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Effect of metal ions on growth and biosurfactant production by Halophilic bacteria

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ABSTRACT

Halophilic microorganisms live in saline environment and have the ability to produce biosurfactant. These organisms have particular adaptation to increase stability in adverse environment that can be used in various fields of biotechnology. In present research work, the biosurfactant producing halophiles were isolated from sea water sample from coastal areas nearby Mumbai. The effect of different metal ions on growth and biosurfactant production was studied. 15 different bacterial colonies were obtained, among them 8 isolates showed capacity to produce biosurfactant. J1 strain from Juhu exhibited highest emulsification activity and was identified as Halobacterium salinarum. Supplementation of metal ions in medium significantly affected the growth of the bacteria. The growth and biosurfactant production was found to be maximum with magnesium ion (5mM). The study suggests that metal ions enhances the growth of halobacteria as well as increase the biosurfactant production.

Key words: *Biosurfactant, Emulsification, Halobacteria, Metal ions.*

INTRODUCTION

Biosurfactants are a diverse group of surface active agents produced by many living organisms [10].They have many industrial and environmental applications related to emulsification, foaming, detergency, wetting, dispersion, bioremediation, microbial enhanced oil recovery and solubilization of hydrophobic compounds [12]. Biosurfactant have advantages of ecofriendly, biodegradability, easily produced using renewable sources, possible regeneration, high specificity and less toxicity as compared to chemical surfactant [7]. Biosurfactant can be synthesized by different microorganisms such as bacteria, fungi, yeast and actinomycetes. Majority of the biosurfactants are produced by bacteria. Among bacteria, halophiles have an important role in production of biosurfactant. As the halophilic bacteria occur in highly saline environment, the biosurfactant produced by them can show high activity at extreme temperature, pH and salinity condition and hence can be exploited under extreme condition [2]. Biosurfactant production by halobacterium is highly influenced by media composition such as metal ions, carbon source, nitrogen source etc. Metal ions affect stability of the cell wall and the protein synthesizing apparatus [3]. Most enzymes involved in biosurfactant production require high concentration of metal ions for both activity and stability, for example malic enzyme involved in metabolic reaction show highest activity in presence of magnesium, cobalt and manganese [4].

The present study aims to isolate biosurfactant producing extreme halophiles and evaluate the effect of metal ion on its growth and biosurfactant production.

MATERIALS AND METHODS

Sample collection
Sea water samples were collected from coastal areas nearby Mumbai region such as Kalyan, Airoli, Juhu, Mumbai (Fort) and Bhayander. All samples were collected in sterile glass bottles, kept in ice box and immediately transported to laboratory for further analysis.

Chemicals and Media
Microbiological media and medium ingredients were purchased from Hi-media laboratories (Mumbai, MH, India).

Enrichment of Halobacterium
Halobacterium salinarum (HS) medium was used for enrichment of *Halobacterium*. 20 ml of each sample was inoculated into a 100 ml sterile HS medium. The flasks were incubated at 37^0C for 1-2 weeks under shaking condition (150 rpm) until the growth is observed. 10 ml of primary enriched culture were transferred to a new flask with respective media with same incubation condition [9].

Isolation of Halobacterium
The enriched culture was diluted and plated on HS media for viable count. The plates were incubated at 37^0C for 5-7 days. After incubation colonies with different morphology were selected and purified.

Biosurfactant production
The selected isolates were subjected to biosurfactant production. It was performed using Standard medium (250 gm of NaCl, 160 gm of $MgCl_2.6H_2O$, 5.0 gm of K_2SO_4, 0.1 gm of $CaCl_2.2H_2O$, 1.0 gm of yeast extract , 1.0 gm of casamino acids and 2.0 gm of starch, 1000 ml D/W). 2 ml of saline suspension of each isolate (O.D. 0.9 at 600nm) was inoculated in 100 ml of Standard medium. The flask was incubated on shaker at 37^0C for seven days.After incubation the broth was centrifuged at 2000 rpm for 20 min.

Drop collapse test for qualitative analysis
Microtitre plates were used to check drop collapse test. Wells of plate were coated with 2 µl of mineral oil, 5 µl of the culture supernatant was added to the surface of oil. The shape of the drop on the oil surface was inspected after a minute. Biosurfactant producing cultures giving collapsed drop on oil were scored as positive '+'. Those cultures that gave rounded drops were scored as negative '-', indicative of the lack of biosurfactant production [2].

Emulsification activity assay for quantitative analysis
Emulsification activity was checked by kerosene. In a sterile test tube, 4 ml of Kerosene were added to 4 ml of cell free supernatant, and vortexed at high speed for 5 min. The mixture was allowed to stand for 24 hrs. Formation of emulsion indicates the production of biosurfactant. Then emulsification activity was measured after 24 hrs by dividing the height of emulsion formed by total height of solution in test tube and expressed as percentage [11].

Identification of maximum biosurfactant producing organism
The isolate giving maximum production of biosurfactant was identified on the basis of morphology and biochemical characteristics as described in Bergey's manual [13].

Effect of metal ions
Effect of metal ions on the growth and biosurfactant production of selected isolate was studied.

Effect on growth
HS medium was supplemented with different metal compounds (5mM) such as $MnSO_4$, $ZnSO_4$, $CaCl_2$, $FeSO_4$, $CoCl_2$, and $MgSO_4$ separately. 1ml saline suspension of selected isolate (O.D= 0.3 at 540nm) was inoculated in 100 ml of media in 250 ml side arm flask incubated on shaker at 37^0C and growth pattern was observed at 540 nm at regular intervals of 24 hours.

Effect on biosurfactant production
Emulsification activity was measured using standard medium was supplemented with different metal compounds (5mM) such as $MnSO_4$, $ZnSO_4$, $CaCl_2$, $FeSO_4$, $CoCl_2$, and $MgSO_4$.

RESULTS AND DISCUSSION

Isolation of Biosurfactant producing Halobacterium

From the samples collected from five different places 15 isolates were obtained. 2 isolates from Kalyan, 4 from Airoli, 2 from Gateway of India, 3 from Bhayander and 4 from Juhu area (Table -1). These 15 bacterial isolates with distinct morphology were studied for biosurfactant production. Among them 8 isolates showed positive results for biosurfactant production by the drop-collapse test (Table-1).

Table 1: Qualitative analysis (Drop- collapse test) of biosurfactant production by isolate

Sr. No.	Sample	Isolates	observation	Sr.No.	Sample	Isolates	Observation
1	Kalyan	K1	+	4	Juhu	J1	+
		K2	-			J2	+
2	Airoli	A1	+			J3	-
		A2	+			J4	-
		A3	-	5	Bhayandar	B1	+
		A4	-			B2	-
3	Gateway	G1	+			B3	-
		G2	+				

+ : collapsed drop (biosurfactant producer) *- : intact drop (non biosurfactant producer)*

Biosurfactant production: These 8 bacterial isolates were screened for their ability to produce biosurfactant with emulsification activity test (EA). All the isolates were able to produce biosurfactant with more than 25% EA. Maximum biosurfactant production was shown by J1 isolate (56%) and minimum by K1 isolate (26%). The bacteria isolated from Juhu was having greater ability of biosurfactant production compare to other bacteria. It may be due to environmental condition in this area which supported the growth for biosurfactant producing organism (Fig1).

Identification of maximum biosurfactant producing organism

The selected organism was found to be Gram negative rod shaped bacteria. It was showing positive results for oxidase, catalase, gelatinase and negative for urease. The strain utilized sugars like maltose, glucose, fructose, mannitol and mannose. On the basis of biochemical results the isolate was identified as *Halobacterium salinarum.* Similar studies at different sites are also reported by research workers. Biosurfactant producing halophilic bacteria isolated from the solar evaporated salt pond of Tuticorin, Tamilnadu, India and was found to be *Halobacterium halobium* [13]. Similarly biosurfactant production by *Pseudomonas spp.* isolated from oil well, Iran was studied by Hameed *et al.* [5].

Effect of metal ions on selected bacteria

Effect on growth

Supplementation of metal ions in cultivation medium (HS medium) improved the bacterial growth as compare to that in HS medium. $MnSO_4$ and $CoCl_2$ showed almost similar growth as compared with HS medium. A slight increase in growth was observed with $CaCl_2$ and $FeSO_4$ whereas growth was doubled with $ZnSO_4$. Among all the metal compounds $MgSO_4$ (5mM) gave maximum bacterial growth (3 times more than in HS medium) (Fig.2).

Effect on Biosurfactant production

From figure 3, it can be seen that biosurfactant production was increased with addition of metal ions in standard medium. All the metal ions such as zinc, calcium, manganese, iron and cobalt showed increased production in some extent (58%, 60%, 63%, 62% and 57%). Maximum production was observed in presence of magnesium ion (68%). Similar results with *Pseudomonas sp*. isolated from marine water was reported by Ibrahim *et al*. [6], wherein the culture media supplemented with different metal ions improved the bacterial growth and biosurfactant production. However he also reported that among the different metals, $MnSO_4$ showed increased biosurfactant production at concentrations of 5mM. According to [13], incorporation of Ca, Mn and Mg (200 mM) together in the growth medium showed higher biosurfactant production by *Halobacterium spp.* The results suggest that addition of Mg ions definitely improve the growth and biosurfactant production. The biosurfactant produced by marine microbes plays a crucial role in removal of pollutant metals and toxic elements from contaminated solutions. Their emulsification and surface active property makes them applicable for utilization in bioremediation and other oil recovery applications [8].

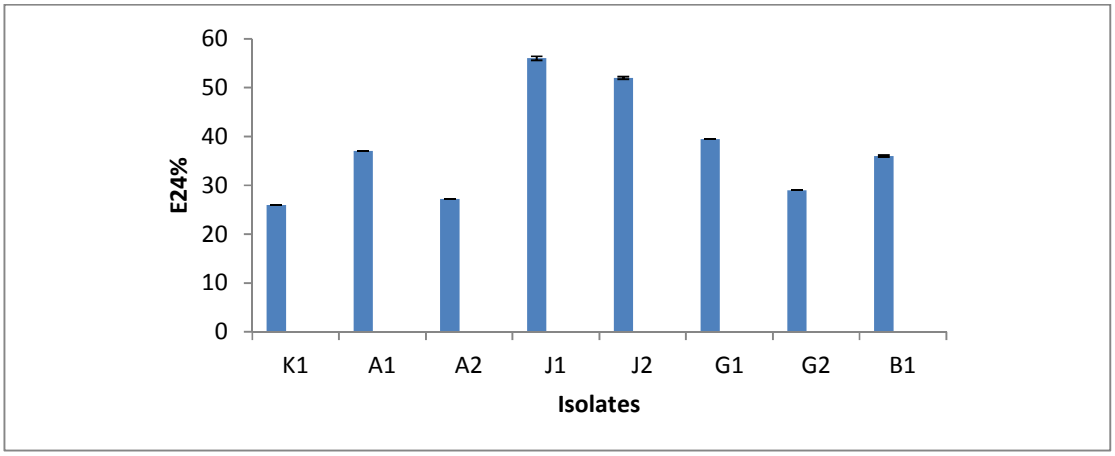

Fig.1: Emulsification activity of bisurfactant producing halophiles

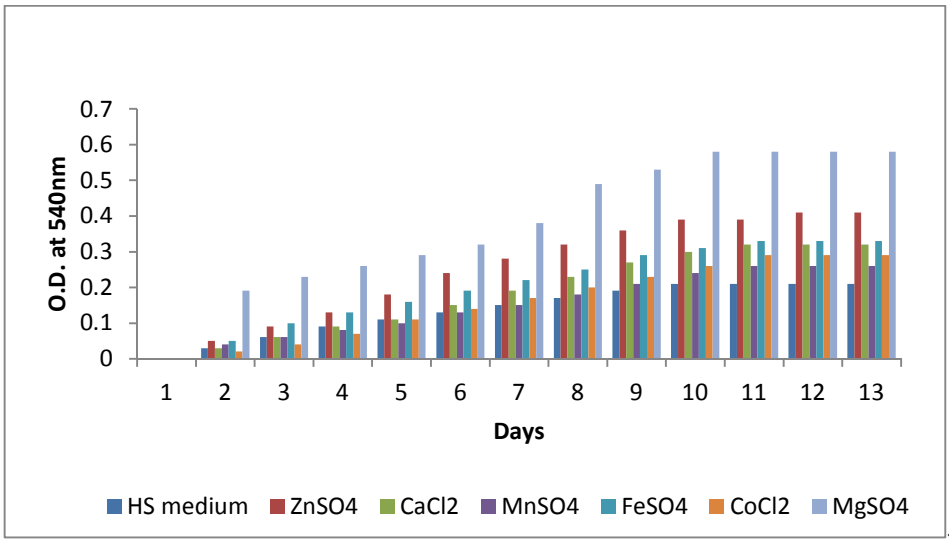

Fig.2: Effect of metal ions on growth of Isolate J1

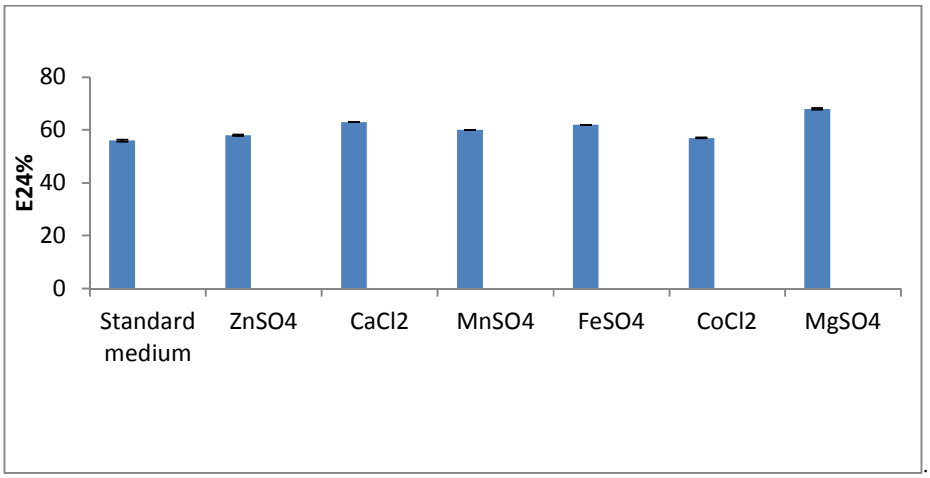

Fig.3: Effect of metal ions on emulsification activity of Isolate J1

CONCLUSION

The study showed that addition of different metal ions in HS medium and standard medium have a significant influence on growth and biosurfactant production of *Halobacterium salinarum.* In future it may be necessary to find out enzymatic system and its genetic characterization responsible for biosurfactant production which may play a significant role in bioremediation and microbial enhanced oil recovery.

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