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Optimization of culture conditions for lipase production by *Micrococcus* sp. isolated from farmed *Clarias gariepinus* intestine

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Abstract
The culture conditions for maximum lipase activity by *Micrococcus* sp. previously isolated from *Clarias gariepinus* intestine were optimized. The varied culture parameters assessed were incubation time (12 hourly for 60hours), temperature (25, 30, 34, 37, 40 and 45^{o}C), medium pH (4, 5, 6, 7, 8 and 9), additional carbon source (glucose, sucrose, lactose, maltose and mannitol), nitrogen source (peptone, urea, yeast extract, ammonium nitrate, potassium nitrate, calcium nitrate and ammonium hydrogen phosphate) and metal ions (Mn^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+}). The lipase activity was measured using titrimetric method and olive oil as substrate. Maximum lipase activity of 27.6U/ml was found in 48h, 37^{o}C and pH 7.0. Among all the additional carbon sources, maltose gave the maximum activity of 30.6U/ml and among all the nitrogen sources and metal ions peptone and Ca^{2+} gave maximum activity of 27.6U/ml and 27.7U/ml respectively. The present study has revealed *Micrococcus* sp. from a freshwater fish intestine as a candidate for potential industrial production of lipase.

1. Introduction

Lipases are glycerol ester hydrolases that hydrolyze long chain water-insoluble triglycerides into diglycerides, monoglycerides, glycerol and fatty acids [1, 2]. They constitute the most prominent group of biocatalysts for biotechnological applications [3]. As inducible enzymes, lipases are generally produced in the presence of a lipid such as oil or any other inducer such as triacylglycerols, fatty acids, glycerol, Tweens, bile salts and hydrolysable esters [4, 5]. These enzymes are ubiquitous in nature and are widely distributed in plants, animals and microorganisms such as bacteria, yeasts and fungi [6, 7, 8].

Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oil seeds and decaying food, compost heaps, coal tips and hot springs [9]. Lipase-producing bacteria have also been found in fish intestine [10]. These intestinal bacterial isolates have potentials that could be exploited to assist in lipid degradation in various industrial processes.

Enzymes from microbial source are more stable than their corresponding plant and

animal enzymes and their production is more convenient and safer [11]. Microbial lipases have gained special industrial attention due to their stability, selectivity and broad substrate specificity [12]. Microbial lipases have immense applications in waste water treatment and in synthesis of fine chemicals and new polymeric materials as well as in various fields such as food, pharmaceutical, cosmetic, agrochemical, feedstock, detergent, textile, biodiesel and processing industries [8, 13, 14, 15]. The industrial demand for lipolytic enzymes continues to stimulate the search for new enzyme source [16].

Many bacteria and other microorganisms that have potential for lipase production are yet to be isolated, identified and characterized. The production of microbial lipases is greatly influenced by medium composition and physicochemical factors. Consequently, temperature, pH, dissolved oxygen, carbon sources, nitrogen sources and essential micronutrients are carefully considered for cell growth [17] and the bioprocess parameters need to be optimized for industrial production.

Therefore, the aim of this present study was to optimize the different bioprocess parameters for maximum lipase activity by a lipolytic bacterium (*Micrococcus* sp.) previously isolated from cultured fish intestine [10].

2. Materials and Methods

Source of Lipolytic Bacterium (*Micrococcus* sp.): *Micrococcus* sp. was previously isolated from fish (*Clarias gariepinus*) intestine [10] and maintained as part of culture collection in Microbiology Laboratory, University of Port Harcourt, Nigeria.

Lipase Production: The modified liquid culture medium of [3] containing (% w/v): peptone 0.5, yeast extract 0.5, NaCl 0.5, $CaCl_2$ 0.005 and olive oil (1.0 emulsified with gum acacia 0.5), pH 7.5 was used for lipase production. One millilitre of a 24h nutrient broth culture of the tested bacterium was inoculated into 100ml of production medium in 500ml Erlenmeyer flask. The inoculated flasks were then incubated for a period of 48hours at 37^{o}C. Cell-free supernatant was recovered by centrifugation at 5,000 rpm for 15 min and used as the source of extracellular enzyme.

Lipase Assay: The method of [18] described by [19] was employed. 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 100mM potassium phosphate buffer pH 7.0. Then 100µl of crude enzyme was added to the emulsion and incubated for 15 minutes at 37^{o}C. The reaction was stopped and fatty acids were extracted by addition of 1.0ml of acetone:ethanol solution (1.1). The amount of fatty acids liberated was estimated by titrating with 0.05M NaOH until pH 10.5 using a phenophathelin indicator [18]. One unit of enzyme is defined as the amount of enzyme required to hydrolyse µmol of fatty acids from triglycerides.

$$\text{Lipase Activity (Units/ml)} = \frac{\text{Vol. of alkali consumed X Normality of NaOH}}{\text{Time of incubation X Vol. of enzyme solution}}$$

2.1. Optimization of Culture Parameters for Lipase Production

Effect of Incubation Period: The tested bacterium was cultured in production medium at 37^{o}C for 96hours. The culture broth was harvested at 12 hourly intervals by centrifugation at 5,000 rpm for 15 min. The supernatant collected was used as crude enzyme and was assayed for lipase activity.

Effect of Temperature: *Micrococcus* sp. was cultured at temperatures ranging from 25^{o}C to 45^{o}C to select the optimum temperature for maximum lipase production while keeping the remaining parameters constant. Incubation was for 48hours. Each supernatant collected after centrifugation was used as crude enzyme which was assayed for enzyme activity.

Effect of Medium pH: The effect of pH of the production medium on lipase production was performed by varying pH of the medium from 4 to 9 while the other parameters were unaltered. Incubation was for 48hours at 37^{o}C and each crude enzyme obtained after harvesting each culture broth was assayed for lipase activity.

Effect of Carbon Sources: The effect of additional carbon sources on lipase production by *Micrococcus* sp. was analysed by supplementing the production medium with different carbon sources. Glucose, sucrose, lactose, maltose and mannitol at a concentration of 1% (w/v) were added into the production medium in 500ml Erlenmeyer flasks containing 100ml of liquid medium. Each crude enzyme was assayed for lipase activity after incubation at 37^{o}C for 48hours.

Effect of Nitrogen Sources: The effect of nitrogen sources on the lipase production was studied by replacing the nitrogen source with organic and inorganic nitrogen sources. Peptone, urea, yeast extract, ammonium nitrate, potassium nitrate, calcium nitrate and ammonium hydrogen phosphate at a concentration of 1% (w/v) were added into the production medium in 500ml Erlenmeyer flasks containing 100ml of liquid medium. Each crude enzyme was assayed for lipase activity after incubation at 37^{o}C for 48hours.

Effect of Metal Ions: To evaluate the effect of different divalent cations on enzyme activity, manganese sulphate, copper sulphate, ferrous sulphate, zinc sulphate, magnesium sulphate and calcium chloride were separately added into the production medium at a final concentration of 1mM.

Statistical Analysis: Standard deviations for each of the experimental results were calculated using Excel Spreadsheets, with Microsoft excel software.

3. Results and Discussion

In this study we assessed the effects of culture conditions on lipase producing ability of *Micrococcus* sp. previously isolated from the intestine of a freshwater fish (*Clarias gariepinus*) [10]. Many microorganisms such as bacteria, yeast and fungi are known as potential producers of extracellular lipases [20] and bacteria of the digestive tract of aquatic animals participate with their enzymes in the process of degradation of nutrients [21].

The effect of incubation time on lipase production is shown in Fig. 1. Maximum lipase activity of 27.6U/ml was observed at 48hours. The lipase production decreases after 48hours. Similarly, [17] and [22] reported a maximum lipase production at 48hours for *Pseudomonas gessardii* and *Staphylococcus* sp. respectively. The incubation time for enzyme production is governed by the characteristics of the culture and is based on growth rate [17].

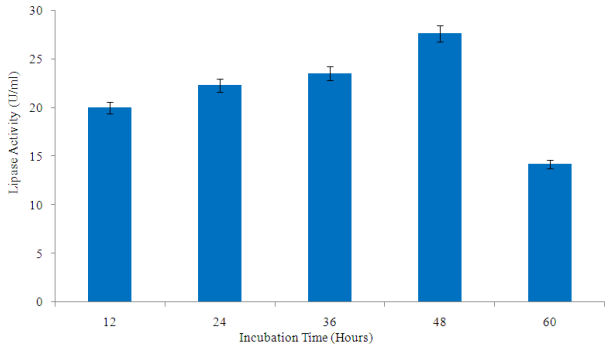

Fig. 1. *Effect of Incubation time on lipase production by Micrococcus sp.*

The effect of medium temperature on lipase production is shown in Fig. 2. The temperature of 37°C was found to be optimum with maximum of 27.6U/ml lipase activity. The enzyme production decreased beyond 37°C. Similarly, the maximum lipase production by *Pseudomonas xinjiangensis* [23] and *Pseudomonas gessardii* [17] was at 37°C. The decrease in lipase production beyond 37°C reported by these researchers and also observed in this work proved that temperature plays a major role in enzyme production. Temperature, as a critical parameter that has to be controlled, influences secretion of extracellular enzymes by changing the physical properties of the cell membrane [17].

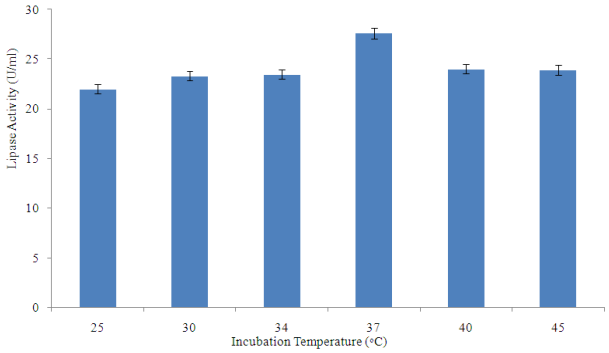

Fig. 2. *Effect of Incubation temperature on lipase production by Micrococcus sp.*

The effect of pH on lipase production is shown in Fig. 3. Maximum lipase activity of 27.6U/ml was observed at pH 7.0. Lipase production declined beyond pH 7.0. Similarly maximum lipase activity was observed at pH 7.0 for *Staphylococcus* [22] and *Pseudomonas gessardii* [17]. These results show that initial pH of growth medium influences the rate of lipase production.

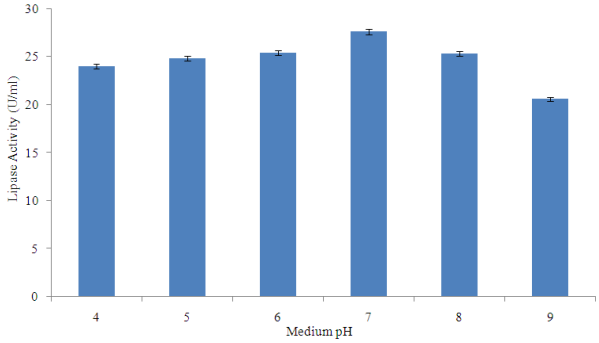

Fig. 3. *Effect of medium pH on lipase production by Micrococcus sp.*

The effect of additional carbon source on lipase production is shown in Fig. 4. Sugars as additional carbon sources have influence on lipase production. Among five sugars, maltose (30.6U/ml), lactose (29.0U/ml) and sucrose (28.0U/ml) influenced lipase production. On the other hand, glucose and mannitol caused a reduction in enzyme production in comparison with the control. This reduction in enzyme production in the presence of sugars as carbon sources could be due to catabolite repression by readily available carbon sources in the medium [24, 25].

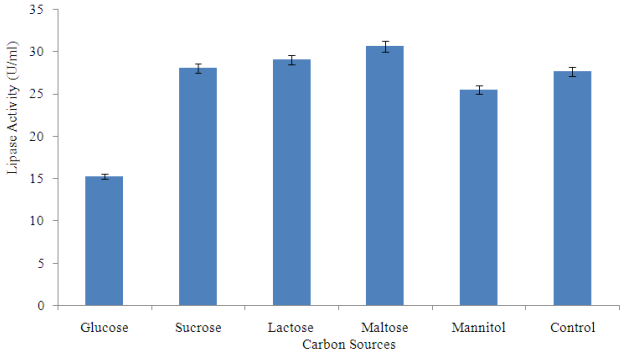

Fig. 4. *Effect of additional carbon sources on lipase production by Micrococcus sp.*

The effect of nitrogen sources on lipase production is shown in Fig. 5. Among the nitrogen sources employed organic nitrogen sources enhanced lipase production whereas lipase production was low with inorganic nitrogen sources. Other authors have reported that microorganisms provide high yields of lipase when organic nitrogen sources such as peptone and yeast extract are used [26, 27]. Maximum lipase activity of 27.6U/ml was observed when peptone was used as nitrogen source. Peptone has been found to be the most suitable source for maximum lipase activity by *Pseudomonas aeruginosa* [28].

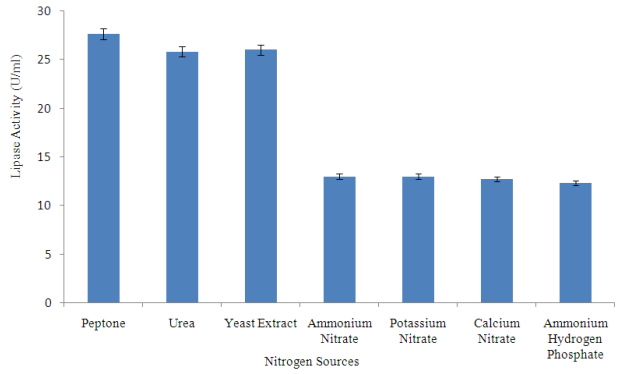

Fig. 5. *Effect of nitrogen sources on lipase production by Micrococcus sp.*

The effect of metal ions on enzyme activity is shown in Fig. 6. *Micrococcus* sp. showed maximum enzyme activity in presence of Ca^{2+}. Lipase activity was reduced on addition of other metal ions employed. Sayari [29] demonstrated that the activity of staphylococcal lipase may depend on the presence of Ca^{2+}. However, it has been reported that *Staphylococcus* sp. isolated from waste contaminated oil showed maximum enzyme activity in presence of Cu^{2+} [30]. Lipase activity is in general inhibited drastically by heavy metals like Co^{2+}, Ni^{2+}, Hg^{2+} and Sn^{2+} and slightly inhibited by Zn^{2+} and Mg^{2+} [31]. These results suggest that lipase production by *Micrococcus* sp. is strongly influenced by medium components as well as cultural parameters.

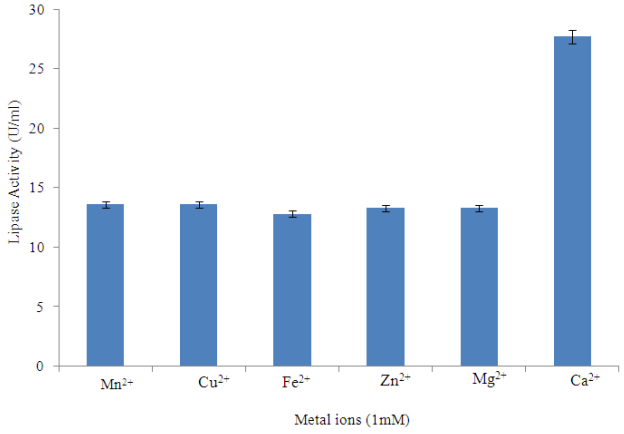

Fig. 6. *Effect of metal ions (1mM) on lipase production by Micrococcus sp.*

4. Conclusion

The study has revealed that *Micrococcus* sp. isolated from fish intestine is a potential producer of lipase. The optimized culture conditions developed in this study can be used for large scale production of lipase to assist in lipase degradation in various industrial applications. Further optimization of lipase production under different nutritional and environmental conditions can be employed to improve the lipolytic activity of the indigenous *Micrococcus* sp. for possible usage in bioremediation of contaminated site.

References

[1] Gilham, D. and Lehner, R. (2005). Techniques to measure lipase and esterase activity *in vitro*. *Methods*, 36:139-147.

[2] Angkawidjaja, C. and Kanaya, S. (2006). Family 1.3 lipase: Bacterial lipases secreted by the type I secretion system. Cell. Mol. Life Sci. 63: 2804-2817.

[3] Ghasemi, Y., Rasoul-Amini, S., Kazemi, A., Zarrini, G., Morowvat, M. H. and Kargar, M. (2011). Isolation and Characterization of Some Moderately Halophilic Bacteria with Lipase Activity. *Microbiology*, 80(4): 483-487.

[4] Sharma, R., Chisti, Y. and Banerjee, U. C. (2001). Production, purification, characterization and applications of lipases. *Biotechnology Adv*ances 19: 627-662.

[5] Gupta, R., Gupta, N. and Rathi, P. (2004). Appl. Microbiol. Biotechnol. 64: 763-781.

[6] Cho, A., Yoo, S. and Kim, E. (2000). Cloning, Sequencing and Expression in *Escherichia coli* of a Thermophilic Lipase from *Bacillus thermoleovorans* ID-1, *FEMS. Microbiol. Lett*., 186(2): 235-238.

[7] Kumar, S., Kikon, K., Upadhyay, A., Kanwar, S. and Gupta, R. (2005). Production, Purification and Characterization of Lipase from Thermophilic and Alkaliphilic *Bacillus coagulans* BTS-3, *Protein. Expr. Purif*., 41(1): 38-44.

[8] Li, H. and Zhang, X. (2005). Characterization of Thermostable Lipase from Thermophilic *Geobacillus* sp. TW1, *Protein. Expr. Purif*. 42(1): 153-159.

[9] Wang, Y., Srivastava, K.C., Shen, G.J. and Wang, H.Y. (1995). Thermostable alkaline lipase from a newly isolated thermophilic *Bacillus* strain, A30-1 (ATCC 53841). *J.Ferment Bioeng*. 79: 433-438.

[10] Ariole, C.N., Nwogu, H.A. and Chuku, P.W. (2014). Enzymatic Activities of Intestinal Bacteria Isolated from Farmed *Clarias gariepinus*. *International Journal of Aquaculture*, 4(18): 108-112.

[11] Wiseman, A. (1995). Introduction to Principles. In: Handbook of Enzyme Biotechnology, Wiseman, A. (ed.), 3rd ed. Ellis Horwood Ltd., T. J. Press Ltd., Padstow, Cornwall, UK, pp. 3-8.

[12] Griebeler, N., Polloni, A.E., Remonatto, D., Arbter, F., Vardanega, R., Cechet, J.L. (2011). Isolation and screening of lipase producing fungi with hydrolytic activity. *Food and Bioprocess Technology*. 4: 578-586.

[13] Ertuğrul, S., Dönmez, G. and Takac, S. (2007). Isolation of lipase producing *Bacillus* sp. from olive mill wastewater and improving its enzyme activity. *J. Hazard Mater*, 149(3): 720-724.

[14] Amoozegar, M., Salehghamari, E., Khajeh, K., Kabiri, M. and Naddaf, S. (2008).

[15] Production of an Extracellular Thermohalophilic Lipase from a Moderately Halophilic Bacterium, *Salinivibrio* sp. Strain SA-2, *J. Basic. Microbiol*., 48(3): 160-167.

[16] Doukyu, N. and Ogino, H. (2010). Organic solvent-tolerant enzymes, *Biochem. Eng. J*. 48(3): 270-282.

[17] Sahu, G.K. and Martin, M. (2011). Optimization of growth conditions for the production of extracellular lipase by bacterial strains from dairy industry effluents. *Biotechnol. Bioinf. Bioeng*. 1(3): 305-311.

[18] Veerapagu, M., Narayanan, A. S., Ponmurugan, K. and Jeya, K.R. (2013). Screening selection identification production and optimization of bacterial lipase from oil spilled soil. *Asian Journal of Pharmaceutical and Clinical Research*. 6(3): 62- 67.

[19] Jensen, R.G. (1983). Detection and determination of lipase (acylglycerol hydrolase) activity from various sources, *Lipids*, 18: 650-657.

[20] Kumar, A., Parihar, S.S. and Batra, N. (2012). Enrichment, isolation and optimization of lipase-producing *Staphylococcus* sp. from oil mill waste (Oil cake). *Journal of Experimental Sciences*. 3(8): 26-30.

[21] Abada, E. A.E. (2008). Production and characterization of a mesophilic lipase isolated from *Bacillus stearothermophilus* AB-1. *Pakistan Journal of Biological Sciences*. 11: 110-1106.

[22] Šyvokienė, J., Stankus, S. and Andreikėnaitė, L. (2011). Bacterioflora of digestive tract of fishes *in vitro*, *Veterinarija ir zootechnika*, 56(78), 93-101.

[23] Sirisha, E., Narasu, M.L. and Rajasekar, N. (2010). Isolation and optimization of lipase producing bacteria from oil contaminated soils. *Advances in Biological Research*. 4(5): 249-252.

[24] Lomthaisong, K., Buranarom, A. and Niamsup, H. (2012). Investigation of isolated lipase producing bacteria from oil-contaminated soil with proteomic analysis of its proteins responsive to lipase inducer. *Journal of Biological Sciences*. 12: 161-167.

[25] Kiran, G.S., Shanmughapriya, S., Jayalakshmi, J., Selvin, J., Gandhimathi, R., Sivaramakrishnan, S., Arunkumar, M., Thangavelu, T., Natarajaseenivasan, K. (2008). Optimization of extracellular psychrophilic alkaline lipase produced by marine *Pseudomonas* sp. (MSI057). *Bioprocess Biosyst. Eng*. 31: 483-492.

[26] Yu, H., Han, J., Li, N., Qie, X. and Jia, Y. (2009). Fermentation performance and characterization of cold-adapted lipase produced with *Pseudomonas* Lip35. *Agricultural Sciences in China*. 8(8): 956-962.

[27] Sugihara, A., Tani, T. and Tominaga, Y. (1991). Purification and characterization of a novel thermostable lipase from *Bacillus* sp. *J. Biochem*. 109: 211-215.

[28] Sharma, R., Soni, S.K., Vohra, R.M., Jolly, R.S. Gupta, L.K. and Gupta, J.K. (2002). Production of extracellular alkaline lipase from a *Bacillus* sp. RSJ1 and its application in ester hydrolysis. *Ind. J. Microbiol*. 42: 49-54.

[29] Kasra-Kermanshahi, R., Mobarak-Qamsari, E. and Moosavi-nejad, Z. (2011).

[30] Isolation and identification of a novel lipase producing bacterium, *Pseudomonas aeruginosa* KM110 *Iran. J. Microbiol*. 3(2): 92-98.

[31] Sayari, A., Agrebi, N., Jaoua, S. and Gargouri, Y. (2001). Biochemical and molecular characterization of *Staphylococcus simulans* lipase, *Biochimie*, 83(9): 863-871.

[32] Sagar, K., Bashir,Y., Phukan, M.M. and Konwar, B.K. (2013). Isolation of lipolytic bacteria from waste contaminated soil: A study with regard to process optimization for lipase. *International Journal of Scientific & Technology Research*, 2(10): 214-218.

[33] Patkar, S. A. and Bjorkling, F. (1994). Lipase inhibitors In: Woolley, P. and Peterson, S. B. (eds.) Lipases – their structure, biochemistry and application. Cambridge University Press, Cambridge, pp. 207-224.