

Coproduction of biosurfactants, lipases, alpha-amylases and proteases by *Bacillus sp.* 0G grown in modified Landy's medium

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Introduction

Nonribosomal peptides (NRPs) are important secondary metabolites produced by some bacteria and Fungi.¹ *Bacillus sp.* 0G produces a number of cyclic NRPs that have antifungal and biosurfactant properties.² The latter is an important feature considering the applicability of surfactants for the chemical and pharmaceutical industries. However, production yields are low, requiring cultivation of several liters of *Bacillus* in order to isolate the product of interest.³ Therefore, the isolation of a biosurfactant such as surfactin is expensive. The aim of this work is to present a strategy to produce and separate biosurfactants and enzymes such as lipases, alpha-amylases and proteases, which could be sold concomitantly in order to reduce the production costs of biosurfactants.

Results and Discussion

Bacillus sp. 0G was grown in a modified Landy's medium⁴ containing broths prepared with corn and sausage (Sadia™) to replace the carbon and nitrogen sources, which are expensive. Broths were prepared by adding 500 g of solids to 500 mL of water and cooking for 20 min. Then 250 mL of the sausage broth was added to 750 mL of the corn broth. This was mixed to dissolve micronutrients and autoclaved. This medium was inoculated with strain 0G and cultivated for 45 h at 37°C. At the end of growth, cells were collected by centrifugation and the supernatant was characterized for the production of enzymes and biosurfactants. In 1 L of centrifuged medium, the total protein was 105 mg/mL. Lipase activity was 0,40 u/mL; alfa-amylase (66 u/mL) and protease (0,08 units/mL). Proteins were precipitated by acetone, and lipopeptides were analyzed in the acetone liquor after evaporation. Figure 1 shows the determination of the the critical micelle concentration (CMC) of the lipopeptide extract dissolved in water, pH 8,0. Figure 2 shows the characterization of this extract by mass spectrometry. Table 1 shows the antibacterial activity of the lipopeptide extract (in water) against *Streptococcus mutans*, the causative agent of dental caries.

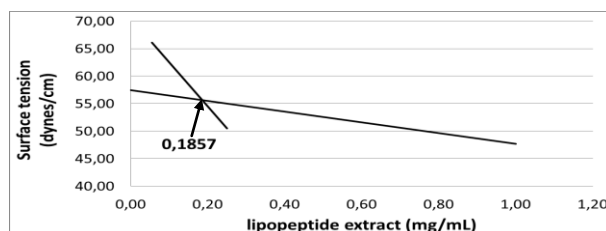


Figure 1 – CMC of the lipopeptide (biosurfactant) extract.

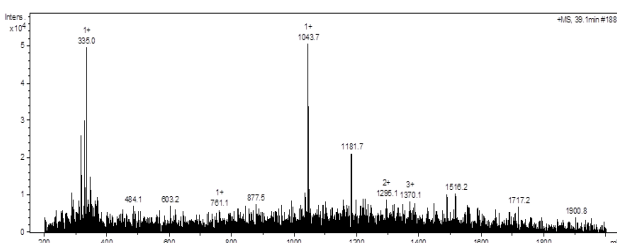


Figure 2 – Mass spectrometry analysis of the lipopeptide extract.

Table 1 – Comparative antibacterial activity of the biosurfactant extract against *Streptococcus mutans*.

| Substance | Growth inhibition (%) |
|-----------------------------|-----------------------|
| Melittin 0,1 mg/mL | 14 |
| Acetone pellet 0,6 mg/mL | 0 |
| Lipopeptide extract 1 mg/mL | 36 |
| SDS 1 mg/mL | 54 |

Conclusions

Bacillus sp. 0G produces lipopeptides and digestive enzymes in Landy's medium containing recyclable substrates. Using acetone it is possible to separate a lipopeptide extract from important enzymes that are useful for the food, biofuel, and detergent industries. The coproduction of biosurfactants and enzymes could lower the production costs of biosurfactants.

Acknowledgments

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