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SHORT COMMUNICATION

Investigation of antimicrobial effects of a *Pseudomonas*-originated biosurfactant

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Abstract: The aim of this work was to investigate the antimicrobial effects of biosurfactant (rhamnolipid) produced from *Pseudomonas* sp. Eight clinical test microorganisms were chosen, which were different groups, for the antimicrobial assays. Antimicrobial activity was evaluated according to the minimal inhibitor concentration (MIC) and disc-diffusion method. The highest activity for the rhamnolipid discs was obtained for β-hemolytic *Streptococcus* sp., whereas the lowest activity was found for *Pseudomonas aeruginosa.* The biosurfactant (rhamnolipid) showed very strong antimicrobial activity against the microorganisms tested.

Key words: *Pseudomonas* sp., biosurfactant, rhamnolipid, antimicrobial effect.

Abbreviations: MIC, minimal inhibitor concentration; OOME, olive oil mill effluent.

Introduction

Biosurfactants are surface-active agents synthesized by some bacteria, yeast and filamentous fungi (FIECHTER, 1992). They are amphipathic compounds with both a hydrophobic and a hydrophilic domain. Their properties of interest regard changing surface-active phenomena, wetting and penetrating actions, spreading, hydrophobicity and hydrophilicity, emulsification and deemulsification, detergency, gelling, foaming, metal sequestration, and antimcrobial action (HAFERBURG et al., 1986; KOSARIC et al., 1987; LANG et al., 1989; COOPER & ZAJIC, 1990; LIN, 1996).

Many microorganisms can synthesise biosurfactants during growth on hydrocarbon substrates (DESAI & BANAT, 1997). Especially, renewable and cheaper substrates, such as olive oil mill effluent (OOME) is preferred for production (MERCADÉ et al., 1993; CAMEOTRA & MAKKAR, 1998; SIDAL & OZKALE-TASKIN, 2003). Some biosurfactants are known to have therapeutic applications as antibiotics and antifungal or antiviral compounds. Several surfactant-resistant and sensitive strains of bacteria have been isolated by artificial or spontaneous mutagenesis and reported to posses altered structures and functions in their cell membranes (NISHIKAWA et al., 1979; VAARA, 1981; ISHIKAWA et al., 2002). Biosurfactants may disturb the membrane structure through interaction with phospholipids as well as membrane proteins. The biological function of biosurfactants has not been completely understood yet; e.g., these substances, when excreted into the medium, they emulsify hydrocarbons, and when located in the structure of the cell wall, they facilitate the penetration of hydrocarbons to the periplasmic space.

The main aim of this study was to obtain some understanding concerning the mechanism of the antimicrobial action of biosurfactant rhamnolipid.

Material and methods

Microorganism
The *Pseudomonas* sp. showed the highest ability to synthesize the biosurfactant rhamnolipid among the 10 *Pseudomonas* rhamnolipid-synthesizing strains, so that this strain was chosen for biosurfactant production. Stock culture was maintained on nutrient agar slants at 4 °C and subcultured every 15 days.

Medium
The optimal media used for the growth of the *Pseudomonas* sp. strain that would produce rhamnolipid were the Medium A (45 mL; g/L): $NaNO_3$, 1.28; K_2HPO_4, 0.87; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 0.1; KCl, 0.2; Tris (hydroxymethyl) aminomethane, 6.5; glucose, 20; and the Medium Mineral Salt Solution (5 mL; g/L): $FeSO_4\ (NH_4)_2SO_4 \cdot 6H_2O$, 0.116; H_3BO_3, 0.232; $CoCl_2 \cdot 6H_2O$, 0.41; $CuSO_4 \cdot 5H_2O$, 0.008; $(NH_4)_6Mo_7O_{24} \cdot 4H_20$, 0.022; $ZnSO_4 \cdot 7H_2O$, 0.174 (RAMANA & KARANTH, 1989). Initial pH of each of the

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