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BIOSURFACTANTS PRODUCED BY METAL-RESISTANT *Pseudomonas aeruginosa* ISOLATED FROM *Zea mays* RHIZOSPHERE AND COMPOST

BIOSURFACTANTES PRODUCIDOS POR LA *Pseudomonas aeruginosa* AISLADA DE LA RIZÓSFERA Y COMPOSTA DE LA *Zea mays*

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ABSTRACT

Contextualization: *Pseudomonas aeruginosa* is capable of producing biosurfactants which have many uses in bioremediation and the production of antiviral, antibacterial, antiparasitic, sporicidal and antifungal agents, among others.

Knowledge gap: This study describes the production of mono and di-rhamnolipid biosurfactants by *P. aeruginosa* strains isolated from Zea mays rhizosphere and composts in the state of Guerrero, Mexico.

Purpose: The overall aims were to investigate biosurfactant, pyocyanin production, and tolerance to heavy metals and antimicrobial activity capacity than biosurfactants produced from *P. aeruginosa* strains from corn rhizosphere and compost in Mexico.

Methodology: Biosurfactant production was determined based hemolysis on blood agar, blue halos in CTAB-Methylene blue agar, drop collapse test and production of foam on PPGAS

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broth, the emulsion index (IE24) and antibacterial capacity. The strains were identified by sequence of the 16S rDNA gene and their resistance to heavy metals were also evaluated.

Results and conclusions: Two strains isolated from Zea mays rhizosphere (PAM8, PAM9) were the best biosurfactant producers and their extracts showed antimicrobial activity against Grampositive and Gramnegative bacteria. PAM8 and PAM9 showed >30% of cellular hydrophobicity to hydrocarbons, and were capable of emulsifying toluene, cyclohexane, petroleum, diesel and oils. All strains showed the same profile of heavy metal tolerance (As5+ >As3+ >Zn2+ >Pb2+ >Fe3+ >Cd2+ >Cu2+ >Cr6+ in concentrations of 20, 10, 10, 6, 4, 4, 2 and 2 mM., respectively). The isolation of biosurfactant-producing and heavy-metal tolerant bacteria from Zea mays rhizosphere and compost in Guerrero demonstrates the capacity for this region to harbor potentially important microbial strains for industrial or bioremediation applications.

Keywords: Rhamnolipids, hydrocarbons, bioremediation, biotechnological applications.

RESUMEN

Contextualización: la *Pseudomonas aeruginosa* es capaz de producir biosurfactantes que tienen muchos usos en la biorremediación y producción de agentes antivirales, antibacterianos, antiparasitarios, esporicidas y antifúngicos, entre otros.

Vacío de conocimiento: este estudio describe la producción de biosurfactantes mono y di-ramnolípidos por cepas de *P. aeruginosa* aisladas de la rizosfera y compostas de *Zea mays* en el estado de Guerrero, México.

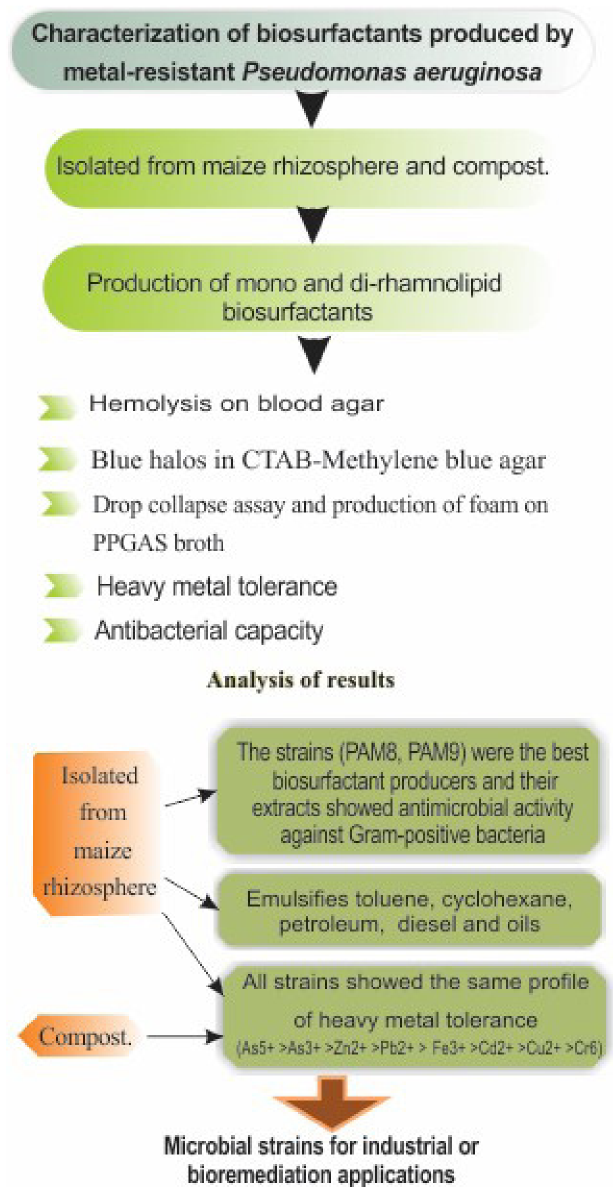
Propósito del estudio: los objetivos generales fueron investigar la producción de biosurfactantes, piocianina y tolerancia a metales pesados y la capacidad de actividad antimicrobiana de los biosurfactantes producidos por cepas de *P. aeruginosa* aisladas de rizosfera y composta en México.

Metodología: Se determinó la producción de biosurfactantes con base en la hemolisis agar sangre, halos azules en agar CTAB-azul de metileno, ensayo de colapso de gotas, producción de espuma en medio PPGAS, índice de emulsión (IE24) y capacidad antibacterial, las cepas se identificaron por la secuencia del gen del 16S y también se evaluó la resistencia a metales pesados.

Resultados y conclusiones: Dos cepas aisladas de la rizosfera de *Zea mays* (PAM8, PAM9) fueron las mejores productoras de biosurfactantes, y sus extractos mostraron actividad antimicrobiana frente a bacterias Grampositivas y Gramnegativas. PAM8 y PAM9 mostraron >30% de hidrofobicidad a hidrocarburos y fueron capaces de emulsionar tolueno, ciclohexano, petróleo, diésel y aceites. Todas las cepas mostraron el mismo perfil de tolerancia a metales pesados ($As^{5+} > As^{3+} > Zn^{2+} > Pb^{2+} > Fe^{3+} > Cd^{2+} > Cu^{2+} > Cr^{6+}$ en concentraciones de 20, 10, 10, 6, 4, 4, 2 y 2 mM., respectivamente). El aislamiento de bacterias productoras de biosurfactantes y tolerantes a metales pesados de la rizosfera y composta de *Zea mays* en Guerrero demuestra que la región alberga cepas microbianas potencialmente importantes para aplicaciones industriales o de biorremediación.

Palabras clave: Ramnolípidos, hidrocarburos, biorremediación, aplicaciones biotecnológicas.

GRAPHIC ABSTRACT



1. INTRODUCTION

Biosurfactants are molecules that lower the surface tension and increase the solubility between solutions that are non-miscible (Toribio Jimenez et al., 2014). Among their many applications, they may act as emulsifying, foaming agents and wetting agents (Pacwa-Płociniczak et al., 2011; Satpute et al., 2010)2011; Satpute et al., 2010. Biosurfactants are produced by microorganisms such as bacteria, yeast and filamentous fungi, and are often considered to be environmentally friendly compounds. They have been mostly studied in the context of microbial enhanced oil recovery (MEOR) and in the bioremediation of water and soils contaminated by hydrocarbon spills and heavy metals (Amani et al., 2013; Darvishi et al., 2011; Wu et al., 2008). Among the better-known biosurfactants are the rhamnolipids (RLs), mainly produced by the bacterium *P. aeruginosa*.

Strains of *P. aeruginosa* can metabolize hexadecane and other polycyclic aromatic hydrocarbons to use them as a carbon source for growth, for synthesis of rhamnolipids (Beal and Betts, 2000; Müller et al., 2012; Nie et al., 2010) and to eliminate heavy metals from wastewater (El Zeftawy and Mulligan, 2011).

Rhamnolipids (the class glycolipids) from this species are known to function as antibacterial, antiviral, antiparasitic, sporicidal and antifungal agents (Abdel-Mawgoud et al., 2010; Bharali et al., 2013; Wang et al., 2005)alpha-L-rhamnopyranosyl-alpha-L-rhamnopyranosyl-beta-hydroxydecanoate (Rha-Rha-C(10. Another compound related to biosurfactants is pyocyanin, which is a blue, secondary metabolite with the ability to oxidize and reduce other molecules (Hassan et al., 1980). Biosurfactants and pyocyanin exhibited a peculiar correlation, as the culture supernatants containing both components emulsified hydrocarbons in a higher amount than that of the biosurfactant alone.

Enhancement in emulsification also occurred by mixing pyocyanin from PPGAS medium and biosurfactant (Das and Ma, 2013). *P. aeruginosa* is a free-living bacterium that has been isolated from soils, contaminated water, plant rhizospheres, animal skin, leachates, and human environments, including hospitals (Soberón-Chávez, 2001). Resistance of many *P. aeruginosa* strains to antibiotics, antiseptics, chemical disinfectants, heavy metals and metalloids suggest that the species is generally robust in nature and may be applied for human uses in certain cases. The overall objective was to investigate biosurfactant, pyocyanin production, and tolerance to heavy metals and antimicrobial activity capacity than biosurfactants produced from *P. aeruginosa* strains from corn rhizosphere and compost in Mexico.

2. MATERIALS AND METHODS

See Annex.

3. RESULTS AND DISCUSSION

RESULTS

Selection and identification of biosurfactant-producing bacteria.

A total of seven bacterial strains were characterized genetically and identified as the genus *Pseudomonas*. Four isolates were found in *Zea mays* rhizosphere (PAM7, PAM8, PAM9 and PAM 10) and the rest were isolated from compost (PAB11, PAB12 and PAB13). The strains showed 99% identity to *P. aeruginosa* PAO1 (reference strain); however, PAM79 and PAM810 isolates are genetically related to strains of *Pseudomonas sp* (Fig 1). All strains exhibited the ability to produce biosurfactants, which was confirmed by beta hemolysis in blood agar plates and dark halos in CTAB-Methylene blue agar.

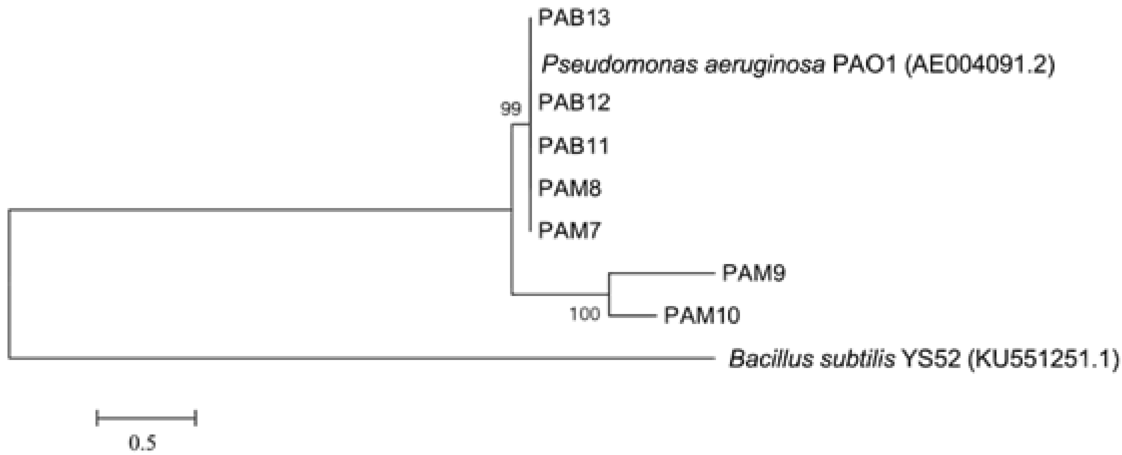


Figure 1. Phylogenic analysis by neighbor-joining method on 16S rDNA gene sequences. Scale bar = 0.5 substitutions per nucleotide position. *Bacillus subtilis* YS52 (HQ202539) was used as an outgroup sequence.

Source: Authors

The surface activity of biosurfactants present in the cultures was positive for the tests of foaming activity in PPGAS broth, drop collapse test and oil displacement test (Fig. 2).

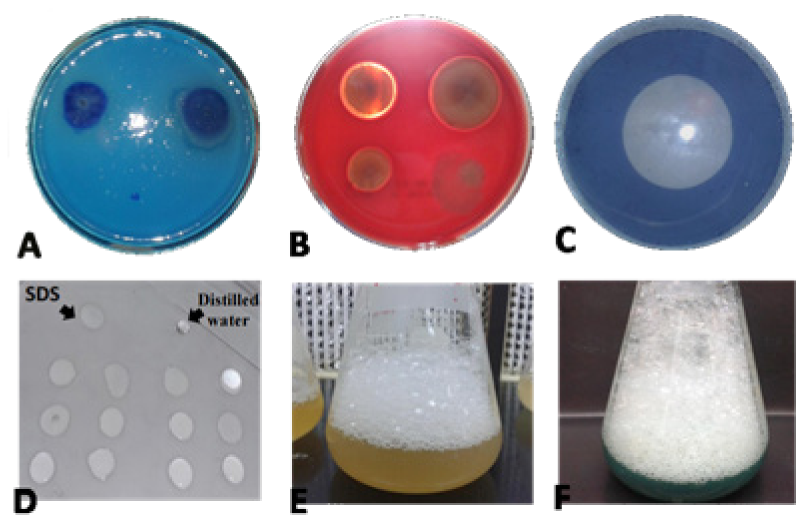


Figure 2. Evaluation of the production of biosurfactants. A) Blue halos formation in CTAB-Methylene blue agar. B) Hemolysis in blood agar. C) oil displacement assay. D) drop collapse test. E-F) Foaming activity in PPGAS broth.

Source: Authors

Emulsifying properties and cellular hydrophobicity associated with biosurfactants production

The biosurfactants produced can emulsify petroleum, diesel, toluene, xylene, cyclohexane and oils (mineral and vegetable) in different degrees, as shown on Table 1. The xylene hydrocarbon was mostly emulsified by the strains (average = 58%), while mineral oil did not exceed the 32% of average emulsification. *P. aeruginosa* strains with highest IE_{24} values were PAM8, PAM9, PAB11 and PAB13.

The strain PAM8 has an average IE_{24} of 64% and PAM9 emulsifies cyclohexane up to 70% in vegetable oil. PAB11 emulsifies petroleum to 79% and PAB13 emulsifies xylene to 67%. The *Zea mays* rhizosphere strains showed better emulsification in diesel compared to the compost strains, and did not present significant differences in the other substrates. The strongly hydrophobic (% Cellular Hydrophobicity or BATH) *P. aeruginosa* strains were PAM8 and PAM9 with 38% and 30% of BATH values, respectively (Table 1).

Table 1. Percent Index of emulsification (IE_{24}) and cellular hydrophobicity by biosurfactants of *P. aeruginosa*.

Table 1. Percent Index of emulsification (IE 24) and cellular hydrophobicity by biosurfactants of *Pseudomonas aeruginosa*.

		Emulsification Index (IE24) %							
Sample	*P. aeruginosa*	Diesel	Toluene	Xylene	Cyclohexane	Mineral Oil	Vegetable oil	Petroleum	% Cellular Hydrophobicity
Maize rhizosphere	PAM7	28	66	57	54	24	45	20	22
	PAM8	60	24	61	64	33	52	26	38
	PAM9	56	66	67	65	23	70	26	30
	PAM10	40	19	42	54	17	55	56	18
Compost	PAB11	27	63	47	54	21	50	79	22
	PAB12	12	54	63	44	60	57	22	18
	PAB13	25	24	67	48	44	45	44	26
Control	PAO-1	57	54	65	45	42	54	73	32
	p value	0.04*	0.86	0.65	0.25	0.21	0.48	0.42	0.06

*Statistically different accorded to unpaired Student's t-test ($p \leq 0.05$)

Source: Authors

Quantification and physicochemical characterization of biosurfactants.

The *P. aeruginosa* strains with the highest biosurfactant titers were PAM8 (187± 5 mg/L), from *Zea mays* rhizosphere, and PAB13 (162±7 mg/L), from compost (Fig.3). The strain PAB12 showed poor performance, with 125±4 mg/L of biosurfactant. All biosurfactants obtained from the isolated *Pseudomonas* strains were negatively charged and retained their capacity to disperse and emulsify oil after treatment at ≥ 100 °C, salinity at 10% and ≥ pH10 (Fig. 3). Significant differences were observed between compost and *Zea mays* rhizosphere strains in terms of amount of biosurfactant produced but not in their physicochemical properties.

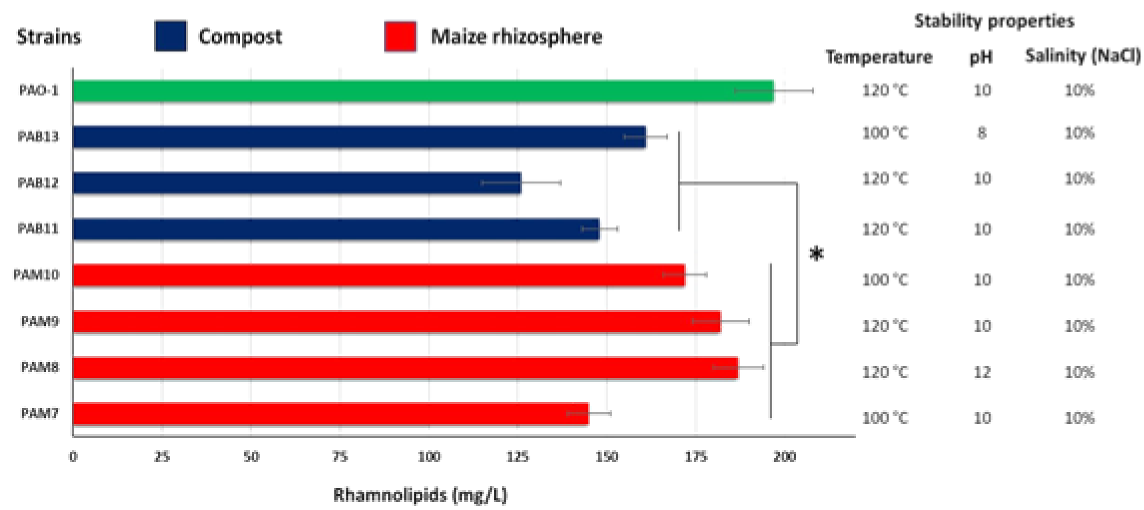


Figure 3. Comparison of production and physicochemical properties of rhamnolipids. *Statistically different between compost and rhizosphere strains accorded to unpaired Student's t-test ($p \leq 0.05$)

Source: Authors

Surface activity of biosurfactants in presence of pyocyanin pigment.

The production of pigments was observed at the same time as formation of abundant foam in PPGAS culture so, due to its blue coloration, pyocyanin was quantified (Fig. 4). The highest production of pyocyanin was observed in *Zea mays* rhizosphere strains: PAM8 (11.9 ± 2.3 µL mL^{-1}), PAM9 (7.8 ± 1.3 µL mL^{-1}) and PAM10 (7.8 ± 1.0 µL mL^{-1}).

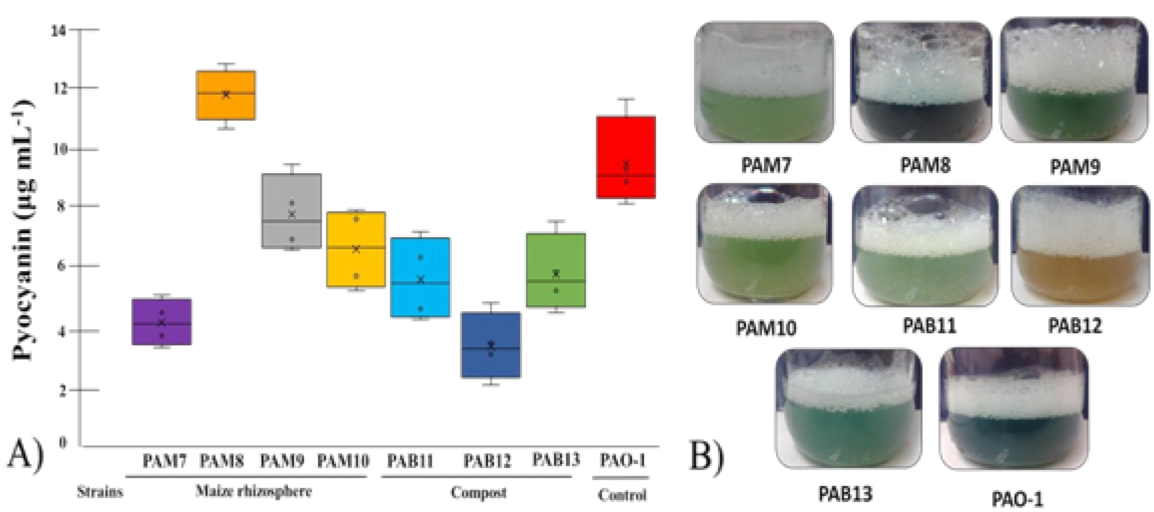


Figure 4 Comparison of pyocyanin pigment between compost and rhizosphere strains. A) Quantification by colorimetric method. B) Intensity of pigment in PPGAS broth.

Source: Authors

Antibacterial activity of biosurfactants.

The produced rhamnolipids have antimicrobial activity against two Grampositive and two Gramnegative bacteria evaluated (Table 2). Maize rhizosphere strains inhibit the bacterium *Bacillus subtilis* better than compost strains, and no significant differences in their antibacterial effect were observed for other bacteria. *P. aeruginosa* PAM8 showed the strongest activity against *B. subtilis, S. aureus, S. paucimobilis* and *K. pneumoniae* with inhibition halos of 26±6 mm, 22±4 mm, 32±5 mm and 24±3 mm, respectively.

Table 2. Comparison of antibacterial activity of biosurfactants produced by *Pseudomonas aeruginosa*.

	Inhibition zone diameter (mm)							
	P. aeruginosa	***B. subtilis***	***S. aureus***	***S. paucimobilis***	***E. cloacae***	***P. vulgaris***	***K. pneumoniae***	***E. coli***
Maize rhizosphere	PAM7	22	20	21	ND	ND	13	ND
	PAM8	26	22	32	ND	ND	24	ND
	PAM9	23	13	22	ND	ND	14	ND
	PAM10	23	17	26	ND	ND	18	ND
Compost	PAB11	11	12	31	ND	ND	17	ND
	PAB12	13	19	12	ND	ND	13	ND
	PAB13	21	17	16	ND	ND	19	ND
Control	PAO-1	26	21	30	ND	ND	23	ND
	p value	0.03*	0.94	0.82	-	-	0.84	-

*Statistically different between compost and rhizosphere strains accorded to unpaired Student's t-test ($p \leq 0.05$). ND=Not detected.

Source: Authors

Heavy-metal resistance profile of P. aeruginosa strains.

The petroleum and its derivatives contain traces of heavy metals and metalloids that give rise to the formation of organometallic compounds; therefore, we evaluated the heavy-metal resistance profile of biosurfactant-producing *Pseudomonas*. All the strains have similar MIC to heavy metals and metalloids in the following order: As^{5+} (>20 mM), As^{3+} (10 mM), Zn^{2+} (10 mM), Pb^{2+}(6 mM), Fe^{3+}(4 mM), Cd^{2+}(4 mM), Cu^{2+}(2 mM) and Cr^{6+}(2mM). The colonies were hyper mucoid when grown in high concentrations of metals especially in Pb, Fe and Cu.

DISCUSSION

The bacterium *P. aeruginosa* is known for its ability to produce several bioactive compounds. In this study, report is made on seven strains isolated from the rhizosphere corn or compost; all isolates produced biosurfactants with emulsification indexes on the different hydrophobic substrates as shown on Table 1. Highlighted in the work by Pirôllo et al. (2008), a *P. aeruginosa* LBI strain was isolated from places that were contaminated by hydrocarbons. The biosurfactant produced maintained the physicochemical properties after treatment at 120 °C, 10% salinity and only PAM8 biosurfactant is active under alkaline conditions (pH 12).

Strains synthetized both mono-rhamnolipids and di-rhamnolipids, except PAB12 that only produces mono-rhamnolipid, probably lacks some gene responsible for incorporating rhamnose during the biosynthetic pathway. Our results of IE_{24} agree with the results obtained by Janek et al., (2013), where it was reported that the rhamnolipid produced by a strain of *Pseudomonas* BD2 isolated in the Arctic, is capable of reducing the surface tension from 71 to 31 mN/m and emulsifies 70% olive oil, meanwhile for n-hexane, xylene, hexadecane and petroleum ether it emulsifies in a range from 51 to 65%.

It was observed that strains that synthesize more pigment also produce more biosurfactant and are better emulsifiers. Das and Ma (2013) report that the pyocyanin pigment enhances the biosurfactant-mediated hydrocarbon emulsification in clinical and environmental strains of *P. aeruginosa.* In the same way, the hydrocarbonoclastic activity (involved in the mineralization of hydrocarbon pollutants) is positively correlated with the pyocyanin production by *P. aeruginosa* strains isolated from gas stations, activated sludge and wastewater (Viana et al., 2018). Since the strains isolated in the present study are biosurfactant producers, they could potentially be used for Microbial Enhanced Oil Recovery (MEOR) in contaminated environments, in oil wells (Brown, 2010) or in agricultural soils (Sachdev and Cameotra, 2013). The production and concentrations the biosurfactants vary depending on the culture conditions and nutrients, especially the carbon source, as reported by Vanavil et al. (2013) It was reported that the concentration of rhamnolipids obtained when growing the strain in glucose was 88 mg/L, and 15-fold higher when they added Mg^{2+} and Fe^{2+} to the media. In the case of this study, it is still needed to find conditions for the improvement of the biosurfactant production.

We propose PAM8, PAM9 and PAM13 strains as candidates for use in bioremediation of marine environments and other systems by biosurfactants that are stable in hypersaline and alkaline conditions. The strains in this study are similar to those that the microbial consortium reported by Darvishi et al. (2011). That consortium produces a biosurfactant capable of resisting temperatures of over 70°C, salt concentrations higher than 15%, and a range of pH from 4 to 10. It also breaks down hydrocarbons, a fact that points it as a good candidate for the remediation of contaminated sites with these compounds.

The strains PAM8 and PAM9 are capable to emulsify different hydrocarbons and have highest cell hydrophobicity, these are good features for the bioremediation of sites contaminated with oil. Kaczorek and Olszanowski (2010) evaluated the binding of hydrocarbons by *Pseudomonas sp.* when growing in different carbon sources. They observed that *P. fluorescens* PI growing in glucose had 8% hydrophobicity but when grown in ethylbenzene, its hydrophobicity increased by 30% similar to the hydrophobicity showed by the strains *P. aeruginosa* PAM8 (28%) and *P. aeruginosa* PAM9 (20%). PAB12 is the smallest emulsifier probably because it only produces mono-rhamnolipid in small quantities (Fig 3), so the critical micellar concentration is not easily achieved.

The crude extracts of strain PAM8 showed high activity against *B. subtilis, S. aureus, S. paucimobilis* and *K. pneumoniae.* These results are consistent with Bharali et al. (2013) where the antibacterial activity of the *P. aeruginosa* OBP1 rhamnolipids on *S. aureus* and *K. pneumoniae* was shown. The rhamnolipids produced by *P. putida* 21BN against *B. subtillus* (Tuleva et al., 2002). Another study showed the effect of the biosurfactant produced by *P. koreensis* M9b on Grampositive bacteria (Toribio et al., 2011). The antibacterial producers included in the CLSI (Clinical and Laboratory Standards Institute) to date are biosurfactants belonging to the class glycolipids and cyclic lipopeptides; for this reason, the biosurfactants produced by our isolates represent good candidates as antibacterial products.

The colonies showed fluorescence and were hyper-mucoid in presence of Pb, Zn and Fe. This was probably in response to the stress they underwent, since the exopolysaccharides have anionic functional groups as phosphates, sulfates, and carboxilic acids that bind to metals, sequestering them and avoiding the interactions of the metal inside the cell. Fluorescence was caused by the production of siderophores that could be related to the presence of specific metals in the soils that our isolates came from. Siderophores as the pioverdine and piochelin are able to bind Fe, Cd, Cu and Zn, reducing their toxicity for bacteria (Dao et al., 1999; Dimkpa et al., 2009).

These data agree with Raja et al. (2006), who reported on a strain of *P. aeruginosa* FC1 being capable of growing in 800 ppm of Pb, 700 ppm of Ni, 500 ppm of Cd and 400 ppm of Cr. On the other hand, studies conducted by Hassan et al. (2008) showed bacterial resistance to heavy metals in contaminated soils in Egypt. However, 89% of the isolates were inhibited by 3.5 mM of the metal, which represents a lower concentration than our isolates could resist. Bodour et al. (2003) This shows the production of associated biosurfactants with resistance to heavy metals, which is similar to the yield of this study.

4. CONCLUSIONS

Seven endemic strains of *P. aeruginosa* were successfully isolated from the rhizosphere from pure breed *Zea mays* and compost. The strains were able to produce rhamnolipid and has antimicrobial activity against Grampositive and Gramnegative bacteria. Moreover, they showed resistance to heavy metals. Strains PAM8 and PAM9, are the best candidates to use them for bioremediation and other biotechnological applications.

AUTHOR’S CONTRIBUTION

First author: Methodology, research, data analysis, conceptualization, writing, original draft. **Second author**: Research, conceptualization, data analysis, writing, review and editing. **Third author**: Logistics, review and edition. **Fourth author**: data analysis, review and editing. **Fifth author**: Resource acquisition, project manager, supervision, conceptualization, writing, review and editing.

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LITERATURE CITED

Abdel-Mawgoud, A., Lépine, F. and Déziel, E., (2010). Rhamnolipids: diversity of structures, microbial origins and roles. Applied Microbiology and Biotechnology, 86(5), pp.1323-1336. doi: 10.1007/s00253-010-2498-2.

Amani, H., Müller, M., Syldatk, C. and Hausmann, R., (2013). Production of Microbial Rhamnolipid by Pseudomonas Aeruginosa MM1011 for Ex Situ Enhanced Oil Recovery. Applied Biochemistry and Biotechnology, 170(5), pp.1080-1093. doi: 10.1007/s12010-013-0249-4.

Beal, R. and Betts, W., (2000). Role of rhamnolipid biosurfactants in the uptake and mineralization of hexadecane in Pseudomonas aeruginosa. Journal of Applied Microbiology, 89(1), pp.158-168. doi: 10.1046/j.1365-2672.2000.01104.x.

Bharali, P., Saikia, J., Ray, A. and Konwar, B., (2013). Rhamnolipid (RL) from Pseudomonas aeruginosa OBP1: A novel chemotaxis and antibacterial agent. Colloids and Surfaces B: Biointerfaces, 103, pp.502-509. doi: 10.1016/j.colsurfb.2012.10.064.

Bodour, A., Drees, K. and Maier, R., (2003). Distribution of Biosurfactant-Producing Bacteria in Undisturbed and Contaminated Arid Southwestern Soils. Applied and Environmental Microbiology, 69(6), pp.3280-3287. doi: 10.1128/AEM.69.6.3280-3287.2003

Boles, B., Thoendel, M. and Singh, P., (2005). Rhamnolipids mediate detachment of Pseudomonas aeruginosa from biofilms. Molecular Microbiology, 57(5), pp.1210-1223. doi: 10.1111/j.1365-2958.2005.04743.x.

Brown, L., (2010). Microbial enhanced oil recovery (MEOR). Current Opinion in Microbiology, 13(3), pp.316-320. doi: 10.1016/j.mib.2010.01.011

Dao, K., Hamer, K., Clark, C. and Harshman, L., (1999). Pyoverdine production by Pseudomonas aeruginosa exposed to metals or an oxidative stress agent. Ecological Applications, 9(2), pp.441-448. doi 10.1890/1051-0761(1999)009[0441: PPBPAE]2.0.CO;2",

Darvishi, P., Ayatollahi, S., Mowla, D. and Niazi, A., (2011). Biosurfactant production under extreme environmental conditions by an efficient microbial consortium, ERCPPI-2. Colloids and Surfaces B: Biointerfaces, 84(2), pp.292-300. doi: 10.1016/j.colsurfb.2011.01.011.

Das, P. and Ma, L., 2013. Pyocyanin pigment assisting biosurfactant-mediated hydrocarbon emulsification. International Biodeterioration & Biodegradation, 85, pp.278-283. doi 10.1016/j.ibiod.2013.07.013

Dimkpa, C., Merten, D., Svatoš, A., Büchel, G. and Kothe, E., (2009). Siderophores mediate reduced and increased uptake of cadmium byStreptomyces tendaeF4 and sunflower (Helianthus annuus), respectively. Journal of Applied Microbiology, 107(5), pp.1687-1696. doi: 10.1111/j.1365-2672.2009.04355.x.

El Zeftawy, M. and Mulligan, C., (2011). Use of rhamnolipid to remove heavy metals from wastewater by micellar-enhanced ultrafiltration (MEUF). Separation and Purification Technology, 77(1), pp.120-127. doi:10.1016/j.seppur.2010.11.030

Hassan, H. and Fridovich, I., (1980). Mechanism of the antibiotic action pyocyanine. *Journal of Bacteriology*, 141(1), pp.156-163. doi: 10.1128/JB.141.1.156-163.1980

Hassan, S., Abskharon, R., Gad El-Rab, S. and Shoreit, A., (2008). Isolation, characterization of heavy metal resistant strain of *Pseudomonas aeruginosa* isolated from polluted sites in Assiut city, Egypt. Journal of Basic Microbiology, 48(3), pp.168-176. doi: 10.1002/jobm.200700338.

Janek, T., Łukaszewicz, M., & Krasowska, A. (2013). Identification and characterization of biosurfactants produced by the Arctic bacterium *Pseudomonas putida* BD2. Colloids and Surfaces B: Biointerfaces, 110, 379–386. doi: 10.1016/j.colsurfb.2013.05.008.

Kaczorek, E., & Olszanowski, A. (2010). Uptake of Hydrocarbon by *Pseudomonas fluorescens* (P1) and *Pseudomonas putida* (K1) Strains in the Presence of Surfactants: A Cell Surface Modification. Water, Air, & Soil Pollution, 214(1–4), 451–459. Doi:10.1007/s11270-010-0436-7

Maier, R. M., & Soberón-Chávez, G. (2000). *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. Applied Microbiology and Biotechnology, 54(5), 625–633. Doi:10.1007/s002530000443

Müller, M. M., Kügler, J. H., Henkel, M., Gerlitzki, M., Hörmann, B., Pöhnlein, M., Syldatk, C., & Hausmann, R. (2012). Rhamnolipids—Next generation surfactants? Journal of Biotechnology, 162(4), 366–380. doi: 10.1016/j.jbiotec.2012.05.022

Nie, M., Yin, X., Ren, C., Wang, Y., Xu, F., & Shen, Q. (2010). Novel rhamnolipid biosurfactants produced by a polycyclic aromatic hydrocarbon-degrading bacterium *Pseudomonas aeruginosa* strain NY3. Biotechnology Advances, 28(5), 635–643. doi: 10.1016/j.biotechadv.2010.05.013

Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental Applications of Biosurfactants: Recent Advances. International Journal of Molecular Sciences, 12(1), 633–654. doi: 10.3390/ijms12010633.

Pirôllo, M. P. S., Mariano, A. P., Lovaglio, R. B., Costa, S. G. V. A. O., Walter, V., Hausmann, R., & Contiero, J. (2008). Biosurfactant synthesis by *Pseudomonas aeruginosa* LBI isolated from a hydrocarbon-contaminated site. Journal of Applied Microbiology, 105(5), 1484–1490. doi: 10.1111/j.1365-2672.2008.03893.x.

Raja, C. E., Anbazhagan, K., & Selvam, G. S. (2006). Isolation and Characterization of a Metal-resistant *Pseudomonas Aeruginosa* Strain. World Journal of Microbiology and Biotechnology, 22(6), 577–585. Doi: 10.1007/s11274-005-9074-4

Sachdev, D. P., & Cameotra, S. S. (2013). Biosurfactants in agriculture. Applied Microbiology and Biotechnology, 97(3), 1005–1016. doi: 10.1007/s00253-012-4641-8

Satpute, S. K., Banpurkar, A. G., Dhakephalkar, P. K., Banat, I. M., & Chopade, B. A. (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. Critical Reviews in Biotechnology, 30(2), 127–144. doi: 10.3109/07388550903427280.

Soberón-Chávez, G. (2001). *Pseudomonas aeruginosa*. In: Microbios en línea, capítulo 3. Martínez Romero E. and Martínez Romero J. (eds). DGSCA, UNAM, http:// www.microbiologia.org.mx/microbiosenlinea/.

Toribio, J., Escalante, A. E., Caballero-Mellado, J., González-González, A., Zavala, S., Souza, V., & Soberón-Chávez, G. (2011). Characterization of a novel biosurfactant producing *Pseudomonas koreensis* lineage that is endemic to Cuatro Ciénegas Basin. Systematic and Applied Microbiology, 34(7), 531–535. doi: 10.1016/j.syapm.2011.01.007.

Toribio-Jiménez, J., Rodríguez-Barrera, MA., Valdez, M., Barrera, A., Segura, D., Wilson-Corral, V and Romero Y. (2014). Production of biosurfactants by bacteria isolated from a mine tailing zone in Southern Mexico and their resistance to heavy metals. J. bacteriol research, 6(2), 13-16. DOI: 10.5897/jbr2014.0128

Tuleva, B. K., Ivanov, G. R., & Christova, N. E. (2002). Biosurfactant Production By a New *Pseudomonas Putida* Strain. Zeitschrift Für Naturforschung C, 57(3–4), 356–360. doi: 10.1515/znc-2002-3-426.

Vanavil, B. (2013). Biosurfactant Production from Novel Air Isolate NITT6L: Screening, Characterization and Optimization of Media. Journal of Microbiology and Biotechnology, 23(9), 1229–1243. DOI: 10.4014/jmb.1212.12031

Viana, A. A. G., Oliveira, B. T. M. de, Cavalcanti, T. G., Sousa, K. A. de, Mendonça, E. A. de M., Amaral, I. P. G. do, & Vasconcelos, U. (2018). Correlation between pyocyanin production and hydrocarbonoclastic activity in nine strains of *Pseudomonas aeruginosa*. International Journal of Advanced Engineering Research and Science, 5(7), 212–223. DOI: 10.22161/ijaers.5.7.28

Wang, X., Gong, L., Liang, S., Han, X., Zhu, C., & Li, Y. (2005). Algicidal activity of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa*. Harmful Algae, 4(2), 433–443. DOI: 10.1016/j.hal.2004.06.001

Wu, J.-Y., Yeh, K.-L., Lu, W.-B., Lin, C.-L., & Chang, J.-S. (2008). Rhamnolipid production with indigenous *Pseudomonas aeruginosa* EM1 isolated from oil-contaminated site. Bioresource Technology, 99(5), 1157–1164. doi: 10.1016/j.biortech.2007.02.026.

Conflicto de Intereses
Los autores declaran no tener ningún conflicto de intereses