The Bioscientist Journal Vol. 11 (1): 37 - 42, January, 2023
Available online at https://www.bioscientistjournal.com



OPEN ACCESS

Full Text of Paper Presented at the 2nd International Conference on Post-Covid-19 Pandemic Resilience: Role of the Biosciences, 10 – 11 January, 2023, Nnamdi Azikiwe University, Awka, Nigeria

SCREENING FOR LIPASE PRODUCING BACTERIA FROM ABATTOIR SOIL

Mathew, C. E[1]. & Izomor, R. N[1].
[1]*Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.*
Correspondence: rn.izomor@unizik.edu.ng

ABSTRACT

Industrial applications of lipase enzymes make them one of the most important enzymes in the biomanufacturing sector. Microorganisms contribute a huge percent of lipases in use today and the search for microorganisms from various habitat for lipase production is continuous. In this study, spread plate method on nutrient agar was used to isolate bacteria from abattoir soil collected from Kwatta Road in Awka, Anambra State. Five (5) Bacterial isolates were processed and screened for their ability to produce lipase using chromogenic plates that were supplemented with olive oil and phenol red as an indicator. Change in colour and zone clearance by isolates were the two (2) factors that were used to determine the results of lipase assay. It was observed that three (3) bacterial isolates showed positive lipase activity at the end of 24h incubation. The colour of the assay-plates were observed to change from yellow to pink and then to fuchsia pink after 48h incubation indicating high lipolytic activity. Morphological and biochemical characterization of the three (3) isolates revealed that they are members of *Bacillus* species. This shows that abattoir soil could be a potential source for bacterial lipase enzymes.

Keywords: Lipase enzyme, Abattoir, Lipolytic activity, Biomanufacturing sector, Phenol red

INTRODUCTION

Lipases are serine hydrolases known as triacylglycerol acyl hydrolases (E.C. 3.1.1.3) which hydrolyze the carboxyl ester bonds in the triacylglycerol at the lipid-water interface to produce fatty acids and glycerol (Gupta, *et al*., 2004; Fickers *et al*., 2011). Biochemical reactions initiated by lipases include esterification, acidolysis, and aminolysis (Patil *et al*., 2011).

Lipases stand out as important enzymes in demand by the biomanufacturing and biotechnology industry today. Examples of such industries include pharmaceuticals,

bioenergy, and food. Lipases are naturally found in plants, animals, and microorganisms. However, lipases from bacteria are most preferred because of their stability (Bharathi *et al*., 2019; Fatima *et al*., 2020). Efforts are currently being made to search for bacterial lipases in different habitats to produce lipase of unique catalytic abilities. Soil and vegetable oil-rich food wastes (Sharma *et al*., 2011). Similarly, lipases have also been isolated from extreme environments (Stathopoulou *et al*., 2013).

In addition, soil impacted with petroleum, waste oil, and related product (Lee et al., 2015; Bharathi *et al*., 2019; Ilesanmi *et al*., 2020) and fats are potential sources of microbial lipase producers possessing desirable and unique catalytic ability. An abattoir is one such place whose operations produce high levels of organic waste made up of suspended solids, liquids, and fat (Adeyemo *et al*., 2002), and soil is mostly the receiving end in addition to water bodies. The negative impact of such waste on the environment has been reported by Akinnibosun and Ayeluyoni (2015), however, such an environment could also be a repository for intriguing microbial lipase producers that can also facilitate cleaning up of the environment. In this study, bacterial isolates from abattoir soil were screened for their ability to produce lipase enzymes, and an attempt was made to identify the isolates.

Materials and Methods

Collection of Samples

Soil samples were collected from different points in an abattoir situated at Kwata road in Awka City of Anambra State, Nigeria. The samples were collected at a depth of 5-10cm, packaged in a sterile container, and samples were transported to the laboratory for further analysis.

Preparation of samples and serial dilution

Sample preparation and serial dilution of soil samples were performed according to Luang-In *et al*., (2019) by homogenizing samples thoroughly, thereafter ten (10) grams were collected and added into 90ml of sterile distilled water to form the stock solution from which serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) was performed in a normal saline (0.9%).

Isolation of bacteria

Nutrient agar plates prepared after autoclaving at 121^0C for 15min at 15psi were inoculated (by spread plate method) with 0.1ml of dilutions (10^{-4}, 10^{-5}, 10^{-6}). Plates were incubated at 37^0C for 24h at an inverted position. Colonies were subcultured subsequently into nutrient agar to obtain pure culture before further analysis. Isolates were then transferred to slant in a bijou bottle for proper storage.

Morphological and Biochemical Characterization of Isolates

Test isolates were subjected to microscopic analysis and Gram staining to determine cell shapes, forms, and arrangements. Biochemical tests such as the catalase test, citrate test, triple sugar test, oxidase test, and Voges-Proskauer test (Cappuccino and Welsh, 2018) were performed on the isolates. To tentatively identify the test isolates, Bergey's Manual of systematic bacteriology was used.

Preparation of Chromogenic Plates and Screening for Lipase Producers

Chromogenic plates were prepared according to Ilesanmi *et al*., (2020) with slight modification using peptone 0.5%, agar 2.0%, olive oil 1%, $CaCl_2$ 0.1%, and phenol red 0.01%. The pH was adjusted with 0.1M of NaOH to 7.4. Test isolates were streaked on the chromogenic plates and incubated for 48h. Plates were observed for the colour change to determine the activity of lipase enzymes on the chromogenic plates.

RESULTS AND DISCUSSION

Lipase catalyzes the hydrolysis of long-chain triacylglycerol to fatty acids and glycerol. The ability of lipase to be active in both aqueous and non-aqueous environments, as well as the non-essentiality of cofactors in lipase, makes them a valuable asset in the biomanufacturing and biotechnology industry. Although soil harbours numerous microorganisms producing lipase. However, when such an environment is impacted with oil and fat they tend to have the advantage of reshaping the microorganisms domicile in that soil to produce lipase of industrial standard.

The morphological and biochemical characteristics of the five isolates from abattoir soil designated as N1, N2, N3, N4, and N5 are presented in Table 1. Lipase enzyme activities of test isolates on chromogenic plates revealed that only three of the isolates (Table 2)gave positive results for lipase activity by changing the colour of the chromogenic plate. It was observed that after 48h the colour changed from yellow-orange to pink and then fuchsia pink. N3 gave the highest colour changecompared to N5 Plate 1. This result is similar to the work of Ilesanmi *et al* (2020) who observed that *Pseudomonas aeruginosa* isolated from oil-contaminated soil produced lipase that gave pink colouration in a chromogenic plate.The isolates used in this study were all found to be members of the *Bacillus* species. Other researchers have also reported lipase production by *Bacillus* species (Chen *et al* 2004; Al-Dhumri, and Bayoumi 2019; Layly *et al*., 2021). Production of lipase by microorganisms is enhanced bythe presence of natural oils such as olive oil (Bornscheur, 2002; Bharathi *et al*., 2019; Fatima *et al*., 2020). In this study olive oil was equally used to stimulate lipase enzyme.

Table 1: Morphology and Biochemical Results of Isolates

Test	N1	N2	N3	N4	N5
Gram's Stain	+	+	+	+	+
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Citrate	+	-	+	-	+
Triple Sugar Iron	-	-	-	-	-
Oxidase	+	-	+	+	+
Maltose	AG	AG	AG	AG	AG
Sucrose	A	AG	AG	A	A
Glucose	AG	AG	AG	AG	AG
Fructose	AG	AG	AG	AG	AG

Key: - = Negative; + = Positive; A = Positive with acid production; AG= Positive with acid and gas production

Table 2: Lipolytic Activities of Isolates

Isolates	Lipolytic Activity
N1	-
N2	+
N3	+
N4	-
N5	+

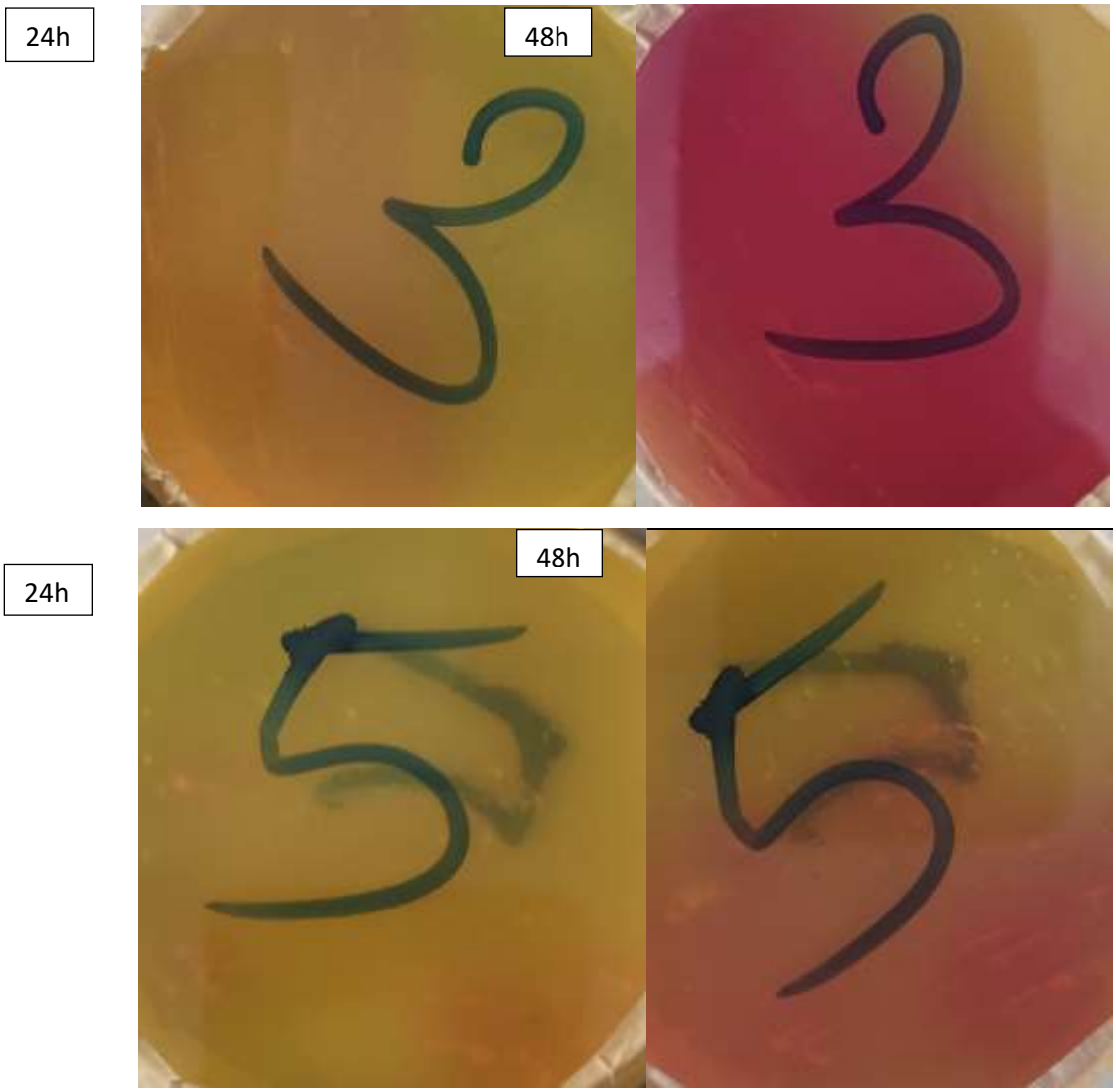


Plate 1: Lipolytic activity on chromogenic media for N3 and N5 after 24h and 48h respectively.

CONCLUSION

In developing nations, abattoirs are known for their deplorable environmental conditions as a result of the wastes generated from their activities. The surrounding soil is impacted by organic

wastes, including fats from slaughtered animals. Bacillus species isolated from abattoir soil were able to demonstrate lipolytic activity on chromogenic plates. This shows that abattoir soil could be a repository for potential lipase with unique catalytic characteristics.

ACKNOWLEDGMENT

The authors wish to thank the Department of Applied Microbiology and Brewing for their assistance.

REFERENCES

Adeyemo, O.k., Ayodeji, I.O., and Aiki-Raji, C.O. (2002). The water quality and sanitary conditions in a major abattoir (Bodija) in Ibadan, Nigeria. *Africa Journal of Biomedical Research* 5: 51-55.

Akinnibosun, F.I., and Ayejuyoni, T.P. (2015). Assessment of microbial population and physico-chemical properties of abattoir effluent-contaminated soil in Benin City, Nigeria. *Journal of Tropical Agriculture, Food, Environment and Extension* 14(3):1-6.

Al-Dhumri, S.A. and Bayoumi, R.A. (2019). Bacterial hyperthermostable alkaline lipase production by B. stearothermophilus isolated from oil polluted soil. *International Journal of Advanced Research in Biological Sciences* 6(2): 166-184.

Bharathi, D., Rajalakshmi, G., and Komathi, S. (2019). Optimization and production of lipase enzyme from bacterial strains isolated from petrol spilled soil. *Journal of King Saud University- Science* 31: 898-901.

Cappuccino, J.G., and Welsh, C. (2018). Microbiology: A laboratory manual 11th edition, Pearson, Harlow, England pp 63-217.

Chen, L., Coolbear, T. and Daniel, R.M. (2004). Characteristics of proteinases and lipases produced by seven *Bacillus* sp. isolated from milk powder production lines. *International Dairy Journal* 14:495-504.

Fatima, S., Faryad, A., Ataa, A., Joyia, F.A., and Parvaiz, A. (2020). Microbial lipase production: A deep insight into the recent advances of lipase production and purification techniques. *Biotechnology and Applied Biochemistry* 68(3): 445-458.

Fickers, P., Marty, A., and Nicaud, J.M. (2011). The lipases from *Yarrowialipolytica*: Genetics, production regulation, biochemical characterization and biotechnological applications. *Biotechnology Advances* 29:632-644.

Gupta, R., Gupta, N., and Rathi, R. (2004). Bacterial lipases: An overview of production, purification and biochemical properties. *Applied Microbiology and Biotechnology* 64:763-781

Ilesanmi, O.I., Adekunle, A.E., Omalaiye, J.A., Olorode, E.M. and Ogunkunmi, A.I. (2020). Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil *Scientific African* 8:e00279.

Layly, I.R., Meryandini, A., Helianti, I., and Astuti, R. I. (2021). Identification of lipase producing bacteria from palm oil sewage sludge processing plant at Malimping, Barten, Indonesia. *Biodiversitas*22(10):4512-4524.

Lee, L. P., Karbul, H.M., Citartan, M., Gopinath, S.C., Lakshmipraye, T., and Tang, T.H. (2015). Lipase-secreting *Bacillus* species in an oil-contaminated habitat: Promising strains to alleviate oil pollution. Biomed Research International 2015: 820575.

Logan, A.N., and DeVos, P. (2009). Genus Bacillus: In:Devos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K-H., and Whitman, W.E. (Eds.), Bergey's Manual of Systematic Bacteriology 2nd Edition, Springer, New York, Vol3, Pp21-128.

Luang-In, V., Yotchaisara, N., Soengla, W., Udomwomg, P., Deeseenthum, S. and Maneewam, K.(2019).Isolation and identification of amylase-producing bacteria from soil in Nasinuan community forest, MahaSarakham, Thailand. *Biochemical and pharmacology Journal* 12(3):1061-1068.

Patil, J.K., Chopda, Z.M., and Mahajan, T.R. (2011). Lipase biodiversity. *Indian Journal of Science and Technology* 4(8):971-982.

Sharma, D., Sharma, B., and Shukla, A.K. (2011) Biotechnological approach of microbial lipase: A review. *Biotechnology* 10(1):23-40

Stathopoulou, P.M., Savvides, A.L., Laragouni, A.D. and Hatzinikolau, D.G. (2013).Unraveling the lipolytic activity of thermophilic bacteria isolated from a volcanic environment. *Biomed Research International* 2013:703130.