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Production and Stability Studies of the Biosurfactant Isolated from Alkaliphilic Bacterium SJS1

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Abstract:

Background: Mostly oil spills occurs in sea ocean and coastal areas, required the best candidate for the degradation of hydrocarbons such as chemical surfactant and synthetic chemicals to minimize such oil spills are primarily effective strategies with environmental risk so the aims of these studies the isolation and characterization of a alkaline and halotolerant bacteria which was isolated from Lonar Crater and able to utilize different oil as carbon source and produces biosurfactant.
Methodology: In present investigation was to determine surfactant substance producing bacterium of Lonar Lake using minimal salt medium with various oil as a carbon sources. Biosurfactant-producing microorganisms were isolated and screening was done on the basis of Drop collapse test, Oil spread and emulsification index.
Result: A total of six bacteria were isolated from the water and sediment samples collected from the of Lonar crater, India. Out of them SJS1was selected for production and, partial characterizations of biosurfactant. A Gram negative bacterium was studied by morphological, physiological and biochemical characterization. The bacterium SJS1 grew in medium containing sodium chloride (NaCl w/v) from 0.5 to 7% and at pH 7–12. The production of a biosurfactant by Bacteria SJS1 was studied to evaluate the influence of the concentration of different oil. SJS1 was able to grow at high salinity conditions and produce biosurfactants. The organism grew and produced biosurfactant when cultured in salinities up to 3 g l-1and temperatures up to 60°C. The biosurfactant was highly stable over broad temperature, pH and NaCl, showing excellent thermostablity, and haloalkaline tolerant nature. The biosurfactant produced by the organism emulsified a range of oil with as Ground nut and Coconut oil best substrate whereas Sesame oil was the poorest.
Interpretation: This is valuable information for biosurfactant production and optimization has bright future towards the improvement and production of novel biosurfactant for entirely new areas of environmental and biotechnological applications. The results confirmed, their enhancing capability on both efficiency and rate of hydrocarbon degradation from water and soil.

Keywords: Haloalkaliphiles, Biosurfactant, Bioremediation, SJS1

INTRODUCTION

Biosurfactants is environment friendly, structurally diverse surface active compounds synthesize from biological origin at particular physiochemical parameters (Mukharjee et al., 2006). Use of commercially synthesize chemical based surfactant are the part of our daily routine but the excessive use of such chemically derived surfactant produce environmental toxicity (Ron et al., 2001; Mukharjee et al., 2006). Nowadays biosurfactant took position in every part of human life. Biosurfactant have been used in different fields like pharma industries, food industries and chemical industries, etc. Since a class of amphipatic molecules have ability to reduce surface or interfacial tensions between solids, liquids and gases and beyond this activity the biosurfactant shows anti-fungal, anti-viral, metal-sequestration capacities and also have wide applications in the petroleum industries oil recovery procedures (Mulligan et al., 2001).

A huge number of microbes produce a variety of biosurfactant differing in their chemical structures and properties which depends upon the physical and chemical conditions provided for their growth (Ghazali et al., 1997). Number of biosurfactant are complex molecules that may be made up of peptides, glycolipids, fatty acids, phospholipids, etc. (Desai et al.,1997) and apart from these they can be produced from various microbes using proteins, lipids and carbohydrates which are renewable substrates for biosurfactant production (Fiechter et al.,1992). The productions of sophorolipids by *Candida bombicola* by using glucose and palm oil (Sasidharan et al., 1993). The oils of Castor, Sunflower and Soyabean has been used as a substrate by *Serretia marcescens* to produce biosurfactants (Ferraz et al., 2002). It is found that glycerol, fructose, glucose, n-paraffins and vegetable oils are used as substrates by *Pseudomonas* to produce rhamnolipid type biosurfactants (Koch et al., 1991). Actinomycete, which are abundantly present in the soil and also plays an important role in the recycling of materials in nature, can be used for major production of biosurfactants and antibiotics, (Oskay et al., 2004; Augustine et al., 2005; Imasda, 2005; Richter et al., 1998). Many biosurfactant producing microbial strains have been isolated from various heavy metal- and oil contaminated substrates like soil, seawater and marine sediments (Olivera et al., 2009; Das et al., 2008; Yakimov et al., 1998) and few species of *Bacillus, Pseudomonas, Aeromonas, Cellulomonas, Serratia,* and fungi that grow in green coffee beans (Silva et al., 2000) are been found to produce biosurfactants (Desai and Banat, 1997). Hopefully microorganisms can overcome these issues on choosing potent microbial strains producing a higher yield of biosurfactant. Oil spill is the release of a liquid petroleum hydrocarbon into the environment, especially marine area, sea, ocean, due to human activity. These oil spills are affects on the habitat and ecosystem so it is required to deterioration of hydrocarbon. Mostly oil spills occurs in sea ocean and coastal areas, required the best candidate for the degradation of hydrocarbons such as chemical surfactant and synthetic chemicals to minimize such oil spills are primarily effective strategies with environmental risk so the aims of these studies the isolation and characterization of a alkaline and halotolerant bacteria which was isolated from Lonar Crater and able to utilize different oil as carbon source and produces biosurfactant.

MATERIALS AND METHODS

Sampling site description:- The Alkaline Lonar Soda Crater (ALSC) in Deccan Trap of India is unique extraterrestrial ecosystem and paradise of microbial biodiversity. Sediment sample of Lonar Crater was collected by using scoop from 5 meters distance from shoreline in 3 meters water depth and kept in sterile sampling bag. Surface water sample collected from the same area was collected in sterile plastic container from defined sampling sites (Dhundale and Hemke, 2015; Tambekar and Dhundale 2010; Joshi *et al.,* 2007).

Enrichment and isolation of bacterium

A standard enrichment strategy was used to isolate alkalitolerant and saline tolerant hydrocarbon-degrading microorganisms from sediment and water samples collected from Lonar crater. Ten grams of soil sample was transferred to 500 ml Erlenmeyer flask containing 100 ml of Minimal Salt Medium (MSM) (Kumar et al. 2008), with 2% (V/V) of crude oil as carbon source. Flasks were incubated at 37^{o}C on a rotary shaker (200 rev. min $^{-1}$) for 7 days. After 7 days 5.0 ml of the culture was transferred to fresh media containing crude oil and re-incubated for another 7 days. Following four cycles of such

enrichment, 1.0 ml of culture was diluted and plated on minimal salt agar plates containing crude oil as sole carbon source.

Isolation and biochemical characterization

After enrichment the samples were inoculated on solid nutrient agar plate and well isolated and morphologically different colonies were selected and stored in glycerol stock at -80°C. All these isolates were further characterized by standard biochemical test according to Bergey's manual of Determinative bacteriology

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Preliminary screening for biosurfactant production

After enrichment, the broth was centrifuged at 5000rpm for 20min then oil layer was discarded and supernatant were subsequently subjected for the preliminary screening (Tambekar *et al.,* 2012).

Detection of biosurfactant activity

Drop collapse test and oil spread test was carried out according to Youssef et al. (2004). Emulsification index (E 24) was determined by the addition of hydrocarbon to the same volume of cell free culture broth, mixed with a vortex for 2 min and left to stand for 24 h. The emulsification activity was determined as the percentage of height of the emulsified layer [mm] divided by the total height of the liquidcolumn [mm]. To study the stability of emulsion, emulsified solutions were allowed to stand at room temperature (Kumar *et al.,* 2008).

RESULTS AND DISCUSSION

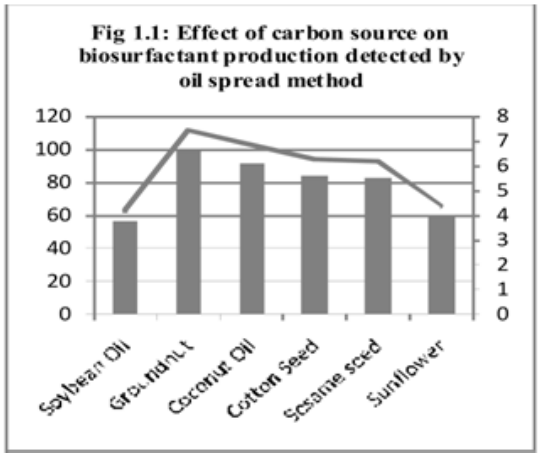
Table 1: Morphological, cultural and Biochemical Characterization of SJS1

Isolates	SJS1
Source	Sediment
Shape	Circular
Size	Pinpoint
Elevation	Convex
Margin	Entire
Opacity	Opaque
Consistency	Non sticky
Pigmentation	Pale yellow
Gram character	-
Motility	Motile
Catalase test	+
Oxidase test	-
Indol test	-
Vogas-prosker test	-
Methyl red test	-
Citrate utilisation test	-
Nitrite reduction test	+
Dextrose sugar	+
Lactose sugar	+
Maltose sugar	+
Sucrose sugar	+
Fructose sugar	+

Total six bacterial isolates obtained in the isolation exercise; cultural and morphological characteristics of all the strains were studied. The isolates were screened on the basis of biochemical

characteristics as described earlier. Out of these six bacterial cultures, SJS1 was revealed to negative Gram reaction, nonspore bearing and rods in cell morphology (Table 1). Then a various conventional phenotypic tests were used to characterization of bacterium SJS1. In present investigation was to determine surfactant substance producing bacterium of Lonar Lake using minimal salt medium with various oil as a carbon sources. All bacteria were tested for biosurfactant activity on the basis of Oil spread and emulsification index. The highest emulsifications index was achieved with bacterium SJS1 and screened for further studies. The effect of different carbon sources on production biosurfactant were synthesized by SJS1 studied. The maximum biosurfactant were produced when the ground nut oil was used as a carbon source for the SJS1 performed by oil spread method (7.5). Whereas, coconut oil (6.9) were found maximum biosurfactant production was detected by oil spreads test (Fig 1-4). Bacterium SJS1 showed drop collapse test positive. The biosurfactant production by SJS1was investigated for possible enhancement through the use of alternative oil. The coconut oil for SJS1 was found maximum biosurfactant production detected by emulsification index, 51.42 (100%) while the sesame oil cotton seed oil (37) was found minimum production of biosurfactant by SJS1. In the present studies, different carbon sources were used for maximum production of biosurfactant. Vasileva-Tonkova *et al.,* (2011), were studied the effect of rhamnolipid biosurfactant produced by *Pseudomonas fluorescens*. In the present studies the coconut oil was also obtained a well carbon sources for biosurfactant production. In the present study, various factors were investigated such as effect of pH, NaCl, temperature on biosurfactant production by bacterium SJS1.

The optimized bioprocess condition was pH 10 for SJS1 (6.2mm). The similar studies were performed by Khopde *et al.,* (2012). When pH (8 and 12) decreased or the production rate was decreased for the SJS1 while the pH was 12 the productions 7% were decreased on the basis of their ability to oil spread method. The SJS1 were found biosurfactant production on the basis of their ability to emulsification index, the SJS1 was found at pH 12 (52). When pH was decreased the production rate were also minimize due to the alkali-tolerant nature of bacteria and depends on alkaline stipulation. Optimum rate of biosurfactsnt production was also depends on the concentration of NaCl, in the present investigation the SJS 1 required 3% NaCl concentration for the optimum production. Khopade *et al.,* (2012) investigated Production and stability studies of the biosurfactant isolated from marine *Nocardiopsis* sp. B4. The strain B4 was found to be moderately halophilic in nature as maximum biosurfactant production was obtained in presence of 3% NaCl. The similar work was also performed by Thavasi *et al.,* (2008). *Nocardiopsis* sp. B4 were found optimum biosurfactant production at 38^0C while in present studies the SJS1 optimum biosurfactant production were found at 60^0C.



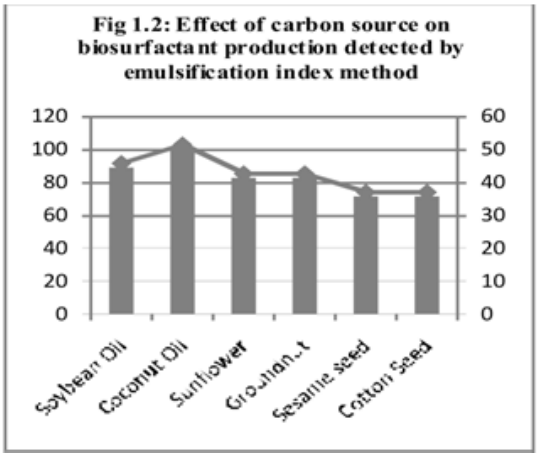
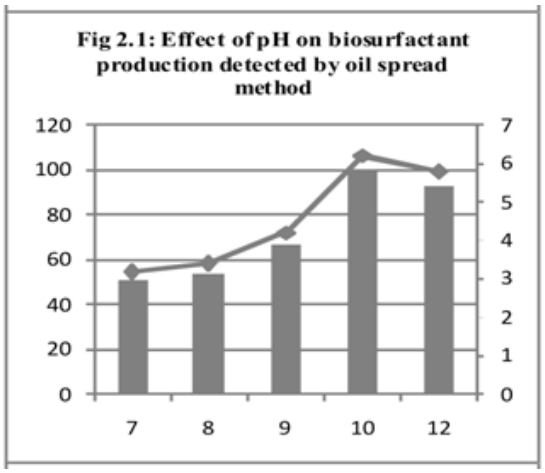
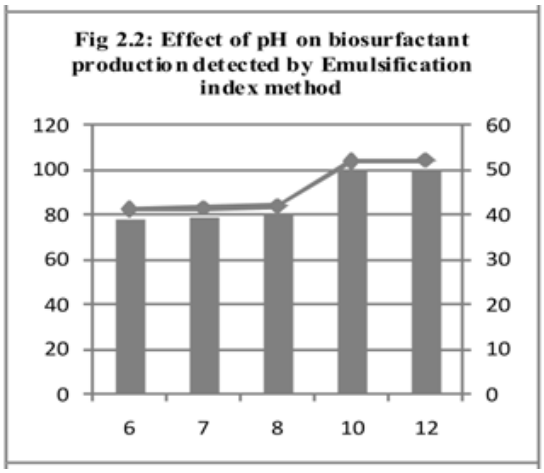


Figure 1:





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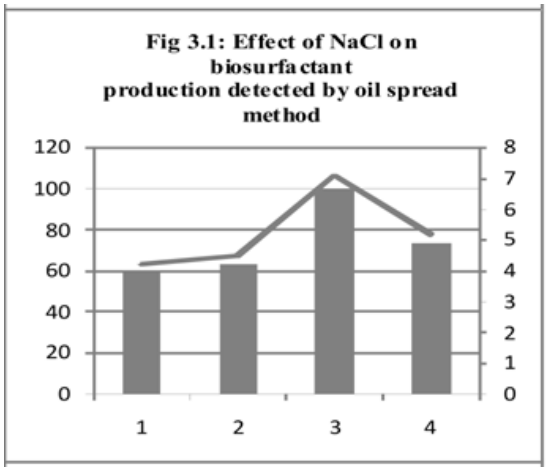

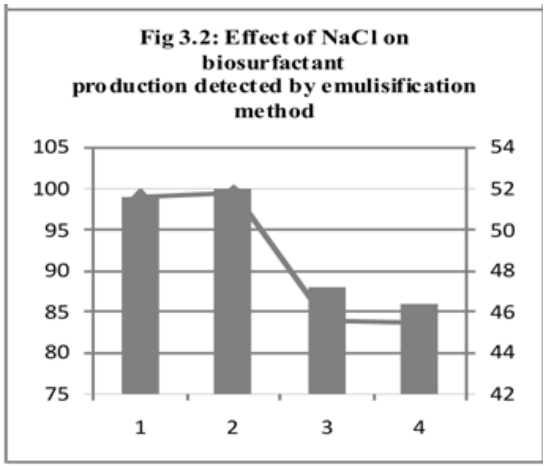

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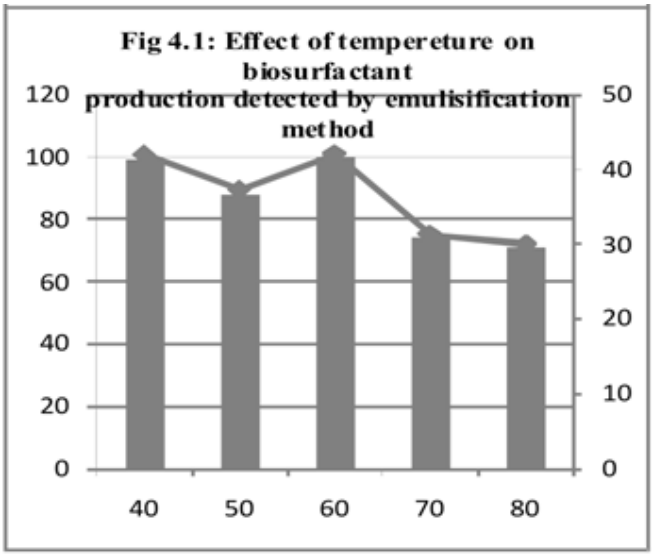

Figure 4:

CONCLUSION

Present investigation evident that the bacteria have a ability to reduce pollution and minimize environmental burden occurred due to manmade activity like increased oil spilled and urbanization. Biosurfactant is best option to overcome on toxicity problem created by commercially developed chemical based surfactants. This present study confirmed that the Lonar Crater is the habitat of Biosurfactant producer microbes and due to extreme condition of Lonar Crater. The biosurfactant showed tolerant to pH, salinity, temperature and production with various types of vegetable oils. It is suggested that the bacteria SJS1 are suitable and making it appropriate candidate for bioremediation of hydrocarbon contaminated soil and marine environment, costal area.

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