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Immobilization of lactic acid bacteria in a polymer matrix

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The sol-gel encapsulation has been proposed as a suitable model system for the study of the effects of crowding and confinement in a living cell.

This work aimed to maximize the production of lactic acid (LA) by immobilizing Lactobacillus plantarum (L.plantarum) genetically transformed to overexpressing the gen of β-galactosidase (β-Gal). Bacteria were encapsulated, in a silicate matrix, by the sol–gel method using tetraethyl orthosilicate (TEOS), to generate an inorganic polymer expecting that it would allow for higher survival rates and improved product yield. In free and immobilized L.plantarum (either wild type or transformed) we determined the cell viability in sol-gel matrices, and also studied the effects of macromolecular crowding in L.plantarum through spectral confocal microscopy using ACDAN as a water polarization sensitive probe.

Fermentation with immobilized L. platarum was carried out in media containing 2% w/v lactose (Lac2%) as a carbon source. LA production was determined at different fermentation temperatures (30°C, 35 °C, 37 °C and 40°C) and at 150 rpm agitation speed of the incubator. Viability d of the immobilized bacteria was determined at 0, 24, 48, 72, 120, and 150 h. Every 36 hours, the fermentation broth was replaced with a fresh medium. All determinations were done in triplicate.

For 36 hours the immobilized *L. plantarum* cells synthesized 20.5 g/L LA, a concentration higher than that produced by the free cells (16g/L). After a period of 150 h incubation period, minimum loss in bacterial count was observed, this showed the effectiveness of capsulation.

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Immobilization of lactic acid bacteria in a polymer matrix

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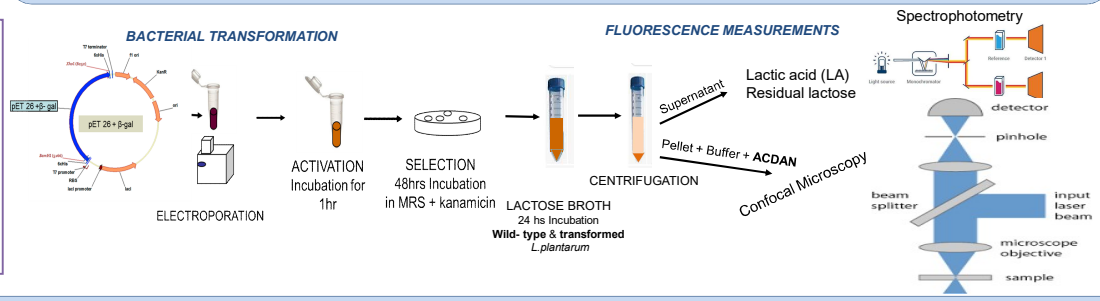
INTRODUCTION

Immoilization is a technique that could offer several advantages such as a higher fermentation rate, the possibility to reuse cells, a protective effect against possible inhibitors, decreased inoculum preparation processing cost, and reduced product contamination.. The aim of this work was to maximize the production of lactic acid (LA) by immobilizing *Lactobacillus plantarum* through the sol–gel process to generate an inorganic polymer that allows for higher survival rates and improved product yield. The cell viability in sol-gel matrices, post-immobilization storage, was determined. Viability was correlated with water structure in the gel and inside cell cytoplasm.

MATERIALS

- Transformed *Lactobacillus Plantarum* (**Pt**)
- Kanamycin Antibiotics
- Lactose broth, whey and lacose
- Isopropyl β-d-1-thiogalactopyranoside (**IPTG**)
- 6-acetyl-2-(dimethylamino) naphthalene (**ACDAN**)
- Phosphate buffer, pH 6.8

METHODS



RESULTS

Comparison of Lactic acid production of free and immobilized cell

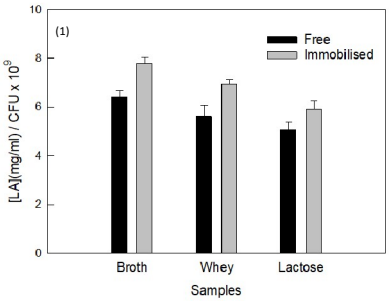


Fig. 1 Production of LA by free and immobilized **Piptg** in broth, whey and lactose.

Fermentation of Broth, Whey and Lactose by immobilized cells and the viability of Immobilised cells

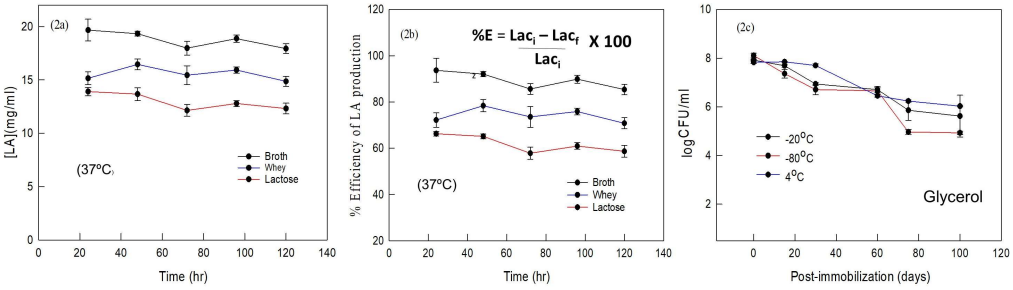


Fig.2 Immobilized Piptg (a) Production of LA in broth, whey and lactose; **(b)** efficiency of LA production in broth, whey and lactose and **(c)** Cell viability at -20, -80 and 4 ºC.

Intracellular Water Dynamics in Immobilised *Lactobacillus Plantarum*

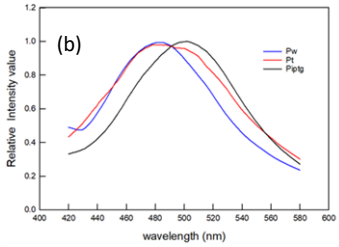


Fig. 3 Fluorescence imaging (a) and emission spectra of ACDAN (b) in immobilised *L.plantarum* wild-type (Pw), transformed without IPTG (Pt) and transformed with IPTG (Piptg)

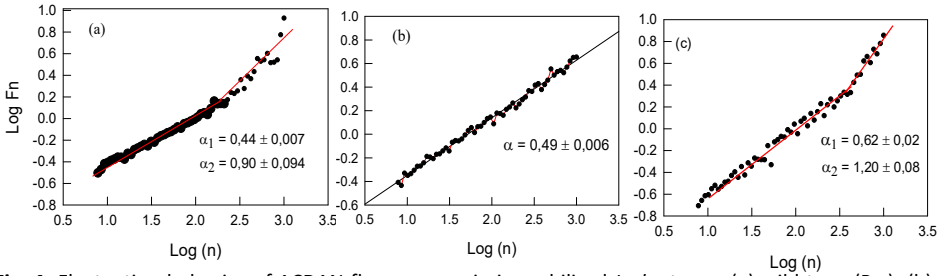


Fig. 4 Fluctuation behavior of ACDAN fluorescence in immobilized *L.plantarum* (a) wild-type (Pw); (b) transformed without IPTG (Pt) and (c) transformed with IPTG (Piptg)

0 < α < 0,5 → Anti-correlated, Antipersistent noise.
α = 0,5 → Uncorrelated, White noise
0,5<α<1 → Long range auto-correlation. Persistent noise.
α = 1 → **1/f** Spectral noise, System near critical state of self-organization.
1 < α < 1,5 → Negative correlation, Brownian noise.

(Eq.2)

$$F(n)=\sqrt{\frac{\sum_{i=1}^{N}(y_i-\hat{y}_{i,k})^2}{N}}$$

CONCLUSIONS

- The immobilized transformed *L.plantarum (Piptg)* was more efficient than free *Piptg* in the production of LA (Fig.1).
- ……(Fig.2).
- From the emission spectra, it is observed that intracellular water in *L.plantarum* transformed (Piptg) is more relaxed in comparison to Pw and Pt (Fig.3).
- Wild type and transformed cells exhibit uncorrelated fluctuations of ACDAN fluorescence. (Fig.4a and 4b). The induction of β-Gal gen expression leads to long range correlated fluctuations within time scales below 5 min (Log n ≅ 2.5) (Fig.4c). The macromolecular crowding may be responsible for spectral and fluctuation behaviors.

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