Production and Characterization of Lipase Enzyme Expressed by Crude Oil Contaminated Soil Isolates

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Abstract Lipases are hydrolytic enzymes responsible for the hydrolysis of triacylglycerol into glycerol and free fatty acids. The biodiversity of crude oil contaminated soil was explored for the isolation of novel potent lipase producing microorganisms. In this current study, microorganisms isolated from crude oil contaminated soils were screened for lipase activity and expression. Six fungal strains namely: Yeast, *Aspergillus flavus*, *Aspergillus niger*, *Verticillus* sp., *Penicillium* sp., and *Microsporum audouini* demonstrated lipase producing potentials and the best two: *Verticillus* sp. and *Penicillium* sp. were selected for enzyme production and characterization. The lipase enzyme was produced in broth medium. Optimization of selected biochemical parameters reveal that optimum temperature for lipase activity was 40 °C, pH 7 with an incubation time of 168 h for lipase expressed by *Verticillus* sp. and 40 °C, pH 8 with an incubation time of 144 h was established for lipase expressed by *Penicillium* sp. The kinetic parameters of the lipase enzyme revealed that the enzymes had low Km (0.19 and 0.30 respectively) for the selected substrate.

Keywords Lipase, Lipase Production, Kinetic Parameters, Optimum PH and Temperature

1. Introduction

Lipases, classified as EC 3.1.1.3 are enzymes that catalyze the complete breakdown of oils and fats by converting them into glycerol and fatty acids (Jaiswal et al., 2017). The principal substrate for lipase enzymes are triacylglycerols. The capacity of lipase to carry out numerous chemical biotransformation is the basis for their increasing popularity in the chemical, detergent, pharmaceutical, food and oil industries. In this regard, they are extensively utilized for lipolysis of cream and fat, ripening of cheese, for the enzymatic hydrolysis of milk

and for the enhancement of flavor etc. in the dairy and food industries (Franken et al., 2009). Lipases are also utilized to augment the absorbency of fabrics in the textile industry, as additives for the production of washing powder in the detergent industries, for diverse transesterification reactions and production of polymers that are biodegradable (Hasan et al., 2006; Jaiswal et al., 2017).

In the oil and gas industry, the use of enzymes has been recently suggested for several applications. A robust of enzymes has been provided for tolerating oil reservoir condition by the modern biotechnology industry. Some applications of enzymes in the oil and gas company were reported by Harris and McKay (1998). Such application includes; breaking of gel in drilling operation to avoid filter cake formation, removal of Sulphur in hydrocarbon, production of “enzyme-based acid” which is used for the purpose of treating formation damage and matrix-acidizing of carbonate, pre-treatment of biopolymers using enzyme to improve the handling characteristics of biopolymer.

Furthermore, lipases have also been employed as catalyst for enhanced production of diverse products utilized in paper, pulp and cosmetic industry (Jaiswal et al., 2017). Many thermostable bacterial lipases have been isolated and characterized (Kumar et al., 2005) from different ecological zones. Among the identified bacteria, *Pseudomonas* sp., *Chromobacterium* sp., *Achromobacter* sp., *Serratia* sp. and *Alcaligenes* sp. have been evaluated for possible production of lipases (Jaiswal et al., 2017).

As a matter of fact, microorganisms that has the capacity to express lipase can be isolated from numerous habitats which include: crude oil contaminated soils, deteriorated food, dairy product industries and wastes from vegetable oil industries to mention but a few (Sharma et al., 2001). According to Koet et al. (2005), soils entertain diverse microorganisms that could be potent producers of lipase and other enzymes of industrial value. The screening of novel microorganisms isolated from distinct ecological

zones for possible production of enzymes could lead to the identification of potent enzyme producers with unique characteristics like stability to diverse pH and temperature ranges, and broad affinity for numerous substrates (Griebeler et al., 2011). To this effect, limited information is available in lipase production capacity of diverse microorganism domicile in numerous ecological areas. Particularly, there is paucity of information about the exploration of crude oil contaminated soils in the Niger delta part of Nigeria. In this regard, we decided to explore the biodiversity of crude oil contaminated soil in Nigeria, here we isolated microorganisms, screened and evaluated their potential and capability for lipase production using standard microbiological techniques. The biochemical characteristics and kinetic properties of the produced enzyme were also evaluated.

2. Materials and Methods

2.1. Sample Collection

The crude oil contaminated soil samples were collected from Tai Local Government Area in Ogoni, River State Nigeria using an Auger as described by Osuoha et al. (2018).

2.2. Isolation and Identification of Lipolytic Microorganisms from Crude Oil Contaminated Soil

A tenfold serial dilution of the soil sample was done till a 10^3 dilutions was obtained according to the technique previously illustrated by Agu (2017). The method reported by Osuoha et al. (2019) was used to isolate the microorganisms under aseptic conditions after serial dilution. In brief, 1 mL from serially diluted 10^3 test tube was inoculated into a molten Saubourand dextrose agar petri dish. The plates were allowed to solidify after mixing on the bench before incubation. Afterwards, the petri dishes were incubated for three days at a temperature of 25 °C to 28 °C. The petri dishes were monitored for production of colonies and they produced colonies were distinguished based on their appearance, elevation, opacity, size, color, and shape. In order to obtain pure cultures, the separated colonies were sub-cultured into different Saubourand dextrose agar. The petri dishes were incubated as described earlier after sub-culturing until the isolates became pure. The obtained pure cultures were reserved in bijoux bottles after due identification via the macroscopic and morphological characteristics.

2.3. Screening for Lipase Producing Microorganisms

Preliminary screening for lipase production of all fungal isolates was done based on the zones of clearance produced

by the organisms on the solid media (Osuoha et al., 2019). All the isolated and identified organisms were cultured on petri dishes containing Saubourand dextrose agar media supplemented with 1 v/v of olive oil as substrate for lipase. The petri dishes were incubated for 72 h as described earlier and then screened for the formation of zones of clearance around the colonies. Organisms that showed zones of 5 mm and above were selected and used for further studies (Osuoha et al., 2019).

2.4. Enzyme Production from Selected Strains

Microbial isolates for lipase production were selected based on the magnitude of zones of clearance formed around the colonies. Standard inoculums of the identified microbial isolates were aseptically inoculated into 500 mL conical flasks containing 500 mL of broth productive medium which contain the following: peptone 5 g, yeast extract 5 g, glucose 10 g, K_2HPO_4 (3g), Na_2SO_4 (2g), $MgSO_4 \cdot 7H_2O$ (0.1g) and olive oil (3 ml) for lipase production. They were incubated at 25 °C to 28 °C on a rotary shaker (150 rpm) for 4 days. The broth was centrifuged to remove viable cells at 3000 rpm for 10 min before used for analysis.

2.5. Lipase Activity

Lipase activity was determined by the technique described by Plou *et al.* (1998) with slight modification. Lipase activity was assayed with Tween 80 as the substrate and measured spectrophotometrically at 450 nm. The reaction mixture contained 800 μ L of 1 % (v/v) Tween 80 in 20 mM Tris-HCl (pH 8.5) and 200 μ L of the enzyme source. The initial absorbance was recorded immediately at 450 nm and the hydrolysis rate of Tween 80 was monitored by recording the change in absorbance after 5 min.

2.6. Optimization of Lipase Production

2.6.1. Effect of Incubation Time

Optimization of the incubation period was achieved by incubating the productive medium as described in section 2.4 for seven days. Lipase activity was monitored periodically (24 h interval) in order to establish the optimum day for lipase production from the inoculated organism.

2.6.2. Effect of Different pH

In order to document the optimum pH for lipase activity, the pH of the 20 mM Tris-HCl buffer was varied (3, 4, 5, 6, 7, 8, 9 10 and 11). Other conditions described in 2.5 were kept constant.

2.6.3. Effect of Different Temperature Ranges

In order to establish the optimum temperature for the activity of lipase expressed by, the temperature of the

reaction medium described in section 2.5 was varied (10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C) for 30 min. Other conditions described in 2.4 were kept constant.

2.6.4. Kinetic Properties of the Produced Enzyme

The V_{max} and K_m the produced lipase enzymes were extrapolated via the Lineweaver-Burk plot. To this end, different concentration (0.2 to 1.80 v/v) of Tween 80 was utilized to perform enzyme-substrate reaction while other reaction conditions illustrated in section 2.5 was kept

constant.

2.7. Statistical Analysis

All the experiments were carried out in triplicates. SPSS version 22 was utilized to obtain the mean of the results obtained.

3. Results

Figure 1. Effect of incubation time on the production of Lipase from *Verticillus sp* and *Penicillium* isolated from crude oil contaminated soil

Figure 2. pH profile of lipases expressed by *Verticillus sp* and *Penicillium* isolated from crude oil contaminated soil

Figure 3. Temperature profile of lipases expressed by *Verticillus sp* and *Penicellum* isolated from crude oil contaminated soil

Figure 4. Enzymes substrate profile for lipases from *Verticillus sp*

Figure 5. Enzymes substrate profile for lipases from *Penicellum*

4. Discussion

In the present study, the biodiversity of crude oil contaminated soil was explored for the isolation of novel potent lipase producing microorganisms. Microorganisms can easily thrive in any polluted environment because of their chemotactic capability (Jorgensen, 2007) to utilize the hydrocarbons in the soil as carbon source. Crude oil contaminated soil has been reported to possess the presence of potential biodegrading microorganism thus, the reason for exploring this biodiversity. In this study, a total of twelve microbial isolates were isolated and was identified based on their macroscopic and morphological characteristics.

Preliminary examination for lipase expression capacity of the isolates was done based of the zone of clearance expressed around the fungal colonies (Osuoha et al., 2019). Out of these twelve isolates, six fungal isolates namely Yeast, *Aspergillus flavus*, *Aspergillus niger*, *Verticillus* sp., *Penicillium* sp., and *Microsporum audouini* demonstrated revealed good lipase producing potentials based on the magnitude of their zones of clearance (Table 1). The production of zones around microbial colonies has been utilized by numerous authors Vermelho et al. (1996), Roy et al. (2014), Dajanta et al. (2009) and more recently Osuoha et al. (2019), for the preliminary screening of enzyme production by microorganisms because it has a direct correlation of enzyme production by the organism (Roy et al., 2014). The enzyme was produced in broth medium and lipase activity was determined to confirm the presence of lipase enzyme, using tween 80 as the substrate. After lipase activity, best two organisms: *Verticillus* sp. and *Penicillium* sp. were selected for lipase production and characterization.

Table 1. Zone of clearance produced around fungal colonies for lipase production

S/N	Name of Organism	Zone of Clearance (mm)
1	<i>Yeast</i>	1.00
2	<i>Aspergillus flavus</i>	7.67
3	<i>Aspergillus niger</i>	6.88
4	<i>Verticillus</i> sp.*	13.45
5	<i>Penicillium</i> sp.*	11.23
6	<i>Microsporum audouini</i>	4.70

*selected for enzyme production

Furthermore, optimization of diverse fermentation conditions was performed in order to improve the activity of the produced enzyme and to establish suitable conditions for enzyme activity. The effects of incubation time on the expression of lipase from the selected isolates are presented in Figure 1. From the results, the optimum incubation time established for lipase production was 168 and 144 h for lipase expressed by *Verticillus* sp. and *Penicillium* sp. A steady decrease in enzyme activity was observed after the optimum day. Microbial enzyme production is not

unconnected to cell growth, for this reason there is a distinctive connection between the production of enzymes by microorganism and incubation period. Microbial enzyme production is usually noticed during the peak of exponential stage or at the starting of stationary stages (Kaur et al., 1998). In this study, highest lipase productions from the selected isolates were observed at the peak of their exponential stages. The recorded decline in the activity of lipase after reaching the peak is likely due to attenuation of nutrients in the production medium which led to slowed cell metabolism and the inactivation of the enzyme (Flores et al., 1997). Our results are in consonance with those reported for lipase by Jaiswal et al. (2017).

Additionally, lipase activity has always been regulated and affected by the temperature, pH as wells the availability of enzyme inducers and the nature of the organism expressing the enzyme (Katz and Betancourt, 1988). Among the significant features for fascinating industrial applications of microbial enzymes, is their capacity to retain strong activity at different physical conditions. In this regard, the optimum conditions for the activity of the produced lipase were established at different pH and temperature ranges. As demonstrated in Figure 2, the optimal pH observed for lipase produced by *Verticillus* sp. and *Penicillium* sp. is 7 and 8 respectively. The optimum pH established in this study for both isolates is in harmony with those reported by Jaiswal et al. (2017) for lipase. Enzymes that have vast activity at different pH ranges are treasured in the biotechnological industries (Singhal et al. 2012).

Currently, one of the characteristics for selecting suitable industrial enzymes is their ability to remain active and perform at diverse temperature ranges. In this study, the influence of diverse temperature ranges on the activity of lipase expressed by *Verticillus* sp. and *Penicillium* sp. was examined. The highest lipase activity was observed at a temperature of 40 °C for *Verticillus* sp. and *Penicillium* sp. respectively as presented in (Figure 3).

The Lineweaver-burk plot demonstrated in (Figure 4 and 5) was used to extrapolate the kinetic properties (V_{max} and K_m) of lipases expressed by *Verticillus* sp. and *Penicillium* sp. The kinetic properties revealed in (Table 2), showed the lipases from *Verticillus* sp. and *Penicillium* sp. had low K_m for their substrates. A low K_m value is an indication of profound affinity for the substrate.

Table 2. Kinetic properties of the produced enzyme

Lipase Source	V_{max} (U/ml/sec)	K_m (mM)
<i>Verticillus</i> sp	0.00456	0.1915
<i>Penicillium</i>	0.00457	0.3012

5. Conclusions

In conclusion, the fungal strains *Verticillus* sp. and *Penicillium* sp. isolated from crude oil contaminated soils

were established to be potent lipase producers. The lipase enzyme had a high activity and was very stable at diverse temperature and pH ranges with vast affinity for numerous substrates. These findings uncover novel sources for microbial lipases that could be applied for numerous industrial applications.

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