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Isolation, Screening and Characterization of Lipase Producing Strain from Oil Contaminated Soil of Hubballi, Karnataka

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Abstract—*The present paper reports the isolation and identification of lipase producing bacterial organism from oil contaminated soil. The production of commercial enzymes, including lipase from bacteria has always been the industrial choice due to its economical and commercial feasibility. Three samples of soil were enriched to isolate lipase producing bacteria and were screened for their lipolytic activity. The best producer (DOD9) was characterized and identified as Bacillus sp. and was studied for lipase activity. Maximal lipase production was detected during the early stationary phase of growth curve i.e. at 48 h of growth. Maximum enzyme activity was recorded at a pH of 7.0 and temperature of 37^{0}C. For lipase production, different substrate and nitrogen source were tested and it was determined that the best substrate was waste groundnut oil and the best nitrogen source was soya peptone. The study provides a good mesophilic bacterial candidate for potential industrial production of lipase.*

1. INTRODUCTION

Lipase is an enzyme of considerable commercial and industrial importance. Lipase(triacylglycerol acyl hydrolases (E.C.3.1.1.3) belongs to the class of hydrolases which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil-water interface and reverse the reaction in aqueous and non-aqueous media. Lipases are reported to be monomeric proteins, having molecular weight in the range of 19-60 kda. The important properties of lipases are substrate specificity (Glycerides are the natural substrate for lipases; they possess a chiral alcohol moiety. It was understood that lipases were particularly useful for the resolution or asymmetrization of esters bearing a chiral alcohol moiety), stero-specificity and the ability to catalyze heterogeneous reactions at the interface of water soluble and water insoluble systems. The lipase enzyme even shows stability to extremes of ph, temperature, region and enantio-selectivity.

Lipases occur widely in nature but only microbial lipases are significant. Microbial lipases are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities available, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media. Genetic and environmental manipulation to increase the yield of cells, to increase the enzyme activity of the cells by making the enzyme of interest constitutive, or by inducing it, or to produce altered enzymes, may be employed easily using microbial cells because of their short generation times, their relatively simple nutritional requirements, and since screening procedures for the desired characteristic are easier.

Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, etc many microorganisms such as bacteria, yeast and fungi are known to secret lipases. Of all these bacterial lipases are more economical and stable. (Mukesh Kumar DJ et.al. 2012**)**

Lipases are used in detergent industry as additive in washing powder, textile industry to increase fabric absorbency, for synthesis of biodegradable polymers or compounds and different trans-esterification reactions. In addition, lipase is used as catalyst for production of different products used in cosmetic industry, in pulp and paper industry, in synthesis of biodiesel, degreasing of leather and in pharmaceutical industry. Bacterial lipases are used extensively in food and dairy industry for the hydrolysis of milk fat, cheese ripening, flavor enhancement and lipolysis of butter fat. (E. Sirisha ***et. al.*** 2010).

Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors, such as temperature, ph, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration. Since soil is a reservoir of a large and diverse microbial population, we investigated the ability of these microorganisms to produce lipases. (Mobarak-Qamsari E, ***et. al. 2011)***

2. MATERIALS AND METHODS

2.1 Collection of soil sample

A soil auger was used in collecting soil sample for analysis. The soil sample for physiological analysis was collected with unused plastic bag sealed with heavy-duty rubber bounds. All samples were labeled with a permanent waterproof maker. (Ajit kumar, et.al.2012)

2.2. Isolation of lipase producers

The soil samples were collected from different oil mills located at Dharwar and Tarihal also from railway mechanical workshop was enriched by periodic sub-culturing of samples in Nutrient Broth (NB) media. They were aseptically subjected to serial dilutions and plated on Nutrient Agar (NA) and incubated at 37°C for 24, 48 and 72 h. After incubation, 50 predominant bacterial colonies were isolated and screened for lipase activity and then subjected to morphological, cultural and biochemical examinations. (Ajit Kumar, et.al.2012)

2.3 Screening for lipase activity by Tributyrin Clearing Zone (TCZ)

The predominant bacteria in the nutrient agar plate were isolated and screened for lipolytic activity. Lipolysis is observed directly by changes in the appearance of the substrate such as tributyrin which are emulsified mechanically in various growth media and poured into a petri dish. The bacterial isolates were screened for lipolytic activity on agar plates containing tributyrin (1%, w/v), agar (2%, w/v), peptone(0.5g), beef extract(0.3g).Lipase production is indicated by the formation of clear halo zone around the colonies grown on tributyrin-containing agar plates.(Kalpana Sagar, et.al.2013)

2.4 Characterization of Bacterial lipase producer

Halos around the colonies on tributyrin agar plates are considered as positive colonies for lipase enzyme production. Such colonies are isolated and identified by phenotypic characterization based on morphological, biochemical and physiological characters according to Bergey's Manual of Systematic Bacteriology.(Kalpana Sagar, et.al.2013)

2.5.Optimization of media components protocol:

Effect of nitrogen sources Effect of nitrogen sources on the lipase production was studied with organic nitrogen sources Beef extract, casein, peptone; yeast extract, soya peptone, and inorganic nitrogen sources urea at a final concentration of 1 % (w/v) were added to the medium and incubated at 37°C for 24 hrs in a rotary shaker (150 rpm).(M.Veeragapa,et.al) Effect of substrate (oil) Effect of different waste oils (substrate) on lipase production was studied using gingelly oil, olive oil, sunflower oil, palm oil, groundnut oil, coconut oil at a concentration of 2.2 ml were added to the medium and incubated at 37°C for 24 hrs in a rotary shaker (150 rpm). (M.Veeragapa, et.al)

2.6.Lipase Enzyme production:

The composition of production medium used in this study was1.5g molasses, 1g soya peptone or yeast extract, 2.2ml waste groundnut oil, 100ml d/w, 0.5g KH2PO4, 0.1g CaCo3, 0.1g (NH4)2SO4, 0.1g MgSO4.7H2O.Overnight cultures were used as the inoculums. Submerged microbial cultures were incubated in 250 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (150 rpm) and incubated at 37°C. After 24 hours of incubation, the culture was centrifuged at 10,000 rpm for 10 min at 4ºC and the cell free culture supernatant fluid was used as the sources of extracellular enzyme. The lipase activity in the supernatant was determined by the titrimetric method. (M.Veeragapa, et.al)

2.7.Lipase assay: Titrimetric method

Lipases can be viewed both as lipolytic and esterolytic enzymes, being catalysts for a large number of esters. The activity of lipases can thus be assayed by monitoring the release of either fatty acids or glycerol from triacylglycerol or fatty acid esters. Further, since lipases act at the oil water interface, change in the properties of the interface is an important criterion for measuring lipolysis. The 1ml crude enzyme is mixed well with 9.9ml of distilled water and 0.1ml of oil. Incubate at room temperature for 15 minutes and then titrate against 0.05N NaOH using phenolphthalein indicator.

$$\text{Enzyme activity (U)} = \frac{(\text{Volume of NaOH consumed})*(\text{normality of NaOH})}{(\text{Time of incubation})*(\text{Volume of enzyme added})}$$

3. RESULTS AND DISCUSSION

DOD9 isolated from Doddawad oil refineries was sub-cultured on nutrient agar slants and used for further experimentatation. This lipase producing micro-organism was characterized as gram positive, spore forming, halophillic, mesophilic, motile, aerobic and showed positive results to catalase test, casein hydrolysis and starch hydrolysis. According to the observation it was classified as *Bacillus sp.* The isolate produced maximum lipase using waste groundnut oil and soya peptone having activity 3.5U after 48 hours of incubation at 37°C pH 7 and 150rpm this was concluded after optimization using different waste oils and nitrogen sources.

Table 1: Isolation and Screening

a	Isolate	Diameter of the halo zone (cm)
1.	HSW1	1
2.	HSW2	0.9
3.	HSW3	1.2
4.	HSW4	1.1
5.	HSW5	0.8
6.	HSW6	0.7
7.	TTH7	2.8
8.	TTH8	2.2
9.	TTH9	1.8
10.	DOD9 (V9)	2.9

Table 2: Morphological, Cultural, Biochemical characterization of DOD9 Isolate

Test		Result
Gram's staining		+
Endospore staining		+
7% NaCl		+
Temperature 5°C		-
Temperature 37°C		+
Temperature 50°C		-
Motility		Motile
Catalase		+
Casein hydrolysis		+
Starch hydrolysis		+

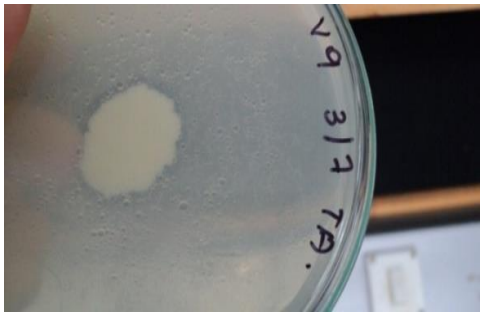


Fig. 1: Isolate DOD9

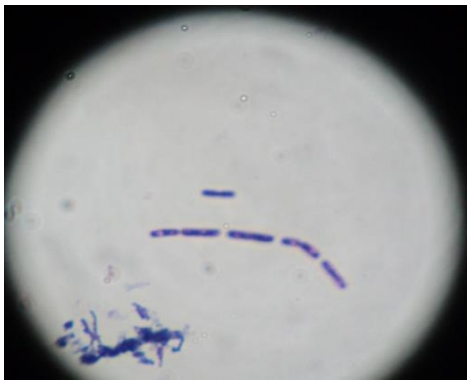
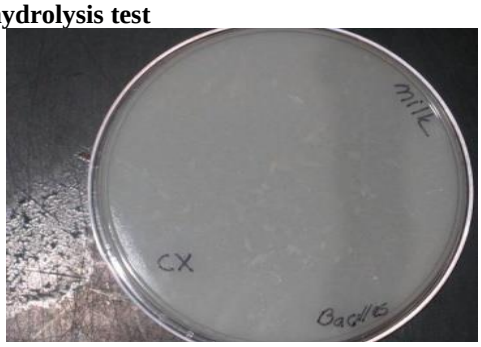


Fig. 2: Morphological, Cultural, Biochemical Characterization of DOD9 Isolate

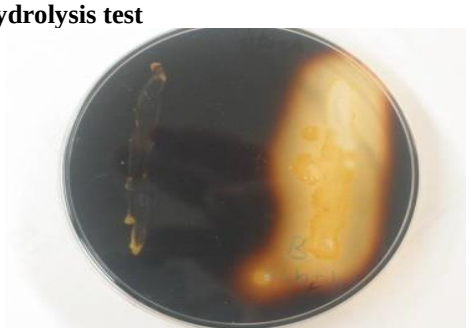
Gram positive short rod chain type and Endospore

Catalase test

Casein hydrolysis test



Starch hydrolysis test



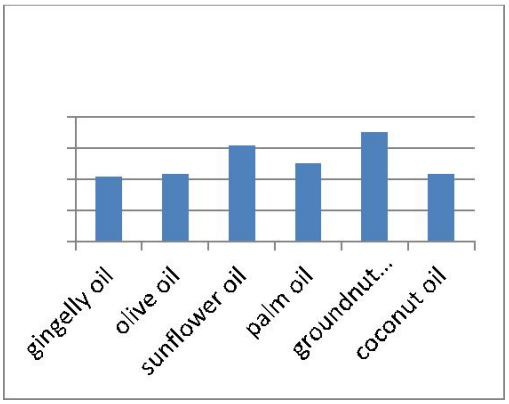


Fig. 3: Effect of substrate (waste oils) on enzyme activity. Enzyme activity(U)

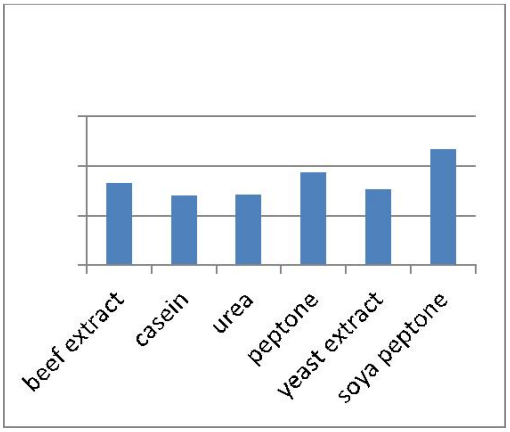


Fig. 4: Effect of nitrogen source on enzyme activity. Enzyme activity(U)

4. CONCLUSION

The bacterial strain *Bacillus* sp. strain DOD9 has been shown to exhibit lipase activities on the basis of the results of qualitative and quantitative analysis. High chemo-, regio- and stereo selectivity have made lipase most important enzyme for pharmaceutical application like resolution of drugs. Experimental results suggest that various media compositions influenced enzyme (lipase) production by indigenously isolated bacterial strains DOD9. Optimization of growth parameters viz., waste oil and nitrogen source etc. had significant effect on lipase activity.

However, the present study requires greater research capacities (further purification of the crude enzyme) for comparison with commercial lipase with regard to specific activity.

5. ACKNOWLEDGEMENT

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