Improved biosurfactant production by a *Pseudomonas aeruginosa* strain using agro-industrial wastes

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INTRODUCTION

Biosurfactants are amphipathic molecules produced by a variety of microorganisms that exhibit pronounced surface and emulsifying activities. These compounds can replace synthetic surfactants in food, pharmaceutical, cosmetics or petroleum industries, as well as in bioremediation. However, their applicability depends on whether they can be produced economically at large-scale. Several efforts have been conducted to reduce production costs, including the use of agro-industrial wastes as substrates, optimization of medium and culture conditions and development of efficient recovery processes.

AIMS: Optimize biosurfactant production by a *P. aeruginosa* strain isolated from a crude oil sample using agro-industrial wastes and evaluate its possible applications.

METHODS

P. aeruginosa #112 was isolated from a crude oil sample[1]. Biosurfactant production was studied in flasks at 37ºC and 180 rpm. Agro-industrial wastes (Corn Steep Liquor, molasses) were obtained from local industries. Surface tension was measured using the Ring method. Emulsifying activity was determined with *n*-hexadecane. Biosurfactants were recovered through acidic precipitation. The antimicrobial activity and the Minimum Inhibitory Concentration (MIC) were determined using the micro-dilution method. Oil recovery assays were performed using artificially contaminated sand containing 10% (w/w) of crude oil.

RESULTS

Several media were used to evaluate biosurfactant production by *P. aeruginosa* #112. The highest biosurfactant production was achieved using the CSLM medium, containing CSL (10%, v/v) and molasses (10%, w/v). The amount of biosurfactant was ten times higher when compared with LB medium (Table 1).

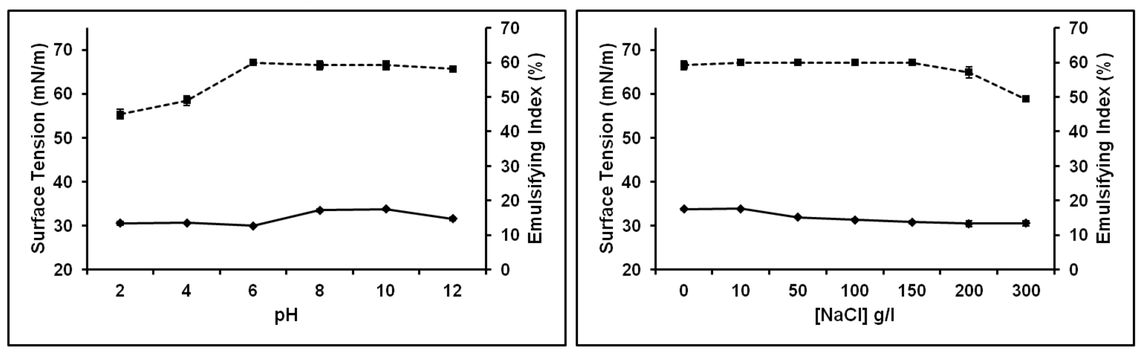
The biosurfactants produced were stable in a wide range of pH values and salinities (Figure 2) and were not affected by exposition to high temperatures (100ºC for 1 h or 121ºC for 20 min).

Table 1. Surface tension values (mN/m), emulsifying indexes (%), biosurfactant yield (mg/l) and critical micelle concentration (mg/l) obtained with *P. aeruginosa* #112 grown in CSLM and LB media at 37ºC and 180 rpm for 72 and 144 hours, respectively. Results represent the average of three independent experiments ± standard deviation.

	LB	CSLM
ST_0 (mN/m)	50.2 ± 0.3	51.1 ± 0.1
ST (mN/m)	32.7 ± 0.4	31.1 ± 1.0
ST^{-1} (mN/m)	34.1 ± 0.1	32.7 ± 0.7
ST^{-2} (mN/m)	46.6 ± 0.3	38.7 ± 2.3
E_{24} (%)	60.5 ± 0.7	61.7 ± 1.2
E_{24}^{-1} (%)	0.0 ± 0.0	58.3 ± 0.0
E_{24}^{-2} (%)	0.0 ± 0.0	28.2 ± 4.4
[BS] (mg/l)	469 ± 63	4861 ± 245
cmc (mg/l)	1600	200

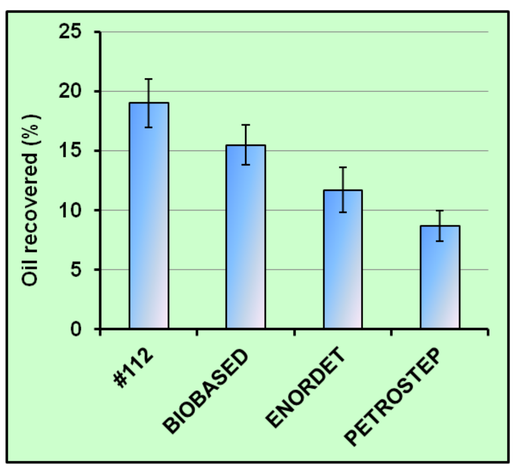
Figure 1. *P. aeruginosa* #112 grown in CSLM medium after 144 hours.

Figure 2. Effect of pH and salinity on surface tension values (—) and emulsifying indexes (----) of cell-free culture broth supernatants obtained at the end of the fermentation of *P. aeruginosa* #112 in CSLM medium.



The biosurfactant produced by *P. aeruginosa* #112 was more effective in oil recovery assays when compared with the chemical surfactants studied (Figure 3).

Figure 3. Percentages of oil recovered by the different chemical surfactants and the biosurfactant produced by *P. aeruginosa* #112 at a concentration of 1 g/l. Results represent the average of three independent experiments ± standard deviation.



Furthermore, the biosurfactant produced by *P. aeruginosa* #112 exhibited antimicrobial activity against all the microorganisms tested, being more active against Gram-positive bacteria (Table 2).

Table 2. MICs and percentages of growth inhibition (at a concentration of 25 mg/ml) obtained with the crude biosurfactant produced by *P. aeruginosa* #112.

Microorganism	MIC (mg/ml)	Microorganism	% inhibition
Bacillus subtilis	0.39	*Candida albicans*	30.7 ± 1.9
Staphylococcus aureus	0.78	*Escherichia coli*	55.2 ± 6.4
Streptococcus agalactiae	0.39	*Pseudomonas aeruginosa*	68.7 ± 10.4
Streptococcus oralis	0.78	*Staphylococcus epidermidis*	89.8 ± 10.3
Streptococcus pyogenes	0.39	*Streptococcus mutans*	65.8 ± 7.9
Streptococcus sanguis	0.19		

CONCLUSIONS

In this study, biosurfactant production by a *P. aeruginosa* strain was improved using agro-industrial wastes as culture medium. The crude biosurfactant exhibited a better performance in oil recovery assays when compared with chemical surfactants, being useful for application in the oil industry or bioremediation processes. Furthermore its antimicrobial activity suggests its possible application as an alternative to conventional antibiotics.

REFERENCES

[1] Gudiña et al. 2012. Isolation and study of microorganisms from oil samples for application in Microbial Enhanced Oil Recovery. *Int. Biodeter. Biodegrad.* 68: 56-64.

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