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

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Abstract:

In the present study, six fungal strains were isolated from different oil contaminated soil and fruits from the region of southern Punjab Pakistan. One of the high yielding strain (≥20 g/l of glycolipids) namely Aspergillus niger EH6o was selected for further study. The ability of Aspergillus niger EH6o to grow and yield biosurfactant was studied using different carbon sources (olive oil, almond oil, amla oil, canola oil, peanut oil, vegetable oil, soya+palm oil (1:1), rapeseed oil, coconut oil) at temperatures (20-60°C), pH (2.5-8.0) and incubation periods (24- 120 h). Aspergillus niger EH6o gave maximum biosurfactant productivity (25g/l) using a nutrient medium containing 0.1% olive oil supplemented with 0.5% $NaNO_3$ (initial pH 5.5) when incubated at 27°C for 96h. In Conclusion, taking the economical aspect into account the biosurfactants produced by Aspergillus niger EH6o were found to have better as compared to the chemical surfactants like SDS and CTAB.

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Key words: Biosurfactant, vegetable oils, emulsification, Aspergillus niger, EH6o.

Introduction

Surfactants are the compounds that have unique properties such as lipophilic and hydrophilic ends. Surfactants certainly present in on the oil-air, oil-water or air-water interfaces and decrease the surface tension and interfacial tension (Fathabad *et al.,* 2011). Microorganisms are the big source of biosurfactants like bacteria, fungi and yeasts (Xu *et al.,* 2011). Hydrocarbons are the chief substrate to get the higher production of biosurfactants from microorganisms and rarely carbohydrates are used. Culture medium composition certainly plays a key role in cell growth and accumulation of metabolic products (Techaoei *et al*., 2011).

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,	528°	20-11-10;

			071°28.790 NW		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 as concentrations yeast extract 5.0 g/l, peptone 2.5 g/l, starch 10 g/l, agar 18 g/l (Strijbosch *et al.,* 1990). Sub-culturing of individual isolates was carried out every 2-3 weeks regularly. The different carbon sources such as olive oil, canola oil, coconut oil, rapeseed oil, amla oil, peanut oil, soya+palm oil and vegetable oil as concentration of 0.1%), incubation period (24-120, hour), temperature (20-60^{o}C), pH (2.5-8.0) were studied by solid substrate fermentation (Praveesh *et al.,* 2011).

CTAB-MB Agar Method: CTAB-MB agar techniques for the purpose of biosurfactants production by (Siegmund & Wagner, 1991); (Pinzon & Lu, 2009) were used with some modifications. Uncultured plates with & without sodium dodocylsulphate (SDS) were used as positive and negative control respectively. The diameter of the clear halo was observed using a millimeter scale (Swordfish Brand, China) after 24, 48, 72, 96, and 120 hours of incubation. The average diameter of triplicate samples was worked out for each strain.

Results

Nine different vegetable oils (carbon sources) were investigated for the production of biosurfactant by diverse fungal isolates. The highest biosurfactant yield of (25g/l) by *Aspergillus niger* EH6o was obtained after 72 h of incubation using (0.1%) olive oil as the carbon source. Except for the amla oil and rape oil, all

other vegetable oils gave surfactant yield higher than (20g/l). There is no significance difference in biosurfactant productivity using the olive oil, almond oil, coconut oil, peanut oil, vegetable oil and soya+palm oil by *Aspergillus niger* EH_{60}. However, *Alternaria alternata* Mtkc1 gave maximum yield of (25g/l) using olive and peanut oil as individual carbon sources. Similarly *Penicillium sp* KE Malta, *Aspergillus fumigatus* SS6da1 and *Candida albicans* SSGbrii gave highest yield of (20g/l) using olive oil and amla oil, (20g/l) using peanut and soya+palm oil, (7.5g/l) using olive and soya+palm oil respectively (Figure 2). This shows that the local strains isolated in this study has the potential for surfactant application and further process development. The local crud surfactant was compared with commercial SDS surfactant Figure 1.

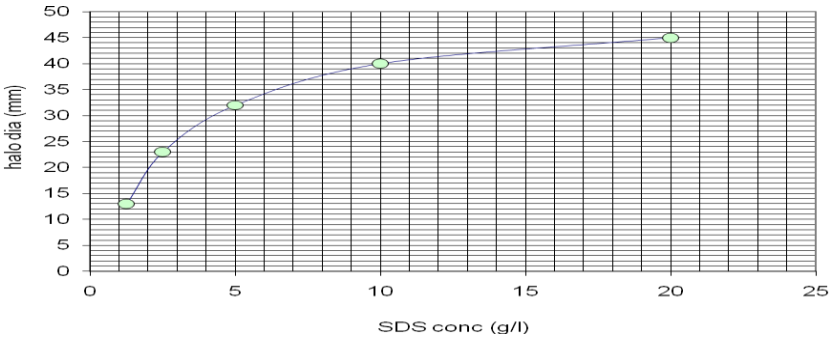

Figure 1: Concentration of SDS vs. diameter of halo on CTAB-MB agar plates.

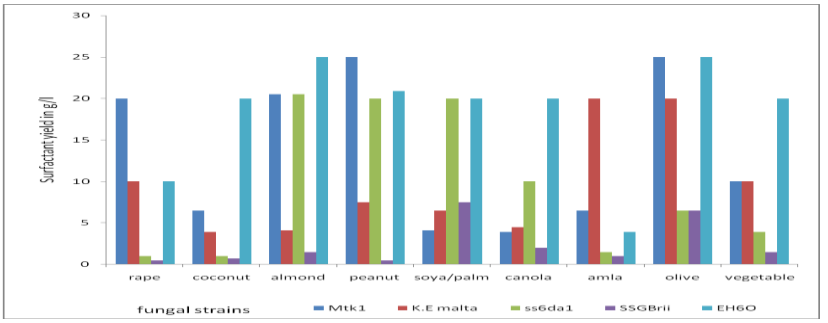

Figure 2: Effect of different carbon sources on different fungal strains.

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, carbon sources 0.1%, KH_2PO_4, Na_2HPO_4 0.9, $CaCl_2$ 0.005, $MgCl_2$ 0.2, Yeast extract 0.3.

Discussion

This study showed that olive oil was the best carbon source at (0.1%) concentration for surfactant production (25g/l) from *Aspergillus niger*. Similar results were found by (Silva *et al*., 2010) who reported that 3% (v/v) glycerol and 0.6% (w/v) NaNO3 for biosurfactant production as carbon and nitrogen respectively by *Pseudomonas aeruginosa* UCP0092 and found (15 g/l) yield of glycolipids surfactants. Present results were better than (Praveesh & coworkers, 2011) who reported (9.2g/l) glycolipids for *Pseudomonas* sp using whey as carbon source incubated for 48h. (Darvishi *et al.,* 2011) noted (1.67g/l) biosurfactants from *Enterobacter* sp using olive oil 1% as carbon source and (NH_4) SO_4 as nitrogen source incubated for 48h. (Zhang *et al*., 2005) reported that the determined biosurfactant production was achieved when *Pseudomonas aeruginosa* was grown in 30 g/L glycerol rather than in glucose, vegetable oil and paraffin oil. The present surfactant amount is nearly equal to that finding. Another study by (Wei *et al*., 2008) stated that *Pseudomonas aeruginosa* J16 when grown 0.32 M glycerol produces a higher production of rhamnolipid than did soy bean oil, sunflower oil and mannitol.

Conclusion

In this study notable surfactant productivity were observed for the strain *Aspergillus niger* EH_6o showed better growth around average room temperature (27C) and produced maximum surfactant yield (25 g/l) using 0.5% $NaNO_3$ as a nitrogen source and 0.1% olive or almond oil as a carbon source. The surfactant

producing local mycoflora of southern Punjab may be used to design and optimize bio control strategies for the further development of sustainable agriculture, economy and the environment for specific purposes.

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