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Influence of immobilization parameters on growth and lactic acid production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* co-immobilized in calcium alginate gel beads

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Abstract

Streptococcus thermophilus and *Lactobacillus bulgaricus* were co-immobilized in different systems with varying calcium (0.1–1.5 M) and alginate (1–2%, w/v) concentrations. Highest lactic acid production was 35 g l^{-1} when both bacteria were in high viscosity beads (1%, w/v alginate) hardened in 0.1 M $CaCl_2$ .The gel bead composition affected size and distribution of entrapped lactic acid bacteria.

Introduction

Lactic acid bacteria are commonly used in the production of cheese, yoghurt, dry sausages, wine and sour-dough breads (Fanema 1996). A mixed culture of lactic acid bacteria, *Streptococuss thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, is used for yoghurt fermentation because the acid tolerance of these organisms is advantageous as they have a competitive advantage over known pathogens (Siegumfeldt *et al.* 2000) and other undesirable bacteria when the concentration of organic acids is high (Russell & Diez-Gonzalez 1998). Their entrapment in gel matrices can protect them from phages, contaminating bacteria and physico-chemical stresses (Trauth *et al.* 2001). The success of the alginate gel entrapment techniques is mainly due to the gentle environment they provide for the entrapped material (Klinkeberg *et al.* 2001, Wenrong & Griffiths 2000).

In the present paper, the impact of immobilization parameters on the production of lactic acid by lactic bacteria *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* co-immobilized in calcium alginate has been evaluated.

Materials and methods

Biocatalyst bead preparation and experimental conditions

A mixed commercial culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (diluted 1/5 in with sterile water) was mixed with 50 ml sterile sodium alginate solution of high (HV), medium (MV) or low viscosity (LV) from Sigma and then dropped into 100 ml sterile $CaCl_2$ to form biocatalyst beads by ionotropic gelation of alginate. The beads were rinsed

with sterile water and transferred to 150 ml sterile milk used as culture medium. Cultures were grown at 40 °C with shaking at 120 rpm for 12 d.

Factorial experimental designs

Factorial experimental designs were performed (Roisin *et al.* 1997) with two independent variables: alginate and $CaCl_2$ concentrations.

Analytical determinations

Total lactic acid content in culture medium was measured using a Boehringer–Mannheim kit with D-lactate dehydrogenase (Sigma) (Trinder 1969).

Results and discussion

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* were co-immobilized in alginate beads as described in Materials and methods. An evaluation of lactic acid production was made after 12 d growth. Highest lactic acid production was 35 g l^{-1} using 1% HV alginate beads hardened in 0.1 M $CaCl_2$ solution (Figure 1a). In MV and LV alginate beads, highest production was 25 g l^{-1} (Figures 1b and c). Lactic acid production with high viscosity alginate beads was 30% higher than that obtained from medium and low viscosity beads under the same culture conditions. It is not surprising to observe different lactic acid production when using different types of alginate, since alginate is a generic name for a range of substances with a wide variety of chemical compositions (e.g. guluronic acid/mannuronic acid ratio) which nevertheless exhibit similar physical properties (Nava Saucedo *et al.* 1996).

At the end of the experiment, biological contamination was observed, specially in low viscosity gel beads, in spite of immobilization seemingly protecting cultures from virulent bacteriophage attack (Trauth *et al.* 2001). Microscopy observations from high viscosity gel bead sections obtained after 12 d fermentation showed that colony size of *L. bulgaricus* was 3–4 fold wider than the cell diameter of *S. thermophilus* round colonies (25 μm). Both type of colonies were located in external crust of the bead (300 μm) and were bigger in size than those located in the center of gel beads.

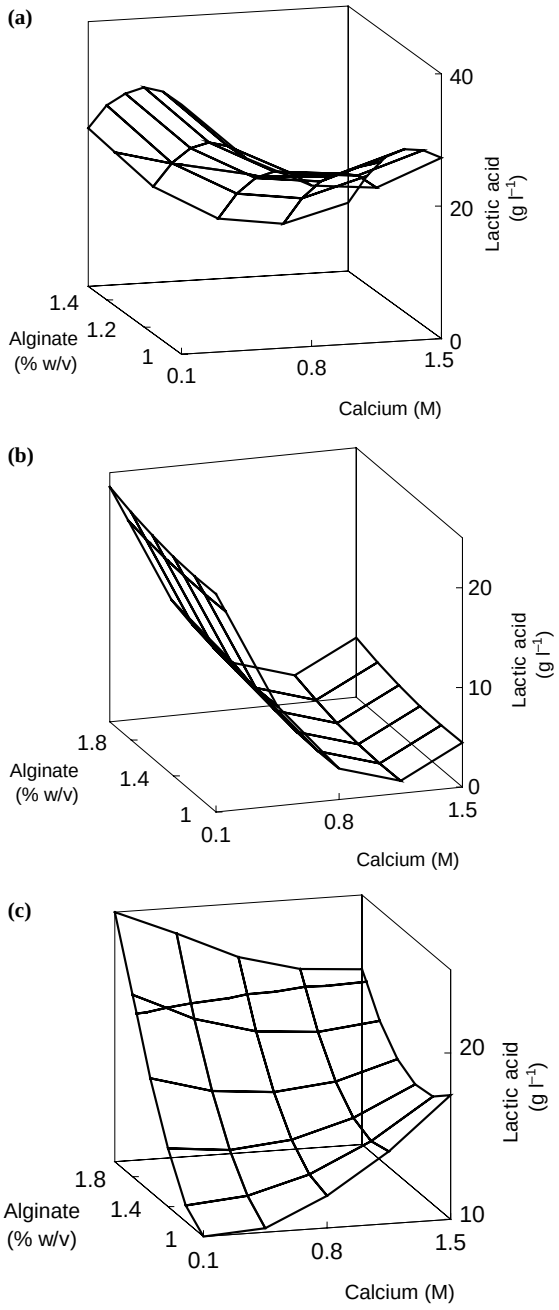

Fig. 1. Response surfaces of lactic acid released into the culture medium by lactic acid bacteria *S. thermophilus* and *L. bulgaricus* immobilized in different viscosity alginate solution as a function of $CaCl_2$ (M) and alginate (% w/v) at 12 d. (a) High viscosity-HV; (b) medium viscosity-MV; (c) low viscosity-LV. Central points were replicated three times.

A heterogeneous size and distribution of the entrapped cell colonies of both lactic acid bacteria was observed. Among the different reasons that can explain the observations above, mechanical

internal forces, exopolysaccharides production by the lactic immobilized colonies and differences in calcium concentration inside the beads worth mentioned (Nava Saucedo *et al*. 1996).

Gel beads are heterogeneous structures presenting different domains of organization that can affect the behavior of entrapped biological materials (Garbayo *et al*. 2002, Smidsrod & Skjak-Braek 1991). This phenomenon led us to hypothesize that immobilization alters cell wall-membrane due to a kind of gel-matrix-structure recognition increasing the permeability (Roisin *et al*. 1997) and lactic acid production. The optimization of the immobilization conditions could be interesting in the applied field.

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