Proceedings of RMBSEFEWS & Indian J.Sci.Res.: 75-80, 2018 ISSN: 0976-2876(Print)
ISSN: 2250-0138(Online)

LIPASE PRODUCTION BY SOLID STATE FERMENTATION UTILIZING AGRICULTURAL BY PRODUCTS

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ABSTRACT

Different parametric conditions for the production of lipase by *Absidia blakesleeana* fungal strain by solid-state fermentation using various agricultural byproducts as substrates were optimized. Maximum lipase activity was obtained after 96 h of incubation under standard conditions. The optimal enzyme activity was found to be at pH 6.5 and 35°C. The enzyme activity was found to be stable between pH 5.0–10·0 and temperature 30–50°C. A maximum of 386 U/gss of lipase activity was found to be at optimal condition of pH 6.5 and 37°C for 96 hrs of incubation.

KEYWORDS: Lipase, *Absidia blakesleeana*, Solid State Fermentation

In the last two to three decades the manufacture, stabilization, packaging and distribution have been carried out on a scale that has taken enzymes from the shelf of exotic specialty reagents in to the holding tank of bulk of useful things. Lipases are ubiquitous enzymes of considerable physiological significance and industrial potential [Joshi and Kuila, 2018] [Erika *et. al.,* 2018]. Lipases (glycerol ester hydrolases EC 3.1.1.3) hydrolyze triacylglycerols to fatty acids, di-acylglycerols, monoacylglycerols and glycerol and under certain conditions, catalyze reverse reactions such as esterification and transesterification [Pandey and Soccol, 2001]. Cost effective large scale applications are made possible by the capacity for producing novel enzymes in large quantity through biotechnological development [Palmer, 1991] [Mahadik *et. al.,* 2002]. Lipases are produced by animals [Shimokawa *et. al.,* 2005], plants [Villeneuve, 2003] and microorganisms [Burkert *et. al.,* 2004] but only microbial lipases are commercially significant [Ionita *et. al.,* 1997] [Sharma *et. al.,* 2001] [Salihu *et. al.,* 2016]. Lipases can be used to accelerate the degradation of fatty wastes [Masse *et. al.,* 2001] and polyurethane [Takamoto *et. al.,* 2001]. In the last decades, the interest in microbial lipase production has increased. Due to the versatility of the molecular structure and catalytic properties, these enzymes have potential application in different industrial sectors such as food, waste water treatment, cosmetics, oleochemical, pharmaceutics, detergents [Castro *et. al.,* 2004] and in the fuel sector, which applies lipase as catalyst for synthesis of esters and for transesterification of the oil for the production of biodiesel [Ranganathan *et. al.,* 2008] [Tamalampudi *et. al.,* 2008]. The interest for biotechnological enzymes lipases, especially from microbial origin, is persistently raising due to their appliances in a wide-ranging multiple applications. They are applied in vast applications in detergent, pharmaceutical food and dairy industries. Lipase producing microorganisms include bacteria [Muthusamy and Beslin, 2018], fungi, yeast and actinomycetes. A large number of lipase producing fungi have been isolated and the most productive species belong to the genera *Geotrichum, Penicillium, Aspergillus, Rhizomucor* and *Yarrowia* [Dominguez *et. al.,* 2003] [Miura and Yamane, 1997] [Mohanasrinivasan *et. al.,* 2009] [Monica *et. al.,* 2008] [Ramachandran *et. al.,* 2007] [Stocklein *et. al.,* 1993] [Vardanega *et. al.,* 2008] [Benjamin and Pandey, 1997] [Varagas *et. al.,* 2008].

Solid state fermentation (SSF), a current development in biotechnology holds tremendous potential for the production of enzymes certain valuable chemicals, fungal toxins by using agricultural wastes such as rice bran, wheat bran, maize bran, soybean bran rapeseed cake etc [Boratyński *et. al.,* 2018]. These substrates provide a rich and complex source of nutrients, which may or may not be need to supplement. Such substrates selectively support mycelial organisms [Sun and Xu, 2008] which can grow at high nutrient concentrations and produce a variety of extracellular enzymes e.g. a large number of filamentous fungi and a few bacteria. Great efforts have been made to reduce production costs of enzymes to broaden their use in industry, and in this context solid-state fermentation (SSF) has attracted attention for use in lipase production. The lower cost of raw materials and inputs, combined with the

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demand for simpler equipment and facilities, are advantages of SSF [Oliveira *et. al.,* 2018].

This paper deals with the screening of potential fungal strain for production of lipase and application of these a lipase in detergent industry. For this agricultural waste residues were investigated for lipase production and application by *Absidia blakesleeana* in solid – state fermentation.

MATERIALS AND METHODS

Isolation of Fungal Strain

Lipase producing fungi were isolated by enrichment culture technique using glucose – yeast extract - peptone (GYP). GYP Agar medium Containing (gL^{-1}) glucose -1.0 peptone 2.5, yeast extract 4.0, starch-1.0 olive oil -10ml, KH_2PO_4 0.5 , $MgSO_4$ -7 H_2O-0.5 ,NaCl -0.25 Agar -18.0 (pH 6.5) and incubated at room temperature for 4 -6 days.

Screening and Selection of Lipolytic Strain

Evaluation of fifty isolates for their lipolytic activity was carried out by point inoculation on a Tributyrin agar plate containing (gL^{-1}) beef extract 3.0, peptone 5.0 tributyrin 15.0ml agar 15.0 plate. The pH of the medium was adjusted to 6.5 and was autoclaved at 15 psi for 15 minutes.

The zone of lipolysis was observed around the fungal colonies on the Tributyrin Agar plates.

Inoculum Preparation

Spores of fungi were harvested from the slant (96 h old culture) by adding 5 ml of sterilized distilled water. The slants were vortexed and conidial suspension thus obtained was filtered to ensure the absence of any hyphal fragment. Conidial Suspension was added in to flasks which contained solid substrate rice bran and mineral growth media (MGM) containing (gL^{-1}) NaH_2PO_4-12.0, KH_2PO_4-2.0, $MgSO_4.7H_2O$-0.3, $CaCl_2$ -0.25 Ammonium Sulfate -10.0 Olive Oil 20ml D.W. 1000 ml pH 6.5.

Inoculation in Solid State Substrate

5ml of mineral growth medium was dispended in 250 ml Erlenmyer flask, containing 5g of rice bran as solid substrate. The flasks were sterilized and inoculated with approximately $5x10^7$ spores/ml which were counted by using heamocytometer (Neubauer, Feinoptik and Kenburg). Flasks were incubated in bacteriological incubator at 37°C. The samples were harvested after every 24 h up to 240 h.

Extraction of Enzyme

At the end of incubation period, 50ml Sorenson buffer (pH 8.0) was added in each flask, shaked the flask for 30 minutes at room temperature and filtered the enzyme by using Whatmann filter paper 1. The clear filtrate was used for lipase assay. The extracellular lipase released into the medium was assayed quantitatively by using 4-nitrophenyl palmitate as the substrate ten milliliters of isopropanol contain – ing 30 mg of p – nitrophenyl palmitate (sigma) was mixed with 90 ml of 0.05 M phosphate buffer (pH 8.0). containing 207 mg of sodium deoxycholate and 100mg gum Arabic .a total amount of 2.4 ml freshly prepared substrate solution was prewarmed at 37°C and mixed with 0.1 ml of enzyme solution .After 15 min of incubation at 37 °C the OD_{410} was defind as I umol ml/min.Protein concentration was determined by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard.

Optimization of Culture Parameters for Maximum Lipase Production

Optimizations of the process parameters for lipase production were aimed to evaluate the effect of a single parameter at a time. The experiments were conducted in triplicate.

Effect of Incubation Time

Lipase production was studied by growing fungal strain up to 240 h and monitoring the production of enzyme at interval of 24 h.

Effect of Solid Substrate

The effect of different solid substrates (wheat bran, rice bran, and coconut cake) for production of lipase was studied. The flasks were inoculated with test organism and incubated at 37°C. The enzyme activity was determined at the end of incubation period.

Effect of Incubation Temperature

Various temperature values viz. 30, 37, 40, 45 and 50°C were tested for their effect on lipase production by growing fungal strain in production medium.

Effect of pH

Various pH values (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, and 10.0) were tested for maximum lipase production.

Potential Application of Lipase in Detergent Formulation

The activity of *A.blakesleeana* lipase was checked in presence of different commercial detergents (Rin, Nirma, Ariel, Fena, Surf Excel and Vim) using titrimetry the enzyme was incubated in the detergent solution (0.5%) at 37±1°C for 30 minutes and then residual activity was determined.

RESULTS AND DISCUSSION

Isolation and Screening of Extracellular Lipase Producing Fungi

Isolation

Fifty fungal strains were isolated from different sources using GYP agar medium. The formation of opaque zone around the colonies is an indication of lipase production by the organisms [Sierra, 1957].

Screening and Selection of Lipase Producing Strain

Fifty fungal strains isolated from different habitats were screened for lipase production on Tributyrin Agar plate at 37^{o}C. Among these isolates only 30 showed lipolytic activities. All the strains demonstrated diverse level of clear zone around the colony. On the basis of diameter of clear zone, five isolates were selected for further study. Culture were identified at IARI, Pure New Delhi i.e. *Absidia blakesleeana*

Quantitative Screening of Screened Isolate under Solid Substrate Fermentation

Since the direct measurement of lipolytic activity under SSF is likely to give more reliable result than the regular plate assay. Five isolates were further tested for their lipolytic activity in modified MGM under SSF. Out of five one isolate gave maximum activity (Table 1).

Table 1: Lipase Secretion Profile of *A. blakesleeana*

S.No.	Isolate No.	Lipase activity (U/gss)
1	Strain A *(A. blakesleeana)*	220.21
2	Strain B	160.11
3	Strain C	109.10
4	Strain D	78.61
5	Strain E	60.11

Optimization of Culture Parameter of Lipase Production

Effect of Incubation Period on Lipase Production

A. blakesleeana was incubated for different time periods from 48 – 240 h under the standard conditions and lipase activity was assayed at the intervals of 24 h.

Maximum lipase production 371.00 U/gss was obtained after 96 h of incubation. There after decline in lipase production was observed (Figure 1).

Effect of solid substrates

Influence of different solid substrates on lipase production was studied and the results are presented as graph (Figure 2). It is clear from the result that rice bran was the best solid substrate for isolate *A. blakesleeana* with lipase activity of 281.70 U/gss.

Effect of Incubation Temperature

Temperatures in the range of 30^{o}C – 50^{o}C were tested for lipase production. The results are presented in Table No. 2.

Table 2: Effect of Temperature on Lipase Production after 96 h and 5.0 ml Moisture Content

Temperature ^{o}C	Dry wt of biomass (mg)	Enzyme activity (U/gss)
30	230	299.80
35	440	361.00
40	380	346.00
45	260	310.00
50	180	263.80

It is clear from Figure 3 that *A. blakesleeana* produced maximum lipase at 37^{o}C, (361.00 U/gss). However, at 40^{o}C there was drop in lipase production.

Effect of pH

A. blakesleeana was grown at pH ranging from 5 to 10 in lipase production medium and lipase activity was estimated after 96 h of incubation. The results presented in Fig. 4 showed that the strain is capable to produce lipase in the pH range of 5 – 10. However, maximum lipase 386.50

U/gss was obtained at pH 6.5. Filamentous fungi are supposed to thrive over a broad range of pH under solid-state culture, because the solid substrate holds a better buffering capacity [Shankar and Mulimani, 2007]. Figure 4 clearly shows that *A. blakesleeana* produces lipase in the broad pH range of 6 – 9, with maximum production at pH 6.5. Significant production was also achieved even at pH 9.0.

CONCLUSION

In the present study *A.blakesleeana* was used to produce lipase enzyme by solid state fermentation. The influence of culture conditions such as incubation time, temperature, pH along with effect of diverse substrates on lipase production was determined. The results of this study show that *A.blakesleeana* produced lipase possesses a good pH stability (pH 6-0-9.0) as well as thermal stability (30-50)°C. After 96 h of incubation, maximum lipase activity 371.00 U/gss was achieved. Also the lipase activity of 386.00 U/gss was obtained for at pH 6.5 and 37°C for 96 hrs of incubation time. The results suggest that agricultural byproducts such as wheat, rice bran and coconut cake can be potentially utilized for the cost-effective substrate for producing industrial enzymes.

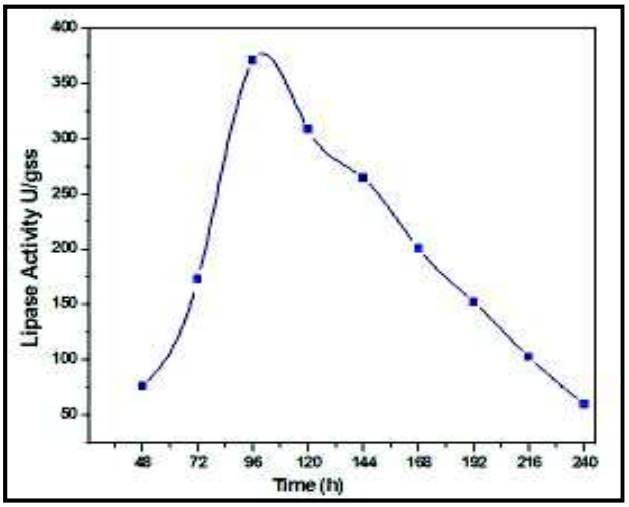

Figure 1: Effect of Incubation Period on Production of Lipase at Temperature 37 °C and pH 6.5.

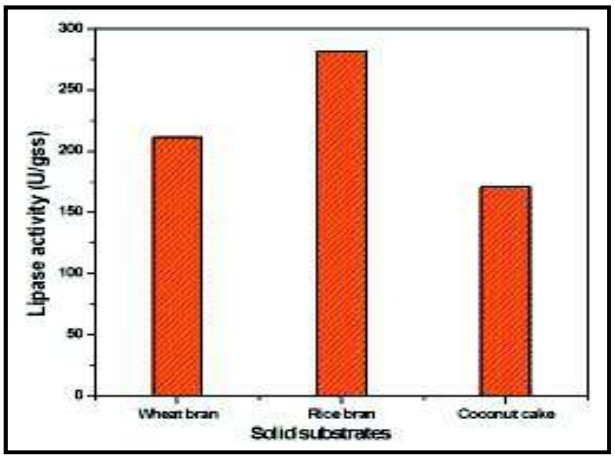

Figure 2: Effect of Different Solid Substrates on Lipase Production at Temperature 37 °C and pH 6.5.

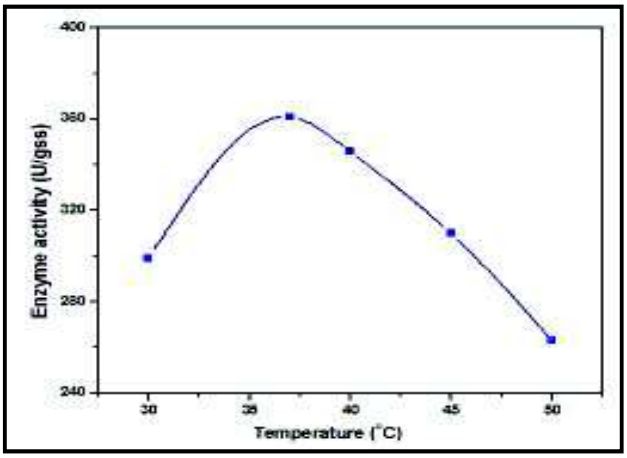

Figure 3: Effect of Different Temperatures on Lipase Production at pH 6.5.

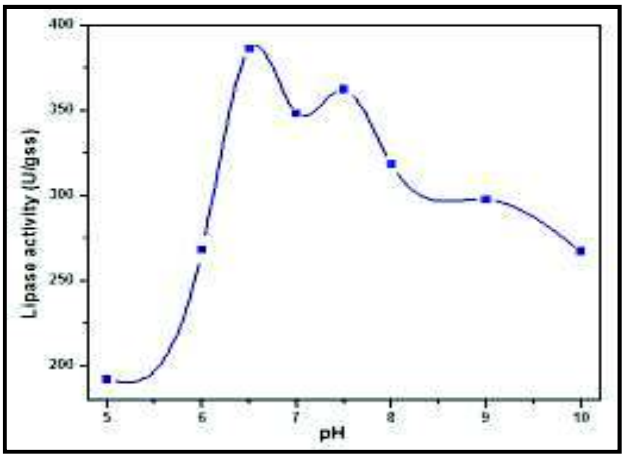

Figure 4: Effect of Different pH on Lipase Production at 37 °C.

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