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Evaluation of Different Nitrogen Sources in Production of Biosurfactant by Mycoflora of Southern Punjab Pakistan

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Abstract:

The study was conducted to evaluate the effect of different nitrogen sources on the production of biosurfactants from locally isolated mycoflora of Southern Punjab Pakistan. Several fresh isolated fungal strains were screened by which Aspergillus niger EH6o was observed to best surfactant production (≥20 g/l of glycolipids) among all the strains. Further optimization was performed to Aspergillus niger EH6o by using different nitrogen sources ($NaHCO_3$, NH_4Cl, $(NH_4)SO_4$, $NaNO_3$, NH_4NO_3) at pH (2.5-8.0) temperatures (20-60°C) and incubation periods (24- 120 h). Aspergillus niger EH6o was observed to gave maximum surfactant yield (25g/l) at 0.5% $NaNO_3$ provided with 0.1% olive oil (initial pH 5.5) when hatched at 27°C for 96h. In Conclusion, as the economical point of view the crude

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surfactant produced by Aspergillus niger EH6o were observed to have improved qualities when tested with commercial surfactants like CTAB and SDS.

Key words: Biosurfactant, Southern Punjab, mycoflora, *Aspergillus niger*, EH6o.

Introduction

Biological surfactants are the microbial products which minimize the surface and interfacial tension between individual molecules at particular surfaces and interfaces. Biosurfactants possessed many advantages at chemical surfactants due to their biodegradable and eco-friendly nature that is the point which make them much more interest in creating the era of green technology (Bhardwaj *et al*., 2013). In recent years, biosurfactants from bacteria and fungi have gained more attention for their antifungal activity, low toxicity to plants and animals, low irritation, compatibility with human skin and high biodegradability (Vijaya Dewaliya and Raashi Jasodani., 2013). The microorganisms which produce biosurfactants can also be used in the diverse bioremediation technologies like removal of oil from contaminated soil, solubilisation and sludge in oil storage tank (Qazi *et al*., 2013). Biosurfactants are not widely used in industry because of their high production cost and complex process development. The critical factors adding to the economic inefficiency of biosurfactants include expensive medium, low yield, impure product and high cost of downstream processing (Syldatk and Hausmann 2010).

Materials and Methods

Sample Collection: Samples from various sources including oil contaminated soil, fermented foods and fruits such as

orange, guava, apple, date palm, corn, mango, rice, were collected from various orchids and markets of Southern Punjab Pakistan in sterilized polythene bags Table 1.

Table 1.The strains isolated from samples collected from different areas of southern Punjab, Pakistan.

Sr.No.	Strain	Source	Sampling location	Elevation	Date & Time
1	SS6da1	*Gossypium* plant	30°11.526SE, 071°28.728 NW	512°	17-10-10; 10:00 am
2	MTKC1	*Psidium* plant	30°11.411SE, 071°28.702 NW	1025°	17-10-10; 10:00 am
3	MTK7Dbii	*Cassia fistula*	30°15.920SE, 071°30.059 NW	369°	15-11-10; 09:00 am
4	SSGBrii	Loamy soil	30°11.494SE, 071°28.790 NW	528°	20-11-10; 10:30 am
5	EH_6Ao	Oil polluted soil	30°16.023SE, 071°30.137 NW	388°	22-11-10; 11:00 am
6	K.E malta	*Citrus indica*	30°15.727SE, 071°30.152 NW	392°	25-11-10; 09:00 am

Samples were collected from the region of Southern Punjab the location of source was noted with the help of GPS (Etrax, GARMIN, 190-00234-01, TAIWAN).

The fungal cells were isolated from these samples by serial dilution method on the yeast extract peptone starch agar (YPSA) medium listed below in Table 2.

Table 2: Composition of YPSA medium, pH 5.5

Ingredient	Concentration (g/l)
Agar	20
Peptone	5.0
Yeast extract	2.0
Starch	18

(Strijbosch *et al.,* 1990), (Waghmode *et al*., 2014)

Isolated colonies were identified on the basis of morphological and microscopic characteristics Figure 3. Sub-culturing of fungal strains was performed at every week regularly. The different nitrogen sources such as Sodium hydrogen carbonate,

ammonium chloride, ammonium sulphate, Sodium nitrate and ammonium nitrate concentrations (0.1-0.5g/l) were studied with solid substrate fermentation by applying culture conditions such as incubation period (24-120hr), temperature (20-60ºC), pH (2.5-8.0). The strains were preserved in sterile liquid paraffin at 4°C (Padmapriya *et al*., 2013).

CTAB-MB Agar Method: CTAB-MB agar methods for the purpose of biosurfactants production by (Siegmund & Wagner, 1991); (Pinzon & Lu, 2009) were used with some optimizations Table 3. Uncultured plates with sodium dodocylsulphate (SDS) were used as positive control and similarly without sodium dodocylsulphate (SDS) plates were used as negative control in the experiment. The diameter of the clear halo was note using a millimeter scale (Swordfish Brand, China) after 24, 48, 72, 96, and 120 hours of incubation. The average diameter of triplicate samples was carried out for individual strain (Hussain *et al*., 2014).

Table 3: Composition of the CTAB-MB agar medium, pH 5.5

Ingredients	**Concentration (g/l)**
Agar	20.0
CTAB	0.2
Methylene Blue	0.1
Starch (soluble)	10.0
KH_2PO_4	2.0
Na_2PO_4	0.9
$CaCl_2$	0.005
$MgCl_2$	0.2
yeast extract	0.3

Results:

The nitrogen source is the critical factor affecting the production of glycolipids (Soniyamby *et al*., 2011). Different nitrogen sources at varying concentrations (0.1-0.5g/l) were screened to find out the best source for biosurfactant

production. Figure 2 shows that sodium nitrate concentration (0.5%) is more effective for surfactant production for *Aspergillus niger* EH6o than ammonium sulfate, ammonium nitrate, ammonium chloride and sodium bicarbonate. This strain is capable of utilizing nitrogen sources in the form of both ammonium and nitrate salts. However, in order to obtain high concentrations of glycolipids it is necessary to limit the amount of this macronutrient. Other nitrogen sources except $NaHCO_3$ had similar effects on the biosurfactant production of *Aspergillus niger* EH6o. Figure-4 showed that Sodium nitrate was the best nitrogen source at (0.5%) concentration for surfactant production (25 g/l) from *Aspergillus niger*. Soniyamby & coworkers (2011) reported (4.6 g/l) of surfactant (rhamnolipids) production from *Pseudomonas aeruginosa* using sodium nitrate as nitrogen source. The local crud surfactant was compared with commercial SDS surfactant Figure 1.

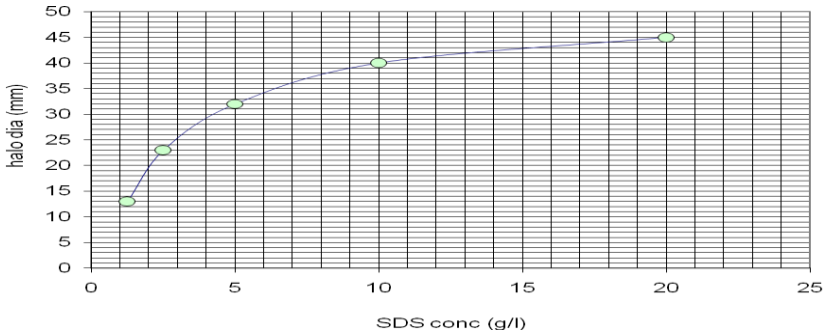

Figure 1: Concentration of SDS vs. diameter of halo on CTAB-MB agar plates

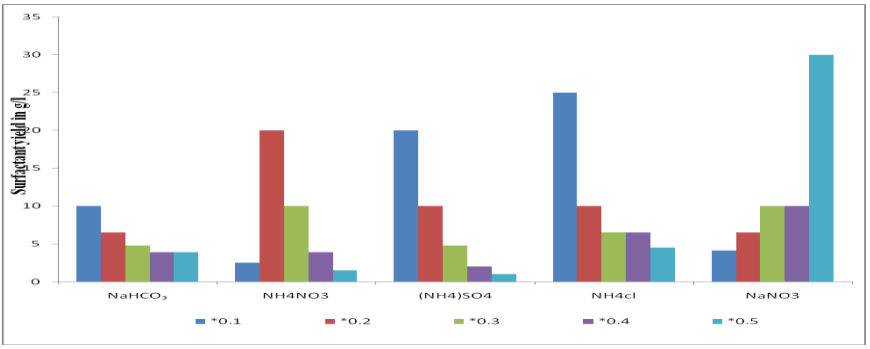

Figure 2: Effect of different nitrogen sources on different fungal strains

Temperature 27°C, initial pH 5.5, incubation period 72h on CTAB-MB agar medium with composition (g/l) agar 20, CTAB 0.2, methylene blue 0.1, nitrogen sources 0.1-0.5g/l, KH_2PO_4, Na_2HPO_4 0.9, $CaCl_2$ 0.005, $MgCl_2$ 0.2, Yeast extract 0.3. *Nitrogen concentration (g/l).

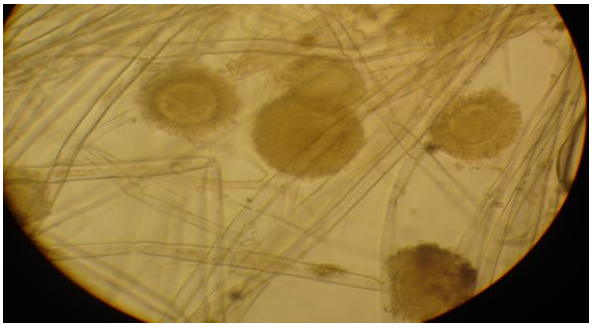

Figure 3- Microscopic view of *Aspergillus niger* EH6O Strain

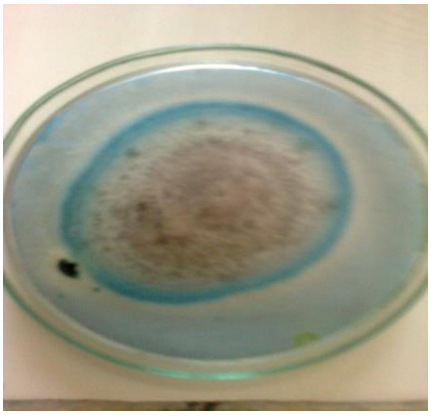

Figure 4-Clear halo on CTAB-MB agar medium

Discussion

This study showed that Sodium nitrate (0.1 %) was the best nitrogen source for biosurfactant production (25g/l) from strain *Aspergillus niger* EH6O. This study showed better results to Patel and Desai, 1997 they observed (0·24 g l^{-1}) rhamnolipid biosurfactants from *Pseudomonas aeruginosa* GS3 when provided cornsteep liquor (5 % v/v) and molasses (7 % v/v) as the primary nitrogen and carbon sources, respectively for 96h growth period. We reported that Sodium nitrate was the best nitrogen source for the biosurfactant production which is verified by the finding of Fonseca *et al*., 2007 they tested *Bacillus subtilis* strain (isolated from contaminated soil of refinery) for biosurfactant production with different nitrogen sources(NH(4))(2)SO(4), urea, residual brewery yeast and NaNO3. They found the highest surfactant yield at 48-h fermentation of ammonium nitrate and crystal sugar 3/3 % w/v. Similar results were found by (Silva *et al*., 2010) who reported (15 g/l) yields of glycolipids when strain *Pseudomonas aeruginosa* UCP0092 was grown with 0.6% (w/v) NaNO3 and 3% (v/v) glycerol for biosurfactant production as nitrogen and carbon source respectively. Onwosi and Odibo. 2012 reported highest biosurfactant production of rhamnolipid (5.46 g/l) by *Pseudomonas nitroreducens* an isolate of petroleum contaminated soil provided the mineral salts medium as the growth medium at C/N (glucose/sodium nitrate) of 22 which is another agreement of our results that we showed sodium nitrate is the best source for biosurfactant production. Abbasi *et al*., 2013 reported that the strain *Pseudomonas aeruginosa* MA01 isolated from petroleum contaminated soil produced highest surfactant (15.68 g/L) when provided with sodium nitrate and soybean oil as nitrogen sources and carbon sources respectively which is agreement of present findings. Our results were much better biosurfactant yield (25 g/l) at cheaper carbon and nitrogen sources than Noparat *et al*., 2014 who reported

the highest biosurfactant production (4.52 g/l) from *Ochrobactrum anthropi* when the strain was grown on a minimal salt medium containing 1 % (w/v) commercial monosodium glutamate and 25 % (v/v) palm oil decanter cake as nitrogen and carbon sources, respectively for 96h at 30 °C.

Conclusion

The present study was an attempt to find economically cheaper nitrogen sources for the large scale production of local microbial biosurfactant. Results obtained in this study recommended the opportunity of industrial production of biological surfactant using local mycoflora on inexpensive nitrogen sources. Local strains tend to give maximal surfactant production have specific properties that would be helpful for process development for the in-country economic production of surfactants.

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