

Original Research Article

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Primary Screening of Actinomycetes in prospects with Biosurfactant Production from Animal fat waste

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ABSTRACT

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Emulsifier is a chemical compounds having water loving hydrophilic head and an oil loving hydrophobic tail which help in uptake of water insoluble liquids by reduction of surface tension. Emulsifier potentially useful in food, Agriculture, Pharmaceutical, Petrochemical industries. Chemical Emulsifier cause harm to environment so need the alternatives Bioemulsifier are the emulsifier naturally produced from the microorganisms like *Bacillus spp*, *Pseudomonas spp*. and *Candida spp*. In the present study Bioemulsifier was produced from the Actinomycetes which is isolated from river sludge deposited along with the animal fat waste. In this study found that A₆ Isolate shows best yield of the emulsifier and it may possible to use for commercial production of the Bioemulsifier. Their Phylogenetic affinity of this isolates was analyze with 16S rRNA sequencing followed with Blast.

Introduction

Bacteria produce a wide range of extracellular products with many properties and applications. Biosurfactants are one such extracellular amphiphilic compounds produced by bacteria, yeast and fungi, especially when they are grown on hydrophobic substrates. The potential use of emulsifier in different areas such as the food industry, agriculture, pharmaceutical, oil industry, petro chemistry, paper & pulp industry makes it a versatile product (Fracchia and Cavallo, 2012).

Earlier the emulsifiers are produced chemically, which was wealthy & causing environmental pollution so that there was need to replace them by only other compound which is alternative for this chemical emulsifier, can be produced within low budget and does not interfere with environment (Colin *et al.*, 2013).

Apart from their potential applications in bioremediation, bio surfactants compounds have wide prospects in medicine as

antimicrobials, anti-adhesives inhibiting bio film formation, as anti-adhesives, biological coatings, fibrin-clot lysing agents etc (Fracchia & Cavallo, 2012; Satpute *et al.*, 2010). Traditionally the bioemulsifier have been produced by using *Bacillus* sp., *Candida* sp. and *Pseudomonas* sp. and their studies are well documented (Chandran, 2010; Ghribi *et al.*, 2012; Padmapriya & Suganthi, 2013; Tambekar & Gadakh, 2013; Vandana & Peter, 2014). However only a few reports are available in comparison that describe bio surfactants or bioemulsifier production from actinomycetes (Khopade *et al.*, 2012). The problem with commercialization of the bioemulsifier is their production cost and the research on the use of cheap renewable substrate for the production has only concentrated on agricultural waste and very little on the animal fat waste (Banat *et al.*, 2014) Also, the studies on actinomycetes in India are in preliminary stages only (Velho-Pereira & Kamat, 2013) and worldwide the studies on aquatic actinomycetes are neglected (Ningthoujam, *et al.*, 2009; Rifaat, 2003; Takizawa *et al.*, 1993). Thus this study was under taken to assess the bioemulsifier production of two actinomycetes isolates from Godavari river sediments using animal fat waste as a substrate.

Materials and Methods

Sample Collection: for the isolation of the Actinomycetes sample was collected from the Gad, and Godavari river area from the river sediment and enrichment was carried out by Using Bushnell Haas agar enriched with 0.1% crude oil. Animal fat waste was collected from the local market, both samples are collected in aseptic bags and processed and used within 24 hrs.

Media Designing: Two sets of media were prepared, Media Ist (Peptone–0.5gm, NaCl–

0.25gm, Yeast extract–0.15gm, Fat–0.5gm, D/W–50ml) Media IInd (L-Asparagine–0.025gm, KH₂PO₄–0.025gm, MgSO₄ –0.01gm, FeSO₄–0.0005gm, Fat–0.5gm, D/W–50ml)

Fermentation Process: Firstly the designed media were divided into two flasks, i.e. 25ml in each and sterilized by autoclaving, further labeled as, Set I A, Set I B, Set II A, Set II B, Then the labeled media were inoculated by developed inoculums, of isolates i.e. A6, S15 In Set I A inoculated with inoculums of A6, Set II A was inoculated with inoculums of S15, Set I B inoculated with inoculums of A6, Set II B inoculated with inoculums S15. After inoculation the flasks were incubated on shaker incubator at 30°C for 7 days, on 120 RPM. After incubation period centrifugation of the fermented broth done at 3000 RPM for 15 min. Then the supernatant were collected. After collection of supernatant, the collected supernatants were used for drop collapse method.

Drop Collapse Test: 2 µl of respective oil was applied to the well regions of the 96-well micro-plate lids and allowed to equilibrate for 24 h. Five micro liters of the cell free culture broth was transferred to the oil coated regions and the drop size was observed 1 min later with the aid of a magnifying glass. A result was considered positive for biosurfactant production when the drop diameter was at least 1 mm larger than that produced by S₁₅ (negative control). SDS (a chemical surfactant) solution prepared at 1mg/ml concentration was used as a standard.

Emulsification Index: Emulsification activity was measured by using mixture of 1 ml culture supernatant along with the respective oil, 4 ml water and 6 ml respective oil vortex for 2 min and then

placed for the emulsification after 48 hrs emulsification index calculated by measuring the height of the emulsion layer and using formula $EI = a/b \times 100$ (where a, height of emulsion layer b, total height).

Result and Discussion

From the 15 samples was collected from the sampling site at October 2014 from which 6

Actinomycetes isolate was identified within them A₆ shows maximum yield about 46.23% this strains may have the industrial prospects in concern with the biosurfactant production using animal waste as an substrate. Taxonomic affinity of isolate S15 and A₆ as established through 16s rRNA searches against RDP Naive Bayesian rRNA Classifier Version 2.10 database and closest relative as identified through

Table.1 Observations for Drop Collapse Method and Emulsification Assay

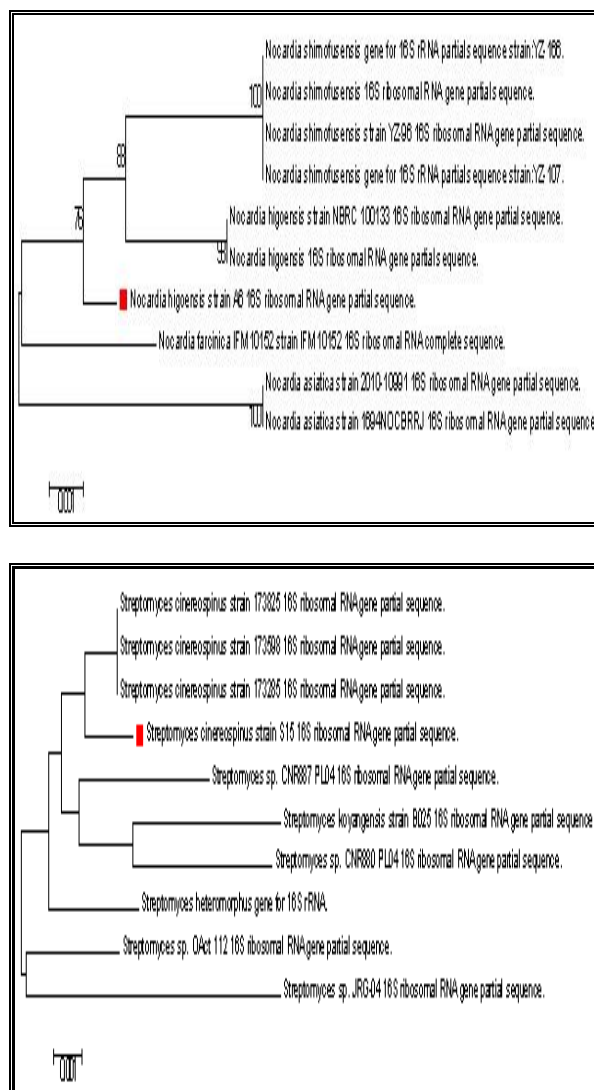
Sample	Drop collapse method			Emulsification Index /ml		
	Kerosene oil	Sunflower oil	Coconut oil	Kerosene oil	Sunflower oil	Coconut oil
SDS (+control)	+++	+++	+++	70.80	69.38	75.26
S ₁₅ (-ve control)	-	-	-	00.10	00.21	00.21
A ₁	-	-	-	-	-	-
A ₂	-	-	-	-	-	-
A ₃	++	+	-	24.00	10.20	-
A ₄	+	-	++	12.10	-	24.12
A ₅	++	-	-	20.12	00.10	00.20
A ₆	+++	+++	++	45.33	46.23	42.12

-:No activity, +: positive activity, ++:weak positive activity, +++: Good activity

Table.2 Taxonomic Affinity of Isolate S15 and A₆ as Established Through 16s Rrn Searches against RDP Naive Bayesian rRNA Classifier Version 2.10 Database and Closest Relative as Identified Through GenBank using the Tool BLAST (blastn)

Name of the Isolate	S 15	A 6
Domain	Bacteria	Bacteria
Phylum	Actinobacteria	Actinobacteria
Class	Actinobacteria	Actinobacteria
Subclass	Actinobacteridae	Actinobacteridae
Order	Actinomycetales	Actinomycetales
Suborder	<u>Streptomycineae</u>	Corynebacterineae
Family	Streptomycetaceae	Nocardiaceae
Genus	<i>Streptomyces</i>	<i>Nocardia</i>
Closest relative	<i>Streptomyces cinereospinus</i> strain 173285 16S ribosomal RNA gene, partial sequence	<i>Nocardia higoensis</i> strain IFM 10084 16S ribosomal RNA, partial sequence
Accession number	EU593556.1	NR_028651.1
% similarity	99%	99%

Figure.1 Showed Neighbor- joining tree



GenBank using the tool BLAST (blastn), to in search of the Phylogenetic position, lab isolate S₁₅ belongs to Streptomycetaceae family and isolate A₆ belongs to Nocardiaceae family

Neighbor- joining tree of closest relatives of S₁₅ and A₆ respectively identified in blastn search The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.03748104 is shown in figure:no:01(a and b)

Figure.1 showed Neighbor- joining tree of closest relatives of S₁₅ and A₆ respectively identified in blastn search The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.03748104.

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