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The Production of Bioemulsifier from Novel Microorganisms from Different Environments

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ABSTRACT
Surface active agents like bioemulsifier find relevance in different industries. Emulsifiers are always synthetic in nature, usually obtained from chemicals that come with a lot of disadvantages. Microorganisms have been established to be good source emulsifier if subjected to the right parameters. There is a dearth of information on the production of biemulsifier from indigenous microorganisms. the present study, 23 isolates were obtained from both soil and marine environments (Bacillus, Peribacillus, Citrobacter, Acinetobacter, Streptomyces, Norcadia, Brevibacterium, Arthrobacter, Lysinibacillus and A. campanulatus) which were screened for their ability to emulsify by subjecting them to series of tests: haemolysis test, ability to grow on hydrocarbon layered plates, Modified drop collapse test, ability to produce enzyme lipase, emulsification assay and emulsification index. Petrol was found to induce bioemulsifier production best in streptomyces sp ATS9. (89EU/mL), while for Lysinibacillus sphaericus, sunflower was the best inducer (67EU/mL). Chemical analysis of the bioemulsifier produced by Streptomyces sp indicates its high carbohydrate content 51.35%, protein – 26.59%. Indigenous microorganisms properly explored would be the source of eco-friendly emulsifier.

Key words: Production, Bioemulsifier, Novel Microorganisms , Different Environments, Biosurfactant

Aims Research Journal Reference Format:
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1. INTRODUCTION

Bioemulsifiers are a type of surface active agents derived from microorganisms, and they are preferable to the synthetic emulsifier because of their numerous advantages over chemical emulsifiers (Niveas et al., 2008). In recent years, bioemulsifiers have received increasing attention because of their role in the growth of microorganisms on water insoluble hydrophobic materials such as hydrocarbons and also because of their commercial potential (Okoro et al., 2011). Surface active agents are widely used in many industries such as agriculture, food production, chemistry, cosmetics and pharmaceutics. (Banat et al., 2010). Since biosurfactant (BS) and bioemulsifiers (BE) both exhibit emulsification properties, bioemulsifiers are often categorized with biosurfactant, although emulsifiers may not lower surface tension (Priya and Usharani, 2009). A biosurfactant may have one of the following structures: mycolic acid, glycolipids, polysaccharide–lipid complex, lipoprotein or lipopeptide, phospholipid, or the microbial cell surface itself (Plocinizak et al., 2011; Rahman et al, 2003)). Isolation and screening of bioemulsifier producing organisms from different environmental samples have not been explored to the maximum locally.Hence, this study aims at this.

2. METHODOLOGY

Isolates were obtained from both the soil and water environment, which were later subjected to various tests to determine their bioemulsification properties.

2.1 Effect of Inducing with different oils on bioemulsifier production by the selected Isolates
The Isolates were inoculated in Bioemulsifier production Broth, with different oils (Petrol, Diesel, Engine oil, Castor oil, Olive oil, Sunflower, Kerosine, Crude oil and Vegetable oil) and then incubated at room temperature. The emulsification activity was studied after every 24hrs and was continued for 120hrs. Emulsification assay was carried out to check the maximum emulsification activity of of isolates after which absorbance was then read using a spectrophotometer at a wavelength of 400nm described by Patil and Chopade, (2001).

2.2 Partial Purification pf Bioemulsifier from Isolates
Partial Purification was carried out using 1l of 96h old broth of both Isolates centrifuged at 8000g for 20min at 30^0C. After centrifugation three volumes of chilled ethanol was added in cell free broth and incubated at 4^0C for 15h. This was subjected to centrifugation at 8000g for 30min at 10^0C and brown precipitate was dissolved in 3ml of sterile distilled water and extensively dialysed against sterile distilled water at 10^0C for 48h. Distilled water was changed after every 12h. (Patil and Chopade, 2001).

2.3 Sugar Content
Total sugar content of the purified bioflocculant was determined by the phenolsulphuric acid method using glucose as the standard solution as described by Chaplin and Kennedy (1986). 200mg of each sample was added to hot 80% ethanol to remove sugar. This was centrifuged (12000 rpm, 3mins) and residues retained. Residues were washed reapeatedly with hot 80% ethanol till the washing no longer gave colour with anthrone reagent. The residues were properly dried over a water bath. To residues were properly dried over a water bath. To the residues, 5ml of water and 6.5ml of 52% perchloric acid were added. Extract was kept for 20mins at 0^0C.

The latter was centrifuged (12000rpm, 3mins) and supernatant was centrifuged and pooled and made to 100ml. 0.2, 0.4, 0.6, 0.8, 1ml was pipette from glucose standard (100mg in 100ml of water) into a series of test tubes.0.1, 0.2ml of the sample solution was pipette out into two separate test tubes. The volume was made up to 1ml with water, in each testtube. A blank was set with 1ml of water. 1ml of phenol was added to each testtube was shaken well. After10mins, the contents in the tubes were shaken and placed in water bath at 25-30^0C for 20mins. Colour was read at 490nm.The amount of total carbohydrates present in the sample solution was calculated from standard graph, prepared using glucose (Zhang, 2003).

2.4 Protein Content determination
Total protein content was measured by Lowry *et al* (1951) method using Bovine Serum Albumin as the standard solution. Bovine serum albumin(BSA) was used as standard in different concentrations(100µg/ml,200 µg/ml,400 µg/ml,600 µg/ml,800 µg/ml and 1000 µg/ml and purified product in the range of 5mg/ml and 1mg/ml in duplicates. 50ml solution of 2g $NaCo_{3+}$ 0.1M NaoH and 50ml solution of 0.5g per 100ml of $CuS0_{4+}$ 1g per 100ml Na – K - tartarate was added. 5ml Folin's reagent was added to it and incubated for 30mins in the dark. Absorbance was recorded at 750nm. The amount of total protein present in the samples was calculated from calibration curve prepared using pure BSA as standard.

2.5 Dry weight determination
About 2g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven and set at 100^oC to dry to a constant weight for 24hrs overnight. At the end of 24hrs, the crucible plus sample was removed from the oven and transferred into desiccators, cooled for 10mins and weighed. If the weight of the empty crucible is W_0, the weight of the crucible plus sample is W_1 and the weight of the crucible plus oven-dried sample is W_3, then

% Dry Matter = $(W_3 – W_0)$ 100/ $(W_1 – W_0)$ (A.O.A.C., 2005). ……(1)

Induced production of bioemulsifier by *Streptomyce*s sp .ATS9 and *Lysinibacillus sphaericus* with different oils resulted in maximum bioemulsifier yield when Sunflower and petrol were used which is in line with Javed *et al*. (2001) where bioemulsifier production from *Streptomyces* sp S22 was induced by sunflower. In addition to this, a high yield of glycolipid was observed in medium supplemented with sunflower oil (Lee and Kim, 1993). Also, bioemulsifier production from *Streptomyces* sp S1 and *Acinetobacter junii* sc14 have been induced by addition of hydrocarbons and oils respectively (Kokare *et al*., 2007 and Javed *et al*., 2011). Bioemulsifiers can be produced by different types of microorganisms. This study shows that the chemical analysis of purified bioemulsifier produced by *Streptomyces sp* and *Lysinibacillus sp.* Revealed to be glycolipids containing both protein and carbohydrate. Most known biosurfactants are glycolipids. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids (Desai and Banat, 1997).

Bioemulsifier produced from *Streptomyces* sp emulsified all the oils except crude oil. This result contradicts where petrol and toluene remained unemulsified. (Doshi *et al*., 2010) which is in line with the work of Kokare *et al*.(2006) who found that the *Streptomyces* sp S1 showed maximum activity with toluene and petrol.The yield of partially purified bioemulsifier obtained from the cell free supernatant of *Streptomyces* sp grown in Malt yeast extract broth was found to be 1.5g/L. It was dark brown which is in tandem with the findings of Kokare *et al*.(2006) in which the yield of partially purified bioemulsifier obtaind from the cell free supernatant of *Actinopolyspora* sp A18 also grown in MYE broth was found to be 1g/L also brownish in colour. Chemical analysis of the bioemulsifier produced by *Streptomyces* sp indicates its high carbohydrate content 51.35%, protein – 26.59%.

Thus it indicated the glycolipopeptide nature of the bioemulsifier which is in line with the work of Doshi *et al*. (2011) and the result finding is in contrast with *Pseudomonas aeroginosa* found to produce peptidoglycolipid type of bioemulsifier (Zheng *et al*.,2009). The chemical analysis of the bioemulsifier produced by *Lysinibacillus* sp indicates its high protein content (73.40%), Carbohydrate (48.64%) which indicate the peptidoglycolipid nature of the bioemulsifier.

3. RESULTS

A total of 23 microorganisms (*Bacilus, peribacillus, Citrobacter, Acinetobacter, Streptomyces, Nocardia, Brevibacterium, Arthrobacter, Lysinibacillus, and Actinomycetes*) isolated from both the marine and soil samples was used in this study. Figure.1 shows the percentage frequency of occurrence of the microorganisms isolated from the samples in which *Streptomyces* sp. (21.73) and *Bacillus*sp.(21.73) had the highest frequency of occurrence and *Actinomycetes, Peribacillus, Lysinibacillus*(4.34%) had the least.

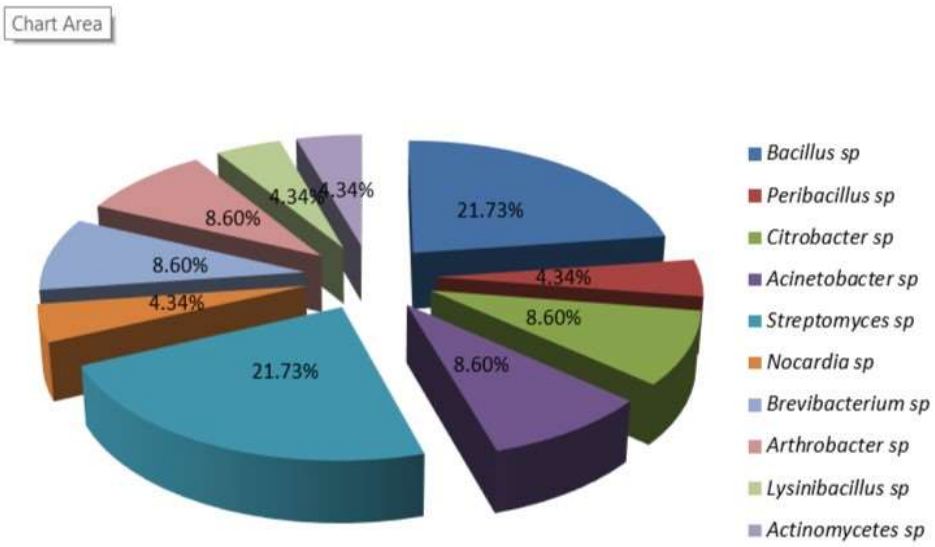

Figure 1: Percentage frequency of occurrence of the isolates

After the preliminary screening six isolates (*Bacillus fusiformis, Streptomyces sp ATS9 Lysinibacillus sphaericus, Arthrobacter, Bacillus licheniformis and Streptomyces sp ATT8*) were selected for further study.

Table 1 shows the bioemulsification assay on different oil(Diesel, Petrol, Engine oil, Vegetable oil, Castor oil and Olive oil respectively. It ranged from 0.124 – 1.551EU/mL, 0.300 – 1.966EU/mL, 0.266 – 1.639EU/mL, 0.092 –0.951EU/mL, 0.800 – 1.967EU/mL, 0.104 – 1.948EU/mL. The best isolates (*Lysinibacillus sphaericus, Streptomyces sp ATS9, Bacillus licheniformis, and Bacillus fusiformis*) were selected base on their emulsification capacity.

Table 1: Bioemulsifier further Screening (growth at 540nm EU/mL).

S/N	Isolate code	Diesel	Petrol	Kerosene	Engine oil	Vegetable oil	Castor oil	Olive oil
1	ATT5	1.551	1.954	0.910	0.951	1.878	1.950	1.866
2	ATT9	0.69	0.300	0.266	0.092	0.800	0.247	0.104
3	ATS3	1.418	1.966	0.950	0.926	1.967	1.456	1.948
4	ATS9	0.76	1.897	1.394	0.868	0.876	1.236	1.007
5	ATT2	1.092	1.292	1.317	0.346	1.561	1.085	1.582
6	ATS4	0.124	1.248	1.639	0.879	1.778	0.969	1.412

PT – Petrol, KR – Kerosene, ENG – Engine oil, CA- Castor oil, VE- Vegetable oil, OL – Olive oil, DI – Diesel oil, CR – Crude oil, SF – Sunflower oil.

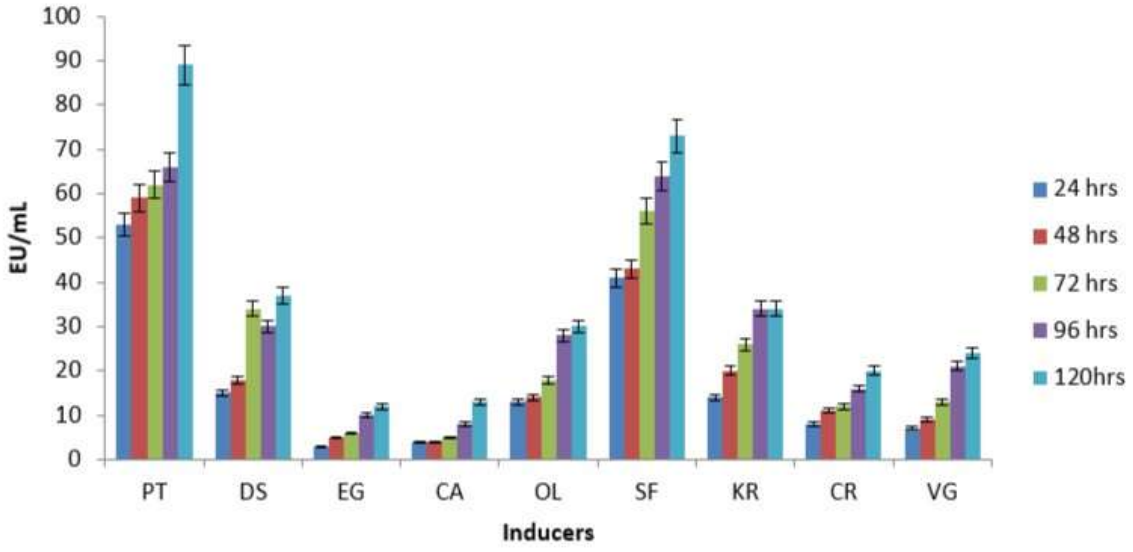

Figure 4.8a: Effect of inducers on bioemulsifier production by *Streptomycetes* sp Ats9
Petrol ,KR – Kerosene ,ENG – Engine oil, CA- Castor oil, VE- Vegetable oil, OL – Olive oil, DI – Diesel oil, CR – Crude oil, SF – Sunflower oil.

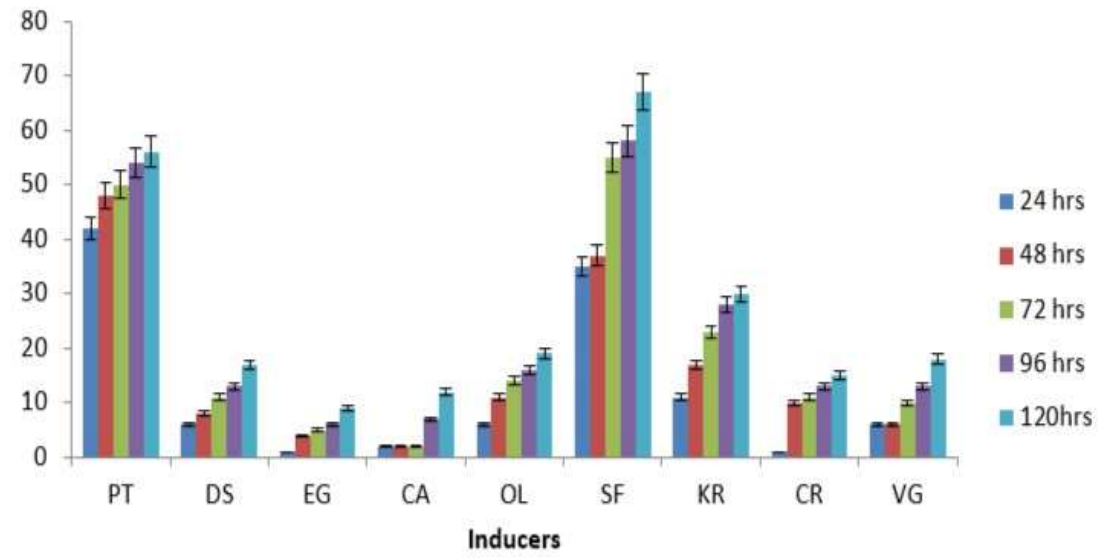

Figure 4.8b: Effect of inducers on bioemulsifier production by*Lysinibacillussphaericus*
Petrol ,KR – Kerosene ,ENG – Engine oil, CA- Castor oil, VE- Vegetable oil, OL – Olive oil, DI – Diesel oil, CR – Crude oil, SF – Sunflower oil.

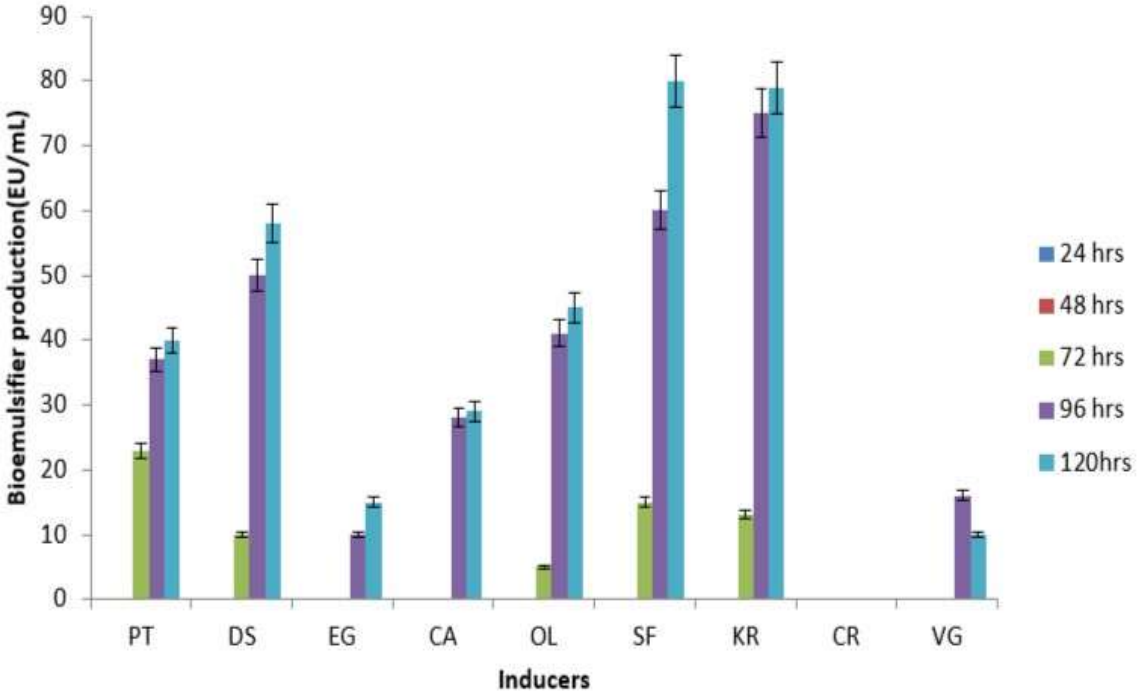

Figure 4.8c. Emulsification index bioemulsifier produced by inducing with different oils on *Streptomycetes* sp. Ats9
Petrol ,KR – Kerosene ,ENG – Engine oil, CA- Castor oil, VE- Vegetable oil, OL – Olive oil, DI – Diesel oil, CR – Crude oil, SF – Sunflower oil.

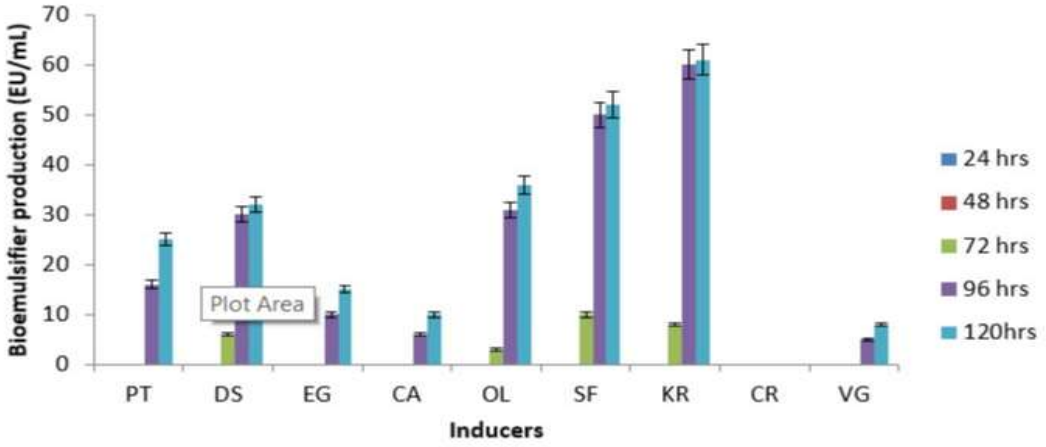

Figure 4.8d. Emulsification index of bioemulsifier produced by inducing with different oils on *Lysinibacillus sphaericus*

Petrol ,KR – Kerosene ,ENG – Engine oil, CA- Castor oil, VE- Vegetable oil, OL – Olive oil, DI – Diesel oil, CR – Crude oil, SF – Sunflower oil.

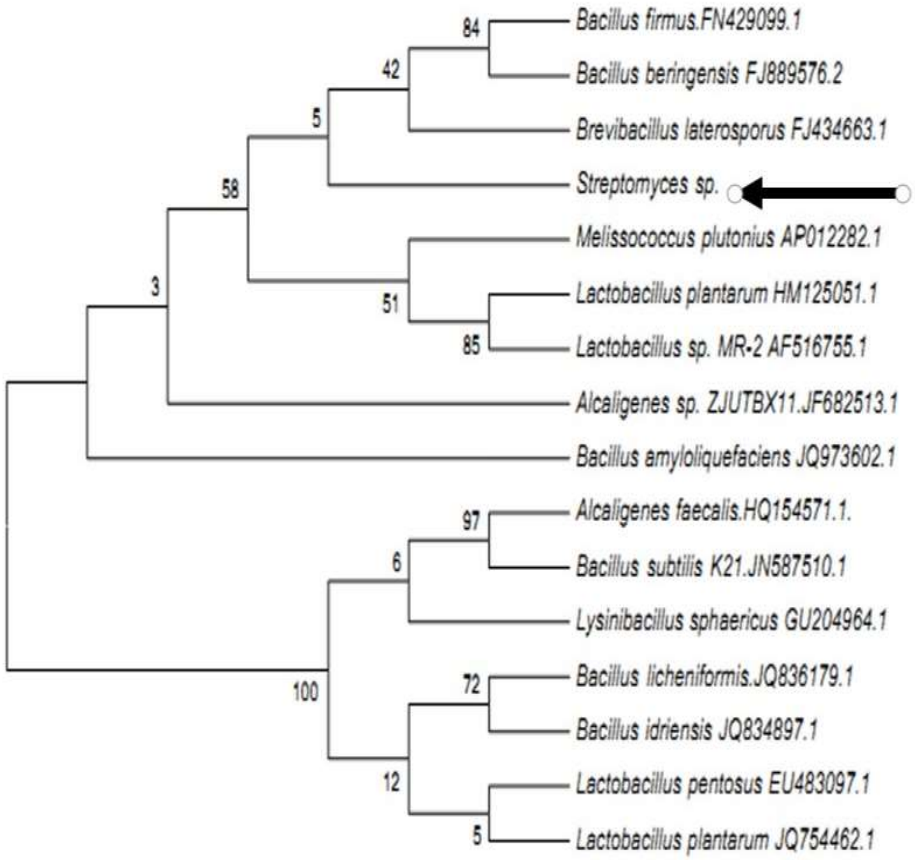

Fig. 4.14a: Phylogenetic tree showing the relationships among the selected isolates (in pointed arrows) and other closely related sequences collected from the Gene Bank.
The dendogram was generated by the neighbor-joining method. Bootstrap values per 100 bootstrap analysis presented for values greater than 30 %.

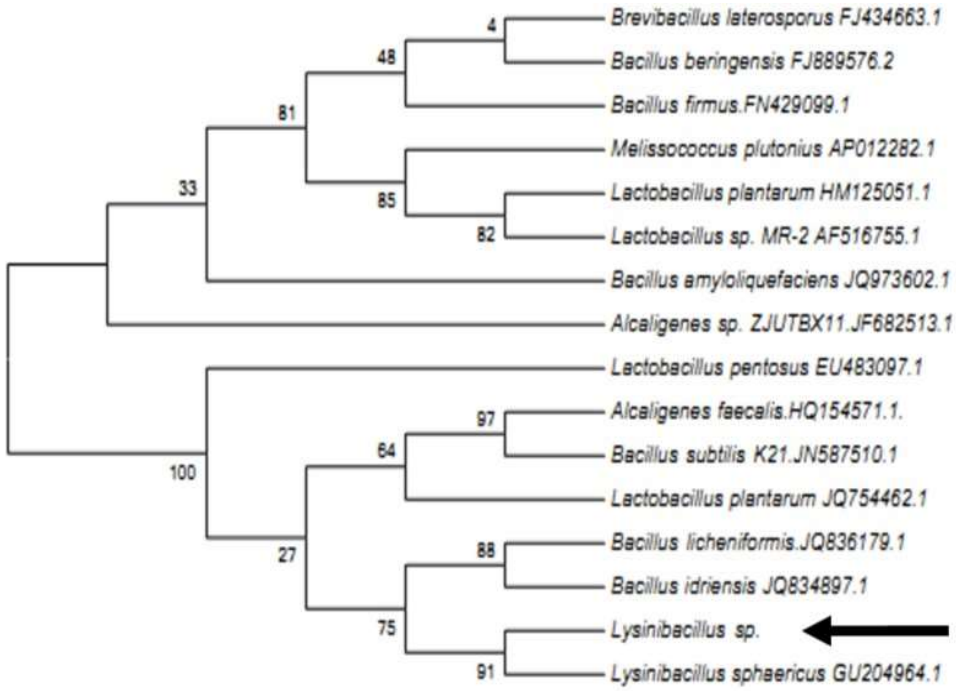

Fig. 4.14b: Phylogenetic tree showing the relationships among the selected isolates (in pointed arrows) and other closely related sequences collected from the Gene Bank. The dendogram was generated by the neighbor-joining method. Bootstrap values per 100 bootstrap analysis presented for values greater than 30 %.

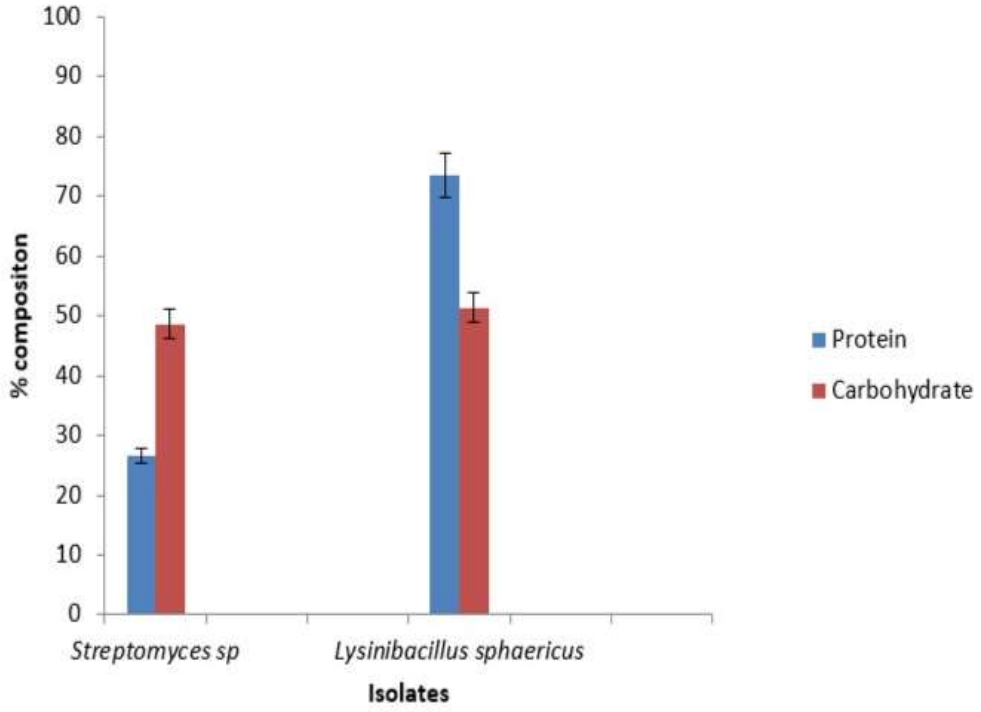

Fig. 4.15: Chemical analysis of the purified bioemulsifier produced by the isolates

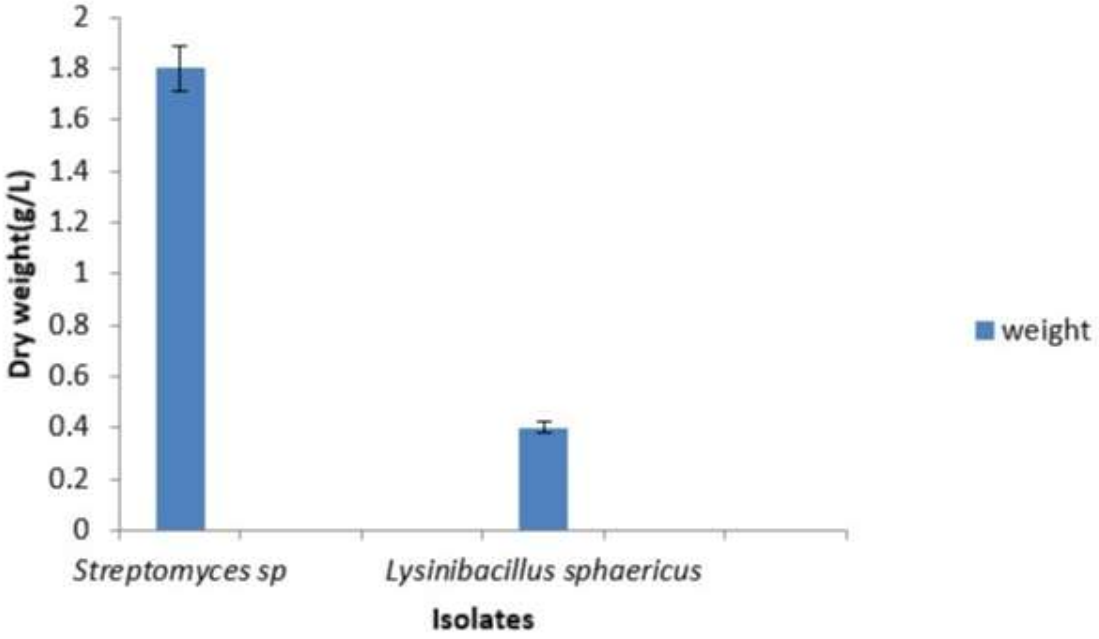

Fig. 4.16: Dry weight of Partially Purified bioemulsifier from the *Streptomyces sp.* and *Lysinibacillus sphaericus*

4. CONCLUSIONS

In conclusion, microorganisms can produce emulsifiers/surfactants (Desai and Banat, 1997). The results of this experiment demonstrated that both isolates grew maximally on peptone when use as the nitrogen source. Maximum bioemulsifier production was achieved at 120hrs of incubation time under agitation and production was supported at a range of pH6 – pH8, which is within appreciable limit for food processing production.The present study revealed that bioemulsifier production was to be accelerated at optimized culture conditions such as medium pH, temperature, agitation, mineral salts and metal ions concentrations, and various substrate concentrations.

The present study revealed that bioemulsifier production was found to be increased at optimized culture conditions such as medium pH, temperature, agitation, salts and various substrate concentrations. From the results, it could be concluded that pH6, temperature 25°C, 2.0g/L Sodium chloride concentration were optimum for maximizing bioemulsifier production by *Streptomyces* sp ATS9, isolates yielding the best bioemulsifier production. The assessment of various substrates for maximizing the production of bioemulsifier by isolates showed that Sunflower oil is the best inducer across all the isolates. Thus adequate study of the organism and the appropriate application of process parameters optimization could improve their possibilities of commercialization.

Chemical analysis of the bioemulsifier produced by *Streptomyces* sp indicates its high carbohydrate content 51.35%, protein – 26.59%. Thus it indicated the glycolipopeptide nature of the bioemulsifier which is in line with the work of Doshi *et al*. (2011) and the result finding is in contrast with *Pseudomonas aeroginosa* found to produce peptidoglycolipid type of bioemulsifier (Zheng *et al*.,2009). The chemical analysis of the bioemulsifier produced by *Lysinibacillus* sp indicates its high protein content (73.40%), Carbohydrate (48.64%) which indicate the peptidoglycolipid nature of the bioemulsifier.

5. RECOMMENDATIONS

The present study demonstrated the production of glycolipopeptide type of bioemulsifier by *Streptomyces* sp and peptidogycolipid type of bioemulsifier by *Lysinibacillus* sp. Thus adding a new perspective to the study of bioemulsifier. These bioemulsifiers emulsified a large number of oils and was partialy soluble in water and hence could find potential applications in food, Pharmaceutical and pesticide industries. For the fact that microorganisms are capable of producing emulsifiers, that far outweighs the advantages of chemically synthesized emulsifiers, It is therefore necessary that much work should be directed to this; not only because of its economics but also of its overall benign nature on our world.

We therefore submit that each potential bioemulsifier producing organisms be studied physiologically, morphologically, genetically and chemically inorder for scientist to fully understand the conditions and practices needed to be in place in a bid to achieving maximum bioemulsifier production at all times at a reduced cost. In addition to this, knowing full well that microorganisms can get their nutrient from a wide array of sources, agro waste or agro product which are cheap or even not in use can be looked into or researched into, inorder to serve as cheap substrates which ultimately leads to gross reduction in production cost of emulsifier from microorganisms.

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