A DESCRIPTIVE MODEL FOR CITRATE UTILIZATION BY
LACTOCOCCUS LACTIS SSP LACTIS BV DIACETYLAETIS.

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ABSTRACT

A model for the use of citrate by Lactococcus lactis ssp lactis bv diacetylaetis CNRZ 125 is proposed. Citrate metabolism by this strain leads to the production of acetate, CO₂ and C₄ compounds (diacetyl, acetoin, 2,3-butyylene glycol). The model furnishes correct simulations, consistent with published results on the pathways used and on lactose-citrate co-metabolism. Citric acid is incorporated independently of growth. The production of flavoring compounds is a complex process, depending on the rate of citrate utilization, on the proportion of pyruvate arising from citrate and which condenses to form α-acetolactate and CO₂, on the rate of transformation of α-acetolactate to diacetyl and acetoin, as well as on the rate of reduction of these compounds to 2,3-butyylene glycol.

INTRODUCTION

Lactococcus lactis ssp lactis bv diacetylaetis is used in the composition of mixed cultures for the dairy industry. The strain is utilized as a flavoring agent, since it has the particularity of metabolizing citrate to produce diacetyl and acetoin. When grown in milk, it uses lactose and citrate at the same time, producing L-lactate, acetate, CO₂ and C₄ molecules (diacetyl, acetoin, 2,3-butyylene glycol). These two pathways for the utilization of citrate and lactose have a common metabolic crossroads, pyruvate, and it has been shown that the key enzymes in these two pathways are under special control, in terms of both their syntheses and activities (Seitz et al., 1963; Jonsson and Petterson, 1977; Cogan, 1981; Kaneko et al., 1990). In addition, there are behavior differences which depend not only on culture conditions, but also on the strains of Lactococcus lactis ssp lactis bv diacetylaetis studied (Cogan, 1982; Kaneko, 1986; Savoy de Giori, 1986; Schmitt et al., 1988).

In prior work (Cachon and Diviès, 1993), growth and acid production kinetics were modeled in strain CNRZ 125 grown in batch culture at pH 6.5 in lactose-containing medium (50 g.l⁻¹) and in the presence of citric acid (2 g.l⁻¹). Taking into consideration the metabolic pathways involved in the utilization of citrate by Lactococcus lactis ssp lactis bv diacetylaetis, a model is proposed at pH 5.5 in which the bioconversion yield of citrate to diacetyl + acetoin is maximal instead of pH 6.5 where low levels of aroma compounds are detected. The model is then compared to published results on the control of the pathways involved and on lactose-citrate co-metabolism.
MATERIALS AND METHODS

Organism
Strain *Lactococcus lactis* ssp *lactis* bv *diacetylactis* CNRZ 125 was used in this study.

Growth conditions and analysis
They have been described in a previous paper (Cachon and Diviès, 1993). The fermentation medium was MRS (de Man et al., 1960) modified by omitting acetate and Tween 80, and substituting lactose to glucose. In experiments reported here, lactose and citrate concentrations were equal to 50 g.l⁻¹ and 2 g.l⁻¹ respectively, and the pH was controlled at 5.5.

Simulations
The optimum parameter values of the model for citrate utilization were calculated by fitting the model to experimental data by least-squares regression. Simulations were performed using the DO2BBF routine (NAG Fortran library) on an IBM RS/6000 (model 550) computer.

RESULTS

Simulation of growth at pH 5.5

A mathematical model characteristic of growth and lactic fermentation has been proposed for this strain grown in conditions of batch fermentation and at pH controlled at 6.5 (Cachon and Diviès, 1993). The constants of this model were recalculated for growth at pH 5.5. Figure 1 shows that the model gives satisfying simulation of the changes in biomass and in lactose and L-lactate concentrations. Fermentation at pH 5.5 is seen to last for 50 hours, while in the prior work at pH 6.5 lactose was totally consumed in 9 hours.

![Graph showing changes in biomass, lactose, citrate, and L-lactate concentrations over time at pH 5.5.](image)

**Figure 1:** Changes in the concentrations of biomass, lactose, citrate and L-lactate vs. time at pH controlled at 5.5 (20°C). Symbols: ◯ biomass, □ lactose, ▲ citrate, ○ L-lactate. Lines represent simulation values (--- represents active biomass \(X_{\text{reg}}\)).

Development of the model for citrate utilization

Citric acid (CIT) is consumed at the onset of fermentation, and these kinetics can be described by an affinity law (Monod, 1942):

\[ q_{\text{CIT}} = q_{\text{CIT}}^\text{m} \cdot \frac{\text{CIT}}{K_{\text{m}} + \text{CIT}} \] (1)

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