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FULL LENGTH ARTICLE

Selection of *Pseudomonas aeruginosa* for biosurfactant production and studies of its antimicrobial activity

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Abstract Biosurfactants are generally microbial metabolites with the typical amphiphilic structure of a surfactant. This study investigated potential biosurfactants production of *Pseudomonas aeruginosa* ATCC-10145 and *Bacillus subtilis* NCTC-1040 using glucose and *n*-hexadecane as substrates separately and compared it with the production in conventional medium. *Pseudomonas aeruginosa* growing in BHMS (Bushnell hass mineral salt) medium with glucose as substrate decreased the surface tension from 72 of distilled water to 32 mN/m, this strain had higher reduction than *Bacillus subtilis* among all the substrates tested. The selection of *Pseudomonas aeruginosa* for the separation of biosurfactant was determined. The crude biosurfactant was extracted from the supernatant and the yield of the crude biosurfactant was about 1 g/l. Some surface properties of rhamnolipids biosurfactant were evaluated. It also showed antimicrobial activity against different bacteria and fungi strains. The crude biosurfactant showed good action as antimicrobial activity against different bacterial and fungal species.

1. Introduction

Biosurfactants are biological amphiphatic compounds consisting of hydrophilic and hydrophobic moieties. where the hydrophobic moiety is either a long chain fatty acid, hydroxy fatty acid, or α-alkyl-β-hydroxy fatty acid and the hydrophilic moiety can be, a carbohydrate, an amino acid, a cyclic peptide, a phosphate, a carboxylic acid or alcohol, etc. [1].

In the past few decades, biosurfactants had gained attention because they exhibited some advantages such as biodegradability, low toxicity, ecological acceptability and ability to be produced from renewable and cheaper substrates [2,3].

The first microbiological biosurfactants on the market were sophorolipids. Of all currently known biosurfactants, rhamnolipids have the highest potential for becoming the next generation of biosurfactants introduced in the market [4].

Microorganisms have been reported to produce several classes of biosurfactants such as glycolipids, lipopeptides, phospholipids, neutral lipids or fatty acids and polymeric biosurfactants [5–7].

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Majority of known biosurfactants are synthesized by microorganisms grown on water immiscible hydrocarbons, but some are produced on water soluble substrates such as glucose, glycerol, and ethanol [8].

Up to now, the most commonly isolated and best studied groups of biosurfactants are those of glycolipid compounds and phospholipids [9]. Rhamnolipids are glycolipid compounds produced by *Pseudomonas* sp. which could reduce water surface tension and emulsify oil [10–12]. These compounds are biodegradable and have a potential industrial and environmental application.

Among the many classes of biosurfactants, lipopeptides from *Bacillus subtilis* were particularly interesting because of their high surface activity and therapeutic potential [13,14].

The present study aimed in:

1. Production of biosurfactant by *Pseudomonas aeruginosa* ATCC-10145 and *B. subtilis* NCTC-1040 grown on two carbon sources separately.
2. Selection of the best bacterial strain which has higher reduction of surface tension.
3. Separation and evaluation of some surface properties of the crude biosurfactant.
4. Tested the biosurfactant activity for antimicrobial activity against a broad spectrum of bacteria and fungi.

2. Experimental

2.1. Bacterial strains

Pseudomonas aeruginosa ATCC-10145 was supplied by the microbial resources center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. *Bacillus subtilis* NCTC-1040 was supplied from Application of Biotechnology in the Field of Petroleum Industry Lab, Department of Process, Design and Development, Egyptian Petroleum Research Institute (EPRI), Cairo, Egypt.

2.2. Inoculum and media preparation

The bacterial strains were streaked on a nutrient agar slant and incubated for 24 h at 30 °C. Two loops of culture were inoculated in 25 ml of nutrient broth in a 50 ml Erlenmeyer flask and incubated in a rotary shaker 150 rpm at 30 °C for 8–12 h until cell numbers reach 10^8 CFU/ml, an aliquot of 2 ml of inoculum was transferred to 100 ml of Bushnell haas mineral salt medium (BHMS) in a 250 ml Erlenmeyer flask and the cultures were incubated on a temperature controlled shaker incubator at 150 rpm and 30 °C for 96 h, the medium contained (g/l): dipotassium phosphate 1.0, magnesium sulfate 0.2, calcium chloride 0.02, potassium dihydrogen phosphate 1.0, ammonium nitrate 1.0, ferric chloride 0.05. The carbon sources carbohydrate (glucose) was added to make the final concentration 1% (w/v). The hydrocarbon (*n*-hexadecane) was added at 1% (v/v). Samples were collected at different time intervals (0, 24, 48, 72 and 96 h) and submitted to analysis. The experiments were conducted in three independent replicates.

2.3. Analytical measurements

a. Biomass determinationAliquots measuring 2 ml at different time intervals of culture were taken in Eppendorf tubes and centrifuged at 10,000×*g* for 10 min. Biomass obtained was dried overnight at 45 °C and weighed [15].

b. Separation of the crude biosurfactantRhamnolipids biosurfactant produced by *Pseudomonas aeruginosa* was recovered from the culture supernatant after the removal of cells by centrifugation at 10,000×*g* for 20 min. Rhamnolipids were then precipitated by acidification of the supernatant to pH 2.0 and allowing the precipitate to form at 4 °C overnight. The precipitate thus obtained was pelleted at 10,000×*g* for 15 min, the precipitate was dissolved in 0.05 M sodium biocarbonate (pH 8.6), reacidified, and recentrifugation at 12,000×*g* for 20 min, following centrifugation, the precipitate was extracted with chloroform/methanol (2/1) three times. The organic solvent was evaporated using a rotary evaporator and a yellowish oily residue was obtained [16].

2.4. Estimation of biosurfactant activity

The activity of the biosurfactant was determined by measuring:

a. Oil displacement test

The oil displacement test is a method used to determine the surface activity by measuring the diameter of the clear zone after 96 h of incubation period, which occurs after dropping a surfactant-containing solution on a thin layer of oil on water. The oil displacement test was done by adding 40 ml of distilled water to a petri dish with a diameter of 10 cm. After that, 15 µl of crude oil was dropped to form a thin oil layer on the surface of the water, and the 10 µl of a test solution was dropped on to the surface of oil. The test was conducted at room temperature. The maximum diameter of the clear zone was observed under light and measured [17].

b. Surface tension values

The surface tension values were measured on a ring tensiometer (krüss-tensiometer K6) using the cell free culture (50 ml) at 28 °C at different time intervals, while a solution of 0.1% by weight was tested at 28 °C when evaluating the crude biosurfactant [18].

c. Foam height

Foaming of biosurfactant in culture medium was determined by shaking vigorously the supernatant (10 ml) after 96 h of incubation period for 2 min and then foaming was calculated according to the following equation [19].

$$\text{Foaming} = \frac{\text{Height of foam}}{\text{Total height}} \times 100$$

d. Critical micelle concentration (CMC)

The critical micelle concentration values of the biosurfactant were determined using surface tension method. The CMC was determined from a semilog plot of surface tension versus rhamnolipid concentrations [16].