Correlation between Growth and Biosurfactant Production in Bacteria Isolated from Oil Contaminated Sites

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Abstract: Biosurfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties and are surface active agent that are produced extracellularly or as a part of cell membrane by bacteria, yeast and fungi.In the present study, four different microorganisms were isolated from oil contaminated soils. Screening of biosurfactant producing potential strain was done using haemolytic activity. Using 16S rDNA sequencing the potential strains were identified as ***Klebsiella pneumoniae, Paenibacillus dendritiformis, Bacillus cereus and Alcaligenes faecalis.*** Growth studies vis-a- vis biosurfactant production was studied. It was found that *Alcaligenes faecalis* produced the biosurfactant in the least time of 48h whereas the other isolates produced the same after 72-96h.
Time required for product formation is an important but less studied aspect of process economics of industrial level production. In the context of this ,the present study shows that Alcaligenes faecalis is the best candidate for biosurfactant production.

Keywords: *Biosurfactant, time effect studies*

I. INTRODUCTION

Growing awareness towards the use of environmentally friendly technology has led to the development of alternatives to chemical surfactants. Biosurfactants (BS) are an example of such environmental friendly options. BS can be obtained from renewable resources, by microbial fermentation processes .BS have several advantages such as lower toxicity, higher biodegradability and diversity, effectiveness at extreme temperatures or pH values and widespread applicability. BS occur naturally in soil, which makes them acceptable ecologically.

Some of these BS are produced by plants also. Compared to plant sources, microorganisms allow a better controlled production in bioreactors, devoid of variation due to physiological state or season encountered for the plants (Bertagnolli et al., 2014) and an easier extraction without any drastic or environmentally toxic compounds. Moreover, microorganisms cultivation in fermenters allows the optimization of the growth and the production yield due to use of well defined medium, controlled environment without the risk for viral or pathogen agents. The advantages of a bacterial source over plant, algal or animal source have made it attractive to obtain macro- molecules for various industrial purposes and strengthened their study The present study analyses the time required for optimum production of BS from various bacterial strains isloted from oil contaminated sites.

II. MATERIALS AND METHODS

A) Collection of Sample

Samples were obtained from following sources for isolation of biosurfactant producing bacteria. Soil from automobile workshop and garages in the Jaipur city, Rajasthan, India .

B) Screening and isolation of biosurfactant-producing bacteria

Bacteria were isolated from above samples as follows:1g sample was taken in 50 ml diluent (1g peptone/ 1). After shaking, on a magnetic stirrer for 1h, serial decimal dilutions were prepared in the same diluents and 0.1 ml of each dilution was spread on the surface of blood agar plates. After incubation at 37°C for 48 hours, colonies were isolated and purified by re-streaking twice. (Carrillo et al.1996).

Biosurfactant producing bacteria were screened on the basis of presence of haemolytic activity as revealed by clearing zone around biosurfactant producing bacterial colonies.. (Mulligan et al., 1984,Carillo et al. 1988)

C) Production of biosurfactant and measurement of surface activity

The production of biosurfactants was conducted by growing cultures in 500-ml Erlenmeyer flasks containing 200 ml sterile medium with the following composition (g/l): Solution A: potassium dihydrogen phosphate (3.0 gm); disodium hydrogen phosphate (6.0gm) ;ammonium chloride (2.0 gm); sodium chloride(5.0 gm); distilled water (800 ml).

Solution B: glucose (8gm); magnesium sulfate(0.1 gm); nutrient broth(15 gm); distilled water(200ml). Suface activity measurements were done according to Iqbal, Khalid and Malik (1995)

D) Growth studies

The bacterial isolates was streaked on a nutrient agar slant and incubated for 24 hours at 37°C. A loopful of each culture was inoculated in 20 mL of nutrient broth in a 50 mL Erlenmeyer flask and incubated in a rotary shaker for 24 hours, 150 rpm at 37°C. These were inoculated in 500-ml Erlenmeyer flasks containing 200 ml sterile medium . Bacterial growth was estimated by measurement of absorbance at a wave length of 540 nm.

E) Time effect studies

Optimum fermentation period for biosurfactant production was determined by carrying out fermentation for upto 5 days .Surface activity of the cell free supernatants were measured on each day.

F) Statistical analysis

Results are presented as mean value ± standard deviation (SD). The Microsoft Excel 2003 and SAS 9.1.3 statistical program were used for data analysis.

III. RESULTS AND DISCUSSION

A) Isolation of biosurfactant producing bacteria

Four bacterial strains showed haemolytic activity (Fig.1).They were identified as *Klebsiella pneumoniae, Paenibacillus dendritiformis , Bacillus cereus and Alcaligenes faecalis* by 16S r-DNA analysis. These have earlier been reported for biosurfactant production. (Fiechter, 1992; Karanth et al.1999; Jiraporn et al., 2003)

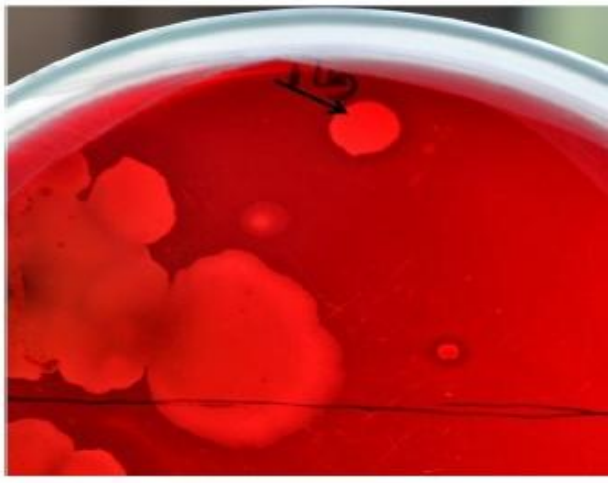


Fig: 1. Zone of clearance on blood agar depicting haemolytic activity

B) Time effect studies

It was interesting to note that the initial rate of biosurfactant production was slow which accelerated greatly after the completion of 72 hours of fermentation. Biosurfactant production was maximum after 72 hours of fermentation in all strains except *Bacillus cereus and Alcaligenes faecalis* in which maximum EI values were observed after 96 h and 48h respectively as depicted by high EI values (Fig.2 a-e). Similar results have been observed by Ray (2012) and Geetha et al.,(2014)in *Bacillus* sp.where biosurfactant production accelerated after 72h.

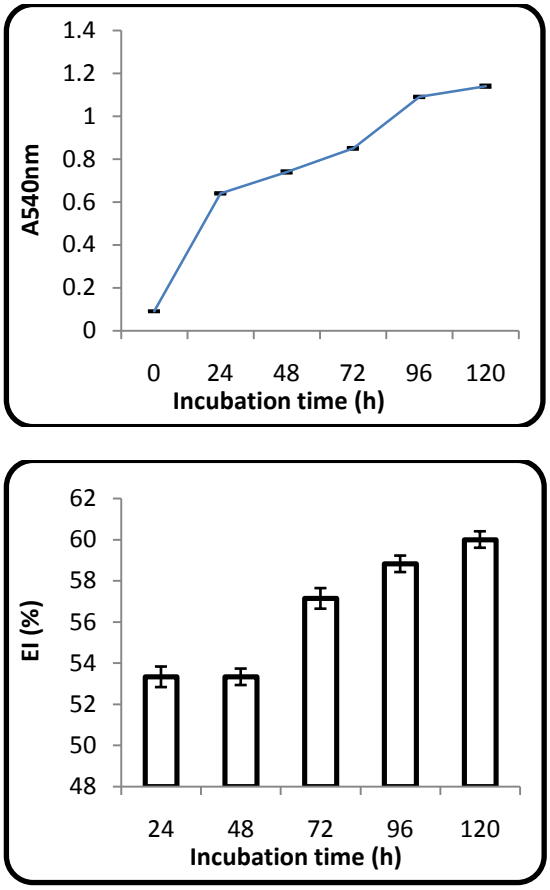
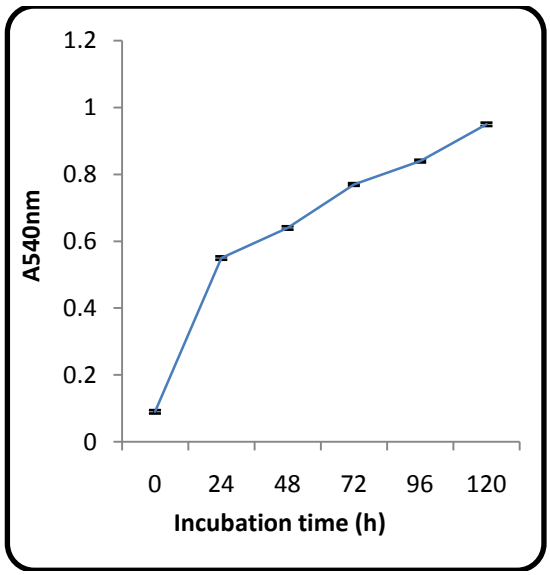


Figure-2a: Growth and Emulsification activity of Klebsiella pneumoniae



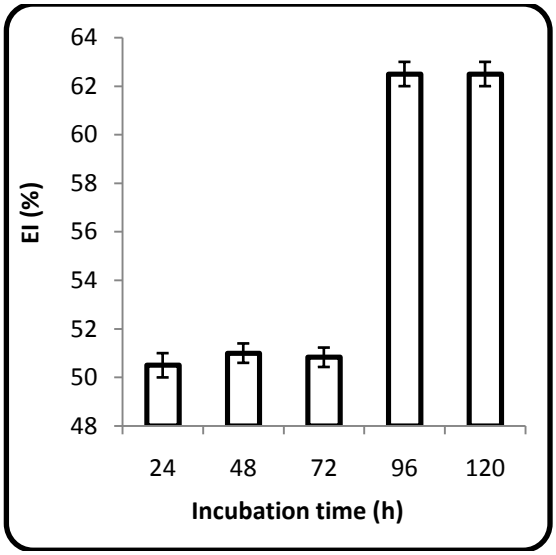
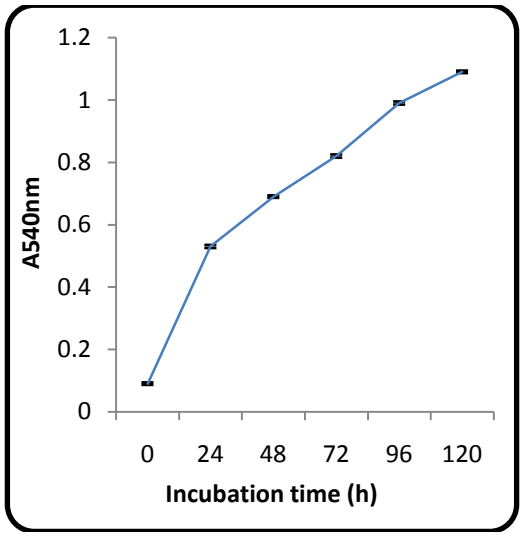


Figure-2b: Growth and Emulsification activity of Paenibacillus dendritiformis



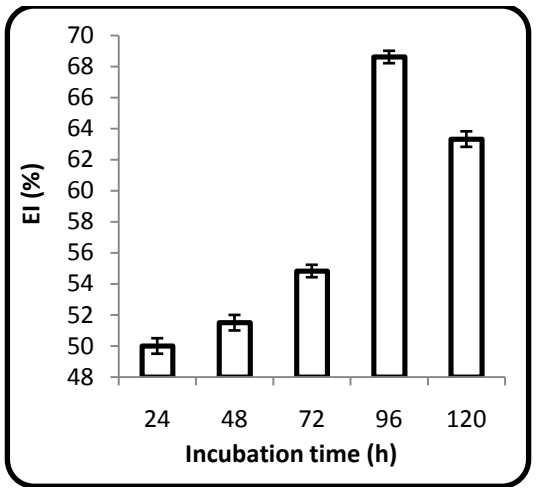
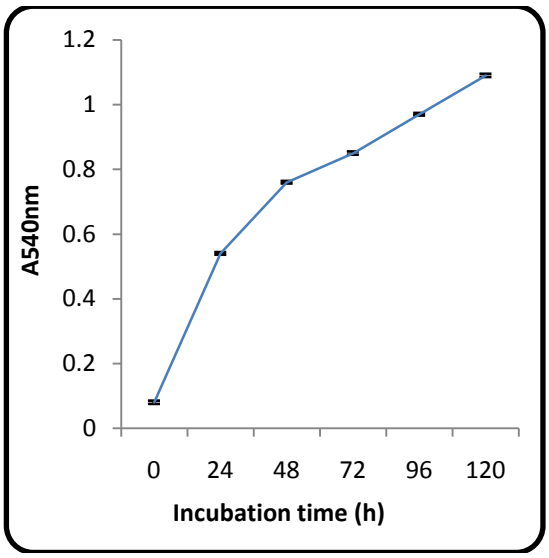


Figure-2c: Growth and Emulsification activity of Bacillus cereus



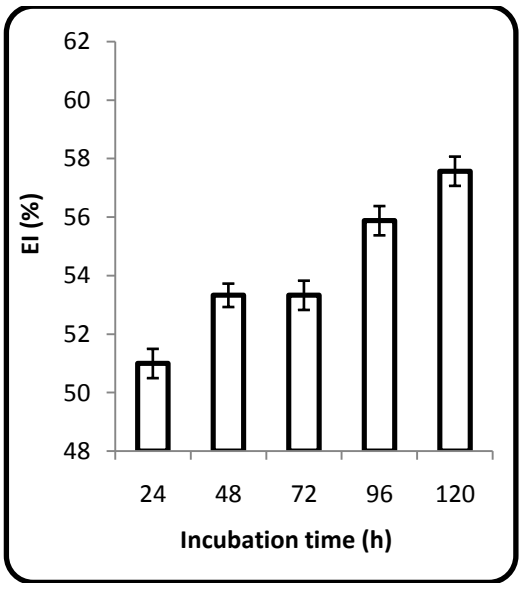
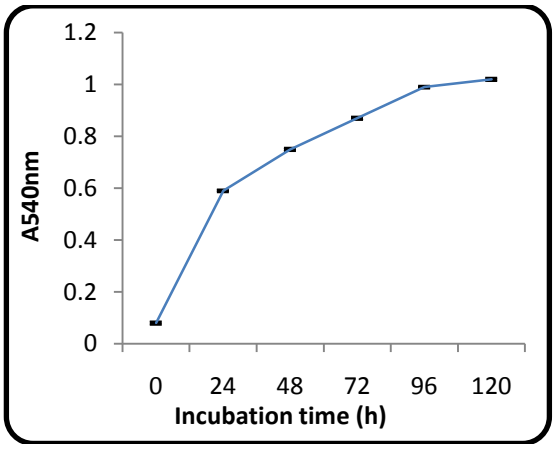


Figure-2d: Growth and Emulsification activity of Bacillus anthracis



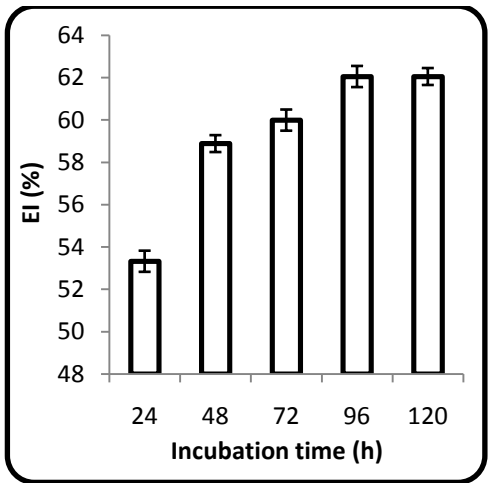


Figure-2e: Growth and Emulsification activity of Alcaligenes faecalis

Figure-2 (a-e): Growth and time effect studies on biosurfactant production estimated as EI of various bacterial isolates

Time taken for the production of BS on a large scale is an important consideration in process economics. There are very few studies on this aspect. The above studies point to the fact that in the search for microbes suitable for mass production of BS, those microbes capable of faster production of BS should be selected. Among the four isolates in this study, *Alcaligenes faecalis* begins production after 48h which is faster that any reports so far.This isolate could therefore be an ideal candidate for industrial production of BS.

IV. ACKNOWLEDGEMENT

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