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ISOLATION OF LIPASE PRODUCING FUNGI FROM GROUNDNUT OIL MILL EFFLUENT SOIL SITE AT NANDYAL

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ABSTRACT

The study is attempted to investigate the production of potent lipase from groundnut oil mill fungi. The fungi was isolated and identified as *Trichoderma sp*. Lipolytic activity of *Trichoderma sp.* was preliminarily confirmed by Rhodamine B method, lipase production was carried out using mineral medium. The extracellular protein concentration and lipase enzyme activity (7.83U/ml) was maximum at 4th day of incubation period. *Trichoderma sp*. was studied by using different carbon, oil and nitrogen sources. It was found that carbon, oil and nitrogen sources like glucose, groundnut oil and sodium nitrate were shown the maximum lipase activity. From the ANOVA analysis, the effect of different sources on lipase production was more significant.

KEY WORDS: ***Trichoderma sps*, Lipase enzyme activity, different sources, production**

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INTRODUCTION

Enzymes are considered as nature's catalysts. Lipase (triacyl glycerol acyl-hydrolases, EC 3.1.1.3) catalyses hydrolysis of long chain acyl glycerol at an oil water interface. These are enzymes belonging to the group of hydrolases that present as main biological function to catalyze the hydrolysis of insoluble triacylglycerols to generate free fatty acids, mono and diacylglycerols and glycerol[1,2]. The ease with which enzymes could be isolated from microbes has made both bacteria and fungi predominant sources of lipase. However, fungi are certainly the best lipase sources and are preferably used for industrial applications [3,4]. Lipase producing fungi are present on a wide range of substrates in the ambient environment and these results could also provide basic data for further investigations on fungal extracellular enzymes [5]. Microbial lipases have already established their vast potential regarding usage in numerous applications. Lipases catalytic potentials are enormous. Lipases of microbial origin which are used in the food, diary, cosmetic, detergent, and tanning industries are particularly attractive. In view of their current and potential applications, lipases are considered to be a promising class of industrial enzymes [6]. Because of the numerous potential uses of lipase enzyme, this study was undertaken to isolate and identify the potential lipase producing fungi.

MATERIALS AND METHODS

(i) Isolation and screening of lipolytic producing fungi:

Lipase producing fungal isolate of *Trichoderma sp.* was isolated from groundnut oil mill effluent soil, Nandyal, Andhra Pradesh, India by serial dilution method. The fungal colonies were sub-cultured on Potato Dextrose agar medium for macro and micro morphological identification [7]. The fungal culture was tested for the production of lipase in solid media containing: Rhodamine B- 0.001%, Olive oil - 2%, Sucrose - 10g, KH_2PO_4- 1g, NH_4NO_3-2g, $MgSO_4$- 2g, $CuSO_4$- 0.06g, Agar - 15g. The plates were incubated at room temperature for 7 days. After incubation period, the plate was irradiated with UV illuminator with a bright pink fluorescent halo confirming lipolytic activity.

(ii) Production of lipolytic enzyme:

The fermentation was carried out in shake flasks using a slightly modified media of Kashmiri et al[8], mineral medium consisting (g/L): olive oil- 20ml (emulsified in 2% gum acacia), Ammonium chloride, 4.0; Magnesium sulphate heptahydrate, 0.25; Dipotassium phosphate, 0.5; calcium carbonate, 5.0. The Erlenmeyer flask containing 50 mL fermentation medium was inoculated with 2 ml spore suspension and incubated at 28±2°C with orbital shaking at l00 rpm for 6 days. In order to estimate the lipase activity, first remove the fungus cells and spores through Whatman No.1 filter paper and then the filtrate was centrifuged at 10,000 rpm at 4 °C for 15 min. The supernatant was collected to perform the lipolytic activity and also for protein estimation. The effects of various carbon (glucose, fructose. Maltose and starch), nitrogen (potassium nitrate, sodium nitrate, ammonium nitrate and peptone) and oils (Palm oil, coconut oil, Ground nut oil and Sunflower oil) were given to the above medium for estimation of lipase activity.

(iii) Lipase assay by titrymetry method:

Lipase activity in the broth or mycelia was determined titrimetrically on the basis of olive oil hydrolysis[9]. One mL sample solution was added to the assay substrate containing 10 mL of 10% homogenized Olive oil in 10% gum acacia, 2 ml of 0.6% CaCl2 solution and 5 ml of 0.2 mol/L citrate buffer, pH 7.0. The enzyme substrate mixture was incubated on orbital shaker with a shaking speed of 100 rpm at 37°C for 1 h. To stop the reaction, 20 ml

ethanol acetone mixture (1:1) was added to the reaction mixture. Liberated fatty acids were titrated with 0.1 mol/L NaOH. Intracellular lipase activity was expressed as units per gram mycelium and extracellular lipase activity as units per mL of the broth. One 'lipase unit' (U) was defined as the amount of the enzyme that released one milli mole fatty acid per min.

(iv) Protein Estimation:

Extracellular protein was estimated according to the method of Lowry et al[10] using Bovine serum albumin as standard.

RESULTS AND DISCUSSION

Trichoderma sps. was isolated from groundnut oil mill effluent soil and it was identified based on macroscopic and microscopic observation with descriptions and illustrations in mycological literature of Domsch et al[7]. It was screened for the lipolytic activity on Rhodamine B plates and it showed zone of fluorescence under U.V transilluminator (figure was not shown). The soil being polluted with oil had very low water activity and low pH (4-5). Fungi, especially moulds are known to adapt best under such environment conditions[11].

Figure 1
Pure culture of Trichoderma sps. on PDA agar medium

The extracellular protein concentration was high at 4th day of incubation period, after which it was gradually decreased. The lipolytic activity was also maximum at 4th day of incubation (7.83±0.07U/ml), later onwards it was decreased (Fig 2).

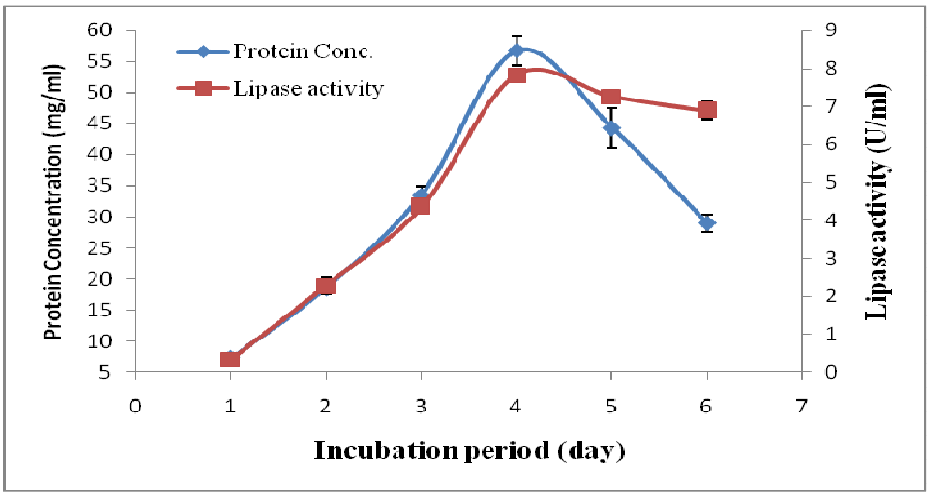

Figure 2
Determination of protein content and lipase activity of Trichoderma sps
****Values are represented as mean ± SD (Standard deviation)***

(i) Effect of different sources on lipase production:

Initially lipase production was carried out in the modified mineral medium[8] supplemented with different substrates. Fermentation was carried out at room temperature (28±2°C) with shaking condition at the rate of 100 rpm for 6 days. Lipase production was determined by assaying lipase activity in crude culture filtrate at standard assay conditions. Different types of oil, carbon and nitrogen sources were tested in order to determine their effects on the lipase production. The best growth was obtained with oil, carbon and nitrogen sources were groundnut oil, glucose and sodium nitrate, respectively. The maximum lipase activity of 7.82 U/ml was observed in the medium supplemented with groundnut oil (Fig 3). The maximum lipase activity was determined as, 7.98 U/ml and 7.81 U/ml on the 4th day in a glucose and sodium nitrate containing medium respectively (Fig 4 and 5). The activity was found to increase slowly upto day 3, after that the lipase production gradually increased in the activity which reached a maximum on day 4. From the day 5 the production rate was found to get decreased, which could be due to proteolysis degradation of enzyme system. Similar type of reports was obtained by *T. reesei*[12]. According to Kashmiri et al[8], the utilization of lipid source with time by *Trichoderma viridae*, the utilization rate increased up to 18 hours, after which, the lipid concentration remained constant. Ely[13] observed that carbohydrates were good source of growth of *Rhizopus oligoporus* but low lipase production was obtained. Hiol et al[14] mentioned that some natural fats or oils were used as inducer for lipase production. Organic and inorganic nitrogen sources play an important role in the synthesis of enzymes. Inorganic nitrogen sources in the form of NO_3 were effective for lipase production[15]. From the results of ANOVA (Analysis Of Variance), based on the value of P (<0.0001), the effect of different sources ie., oil, carbon and nitrogen sources on the lipase production was more significant (Table 1).

Table 1
Analysis of variance for lipase activity

Source	Sum of squares	DF	Mean square	F-Ratio	P-value	Significancy
Oil	43.33352	4	10.83338	28.42126	7.61E-07	Significant
Carbon	57.44997	4	14.36249	200.7524	7.89E-13	Significant
Nitrogen	52.5315	4	13.13288	252.847	1.44E-13	Significant

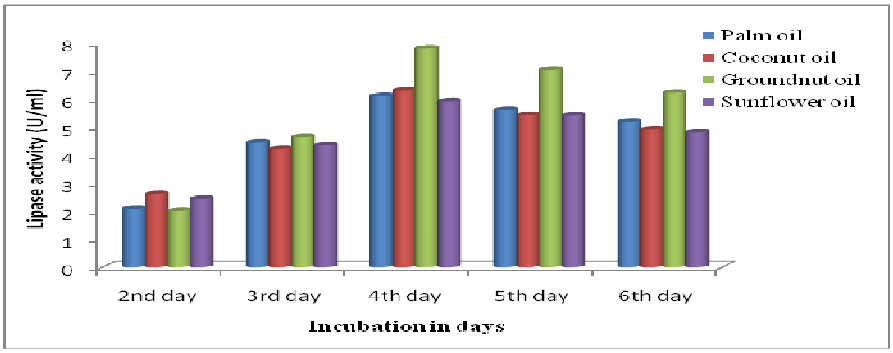

Fig ure 3
Effect of different oil sources on lipase production bu Trichoderma sps

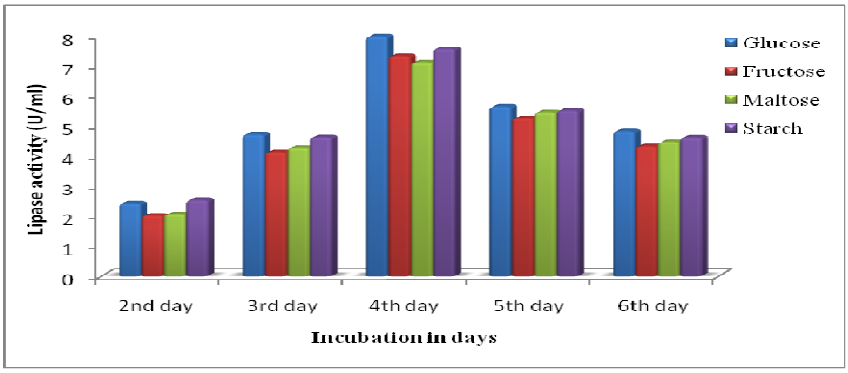

Figure 4
Effect of different carbon sources on lipase production bu Trichoderma sps

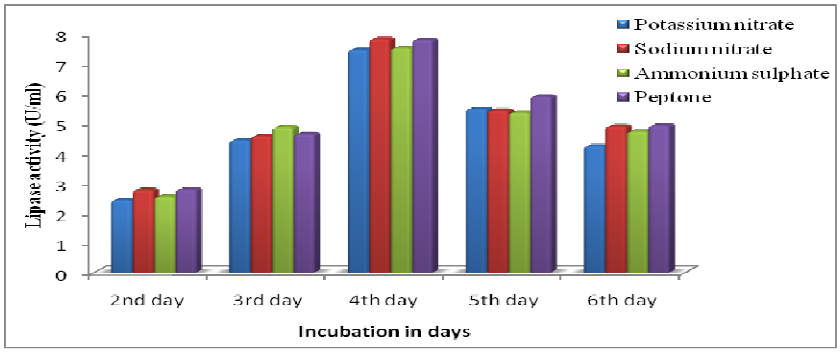

Figure 5
Effect of different nitrogen sources on lipase production bu Trichoderma sps

CONCLUSION

The present study revealed that the potent lipase producing fungi *Trichoderma sps* was isolated and screened from groundnut oil mill effluent soil. The maximum protein concentration and lipase activity (7.83±0.07U/ml) were shown by *Trichoderma sps* at 4th day of incubation period. From the results of the effect of different sources like oil, carbon and nitrogen; groundnut oil, glucose and sodium nitrate were showed maximum lipase activity. Experiments for optimization of nutritive medium composition and cultivation conditions are currently in progress.

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