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The Influence of Vegetable Oils on Biosurfactant Production by *Serratia marcescens*

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Abstract

The production of biosurfactant, a surface-active compound, by two *Serratia marcescens* strains was tested on minimal culture medium supplemented with vegetable oils, considering that it is well known that these compounds stimulate biosurfactant production. The vegetable oils tested included soybean, olive, castor, sunflower, and coconut fat. The results showed a decrease in surface tension of the culture medium without oil from 64.54 to 29.57, with a critical micelle dilution (CMD^{-1}) and CMD^{-2} of 41.77 and 68.92 mN/m, respectively. Sunflower oil gave the best results (29.75 mN/m) with a CMD^{-1} and CMD^{-2} of 36.69 and 51.41 mN/m, respectively. Sunflower oil contains about 60% of linoleic acid. The addition of linoleic acid decreased the surface tension from 53.70 to 28.39, with a CMD^{-1} of 29.72 and CMD^{-2} of 37.97, suggesting that this fatty acid stimulates the biosurfactant production by the LB006 strain. In addition, the crude precipitate surfactant reduced the surface tension of water from 72.00 to 28.70 mN/m. These results suggest that the sunflower oil's linoleic acid was responsible for the increase in biosurfactant production by the LB006 strain.

Index Entries: Biosurfactant, *Serratia marcescens*; fermentation; surface tension; vegetable oils.

Introduction

Surfactants are potentially useful in every industry dealing with multiphase systems. These molecules contain both hydrophilic and lipophilic portions *(1)*. The effectiveness of a surfactant is determined by its

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ability to lower the surface tension, forming microemulsions where hydrocarbons can solubilize in water or where water can solubilize in hydrocarbons *(2,3)*. Such characteristics confer excellent detergency, which makes surfactants some of the most versatile process chemicals *(4–6)*.

Biosurfactants are produced as metabolic byproducts by bacteria, yeast, and fungi. These include a wide variety of chemical structures such as glycolipids, lipopeptides, polysaccharide protein complexes, phospholipids, fatty acids, and neutral lipids *(7–12)*. Cooper *(13)* and Desai and Banat *(6)* showed that these lipids can be produced extracellularly or from the cell wall. Microbial surfactants exhibit high chemical specificity to specifics substrates, effective physical and chemical properties, and temperature stability. They are consequently more suited to new applications. Other advantages of microbial surfactants include biodegradability, reduced toxicity, and a broad range of structures capable of specific applications.

Kosaric et al. *(14)* used a variety of simple, potentially less expensive substrates for the production of biosurfactant. These substrates include *n*-alkanes, carbohydrates, vegetable oils, and wastes. Some organisms can only produce biosurfactants from hydrocarbons or carbohydrates, whereas others are able to utilize several substrates, in combination or separately. However, better yields were obtained from hydrocarbons or carbohydrates or lipids *(15,16)*. In the case of *Torulopsis bombicola*, the addition of soybean oil to a less expensive carbohydrate medium resulted in enhanced yields up to 35-fold *(15)*.

Biosurfactants are environmental friendly because they are nontoxic, biodegradable, and possibly can be used for *in situ* production *(17)*. Moreover, the interest in biosurfactants has been related primarily to enhanced oil recovery process and formation of stable emulsions. However, potentiality of application of biosurfactants is determined by their cost and properties in relation to synthetic compounds. Thus, enhancing biosurfactant yield is very important. The methods used to increase metabolite production may consist of strain selection, mutagenesis, manipulation of environmental and nutritional factors, and genetic manipulations.

In the present study, we reported the utilization of commercial vegetable oils in combination with peptone/glycerol medium to increase the production of biosurfactant by two strains of *Serratia marcescens*. The relationship among biosurfactant production, surface properties, and biomass formation was investigated.

Material and Methods

Microorganisms and Medium

The pigmented *Serratia* sp. strain LB006 was isolated at the Biochemistry Laboratory (UNICAMP, Brazil) from beets obtained from a local market in Campinas, São Paulo, Brazil. The beets were cleaned and slices were incubated in nutrient broth at 30°C for 24 h, after which 0.1 mL was spread on nutrient agar medium and incubated at 30°C for 24 h. Then,

some colonies were isolated. Microorganisms producing biosurfactant were selected using published methods *(18)*. *S. marcescens* 0710 strain was obtained from Tropical Culture Collection, Campinas, São Paulo, Brazil.

Inoculation and Incubation

Cells (10^9 CFU/mL) were inoculated on minimal culture medium with the following composition: 0.5% bactopeptone, 1% glycerol, supplemented with 0.1% different vegetable oils at pH 7.2. Flasks containing 15 mL of minimal culture medium were incubated at 30°C in a shaker incubator at 150 rpm.

Dry Biomass Estimation

Dry biomass was determined by centrifugating of 15 mL of culture broth at 10,000 rpm for 15 min. The pellet obtained was dried overnight at 105°C.

Biosurfactant Isolation

Serratia cultures grown on minimal culture medium were centrifuged for 15 min at 10,000 rpm to obtain the cell-free supernatant. The supernatant was treated with 3 vol of chilled acetone. The precipitate formed was collected by filtration, and dried in an oven at 50°C. This method was described by Pruthi and Cameotra *(19)*.

Analyses

Biosurfactants were analyzed by measuring the surface tension of the cell-free supernatant using a Krüss K12 T processor tensiometer. Critical micelle dilution (CMD), a parameter used as an indirect measure of surfactant concentration, was determined by measuring the surface tension of serial dilutions of the cell-free broth in distilled water at pH 7.0.

Results and Discussion

The microorganism isolated from beets was identified as *S. marcescens* using an API Kit 20 E (BioMérieux) and complementary biochemistry tests. *S. marcescens* is an ubiquitous bacterium inhabiting water, soil, plants, insects, and vertebrates. It is an opportunistic pathogen of humans, and a real threat in hospitals, as described by Grimont and Grimont *(20)* and Matsuyama et al. *(21)*.

Tables 1 and 2 show the influence of vegetable oils in the production of biosurfactant by the LB006 and 0710 strains on minimal culture medium containing peptone and glycerol for 72 h. The decrease in surface tension of the cell-free broth without oil by the LB006 strain was from 64.54 ± 0.04 to 29.57 ± 0.10 mN/m, with CMD^{-1} and CMD^{-2} values of 41.77 ± 0.19 and 68.92 ± 0.18 mNm, respectively. Among all the oils used, sunflower oil gave the best results (29.75 ± 0.07) with a CMD^{-1} value of 36.69 ± 0.18 mNm and a CMD^{-2} value of 51.41 ± 0.03 mN/m. In addition, the crude precipitate

Table 1
Surface Tension for LB006 Strain on Minimal Culture Medium Supplemented with Different Vegetable Oils After 72 h of Incubation at 30°C and 150 rpm

Oil	Surface tension (mN/m) media without inoculum	Surface tension (mN/m)	CMD^{-1} (mN/m)	CMD^{-2} (mN/m)
Oil free	64.54 ± 0.04	29.57 ± 0.10	41.77 ± 0.19	68.92 ± 0.18
Soybean	52.65 ± 0.12	30.76 ± 0.15	43.49 ± 0.19	54.04 ± 0.04
Coconut fat	51.52 ± 0.07	30.33 ± 0.12	41.96 ± 0.20	58.95 ± 0.19
Castor	53.74 ± 0.19	30.43 ± 0.14	42.75 ± 0.18	51.40 ± 0.02
Corn	53.76 ± 0.10	30.73 ± 0.05	43.34 ± 0.20	53.02 ± 0.02
Olive	51.90 ± 0.09	30.98 ± 0.13	40.27 ± 0.17	52.07 ± 0.05
Sunflower	52.70 ± 0.02	29.75 ± 0.07	36.69 ± 0.18	51.41 ± 0.03

Table 2
Surface Tension for 0710 Strain on Minimal Culture Medium Supplemented with Different Vegetable Oils After 72 h of Incubation at 30°C and 150 rpm

Oil	Surface tension (mN/m) media without inoculum	Surface tension (mN/m)	CMD^{-1} (mN/m)	CMD^{-2} (mN/m)
Oil free	64.54 ± 0.04	30.43 ± 0.10	47.52 ± 0.19	58.41 ± 0.04
Soybean	52.65 ± 0.12	30.62 ± 0.08	42.79 ± 0.20	54.18 ± 0.10
Coconut fat	51.52 ± 0.07	29.72 ± 0.10	42.86 ± 0.18	52.11 ± 0.19
Castor	53.74 ± 0.19	31.98 ± 0.12	45.35 ± 0.20	61.14 ± 0.20
Corn	53.76 ± 0.10	31.69 ± 0.08	42.48 ± 0.10	59.06 ± 0.19
Olive	51.90 ± 0.09	31.59 ± 0.14	47.58 ± 0.19	55.87 ± 0.32
Sunflower	54.70 ± 0.02	30.64 ± 0.05	49.72 ± 0.19	56.98 ± 0.03

surfactant showed the ability to reduce the surface tension of water from 72.00 ± 0.01 to 28.70 ± 0.03 mN/m.

The addition of vegetable oils showed low influence on the production of biosurfactant by the 0710 strain, and little difference in surface tension, CMD^{-1}, and CMD^{-2} was observed (Table 2).

By comparing the CMD^{-2} values (Tables 1 and 2), it is evident that LB006 was strongly influenced by the addition of vegetable oils whereas the 0710 strain demonstrated almost the same values for free oil and oil added to the minimal medium.

Sunflower oil contains about 60% linoleic acid, so the minimal culture medium containing peptone and glycerol was supplemented with 0.06% (representative 60% of 0.1% sunflower oil) linoleic acid; the results were remarkable. The decrease in surface tension was from 53.70 to 28.32 mN/m (47%), CMD^{-1} (47%), and CMD^{-2} (38%), suggesting that the addition of linoleic acid stimulated the biosurfactant production by the LB006 strain. These results are comparable with those reported by Matsuyama et al. *(22)* in which three *S. marcescens* strains, NS38, NS25, and NS45, showed a sur-

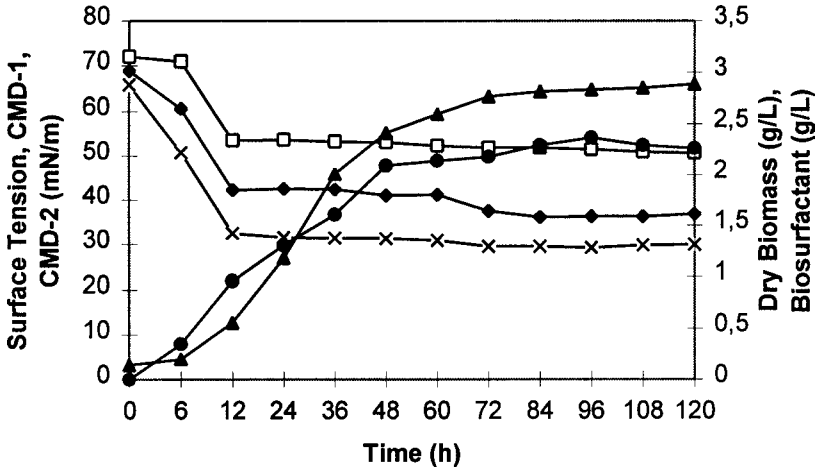

Fig. 1. Profiles of biosurfactant production (●), dry biomass (▲), surface tension (×), CMD^{-1} (◆), and CMD^{-2} (□) vs time during growth of *S. marcescens* (LB006).

face tension of 32.2 ± 0.10, 33.9 ± 0.20, and 28.8 ± 0.30 mNm, respectively, when grown on 0.5% peptone, 1% glycerol, and 1.2% agar.

The production of biosurfactant, biomass, surface tension, and CMD^{-1} and CMD^{-2} profiles during growth of LB006 on minimal culture medium with 0.1% sunflower oil is shown in Fig. 1. These results indicated that biosurfactant and growth-associated biomass production are directly correlated. In 12 h of fermentation, the surface tension showed a maximal decrease, suggesting that a small quantity of crude biosurfactant (0.96 g/L) is responsible for this reduction. At this point, the critical micelle concentration was reached; that is, no further reduction in surface tension was obtained although biosurfactant production continued to increase. The maximal biosurfactant production was achieved after 72 h of fermentation (2.2 g/L) for the LB006 strain, followed by a reduction in both the surface tension and the CMD^{-1} values.

Insufficient information is available on the metabolic pathway involved in biosurfactant production. This knowledge is essential for biosynthesis control. Biosynthesis involves the synthesis of the lipid moiety, the nonlipid moiety (e.g., sugar, peptide), and the subsequent linkage of the two portions. The pathways involved in biosynthesis are dependent on the carbon source and the type of biosurfactant produced.

An aminolipid biosurfactant called serratamolide (cyclodepsipeptide) was isolated from *S. marcescens* NS.38. On hydrolytic cleavage of amide and ester linkages, the molecule is known to yield serine and 3-hydroxy-decanoic acid. Matsuyama et al. *(22,23)* suggested that these two components must contribute to the surface-active properties of this aminolipid. A similar structure of the biosurfactant produced by LB006 is being investigated.

Biosurfactant production by *S. marcescens* LB006 was stimulated by the addition of long chain fatty acids, suggesting that the mechanism of

biosurfactant synthesis is related to the emulsification of these vegetable oils, which enhanced the nutrients' availability for bacterial cell. Further studies are being conducted in order to examine some applications of this biosurfactant production.

Conclusion

The local isolated LB006 strain showed better surface tension reduction than the collection culture 0710 strain. Sunflower oil was the best vegetable oil tested. Linoleic acid was probably the component of sunflower oil responsible for the increase in biosurfactant production.

The addition of vegetable oils showed low influence on the production of biosurfactant by *S. marcescens* 0710, and little difference in surface tension, CMD^{-1}, and CMD^{-2} was observed.

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