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Mining of biosurfactant producing bacteria from soil

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Abstract

Biosurfactants are surface active compounds produced by microorganisms. They have both hydrophobic and hydrophilic domains and can lower the surface tension and the interfacial tension between two liquids or between a liquid and a solid. They have unique properties such as non-toxicity, easy biodegradability, eco-friendliness, high stability, higher foaming, high selectivity and specific activity. Biosurfactants have potential uses in the pharmaceutical, food, cosmetic, pesticide and oil industries. In this study, out of a total of thirty isolates were randomly selected for checking biosurfactant activity. Biosurfactant production was confirmed by oil displacement and drop collapse tests. The Critical Micelle Concentration (CMC) and surface tension reduction were analyzed by the tensiometer. The isolate No. 3 was found to have Critical Micelle Concentration (CMC) as 60 mg/L and lower the surface tension of water by 35.6 mN/m.

The term "surfactant" is derived from the phrase "surface active agent," which is used in every industrial sector, from household detergent to drilling mud and food products to pharmaceuticals[2,8,11,13,16]. Surfactants are surface active compounds that reduce the surface tension and interfacial tension at the interfaces (solid-liquid, liquid-liquid, or vapour-liquid)[7]. An interfacial boundary exists between two immiscible phases[4]. Surfactants are organic amphiphilic compounds that contain both hydrophobic (head part of the surfactant) and hydrophilic (tail part of the surfactant) moieties[10,15]. As a result, surfactant contains both water-insoluble, *i.e.*, water repellent and water-soluble, *i.e.*, water-loving groups. Groups of microorganisms that produce surface-active agents are known as biosurfactants. They could be produced from renewable resources (micro-organisms, plants and animal oils) and petrochemical feedstock[1]. Recently, interest in biosurfactants has increased because of their diversity, selectivity, flexibility in operation, higher ability to produce foam, less toxicity, greater stability under extreme conditions and are more ecofriendly than chemical surfactants[6,12]. Biosurfactants are produced by a variety of microorganisms, mainly bacteria, fungi and yeast and are diverse in chemical composition and nature.

Their amount depends on the type of microbes producing a particular biosurfactant. Microorganisms can carry out biosurfactant production when grown either on insoluble substrates (hydrocarbons, oils and waxes) or soluble ones (carbohydrates). In the present study, efficient biosurfactant-producing bacteria were isolated from soil samples.

Sample collection :

The soil sample was collected in a sterile container. After sample collection, the sample was immediately stored at 4°C till further use.

Bacterial isolation : Bacterial isolates were isolated using the standard procedure described by Saravanan and Vijayakumar[13].

Oil spreading assay : Oil spreading assays were performed as per the standard procedure of Morikawa *et al*.,[9].

Drop collapse assay : Drop collapse assays were performed as per the standard procedure of Bodour and Maier in 1998.

Surface tension analysis : Surfaces were checked as per the standard procedure of Du Noüy *et al*.[5].

Isolation of Bacterial isolates :

The soil samples were enriched by inoculating them in sterile mineral salt medium (MSM) broth. Five gram of soil were inoculated into 100 ml mineral salt medium broth and kept on a rotary shaker at 200 rpm at 37°C. After 72 hours of incubation, the samples were serially diluted and spreaded on Nutrient Agar Medium. On nutrient agar medium plates, thirty bacterial colonies were seen after 72 hrs of incubation in Biological Oxygen Demand (BOD) at 37°C.

Screening for Biosurfactant production :

All bacterial isolates were cultivated aerobically in an Erlenmeyer flask (500 ml) containing 100 ml of mineral salt broth. Inoculating with a loopful of bacterial culture from nutrient agar plates into flasks containing sterilized mineral salt broth were put on shaker at 37°C at 200 rpm for 72hr. After 72hr of incubation, from each flask‘s 50ml of culture broth was centrifuged for 15 minutes at 6000 rpm at 4°C and the supernatant was filtered through a 0.45µm pore size filter (Merck Millipore). After centrifugation, supernatant was collected in sterilized centrifuge tubes and drop assay, oil spreading assay and surface tension assay were performed. All the screening experiments were done in triplicate and the mean values were taken as results (Table-1).

On the basis of a screening experiment, out of thirty bacterial isolates, only three isolates had biosurfactant activity (Table-1). Biosurfactant-producing capacity in liquid medium was found to be associated with oil displacement activity. Oil displacement activity therefore appears to be a good screening criteria for searching for biosurfactant-producing strains. In 72-hour grown culture, the surface tension of the broth was reduced from an initial value of 63 to 30 dyne/cm in cell-free media. Isolate number 3 was formed as the most effective biosurfactant isolate. The surface tension of Isolate number 3 was 30 mN/m and its critical micelle concentration (CMC) was 0.01 g/I,

Table-1. Oil spreading assay, Drop collapse assay and Surface tension measurement

Isolates	Oil spreading assay	Drop collapse assay	Surface tension measurement
Isolate 1	Oil spreading with a clear zone of 1.5 to 3.0 mm	Drop collapse after 1 minute	39 to 49 mN/m
Isolate 2	Oil spreading with a clear zone of 1.5 to 3.0 mm	Drop collapse after 1 minute	39 to 49 mN/m
Isolate 3	Oil spreading with a clear zone of 1.5 to 3.0 mm	Drop collapse within 1 minute	Less than 39 mN/m

indicating its powerful surface tension reducing property.

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