POTENTIAL PRODUCTION OF LIPASES BY *PSEUDOMONAS* AND *STAPHYLOCOCCUS* SPECIES ISOLATED FROM PALM OIL CONTAMINATED TROPICAL SOIL

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ABSTRACT: This study reports on the potential production of lipases by *Pseudomonas* and *Staphylococcus* species isolated from palm oil contaminated tropical soil. The lipase activities of *Pseudomonas* species and *Staphylococcus* species at 37^{o}C and pH 7 were evaluated. Both organisms grew well and produced lipases at the prevailing assay conditions. The lipase activity by *Pseudomonas* species was significantly higher than that of *Staphylococcus* species at P< 0.05. Effect of pH on the enzyme activities of the isolates showed that lipase production varied according to the pH of the assay medium. This shows that pH is a determinant factor in lipase production. There were increases in lipase activities with increase in pH up to optimum pH of 7 in both organisms. The lipase activities decrease progressively after this optimum pH till the last pH of exposure (pH 10). At pH 7 and 8, the two organisms had equal lipase activities (0.64 and 0.63 U/ml/min, respectively), after which, the lipase activity of *Pseudomonas* species reduced gradually with increase in pH unlike *Staphylococcus* species whose lipase activity abruptly decreased with increase in pH. Lipase activities in both organisms were affected differently by varying temperature changes. In *Pseudomonas* species, there was a steady increase in lipase activity with increase in temperature up to the optimum at temperature of 30 ^{o}C (0.67 U/ml/min). The activity decreased slightly at temperature of 35 ^{o}C but effect of temperature against the activity was more pronounced at the temperatures of 40 and 45 ^{o}C. The lipase activity of *Staphylococcus species* was also affected by temperature changes. There was a steady increase in the activity up to the optimum at temperature of 35 ^{o}C. The activity started decreasing steady with further increase in temperature. In conclusion, the study showed that *Pseudomonas* and *Staphylococcus* species isolated from palm oil contaminated tropical soil has a great potential for the production of lipases.
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1. INTRODUCTION

Lipases are water soluble enzymes which have the ability to hydrolyze triacylglycerols to release free fatty acids and glycerol. Lipases constitute a major group of biocatalysts that have immense biotechnology applications. Lipases have been isolated and purified from fungi, yeast, bacteria, plant and animal sources (Joseph *et al* 2008). Of all these, bacterial lipases are more economical and stable (Immanuel *et al*., 2008). Lipases are currently attracting an enormous attention because of their biotechnological applications. Hence, they have become important biocatalysts in various industrial sectors, such as the agrochemical, pharmaceutical, detergent and food industries (Bouke *et al*., 2007). Bacterial lipases are glycoproteins, but some extracellular bacterial lipases are lipoproteins. Most of the bacterial lipases reported so far are constitutive and are non-specific in their substrate specificity and a few bacterial lipases are thermostable. Due to such attributes, lipases are used in detergents, manufacture of food ingredients pitch control in pulp and paper industry (Fariha *et al.*, 2006), production of aromas, production of insecticides and synthesis of drugs such as naxopren and ibuprofen and as a biocatalyst stereo selective transformations. The exponential increase in the application of lipases in various fields, in the past few years, necessitated both qualitative and quantitative improvement in enzyme production. The quantitative enhancement requires strain improvement and medium optimization for overproduction (Immanuel *et al*., 2008).

Microbial lipases constitute an important group of biotechnological valuable enzymes, mainly because of the versatility of their applied properties and ease of mass production (liu *et al*., 2005). Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial application (Ruchi *et al.,* 2008). Lipases are used in

two distinct fashions. They are used as biological catalysts to manufacture other products (such as food ingredients) and by their application such as (in marking fine chemicals). Lipases have received increased attention recently, evidenced by the increasing amounts of information about lipase in the current literature. The renewed interest in this enzymes class is due primarily to investigations of their role in pathogenesis and their increasing use in biotechnological applications (Haki *et al*., 2003). The commercial use of lipases is a billion-dollar business that comprises a wide variety of different applications (Snellman *et al*., 2006).

The limited resources of fossil fuels, increasing prices of crude oil, and environmental concerns have been the diverse reasons for exploring the use of vegetable oil as alternative fuels (Shah *et al*., 2006). The biodiesel fuels from vegetable oil do not produce sulphur oxide and minimize the soot particulate one third times in comparison with the existing one from petroleum. Because of these environmental advantages biodiesel fuel can be exported as a substitute for conventional diesel fuel (Iso *et al*., 2007).

Immobilized *P. cepacia* Lipase was used for the transesterification of soya bean oil with methanol and ethanol (Noureddini *et al*., 2005). Fatty and esters have also been prepared from castor oil using n-hexane as solvent and two commercial lipases, Novozym 435 and lipozyme IM, as catalyst (de Oliveria *et al*., 2004). Fatty acids esters were produced from two Nigerian Lauric oils, palm kernel oil and coconut oil, by transesterification of the oil with different alcohols using PS30 lipase as a catalyst in the conversion of palm kernel oil to alkyl esters (biodiesel), ethanol gave the highest conversion of palm kernel oil to alkyl esters (biodiesel), ethanol gave the highest conversion of 72%. Some of the fuel properties compared favourably with international biodiesel specifications (Abigor *et al*., 2009). This study reports on the potential production of lipases by *Pseudomonas* and *Staphylococcus* species isolated from palm oil contaminated tropical soil.

2. MATERIALS AND METHOD

2.1. Sample Collection and Isolation of Test Organisms

The test organisms were isolated from soil samples contaminated with palm oil. About 1 g of soil sample was collected in a sterile container and transported to the laboratory on ice. The soil sample was serially diluted in sterile water and inoculated onto nutrient agar plates. After incubation at 37 ^{o}C for 18-24 hours, the colonies were isolated, purified and identified using their cultural and biochemical characteristics. Chromogenic substrate plates were prepared were prepared according to the method of Singh *et al* (2006) as reported by Amara *et al* (2009). Briefly, plates were prepared by using 0.01% of phenol red together with 1% olive oil which served as substrate, 2 % Arabic gum, 10 mM CaCl and 2% agar. The pH was adjusted to 7.3–7.4 using 0.1 N NaOH, where 2% olive oil, 4% Arabic gum and 20 mM CaCl were added to water (pH 7.3) and mixed using suitable mixture till complete homogenization was occurred then the mixture was added to the same volume of 4% melted agar (50°C). The phenol red was added in final concentration 0.01% to the mixture to give orange-reddish color. They were distributed to agar plates wells were punched in the agar plates using sterile cork borer (8 mm in diameter). The base of each hole was sealed with a drop of melted sterile water agar. The organisms were grown in nutrient broth and centrifuged after 24 hours of incubation. The supernatants were used to detect lipase activity. Aliquots (50 µl) of the supernatant were introduced into each well while sterile media served as control. Plates were incubated for 30 minutes at 37 ^{o}C. The changes in the color around the wells were taken as indicative of the presence of lipase activity.

2.2. Lipase Assays

Lipase activity was measured by titrimetric method using olive oil as a substrate. Olive oil (10% v/v) was emulsified with gum Arabic (5% w/v) in 100 mM potassium phosphate buffer (pH 7.0). Up to 100µl of supernatant was added to the emulsion and incubated for 15 minutes at 37^{0}C. The reaction was stopped and fatty acids were extracted by addition of 1.0 ml of acetone: ethanol solution and titrating with 0.05M NaOH until pH 10.5 was reached using phenophathelin as the indicator (Jensen, 1983). The unit of enzyme activity was defined as the amount of enzyme required to hydrolyze one µmol of fatty acids from triglycerides per minute.

2.3. Effect of Temperature on Lipase Activity:

For selection of optimum temperature for the production of lipases, the temperatures varying from 20 to 45°C were selected by keeping the remaining parameters same. The lipase assay was performed as reported above.

2.4. Effect of pH on Lipase Activity:

The optimum pH for enzyme production was selected by varying the pH of the assay medium from 4 to 10 whereas the other parameters were unaltered. The lipase assay was performed as stated above.

3. Result Analysis

Microbial lipases have been a kind of favorable enzymes with their actual and potential applications in household detergents, synthesis of pharmaceuticals or agrochemicals, processing of fats and food ingredients, dairy and textile industries and production of surfactants (Gupta et al., 2004). Lipases have been isolated and purified from fungi, yeast, bacteria, plant and animal sources. Of all these, bacterial lipases are more economical and stable (Joseph et al, 2008). The lipase activities of *Pseudomonas* species and *Staphylococcus* species at 37^{o}C and pH 7 were evaluated. Both organisms grew well and produced lipases at the prevailing assay conditions. The lipase activity by *Pseudomonas* species was significantly higher than that of *Staphylococcus* species at $P< 0.05$ (Figure 1). Some bacterial species have been shown to produce lipases. Among these are *Bacillus*, *Pseudomonas* and *Burkholderia* (Svendsen 2000). Patil *et al*, (2011) reported that lipase can be produced by a variety of organisms including bacteria, fungi, plants and animals. The present study evaluated the lipase production potentials of *Pseudomonas* and *Staphylococcus* species isolated from palm oil polluted soil.

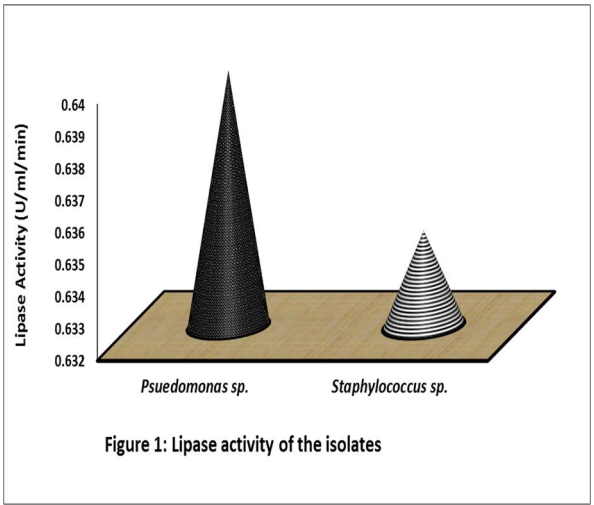

Figure 1: Lipase activity of the isolates

Effect of pH on the enzyme activities of the isolates showed that lipase production varied according to the pH of the assay medium. This shows that pH is a determinant factor in lipase production. There were increases in lipase activities with increase in pH up to optimum pH of 7 in both organisms (Figure 2). The lipase activities decrease progressively after this optimum pH till the last pH of exposure (pH 10). At pH 7 and 8, the two organisms had equal lipase activities (0.64 and 0.63 U/ml/min, respectively), after which, the lipase activity of *Pseudomonas* species reduced gradually with increase in pH unlike *Staphylococcus* species whose lipase activity abruptly decreased with increase in pH. This is at variance with the work of some researchers who reported the optimum lipase activity at alkaline pH (Yuan et al, 2010; Prazeres et al, 2006). But the present study is in consonance with the work of some other researchers whose bacterial species samples isolated from oil contaminated soil had optimum lipase activity at pH 7 (Sirisha et al, 2010; Vijayaraghavan et al, 2011).

Lipase activities in both organisms were affected differently by varying temperature changes. In *Pseudomonas* species, there was a steady increase in lipase activity with increase in temperature up to the optimum at temperature of 30^{o}C (0.67 U/ml/min). The activity decreased slightly at temperature of 35^{o}C but effect of temperature against the activity was more pronounced at the temperatures of 40 and 45 ^{o}C (Figure 3).

The lipase activity of *Stahpylococcus species* was also affected by temperature changes. There was a steady increase in the activity up to the optimum at temperature of 35 ^{o}C. The activity started decreasing steady with further increase in temperature. It has been demonstrated by many authors that temperature is a very strong determinant of enzyme activity (Sirisha et al, 2010; Vijayaraghavan et al, 2011; Prazeres et al, 2006; Yuan et al, 2010). This study agrees with the work of Guzman et al, (2008) who reported an optimum temperature for lipase activity between 35-45 ^{o}C. In conclusion, the study showed that *Pseudomonas* and *Staphylococcus* species isolated from palm oil contaminated tropical soil has a great potential for the production of lipases.

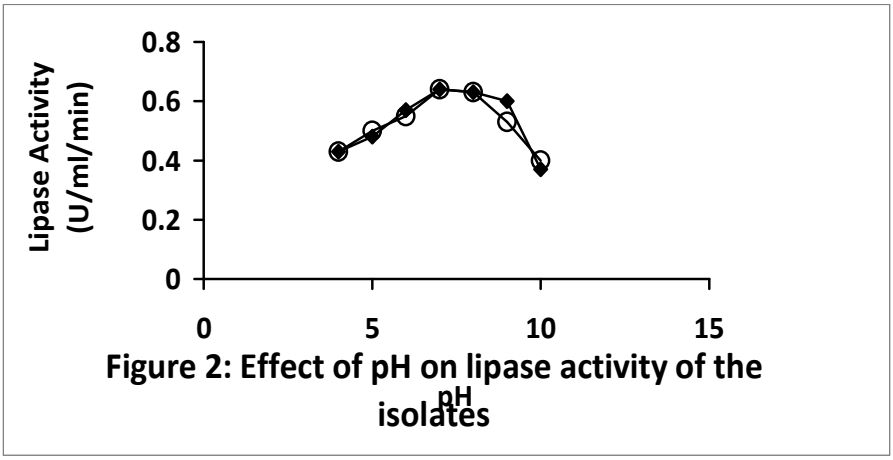

Figure 2: Effect of pH on lipase activity of the isolates

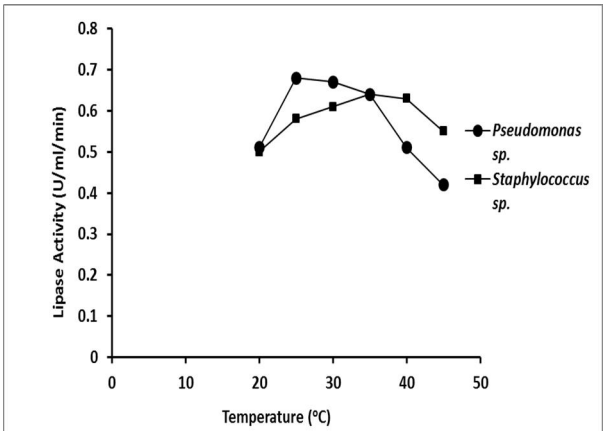

Figure 3: Effect of temperature on lipase activity of the isolates

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