

Isolation of Lipase Producing Fungi from Palm Oil Mill Effluent (POME) Dump Sites at Nsukka

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ABSTRACT

In this study, twelve fungal lipase producing strains belonging to Aspergillus, Penicillium, Trichoderma and Mucor genera were isolated from palm oil mill effluent composts. The Aspergillus spp. were more frequent (42%) and was present in all the samples assayed. Mucor sp. was the least encountered (8.3%). The lipase producing profile showed that Trichoderma (8.07-8.24 u/mL) and Aspergillus (6.25 -7.54 u/mL) spp. were the highest lipase producers while Mucor (5.72 u/mL) was the least.

Key words: Enzymes, Lipases, Triglycerides, Esterification, Waste water treatment

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are a class of enzymes that catalyze the hydrolyses of long chain triglycerides to intermediate and short chain di and mono glycerides, free fatty acids and glycerol (Prazeres et al., 2006; Babu and Rao, 2007). Apart from hydrolyses, lipases are also involved in a wide range of conversion reactions that include esterification, interesterification, transesterification, alcoholysis, acidolysis and aminolysis in non aqueous media (Davranov, 1994; Savitha et al., 2007). Lipase mediated reactions are reversible, hence, the enzyme can catalyze the esterification of glycerol from mono, di and triglycerides. This versatility makes lipases the enzyme of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic and paper industries (Houde et al., 2004).

Lipases are widely distributed in nature and have been found in many species of animals, plants, bacteria, yeasts and fungi (Saeed et al., 2005). Indeed, lipases from a large number of sources have been purified to homogeneity (Saxena et al., 2003). Such lipases from different origins differ widely in physical and biochemical properties such as positional specificity, fatty acid specificity, thermo stability and optimum pH. (Saeed et al., 2005; Kyu et al., 2005). Microbial enzymes are often more useful than enzymes derived from plants and animals because of the great variety of catalytic activities available, the high possible yields, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media (Davranov, 1994) Microbial enzymes are also more stable and safer than their corresponding plant and animal enzymes and their production is more convenient (Wiseman, 1995). Microbial lipases have already established their vast potential regarding usage in numerous

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applications. Specifically, they are employed in waste water treatment (degreasing of lipid clogged drains), pharmaceutical (resolution of racemic mixtures), dairy (hydrolysis of milk, fat), leather (removal of lipids from hides and skin), detergent (removal of oil / fat stains) and medical (diagnostic tool in blood triglyceride assay) industries (Davranov, 1994; Jaeger and Reetz, 1998; Pandey et al., 1999; Rath et al., 2001; Burkert et al., 2004; Kumar, 2005).

Bacterial strains are generally more used as sources of lipases because they offer higher activities compared to yeast (Frost and Moss, 1987). Fungal lipases which are reported to be derived mainly from *Candida* and *Aspergillus* sp (Savitha and Ratledge, 1991) and *Geotrichum* sp. (Ginalska et al., 2004) are particularly important in industrial applications (Essamri et al., 1998).

Because of the numerous potential uses of lipase enzyme, this study was undertaken to isolate and identify lipase producing microorganisms.

MATERIALS AND METHODS

Sample Collection:

Soil samples were collected from four different palm oil mill effluent (POME) dumpsites at Nsukka, Enugu State, Nigeria. Samples were taken from a depth of 2 – 5 cm, transported in sterile plastics bags and stored at 4°C when not used immediately.

Isolation of Lipase Producing Microorganisms:

Ten gram samples (from each site) were suspended in 250mL Erlenmeyer flask containing 100mL sterile physiological saline (PS; Oxoid). This was followed by constant and vigorous stirring for 30 mins at 120 rpm to dislodge soil clumps before allowing settling. The supernatant was decanted and a 10-fold serial dilution made from it. Approximately, 100µL of each dilution (10^{-2} – 10^{-4}) was spread inoculated on a mineral salts agar medium formulated by a modification of the methods of Kashmiri et al., (2006) and Bapiraju et al., (2004). The medium contained (g/l) palm oil 10 (emulsified in Tween 80 at 10mL oil:1mL Tween); $(\text{NH}_4)_2\text{SO}_4$, 5; Na_2HPO_4 , 6; KH_2PO_4 , 2; MgSO_4 , 3; CaCl_2 , 3 and Agar 12. The pH of the medium was adjusted to 5.0 using citrate-phosphate buffer (0.5M). Plates were incubated at 28 °C for 5 – 7 days and checked every day for growth.

Developed colonies were purified and transferred to the mineral salts agar slants for storage. In addition, fungal colonies were subcultured on Potato Dextrose Agar (PDA, Oxoid) plates for macro and micro-morphological identification.

Lipase Production

Suspensions of mature spores were obtained by gently washing the surface of agar slants with 5mL of sterile saline and the resulting suspension adjusted to 10^6 spores/mL in a Neubauer haemocytometer before transferring 5mL of the diluted spore suspension into 250mL Erlenmeyer flask containing 45mL of the mineral salts medium without agar. The flasks were incubated at 28°C for 72 hr at 100 rpm. The culture broth was then filtered in a double layered muslin cloth followed by Whatman No. 1 filter paper. The filtrate was used as the crude enzyme source.

Assay of Lipase Activity

Lipase activity was measured by modification of the titrimetric assay method of Pignede et al., (2000). The substrate (primary) emulsion was prepared with olive oil, gum arabic and water (4:1:2). Appropriate measure of olive oil and gum arabic was triturated with pestle in a clean dry porcelain mortar. When well mixed, the vehicle (water) was added all at once and triturated vigorously to produce a thick white creamy emulsion; the primary emulsion. The supernatant of the cell culture (20µL) was added to 5mL of substrate emulsion and 2ml of 50mM phosphate buffer, pH 7.0 ($\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$) before incubating for 20 mins at 37°C with shaking (120rpm). The reaction was stopped with 4mL of acetone-ethanol (1:1 v/v) containing 2-3 drops of 0.09% thymolphthalein as indicator. Enzymatic activity was determined by titration of fatty acid released with 50mM NaOH. All lipase activity assays were performed at least in triplicate. One unit of lipase was defined as the amount of enzyme that catalyzes the release of 1µmol of fatty acids per minute at 37°C.

Partial Identification of Fungal Isolates

Partial identification of fungal isolates was done by visual observation in petri dish culture and micro-morphological studies in slide culture. For visual observation, the isolates grown in PDA were used. The mode of mycelial growth, colour, odour and changes in medium colour of each isolate were examined daily. In the micro-

morphological studies, a piece of PDA block containing the fungi was detached by a sharp sterile knife. The shape, size, arrangement and development of conidiophores, phialides, conidiospores and phialospores were studied using the taxonomic tools of Hoog et al., (2000).

RESULTS

Fungi were the only organisms isolated. Bacteria did not grow on the isolation medium. Four genera of fungi belonging to *Aspergillus*, *Penicillium*, *Trichoderma* and *Mucor* were identified by their

macro and micro-morphological characteristics which was the basic identification tool. *Aspergillus* was the most frequently isolated fungus because it was conspicuously present in all the soil samples analyzed. *Penicillium* and *Trichoderma* were present in identical number of samples but *Mucor sp.* was isolated only once. The lipase producing profile of the isolates (Table 1) showed that the highest lipase producing strains belong to the *Trichoderma* genera while the lowest was *Mucor sp.* The lipase activity of the *Aspergillus* species was high but varied significantly among the isolates which probably were different species of *Aspergillus*.

Table 1 - Fungi isolated from different POME dumpsites and their lipase producing activity.

Code	Organism	Lipase activity (U/mL)
PC1a	<i>Aspergillus sp.</i>	7.54
PC1b	<i>Penicillium sp</i>	6.21
PC1c	<i>Trichoderma sp.</i>	8.24
PC1d	<i>Aspergillus sp.</i>	6.47
PC2a	<i>Aspergillus sp.</i>	6.25
PC2b	<i>Mucor sp.</i>	5.72
PC3a	<i>Aspergillus sp.</i>	7.08
PC3b	<i>Trichoderma sp.</i>	8.07
PC3c	<i>Penicillium sp.</i>	5.87
PC4a	<i>Aspergillus sp.</i>	7.43
PC4b	<i>Penicillium sp.</i>	6.18
PC4c	<i>Trichoderma sp.</i>	7.19

DISCUSSION

The inability to isolate the bacterial lipase producers did not imply their absence in the POME dumpsites. One factor that could have contributed to the apparent absence of bacterial growth might be associated with the growth conditions prior to sampling. 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. The medium of isolation was selective since only organisms capable of metabolizing lipid as carbon and energy source grew on it. Growth and multiplication of microorganisms on any substrate is often considered as the first step towards its bioconversion (Molla et al., 2002). The fungal organisms isolated were identical to those earlier reported by Ibrik et al., (1995); Ugoji (1997); Toida et al., (1998); Namboodiri and Chattopadhyaya (2000) and Savitha et al., (2007) The use of fungal lipases to facilitate processes and application is gaining grounds and so many of

them have been characterized (Bapiraju et al., 2004, Prazeres et al., 2006, Kashmiri et al., 2006 and Savitha et al., 2007). In the area of waste water treatment, especially the bioremediation of palm oil mill effluent (POME), the yeast *Yarrowia lipolytica* have been successfully utilized. (Oswal et al., 2002). Data on lipase producing profile of isolates suggested that the preponderance of *Trichoderma* and *Aspergillus* spp in the samples analyzed might not be unconnected with their high lipase producing ability since only organisms capable of metabolizing palm oil as carbon source could grow on the medium of isolation. The variation in enzyme activity level within the *Aspergillus* genera could be attributable to species differences.

The use of lipases in industries is enormous and increasing. According to Davranov (1994), extensive and persistent screening for new microorganisms and their lipolytic enzyme will open new, simple routes for synthetic processes and consequently new and faster ways to solve environmental problems.

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Received: October 15, 2009;
 Revised: February 25, 2010;
 Accepted: July 28, 2010.