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RESEARCH ARTICLE

Extracellular lipase production by *Penicillium citrinum* isolated from petroleum contaminated soil

A. Jayaprakash and P. Ebenezer
Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India
aruljaypee@gmail.com; +91 9841378323

Abstract

Penicillium citrinum was isolated from petroleum contaminated soil and screened for its extracellular lipase production in Tween-20 medium. Production of extracellular lipase was substantially enhanced when the pH and temperature of the culture medium, types of carbon and nitrogen sources, oil substances probably stimulating the lipase biosynthesis. The optimum temperature of the enzyme was found to be 35℃ where as the optimum pH was found to be 7.5. Sucrose was found to be the best carbon source for lipase production where as peptone served the best nitrogen source. Olive oil proved to be the best oil substrate which enhanced lipase production. These results are promising because this strain produces lipase in an inexpensive production medium which might be useful in further scaling-up process.

Keywords: *Penicillium citrinum,* petroleum, extracellular lipase, sucrose, peptone, olive oil.

Introduction

Lipases (Triacylglycerol acyl hydrolase E.C.3.1.1.3) are lipolytic serine esterases that are secreted by many fungi, yeasts and bacteria. They are active at their interface between their hydrophobic lipid substrate and the hydrophilic space medium (oil-water interface) cleaving water insoluble glycerides into molecules that can be readily assimilated by cells (Jaegar and Eggert, 2002). Microbial lipases have assumed a great deal of importance as industrial enzymes in view of their potential for use in various biotechnological processes. Fungi are important enzyme producers since; they produce enzymes extracellularly (Ferreira and Peralta, 1999). Lipases are widely exploited due to their low cost of extraction, thermal and pH stability, substrate specificity, and activity in organic solvents. The production of lipases by fungi is influenced by many factors such as pH, temperature, carbon and nitrogen sources (George *et al*., 1999). *Penicillium* spp. are natural 'factories' for the synthesis of extracellular enzymes such as cellulases, xylanases, amylases, proteases, and lipases. For this reason they play an important role in the production of industrial enzymes (Lockington *et al*., 2002).

The industrial demand for lipolytic enzymes continues to stimulate the search for new sources. *Penicillium* spp. are among the most well known lipase producers and their enzymes are suitable for use in many industrial applications (Fu *et al*., 1995). The present study is aimed at screening and identifying the potential fungal strains from a soil sample contaminated with petrol and oils for the production of extracellular lipases by manipulating the physico-chemical environment.

The role of environmental factors such as cultivation temperature, pH, carbon and nitrogen sources, lipid substances and vegetable oils for lipase production has been evaluated.

Materials and methods

Isolation of fungi from soil

Soil sample was collected from the vicinity of a petrol filling station in the Chennai city. One gram soil was collected using a sterile spatula in a sterile petri plate and processed immediately (Plate 1). The fungal isolates from the soil were isolated by soil dilution method (Booth, 1971) and the identified cultures were maintained on Potato Dextrose Agar at 25℃.

Plate 1. Petroleum contaminated soil sample.

Lipase activity on Tween-20 agar plates
The culture medium was prepared by dispensing peptone 100 g, NaCl 5 g, $CaCl_2 \cdot 2H_2O$ 0.1 g and agar 20 g in to 1000 mL water. 10 mL Tween-20 was separately sterilized and added to the autoclaved medium, and the pH was adjusted to 6. The plates were inoculated at the center by a pinpoint inoculum of the test fungus. Lipolytic activity was indicated by the appearance of a visible precipitate, resulting from the deposition of crystals of the calcium salt generated by the fatty acids liberated by the enzyme, or as a clearing of such a precipitate around a colony due to complete degradation of the salt of the fatty acid (Plate 2). At regular intervals of 24 h, each plate was examined and measurements were made to monitor the lipolytic activity (Sierra, 1957).

Plate 2. Lipolytic activity of *P. citrinum* on Tween-20 agar (Note the lipolytic halo).

Lipase production medium
Modified lipase medium was prepared by dispensing 10 g peptone and 1 g yeast extract into 1 L 0.1 M phosphate buffer, pH 7, (Roberts *et al*., 1987). Next, 1% filter sterilized glucose was added to the sterile peptone–yeast extract solution. Sterile Erlenmeyer flasks (250 mL) containing 100 mL sterile yeast-extract-peptone medium with glucose were inoculated with 2 mL spore suspension from 7 d old culture of the test fungi and incubated at 30℃ in a rotary shaker. After 5 day incubation, the cultures were filtered through a filter paper to remove the mycelium. The sterile culture filtrates were stored at -20℃ for further use. The culture filtrate was used as the enzyme source.

Spectrophotometric assay for lipase activity
Lipase activity was assayed quantitatively by using ρ-nitrophenyl palmitate as the substrate (Winkler and Stuckmann, 1979). 10 mL isopropanol containing 30 mg ρ-nitrophenyl palmitate (Sigma) was mixed with 90 mL 0.05 M sodium phosphate buffer (pH 8.0) containing 207 mg sodium deoxycholate and 100 mg gum arabic. A total amount of 2.4 mL freshly prepared substrate solution was prewarmed at 37℃ and mixed with 0.1 m L enzyme solution. After 15 min. incubation at 37℃, absorba nce at 410 nm was measured against a blank. One enzyme unit was defined as 1 µmol of ρ-nitrophenol enzymatically released from the substrate per milliliter per minute (mL/min.).

Effect of carbon sources, nitrogen sources, pH, temperature, lipid substances and vegetable oils
Glucose, sucrose, galactose, lactose and starch were used as carbon sources and different nitrogen sources were amended to the culture medium at 1%. Yeast extract, peptone, ammonium sulphate, ammonium nitrate and ammonium hydroxide were used as nitrogen sources. The experiments were carried out in triplicate, and the results are the mean of three trials.

The test organism inoculated in the lipase production medium were incubated at five different temperatures such as 4°C, 15°C, 25°C, 35°C and 45°C and the effect of pH was studied by adjusting the pH of the lipase production medium to different pH values as 5.5, 6.5, 7.5, 8.5 and 9.5. Different lipid substances at 1% were amended for the extracellular lipase production in the lipase production medium. Tween-20, Tween-80, Cholesterol, Tributyrin and Lecithin were employed as lipid sources. Likewise, different vegetable oils were amended to the lipase production medium at 1% each. Olive oil, Sunflower oil, Castor oil, Mustard oil and Groundnut oil were used as vegetable oil sources.

Results and discussion
The influence of the environmental factors such as temperature, pH, nitrogen, carbon and lipid sources on lipase production has been studied by various investigators. These factors are believed to be crucial for enzyme production in microorganisms and so are true of fungi. After having isolated the test fungi, *Penicillium citrinum* from the soil sample, the same were tested for their ability to produce extracellular lipase in the laboratory, subsequently; the enzyme production was quantified by a spectrophotometric method by manipulating the culture environment. The effects of the environmental variables were investigated.

Fig. 1. Effect of carbon source on extracellular lipase production by *P. citrinum.*

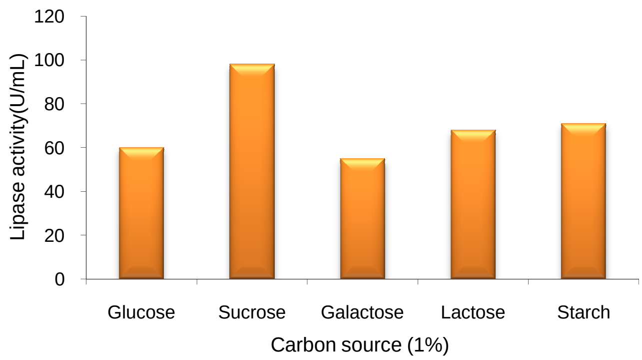


Fig. 2. Effect of nitrogen source on extracellular lipase production by *P. citrinum.*

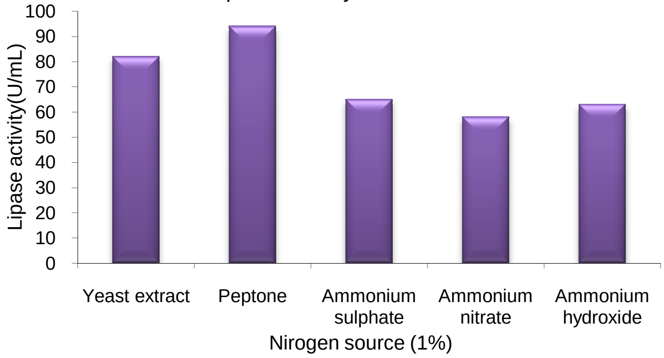


Fig. 3. Effect of hydrogen ion concentration on extracellular lipase production by *P. citrinum.*

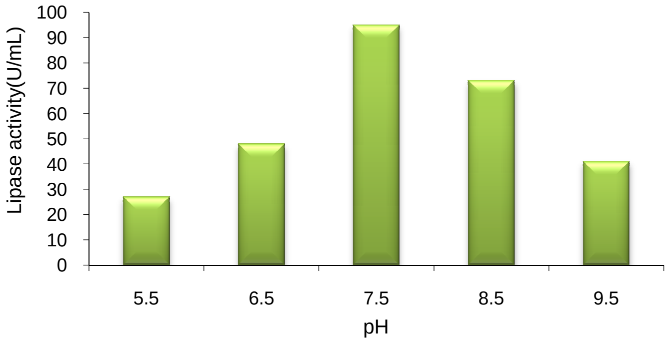


Fig. 4. Effect of temperature on extracellular lipase production by *P. citrinum.*

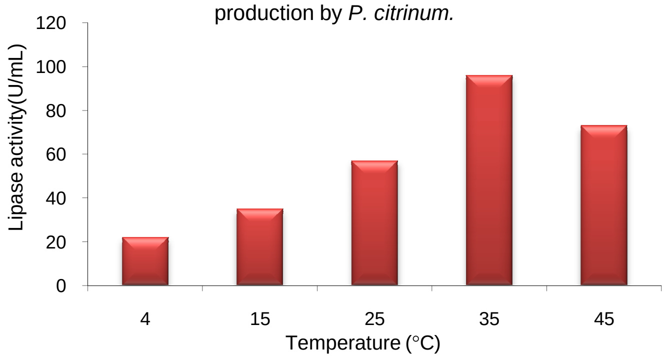
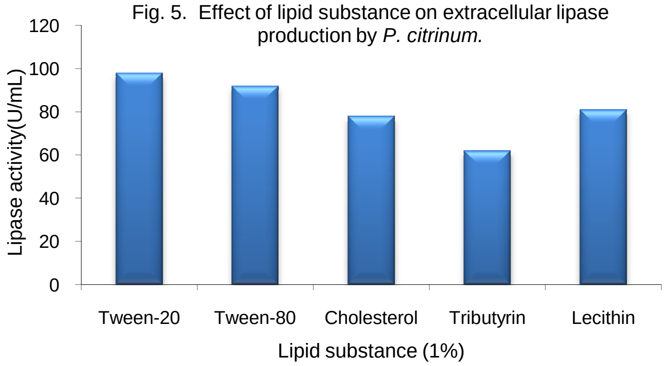
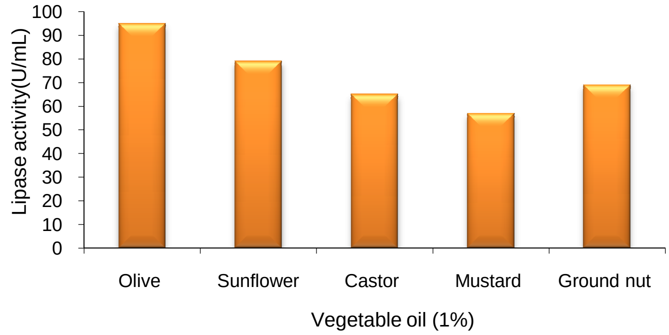


Fig. 5. Effect of lipid substance on extracellular lipase production by *P. citrinum.*



The effects of carbon and nitrogen sources in the culture medium were first studied and it was observed that sucrose, a disaccharide as the carbon source favoured highest lipase production. Glucose and sucrose increase lipase production in *Aspergillus niger* (Kaimi *et al.,* 1998). An organic nitrogen source, peptone favoured highest lipase production in the test organism (Fig. 1 and 2). A near neutral pH (7.5) was best for lipase production. It has also been observed that the enzyme indicated a trend towards thermotolerance since, despite the organisms preference of 35°C, the approximate room temperature for lipase elaboration, a little higher temperature viz. 45°C does not seem to have a pronounced deleterious effect and hence its trend towards thermotolerance. Thermotolerance of these industrial enzymes is a welcome feature in view of the suitability of thermostable enzymes for various applications (Jaeger and Reetz, 1998) (Fig. 3 and 4).

Fig. 6. Effect of vegetable oil on extracellular lipase production *by P. citrinum.*



The substrate that induces enzyme production in any organism is of great influence. In the case of lipases too it is needless to say that the lipid substances provide good raw materials. In this study, although most of the lipids studied more or less had the same effect, Tween-20 favoured best extracellular lipase production in *P. citrinum*. As for the vegetable oils, the trend is the same but olive oil proved to be the best inducer of the enzyme. Sunflower and ground nut oil follows next (fig. 5 and 6).

Conclusion

The brief study revealed that the environment greatly influences enzyme production and that its optimization needs a careful manipulation of the culture environment. The indication that the enzyme showed a tendency towards thermotolerance merits further consideration for developing the same towards this goal for better industrial applications.

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