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Studies on Lipase Producing Bacterial Strains Isolated from Different Soil

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Abstract: Lipases catalyses the hydrolysis of triacylglycerols to free fatty acids, partial glycerides and glycerol. Among the various lipases, bacterial lipases are the most important when compared to animal and plant lipases. In the present study lipase producing bacteria were isolated from different dairy farms and slaughter houses soil of Indore city and identified as Bacillus sp for dairy soil and pseudomonas sp for slaughter house soil. They were grown within the temperature ranging between 25°C to 60°C and pH ranging from 3.0 to 11.0. The optimum condition for lipase production obtained was 37°C for both the sp and pH 5 for Bacillus sp and pH 9 for pseudomonas sp. The best carbon source was olive oil for bacillus sp. and Castor oil for pseudomonas sp. The most effective nitrogen source was urea for both the species. Lipases are widely used in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufacturer and production of cosmetics and pharmaceuticals, therefore industrial fronts have shifted towards utilizing this enzyme for a variety of reactions of immense importance.

Key words: Lipase Enzyme · Bacillus Sp · Pseudomonas Sp · Screening

INTRODUCTION

The demand for industrial enzymes, particularly of microbial origin, is ever increasing owing to their applications in a wide variety of processes. Lipases, triacylglycerols hydrolases, are an important group of biotechnology relevant enzymes and they find immense application in food, dairy, detergent and pharmaceuticals industries [1, 2]. The majority of the industrial enzymes origin is microbial. Microbial enzymes are more useful than enzymes derived from plants or animals because of the great variety of catalytic activities, the high yield possible, cases of genetic manipulations, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer. Lipases are available from many sources however; the most suitable sources for lipase production are microbes including bacteria, fungi and yeast.

These microorganisms can produce high quality lipases in lower cost and shorter time [3]. In order to get the highest yield of lipase, the optimal growth conditions should be considered [4]. Lipase production is dependent upon a number of factors including carbon and nitrogen sources, pH, temperature, aeration and inoculums size [5, 6]. Lipases are widely used in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufacturer and production of cosmetics and pharmaceuticals [7, 8]. Microbial lipases are mostly extracellular and there production is greatly influenced by medium composition besides physicochemical factors such as temperature, pH and dissolved oxygen.

The Major factor for the expression of lipase activity has always been reported as the carbon source, since lipases are inducible enzymes. These Enzymes are generally produced in the presence of a lipid such as oil or any other inducer, such as triacylglycerols, fatty acid, hydrolysable easters, tweens, bile salts and glycerols [6, 9]. It is well established that lipase production in microorganism is greatly influenced by media components [10]. The present investigation is aimed at optimization of growth conditions and other parameters which have been pre-directed to play a significant role in enhancing the production of lipase enzyme. For this various parameters of nutritional and environmental factors were tested and growth and lipase activity were measured.

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MATERIALS AND METHODS

Isolation: The bacteria were isolated from the samples using serial dilution plate technique. One gram soil of each sample was dissolved in 100ml of distilled water. The serially diluted samples were streaked on the Tributyrin agar medium having following composition:- Peptone 2.5gm%, Yeast extract 3.0%, Agar 15.0gm%, Glycerol Tributyrate 10.0 ml pH adjusted to neutral (6.8-7) at 25°C [11]. Plates were incubated at 37°C for 48 hrs. The colonies appeared on the plates were taken on the slant of the same medium. The organisms were purified by repeated sub culturing and maintained on the slants of the same medium. The organism were purified by repeated sub culturing and maintained on the slants of same medium.

Screening: To screen out the best lipase producing Bacterial strains plate assay method was used. In this method bacterial strains were screened on solid agar media containing tributyrin as a substrate by qualitative plate assy. Isolates were grown on tributyrin agar base plates and incubated at 36°C for 2 days. Zone of clearance was observed due to hydrolysis of tributyrin agar by lipase. The isolate showing the maximum zone of clearance was selected for further identification [12].

Production Media and Culture Conditions: The test strains were cultured at 37°C in 100ml Erlenmeyer flasks containing 50ml of the Broth of production media having following composition:-

Peptone 0.2gm %, $NH_4H_2PO_4$ 0.1gm%, NaCl 0.25gm%, $MgSO_4.7H_2O$ 0.04gm%, $CaCl.2H_2O$ 0.04gm%, Olive oil 2ml, Tween 20- 2-3 drops. One loop full of bacterial culture was inoculated in each flask and incubated at 37°C. The pH of the medium was adjusted at pH 7 and the supernatant were collected after centrifugation at 10,000 rpm for 20min at 4°C as the crude enzyme sources.

Lipase Assay: Lipase activity in the culture supernatant was determined using the colorimetric method [13], 0.5ml of the crude enzyme was incubated for 5min with 1ml of olive oil used as the substrate, after 5 min of incubation the reaction was stopped with the addition of 1.0ml of 6N HCL and 1ml Benzene, 1ml of cupric acetate was then added and the absorbency was measured at 715nm. Quantification of fatty acid released by lipase is determined by reference to a standard curve prepared using oleic acid. Olive oil is used a substrate. One unit of enzyme activity was defined as the amount of enzyme required to release 1μmol of free fatty acid in 1min at 37°C under the standard assay conditions.

Optimization of Temperature and pH: The effect of temperature was determined by growing the isolates in production media at varied temperature (25°C- 60°C). The effect of pH on lipase production of the isolates was determined by growing the isolates in production media of different pH ranging from 3-11 using appropriate buffers. Phosphate buffers (pH 3.0-7.0), Tris –HCL buffer (pH 8.0-9.0), Glycine- NaOH buffer (10-11).All the flasks were incubated at 37°C for 4 days. The resulting culture was subjected to centrifugation at 10,000 rpm for 20 min at 4°C. Finally the Lipase activity was assayed.

Optimization of Various Carbon Sources

Effect of Carbon Sources: For selection of optimum carbon source on the production of lipase by test strains of bacteria ALJ.3 and AL.5, different carbon sources like olive oil, Castor oil, mustard oil, soybean oil, Maltose, starch, Galactose were used and were added in lipase production media in the concentration of 2% w/v. The isolates were inoculated into different carbon sources flasks and the flasks were incubated at 37°C for 4 days. The resulting culture was subjected to centrifugation at 20,000 rpm at 4°C for 20 minutes. Finally the lipase activity was assayed.

Effect of Nitrogen Sources: The sterilized production broth was prepared with a pH 7, with various nitrogen sources like Peptone, Yeast extract, Ammonium sulphate and Potassium nitrate. These nitrogen sources were used to replace the nitrogen sources available in the media. The isolated strains ALJ-3 and AL-5 were inoculated into different nitrogen sources flasks and the flasks were incubated at 37°C for 4 days. The resulting culture was subjected to centrifugation at 20,000 rpm at 4°C for 20 min. Finally the Lipase activity was assayed.

RESULTS AND DISCUSSION

The lipase enzyme was synthesized by Bacillus species and pseudomonas species, isolated from the different soil samples of Milk dairies and Slaughter houses from different regions of Indore city. The result obtained in the present study revealed the ability of collected bacillus sp and Pseudomonas sp to produce Lipase enzyme. Different culture conditions were used to obtain the maximum levels of Lipase production by the two isolated species.

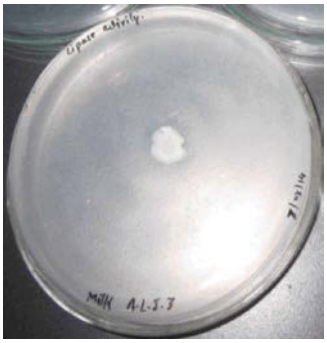

Fig. 1: Photograph showing lipolytic activity of Bacillus species

Fig. 2: Photograph showing Lipolytic activity of pseudomonas species

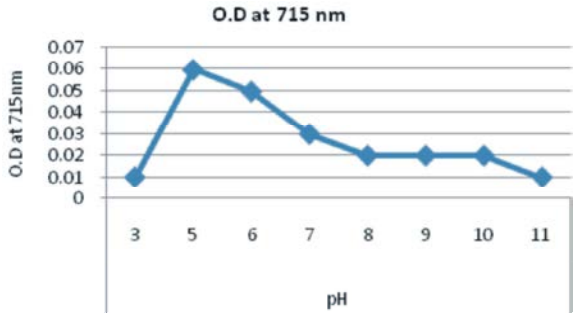

Fig. 3: Effect of different pH on Growth of ALJ.3 (Bacillus sp).

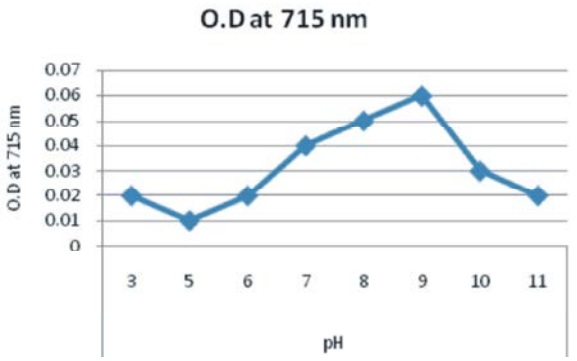

Fig. 4: Effect of different pH on Growth of AL.5 (pseudomonas sp).

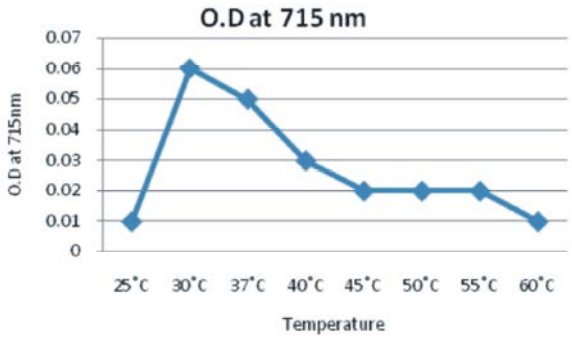

Fig. 5: Effect of different Temperature on Growth of ALJ.3 (Bacillus sp).

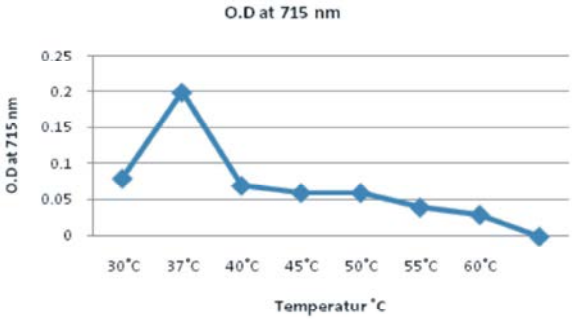

Fig. 6: Effect of different temperature on Growth of AL-5 (pseudomonas sp)

Effect of Temperature and Ph on Growth and Lipase Production: It is known that temperature is one of the most critical parameters that have to be controlled in bioprocess. It is obvious from the results (Fig 1&2) that 37°C was generally more favorable for Lipase production in both the isolated species.

However the temperature below or above 37°C resulted in decrease in Lipase yield as compared to the optimal temperature. It has been noted that the important characteristics of most microorganisms is their strong dependence on the extracellular pH for cell growth and enzyme production [14]. The production medium was adjusted at different pH values of different buffers. The results of pH studies showed (Fig 3 & 4) that the best buffer was Phosphate buffer for Bacillus sp at pH 5 and Tris-HCL buffer for pseudomonas sp at pH 9.

Effect of Carbon Sources: Various carbon sources such as olive oil, castor oil, soybean oil, mustard oil, starch, maltose and Galactose were used in the production media. Results obtained showed that, Galactose brought the highest lipase production for Bacillus sp and olive oil for the pseudomonas sp as compared to the other carbon sources (Fig 7&8).

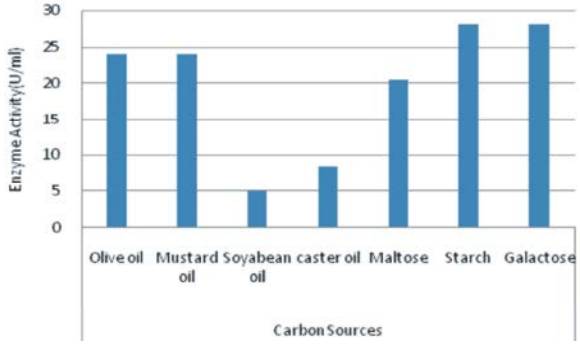

Fig. 7: Effect of different carbon sources on Lipase Production for Bacillus sp

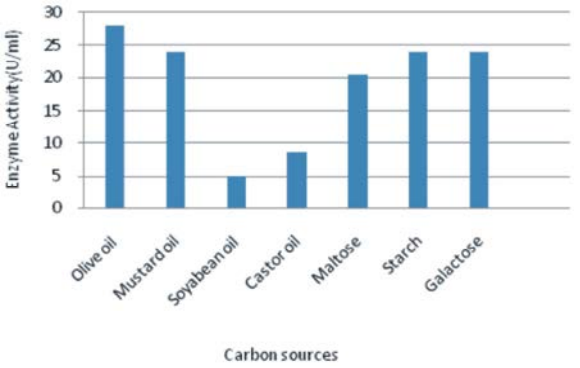

Fig. 8: Effect of Different carbon Sources on Lipase Production for pseudomonas sp.

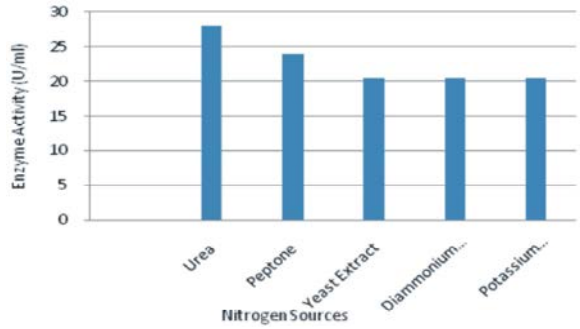

Fig. 9: Effect of Different Nitrogen sources on Lipase Production for Bacillus sp.

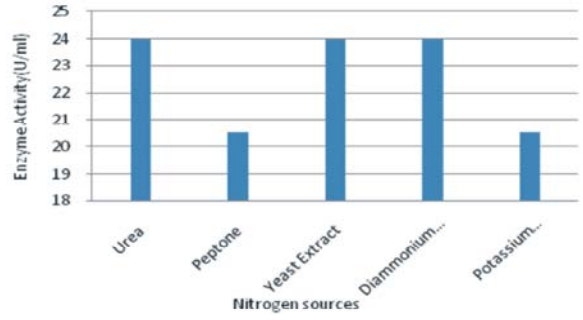

Fig. 10: Effect of different Nitrogen Sources on Lipase production for pseudomonas sp.

Effect of Nitrogen Sources: Generally microorganisms provide high yield of lipase when organic nitrogen sources are used, such as yeast extract and peptone, which have been used for lipase production by various thermophilic bacillus sp. and various pseudomonas [15, 16]. In this research work the effect of different nitrogen sources was studied in the production medium, where different nitrogen sources were used by replacing urea with peptone, yeast extract, diammonium sulphate and potassium nitrate. Among various Nitrogen sources tested Urea as found to be the best Nitrogen source for lipase production for both the Bacillus sp as well as pseudomonas sp (Fig 9&10).

CONCLUSION

It was concluded from the present study that Bacillus and pseudomonas sp which can be used as potential bacterial sources of Lipase production if supplied by media optimization conditions of different carbon and different nitrogen sources showing results for promising bacterial candidates can be used at higher scales of pilot and Industrial level.

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