacteria; Lactate; Acetate; *Lactobacillus plantarum* A6

1. Introduction

Lactobacillus plantarum is a facultative heterofermentative lactic bacteria that can convert lactate to acetate after glucose depletion using oxygen as electron acceptor (Tseng and Montville, 1992). The metabolic pathway involves pyruvate oxydase and lactate dehydrogenase (LDH) and generates H_2O_2 and an extra ATP, but its regulation remains to be elucidated.

It is known that for lactic acid bacteria (LAB), besides oxygen and pH (Montville and McFall, 1989; Tseng and Montville, 1990), the type and concentration of the carbon source can affect the fermentation product patterns (Borch et al., 1991). In some homofermentative lactic acid bacteria as *Lactococcus lactis*, mixed fermentation was observed when grown on maltose (Sjöberg and Han-Hägerdal, 1989)

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or under glucose limitation (Thomas et al., 1979). This has been first related with a low level of fructose 1,6-diphosphate (FDP), that promotes a decreased activity of lactate dehydrogenase (LDH), and with an inhibition of the pyruvate formate lyase (anaerobic conditions) or pyruvate dehydrogenase (aerobic conditions) by glyceraldehyde-3-phospate (GAP) and dihydroxyacetone-phosphate, promoting a shift of pyruvate towards other metabolic pathways. Nevertheless, Garrigues et al. (1997) demonstrated that the inhibition of lactate dehydrogenase was mostly due to the NAD/NADH ratio, which controls the carbon flux through both the GAP dehydrogenase and LDH.

Although *L. plantarum* has been reported to lack of a FDP-dependent LDH (Tseng and Montville, 1990), a similar heterofermentative behavior in alkaline and aerobic environments was observed by Tseng and Montville (1990). A shift from homo- to heterofermentation during glucose limitation has also been reported for *Lactobacillus* sp. in anaerobic culture (Borch et al., 1991). Previous results with an α-amylase-producing strain, *L. plantarum* A6, showed acetate production from lactate during prolonged incubation in agitated batch cultures (Pintado et al., 1999). This conversion of lactate into acetate occurred at a higher degree when the strain was grown with polysaccharides as substrate rather than with glucose.

The aim of this work was to study the influence of carbohydrates on the conversion of lactate to acetate in aerobic condition by an amylolytic *L. plantarum* strain. This fact could play an important role in the control of fermentation of amylaceous raw materials. On one hand, when the production of lactate leads to acidification that inhibits amylases (Giraud et al., 1993), the production of acetate increases pH which could favor amylolytic activity. On the other hand, the production of acetate and oxygen peroxyde can inhibit pathogenic bacteria (Frey and Hubert, 1993). Besides, the pleasant taste in fermented foods is critically dependent on the balance between the concentrations of lactic acid and acetic acid, respectively, in conjunction with the level of sweetness (Adler-Nissen and Demain, 1994). In this sense, aeration can be utilized as a mechanism to control the final concentration of acetic acid in LAB food fermentation processes.

2. Materials and methods

2.1. Microorganisms

L. plantarum A6 (LMG 18053) was isolated from retted cassava in the Congo (Giraud et al., 1991) and maintained in glycerol (30%) at -80 °C.

2.2. Culture media and conditions

Strains were cultivated in MRS medium (Man et al., 1960) inoculated with 5% (v/v) of a 20-h old culture on MRS. After 20 h, cells were collected by centrifugation ($5000 \times g$ for 10 min), washed with isotonic solution and transferred to a basic nutrient medium (Giraud et al., 1998) with lactate (BNML), containing: 8 g of lactic acid (racemic); 10 g of soy peptone obtained by papain digestion; 0.5 g K_2HPO_4; 0.5 g KH_2PO_4; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.05 g $MnSO_4 \cdot H_2O$, 0.5 g $CaCl_2 \cdot 2H_2O$ and 1000 ml distilled water. In some cases, 5 g/l of different carbon sources, such as glucose, maltose, cellobiose, soluble starch (Prolabo, Fontenay-Sous-Bois, France) or glycogen, obtained from mussel processing wastes (Pintado et al., 1999), were added. In fed batch cultures, glucose was added in sterile conditions.

All cultures were incubated at 30 °C. Aerobic cultivation were conducted in 250-ml Erlemeyer flasks with baffles, containing 150 ml of medium, and with orbital agitation (150 rpm). Anaerobic cultivations were conducted in 15-ml tubes with 10 ml of medium in static conditions and nitrogen atmosphere.

Aerobic-fed batch culture was conducted in a 2-l bioreactor (LSL-Biolafitte, Saint Germain en Laye, France) with 1 l of medium, 300 rpm agitation and 27.7 l h^{-1} air flow. Addition of glucose was started at 48 h of culture s at a specific rate of 0.08 g h^{-1} g of biomass^{-1} using a peristaltic pump (Miniplus 2, Gilson, Middleton, WI, USA) with a flow of 2.26 ml h^{-1}.

2.3. Analytical methods

Samples of 10 ml were taken aseptically from aerobic cultures or the whole content of one tube of anaerobic cultures. After appropriate dilution, bio-

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