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AN ENVIRONMENTAL FRIENDLY ALTERNATIVE TO CHEMICAL SURFACTANTS BY EXPLORING BIOSURFACTANT PRODUCED BY *ACINETOBACTER BAUMANNII* ISOLATED FROM DIESEL OIL CONTAMINATED SOIL

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ABSTRACT

In this paper a diesel oil degrading bacteria was studied for the purpose of bio surfactant production and screened by drop collapsing assay and emulsification activity. Molecular compositions of crude bio surfactants evaluated by FT-IR. This indicated the presence of lipid and protein moieties useful for surfactant activities in industrial environments to be replaced instead of chemical surfactants, thus being environment friendly. It is noticed that properties like emulsification specificities of the lipoprotein produced by the isolate can be applied to microbial biosurfactant as possible alternatives to chemical surfactants.

KEY WORDS: Diesel oil, Microbial, Bio surfactants, Emulsification, FT-IR.

INTRODUCTION

The enormous market demand for surfactants is currently met by numerous synthetic, mainly petroleum-based chemical surfactants. These compounds are usually toxic to the environment and hardly degraded by causing damage to the environment. They may bio-accumulate and their production, processes and by-products can be environmentally hazardous. These hazards caused by synthetic surfactants have drawn much attention to microbial biosurfactant (Kiran *et al*., 2009). Tightening environmental regulations and increasing awareness for the need to protect the ecosystem have effectively resulted in an increasing interest and researches in biosurfactant as possible alternatives to chemical surfactants.One of the mechanisms used by these microorganisms for hydrocarbon degradation is through production of extracellular biosurfactant (Korayem *et al*., 2015). A variety of microorganism, including bacteria, fungi and yeasts has been reported to produce biosurfactant (Krishnaswamy *et al*., 2008). Chemically synthesized surfactant have been used in the oil industry to clean up of oil spills as well as to enhance oil recovery from oil reservoir. These compounds are not biodegradable and can be toxic to environment (Ashtapurtre *et al*., 1995 and Jacobucci *et al*., 2009)

Microbial biosurfactant have recently been recognized as important microbial products with properties applicable in a number of industries and processes. Being capable of lowering surface and interfacial-tension, biosurfactant are today thought to be efficient replacers and possible enhancer of chemically synthesized surface-active agents. The absence of toxicity, biodegradability, specificity etc. makes these microbial products both attractive for specific industries and environmentally acceptable (Banat *et al*., 2012).

The microbes present in the soil first recognize the oil and its constituent by biosurfactants and bioemulsifiers, and then they attach themselves and use the hydrocarbon present in the petroleum as a source of energy and carbon. The low solubility and adsorption of high molecular weight hydrocarbons

limit their availability to microorganisms. The addition of biosurfactants enhances the solubility and removal of these contaminants, improving oil biodegradations rates (Bijay Thapa *et al.*, 2012).

Biosurfactants have considerable potential in commercial applications within various industries. They are indispensable component of daily life and are widely used in the pharmaceutical, cosmetic, food, agricultural, public health, health care, waste utilization, environmental pollution control such as hydrocarbon degradation, pesticide degradation, corrosion inhibition, heavy metal detoxification (Desai and Banat, 1997).

MATERIALS AND METHODS

Screening for Biosurfactant Production

YPG medium broth containing yeast extract (5g/l, peptone (5 g/l) and glucose 15 g/l was prepared. The flasks were sterilized, inoculated with the isolate and incubated for 7 days in shaking condition at room temperature. After incubation, the media was centrifuged at 10,000 rpm for 10 min. to obtain a cell free supernatant. The culture supernatant was then tested for the presence of biosurfactant. The following screening assays were carried out for detecting biosurfactant production.

Drop collapsing assay

Qualitative drop collapsing assay was performed by a modified technique described by Boudour and Miller (1998). 2 µl of diesel oil was added to the 96 well microtitre plates. The plates were equilibrated for 1 h at 37 ^{0}C for forming a uniform thin coating of oil in the well. 5 µl of culture supernatant was added to the surface of the oil. The shape of the drop on oil surface was observed after 1 min. Positive result was indicated by collapsing of the oil drop and that drop remain beaded were scored as negative which was examined with distilled water as control.

Emulsification index (EI)

Emulsification activity was measured using two methods: the measurement of optical density at 540 nm and the measurement of emulsion stability after 24 hour (emulsification index; E_{24}). In the first method, 2 ml samples of cell free supernatant were added to as screw-capped tubes containing 2 ml distilled water, and the solution was mixed with 1 ml of diesel. After a vigorous vortex for 2 min, the tubes were allowed to sit for 1 hour to separate aqueous and oil phase, before measuring the absorbance at 540 nm. Aqueous phase was removed carefully and OD at 540 nm was measured and compared with uninoculated broth used as negative control (Patel and Desai, 1997). Emulsification activity was defined as the measured optical density at 540 nm. In the second method, a mixture of 6 mL of studied hydrocarbon (diesel) and 4 mL of the culture supernatant was vortexed at a high speed for 2 min. After 24h, the emulsification index (E_{24}) was calculated by measurement of the height of the emulsion layer (a), divided by the total height (b),multiplied by 100 (Cooper and Goldenberg, 1987).

Characterization of Biosurfactant

Molecular characterization of obtained biosurfactant was done by FTIR. The spectral bands were taken in mid infrared absorption 4000 to 400 cm^{-1} with 30 scan speed. The spectrum was recorded using ATR (Attenuated Total Reflectance). The digitalized spectra were processed using OMNIC® soft ware. The compounds were identified by comparing the data with the standard data.

RESULTS AND DISCUSSIONS

Initial work of this study proved that peptone as a nitrogen source is an essential component for the growth of *A.baumannii*. Hence YPG (Yeast Peptone Glucose) medium containing yeast extract (5 g/l), peptone (5g/l) and glucose 15 g/l was used for studying the production of biosurfactant by *A.baumannii.* The inoculated media was incubated for 7 days in shaking condition at room temperature. After incubation, the media was centrifuged at 10,000 rpm for 10 min. to obtain a cell free supernatant. The culture supernatant was then tested for the presence of biosurfactant. Drop collapsing assay is an indicative of the surface and wetting activities. The addition of culture supernatant on oil coated well lead to the formation of flat drops within a minute. Hence the isolate was scored as biosurfactant producers.

Emulsification activity is one of the criteria to support the selection of potential biosurfactant producers. Emulsifying activities (E_{24}) determine productivity of bioemulsifier OD at 540 nm was found as 0.824. The diesel degrading *Acinetobacter* were tested for their emulsification activity with diesel and kerosene. Water as control showed 32.05% after 24 hr. Emulsification index of the

culture supernatant of *A.baumannii* was measured for diesel as 65.51 % after 10 min which remained almost stable after 24 hr (65.47%) which accounts for good emulsification. However culture supernatant was unable to emulsify and stabilize emulsions with kerosene (23 %) (Figure 1). These results indicate that the biosurfactant produced by the isolates had high emulsification specificity toward diesel oil and a rather very low efficiency with kerosene. This finding suggests that the emulsifier's activity depends on its affinity for specific hydrocarbon substrates.

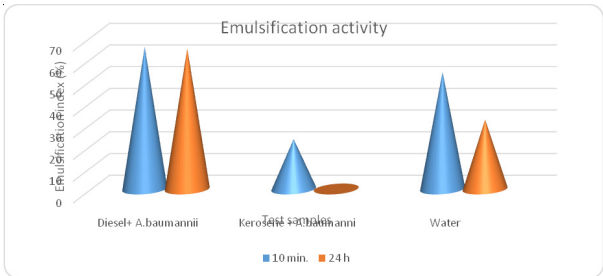

Fig. 1. Comparison between the emulsification indexes of the samples tested.

Most biosurfactant are specific and emulsify different substrate differently. The supernatant obtained on centrifugation of the media containing biosurfactant emulsified diesel and kerosene. Emulsions with diesel were found to be stable for 24hrs. But the emulsions with kerosene were not stable as emulsification activity reduced within 24hrs. This observation emphasizes upon selection of specific biosurfactant for particular hydrocarbon pollution.

Emulsification enhances the biodegradation of hydrocarbons by increasing their bioavailability to the microorganisms involved in the process.

Molecular compositions of crude biosurfactants produced by *Acinetobacter baumannii* were evaluated by FT-IR. The compounds were identified by comparing the data with the standard data available at The South India Textile Research Association (SITRA), Coimbatore and literature reviews. Figure-2 shows the infra red spectrum of the isolated biosurfactant. The wave number 3241 cm^{-1} indicated the presence of amino group. The C-H bond was observed at wave number 2958, 2855 and 1410 cm^{-1}. The wave number 1070 cm^{-1} is because of C-O stretching vibrations and the wave number 607 cm^{-1} is due to O-H bond. The peaks observed altogether indicate the presence of lipid and protein moieties.

From the FT-IR data obtained and literature reviews it is evident that lipoprotein form of biosurfactant is dominant in the *Acinetobacter* sps.

CONCLUSION

The isolate was screened for biosurfactant production. Biosurfactants /bioemulsifiers play a key role in emulsifying hydrocarbons. The isolate

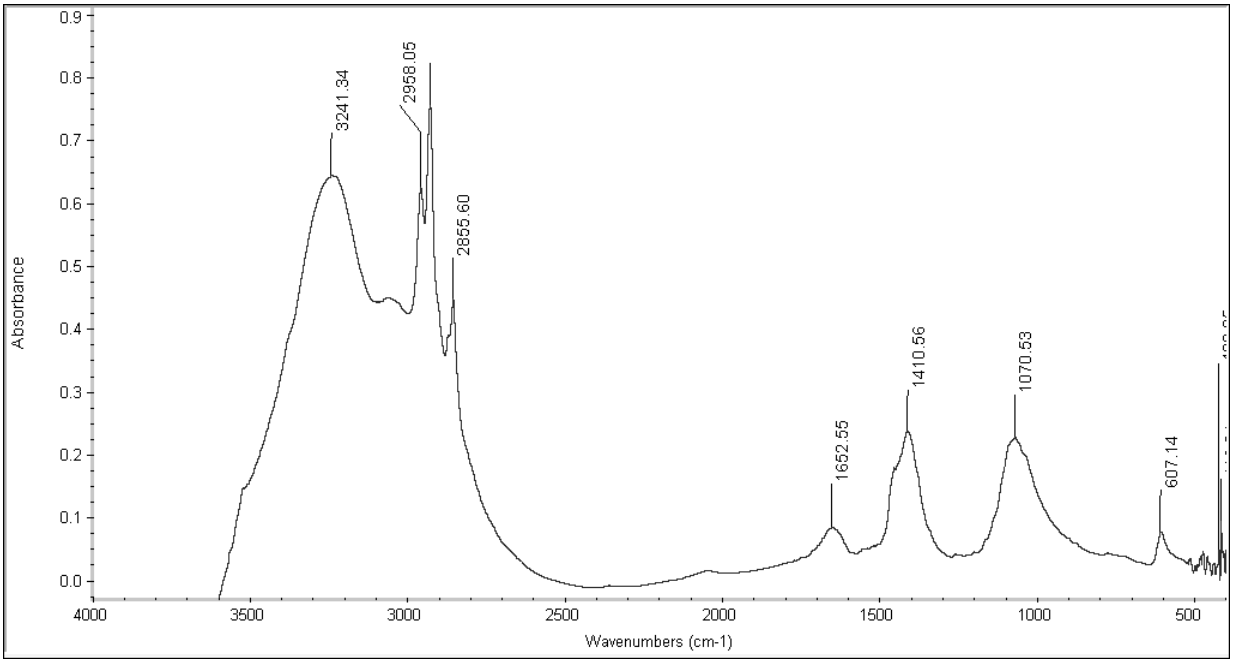

Fig. 2. FT-IR spectra of crude biosurfactant produced by *Acinetobacter baumannii*.

showed good emulsification activity of up to 65% along with positive result in drop collapsing test. *Acinetobacter* sps. manages to uptake diesel effectively for rapid growth via mechanism such as secretion of extracellular emulsifier to increase the solubility of diesel. This surface active product (biosurfactant) enhance the contact between bacteria and hydrophobic hydrocarbon and helps them to utilize hydrocarbon compounds such as diesel which are usually unavailable to them in aqueous phase due to their low solubility. The excellent supernatant emulsification activity for diesel is also a proof of similar strategy for the enhanced uptake of hydrophobic carbon source by this diesel assimilatory isolate.

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