Novel bioemulsifier produced by a *Paenibacilus* sp. strain and applicability in Microbial Enhanced Oil Recovery

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INTRODUCTION

Surface-active compounds (SACs) produced by microorganisms exhibit several advantages over their synthetic counterparts and could replace some of them in many environmental and industrial applications.

Chemical SACs
Derived from petroleum
Toxic to the environment
Low biodegradability
Environmentally hazardous
High structural variation

Microbial SACs
Microbial origin
Low toxicity
High biodegradability
Environmentally friendly
Low structural variation

The large-scale production and the widespread use of microbial SACs has not been achieved due to their high production costs and their limited structural variation.

AIMS: Isolation and study of novel SACs-producing microorganisms.

MATERIALS AND METHODS

The SAC-producing strain was isolated from a crude oil sample and identified through 16S rRNA sequencing. SACs production was studied in flasks using a mineral medium (MSS) at 40ºC and 120 rpm. The surface tension (ST) was measured according to the Ring method, and the emulsifying activity (E_{24}) was determined with *n*-hexadecane. The SAC was recovered by precipitation with ethanol. The toxicity tests were performed using a Microtox® M500 analyzer with *Vibrio fisheri* as indicator microorganism. The biodegradability was assessed by liquid respirometric assays. The chemical characterization was performed using FT-IR, ^{1}H NMR and ^{13}C CP-MAS NMR.

RESULTS

A promising SAC-producing microorganism (strain #510) was isolated from crude oil and identified as *Paenibacillus* sp. according to the sequence of its 16S rRNA gene (accession number KF151179)[1].

The SAC produced by *Paenibacillus* sp. #510 was identified as a bioemulsifier, as it did not significantly reduce the surface tension, but exhibited a high emulsifying activity **(Figure 1)**.

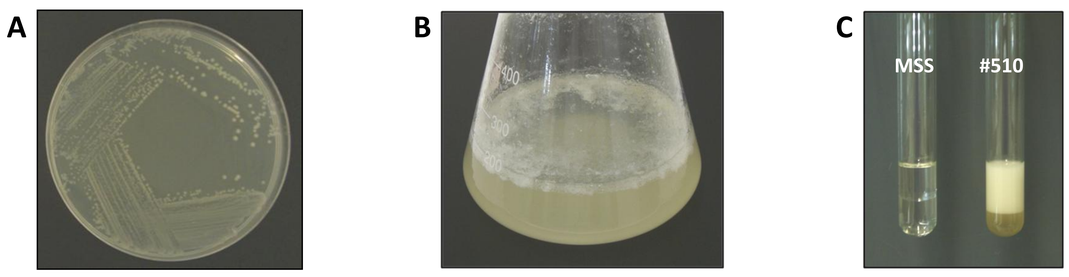

Figure 1. *Paenibacillus* sp. #510 growing in LB medium **(A)** and MSS supplemented with paraffin **(B)**. Emulsifying activity against *n*-hexadecane **(C)**.

Although bioemulsifier production by *Paenibacillus* sp. #510 also occurred under anaerobic conditions, it was higher under aerobic conditions, and it was induced by the presence of hydrocarbons in the culture medium **(Table 1)**.

Table 1. Effect of aeration and hydrocarbons on bioemulsifier production by *Paenibacillus* sp. #510. The ST of MSS was 72.0 ± 0.5 mN/m.

	Medium	ST (mN/m)	E_{24} (%)	[Bioemulsifier] (g/L)
Aerobic	MSS	52.9 ± 1.5	36.4 ± 4.4	5.0 ± 0.5
	MSS + paraffin	51.1 ± 1.8	43.1 ± 3.1	5.6 ± 0.2
	MSS + crude oil	50.6 ± 1.4	42.1 ± 2.1	5.4 ± 0.4
Anaerobic	MSS	51.1 ± 0.9	49.9 ± 2.7	6.1 ± 0.2
	MSS + paraffin	50.1 ± 1.6	64.4 ± 1.6	7.9 ± 0.1
	MSS + crude oil	50.0 ± 0.6	62.1 ± 2.5	7.4 ± 0.3

The bioemulsifier maintained its activity in a wide range of pH values and salinities **(Figure 2)**, and it was not affected by exposure to high temperatures (100-121ºC).

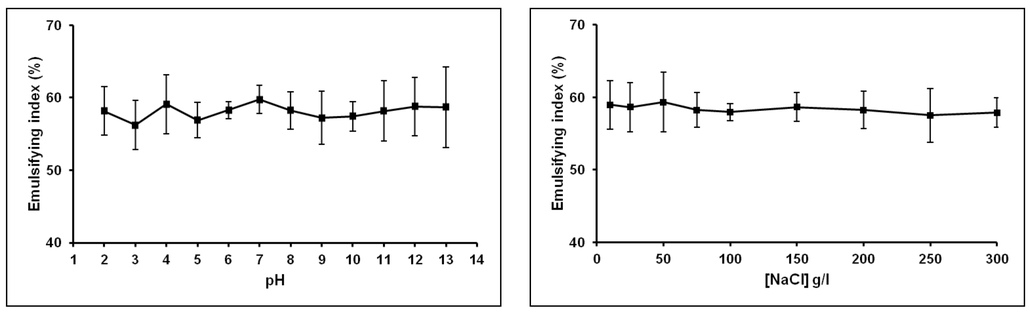

Figure 2. Effect of pH and salinity on the activity of the bioemulsifier produced by *Paenibacillus* sp. #510.

The bioemulsifier formed stable emulsions (up to one month at 40ºC) with different aliphatic and aromatic hydrocarbons, as well as with hydrocarbon mixtures **(Table 2)**.

Table 2. Emulsifying indexes obtained with different hydrocarbons using the bioemulsifier produced by *Paenibacillus* sp. #510.

Hydrocarbon	E_{24} (%)	Hydrocarbon	E_{24} (%)
Chloroform	63.8 ± 0.7	*n*-hexadecane	59.3 ± 1.0
Crude oil	75.1 ± 1.6	*n*-hexane	50.9 ± 1.8
Dichloromethane	66.1 ± 1.9	Paraffin	63.1 ± 1.6
Ethyl acetate	52.7 ± 1.3	Toluene	61.6 ± 1.1
Heating oil	62.7 ± 1.5	Xylene	59.3 ± 1.0

The bioemulsifier exhibited less toxicity and higher biodegradability when compared with commonly used chemical SACs **(Table 3)**.

Table 3. Toxicity and biodegradability data obtained for the different SACs studied.

Compound	EC_{50} (mg/L)	COD (mg O_2/L)	BOD_5 (mg O_2/L)
Glucopone®650	4.9 ± 1.3	335.5 ± 0.5	35.5 ± 1.5
Findet®1214 N/23	9.6 ± 6.2	384.5 ± 3.5	34.0 ± 2.5
LAS	21.1 ± 4.8	345.5 ± 0.5	35.0 ± 1.0
Bioemulsifier #510	> 1000	10.9 ± 0.2	9.5 ± 2.0

A preliminary chemical characterization revealed that the bioemulsifier is a low-molecular weight oligosaccharide-lipid complex.

CONCLUSIONS

- Bioemulsifier production by a *Paenibacillus* strain was reported for the first time.
- Not affected by exposure to adverse environmental conditions.
- Broad spectrum of emulsifying activity.
- Good environmental compatibility: low toxicity and high biodegradability.
- Unusual bioemulsifier: low-molecular weight oligosaccharide-lipid complex.
- Promising product for many environmental and industrial applications.

References

[1] Gudiña EJ *et al.* 2015. Novel bioemulsifier produced by a Paenibacillus sp. strain isolated from crude oil. *Microbial Cell Factories* **14:**14.

Acknowledgements

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