MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

UTILIZATION OF SOME AGRO-INDUSTRY WASTES FOR PRODUCTION OF FUNGAL LIPASE

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Received:27/1/2019
Accepted: 18/2/2019

Abstract: Fungi are used in several industrial processes, such as production of enzymes. Importance of enzymes is increasing especially microbial lipases which are of indefinite industrial purposes. Solid state fermentation is an economical alternative for large scale production of enzymes which produced by fungi. By using solid state fermentation, the production of extra- cellular lipase from filamentous fungi isolated from soil and the oil contaminated soil using agricultural wastes has been studied. Seven fungal strains were isolated from twenty soil samples and then tested for lipase production. Among these fungi, *Aspergillus flavipes* was identified and selected as highest lipase producer using *Nigella sativa* as a waste among seven agricultural wastes. The optimum conditions for lipase production were pH 7.2, temperature 28°c and 6 day incubation period. Maltose and yeast extract were the best carbon and nitrogen source respectively.

Key words: Solid state fermentation, Agro-industrial wastes, Enzyme production, Optimization of lipase production, *Aspergillus flavipes*.

Introduction

Fungi can produce both intracellular in addition to extracellular enzymes; the extracellular enzymes are easier to be extracted [1]. Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are one of the most important classes of industrial enzymes. They hydrolyse triglycerides into diglycerides, monog-lycerides, glycerol and fatty acids. Lipases are considered as important part of many industries such as pharmaceuticals, tea industries, dairy industry, cosmetics, food industry, leather industry, detergents, oleo-chemicals, agrochemicals and of many bioremediation processes. Newer micro-organisms are being selected for the production of lipases having desirable features due to their great applications in industries [2].

The production of lipases from fungi also varies depending upon the conditions of synthesis (composition of medium, pH, temperature, sources of carbon and nitrogen) [3]. In order to obtain high yield of lipases, carbon sources, nitrogen and micronutrients should be selected very carefully. These requirements of nutrients can be satisfied by agro-industrial residues [4]. Over the recent years, research on the selection of suitable substrates for fermentative process has mainly been concentrated on agroindustrial residues, due to their potential benefits. Utilization of agroindustrial wastes delivers different substrates and may help solving pollution problems, which else might be caused by their discarding. Agro-industrial waste obtained while extracting oil from seeds has also been reported to be a possible substrate for producing lipases from microorganisms due to their remaining lipid content, which can induce enzyme production [5, 6].

Solid state fermentation (SSF) has grown significant believability in the biotechnology industry in recent years because of its promising application in the production of biologically active metabolites and its extensive applications in the food, fuel, chemical and pharmaceutical industries. SSF involves the

growth of microorganisms on moist solid material where the spaces between the particles of the material are filled with a continuous gas phase. It is important to note that the majority of SSF processes involve aerobic organisms such as the filamentous fungi.

Since 1980s, the need for lipases has been increased. Their use as an industrial catalyst is increasing day by day due to the advantageous properties like high catalytic efficiency, bio-degradability and high specificity [7]. The present study goals to isolate of some lipolytic fungi from different soil samples and the study was prolonged to evaluate the optimum conditions for lipase production by using *A. flavipes*.

Materials and methods Sampling

Twenty soil samples used for the isolation of lipase producing fungi. The soil samples were collected from different crop fields at different locations and oil contaminated soil sample at El-Behira, El-Gharbiya, El-Dakahlia and Alexandria governorates in Egypt

The agro –industrial wastes

The agro-industry wastes of *Nigella sativa, Eruca sativa, Sesamum indicum, Glycine max, Gossypium barbadense, Linum usitatissimum, Olea europaea* were obtained from El-Nasr for Natural oil in Damanhur El-Behira Governorate.

Chemicals

4-Nitrophenyl palmitate used for lipase assay was purchased from SIGM –ALDRICH, USA and all chemicals used were obtained from EL NASER Company Pharmaceutical and Chemicals Company ,Egypt.

Isolation of lipase producing fungi

By using soil dilution method [8], fungi were isolated from soil samples collected from olive agricultural areas on the following medium: 5ml olive oil, 2 gm. sodium nitrate, 1 gm. potassium dihydorgen phosphate, 0.5 gm. hydrated magnesium sulphate, 0.5 gm. Potassium chloride, 0.01 gm. hydrated ferrous sulphate, 20 gm. agar, distilled water up to 1 liter.

Phenotypic characterization of fungi

Fungal identification was done on the basis of colony and micro morphology characteristics, and was identified using standard taxonomic references. [9-12].

Production of crude lipase in SSF

A sample (2 g) of the agro-industry residue was taken in a series of 250ml Erlenmeyer flasks, triplicate for each residue type moistened with 2ml of water (1:1, substrate: water, w/v) and sterilized for 20 min. at 120 °C. The flasks were inoculated with 1 ml of fungal spore suspension, and incubated at 28°c for 4 days and after 96 h.

- **Lipase assay**

Lipolytic activity was determined spectrophotometrically using para nitro phenyl palmitate (p-NPP). To determine the lipase activity, 1.8 ml of 0.1M tris-HCL buffer (PH 8) containing 0.15M NaCl and 0.5% Triton X-100 was taken and pre incubated at 37 °C with 100 microliter crude enzyme extract. Twenty microliter of 50 micro Molar of p-nitrophenyl palmitate (0.25) gm in 30 ml acetonitrile was added as a substrate to the reaction mixture and it was incubated at 37 °C for 30 min. the amount of liberated p-nitrophenol was measured at 410 nm [13]. One unit of activity is defined as the amount of enzyme releasing 1 nmol of pNp/ml/min under standard assay conditions .lipase activity was expressed in units/ gram(U/g) of dry substrate used for SSF.

Optimization of lipase production of *Aspergillus flavipes* Effect of initial pH:

The effect of initial pH on lipase production of the selected strain was investigated for pH 5.8 - 8 values with sodium phosphate buffer.

Effect of incubation time:

The effect of incubation time on lipase production was calculated at different incubation periods 2, 4, 6, 8, 10 days. Fermentation flasks were incubated at 28°C for these different time intervals by inoculating the selected waste with lipolytic fungus in solid state fermentation and use phosphate buffer at the pH which give maximum productivity.

Effect of temperature on lipase activity:

The effect of cultivation temperature on lipase activity of the selected fungal strain was investigated at 25°C, 28°C, 31°C, and 34°C through 6 day of cultivation.

Effect of carbon source:

The effect of carbon sources [glucose, sucrose, malltose and starch (10 % W/W)]. on lipase activity of the selected fungus was examined.

Effect of nitrogen source:

The effect of nitrogen source on the lipase production was studied using $NaNO_3$,peptone, yeast extract, $(NH4)_2SO4$ and L-asparagine. The equivalent weight of nitrogen present in $NaNO_3$ was calculated and the same equivalent weight of other nitrogen source were used on their weights. The culture was incubated in the same conditions recommended from previous studies.

RESULTS

Isolation of lipolytic fungi

The isolated lipase producing fungi are seven species namely, *Aspergillus terrreus, Aspergillus carenus, Penicillum oxalicum, Aspergillus flavipes, Aspergillus awamori ,Alternaria pluriseptata, Pythium sp.*

Production of extracellular lipase in solid state fermentation of *Aspergillus flavipes*

The seven fungal isolates were tested for the production of extracellular lipases, as shown in figure (1), *Nigella sativa* is the most suitable substrate for production of lipase for all tested fungal species and *Aspergillus flavipes* showed maximum lipase activity on *Nigella sativa*. *Linium ustatissimum* gave high activity with *Aspergillus terreus* and *Aspergillus awamori* followed by *Gossipium barbadense* which gave high lipase activity with the same fungi *Aspergillus terreus* and *Aspergillus awamori*. *Glycine max* showed the minimum activity among all the wastes.

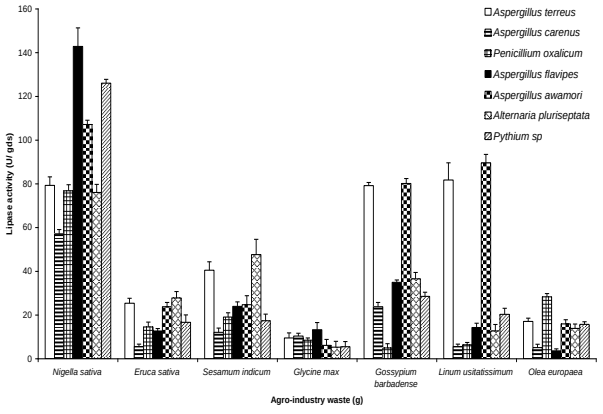


Figure (1): Production of extracellular lipase by different fungal isolates in solid state fermentation

Factors affecting lipase production of the selected *Aspergillus flavipes*

Effect of initial pH on lipase production:

The effect of initial pH on lipase production of selected strain was investigated for pH 5.8 - 8 values. The results illustrated in Figure (2) show that the fungal activity increase from pH 6.2 and the highest level of lipase production by the selected strain was obtained at pH 7.2 then lipase activity decrease gradually as pH increase.

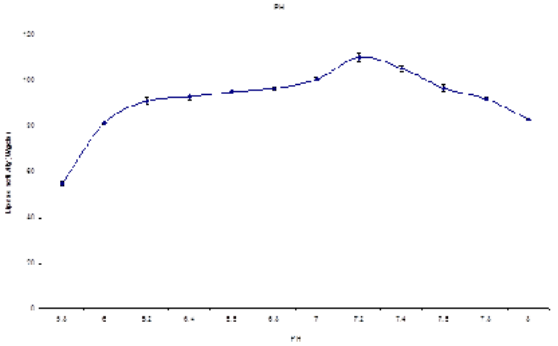


Figure (2): Effect of initial pH on lipase production of *A. flavipes*

Effect of incubation time on lipase production:

Production of lipase was assayed at different incubation periods 2, 4, 6, 8, 10 days. The fermentation was carried out at 28°C. Low level of lipase activity was detected at the beginning of fungal growth, lipase activity was increase gradually as the time increase. The optimum time for lipase production was 6 days incubation period as shown in figure (3).

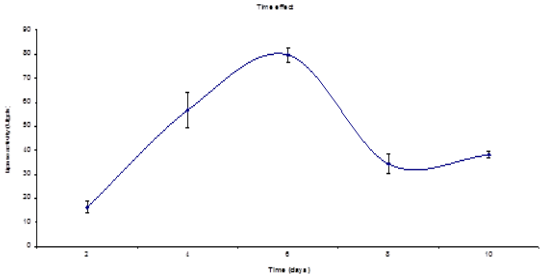


Figure (3): Effect of incubation time on lipase of *A. flavipes*

.Effect of cultivation temperature:

The effect of cultivation temperature on lipase activity of the selected fungal strain was investigated at 25, 28, 31, and 34ºC through 6 days at pH 7.2. The result in Fig (4) show that

temperature significantly affected lipase production by the selected fungus as showed in curve as temperature increase lipase activity increase and the

maximum lipase production was achieved at 28ºC. while at 31 ºC lipase activity decreased. Lipase activity was the minimum at 34°c. Incubation at either lower or higher than the optimum temperature may led to decrease of enzyme production.

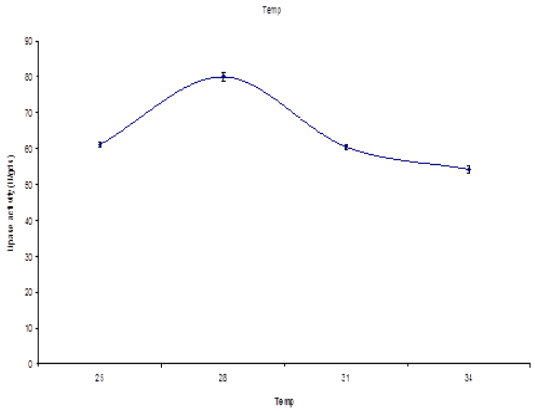
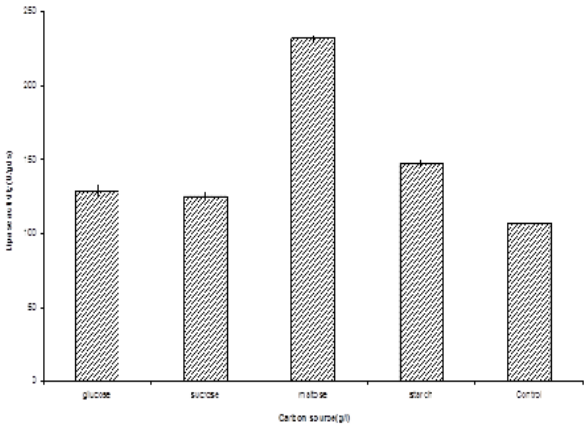


Figure (4) Effect of cultivation temperature on *A. flavipes* lipase production.

Effect of carbon source:

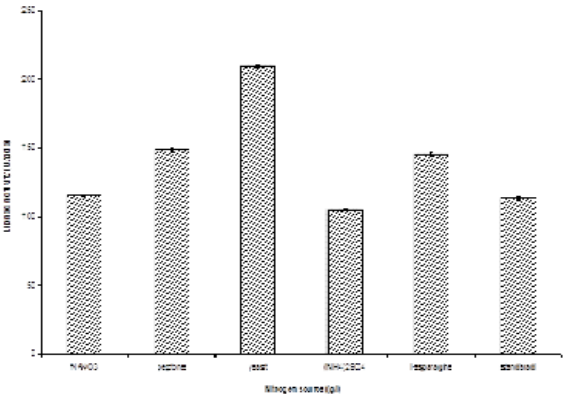
Lipase production from isolated fungal strain was reported in the presence of 10% wt. /wt. of starch, maltose, sucrose and glucose. The suitable carbon source (Fig. 5) show the highest enzyme activity was maltose followed by starch. Glucose gave activity higher than sucrose.



Fgure (5) Effect of different carbon source on *A. flavipes* lipase production.

Effect of nitrogen source:

The type of nitrogen source in the medium also has influence over the lipase production, in our work yeast extract was found to be the most suitable source and maximum production was found (Figure 6) followed by peptone.



Figuer (6) Effect of nitrogen source on *A. flavipes* lipase production.

Discussions

Lipase is an enzyme capable of hydrolyzing lipids into fatty acids and glycerol [14]. In recent years, there has been an increasing attention in the study of lipases mainly due to their potential applications. In this connection, *Nigella sativa* was the agricultural waste which yielded maximum lipase activity among other wastes using the seven fungal strains obtained from the isolation which was 142.86 U/gds. There was no clear definite research on production of lipase from *Nigella sativa* but screening of different agricultural wastes was done by Amin et al. [15], maximum lipase activity (684.02 U/gds) was observed using canola seed oil cake and 363.6 U/gds was obtained by Kamini et al. [16] using gingelly oil cake where Gombert et al. [17] achieved only 30.3 U/gds by babassu oil.

Comparing our results to the others we found that maximum lipase production of 1152 U/gds was obtained after 96 h of incubation by *Aspergillus* species [18] whereas studies of the production of thermostable lipase by *Penicillium simplicissimum* strain grown in castor bean waste with maximum lipase activity of 44.8 U/gds [19] while the maximum lipase activity we obtained were 142.86 U/gds.

The majority of the microbial lipases are extracellular in nature and their production is considerably affected by composition of medium, sources of carbon and nitrogen besides physic-chemical factors such as pH and temperature. Commonly, high yield can be obtained by optimization of culture medium. PH 7.2 gave maximum lipase activity and a

similar result obtained for *A. niger* by Rai et al. [20]. Another study was conducted Abdel-Fattah and Hammad [21] to examine the effect of various pH (2.0-8.0) on production of lipase by *A. niger* and *A. terreus*. Highest production was achieved at pH 6.0 and it decreased above pH 6.0 in this experiment.

Mahmoud et al. [22] studied effect of temperatures on production of lipase by incubating the cultures of *A. terreus* at various temperatures viz. 10 ºc, 20 ºc, 30 ºc and 45 ºc. Highest activity of lipase was obtained at 45 ºc. Where Mukhtar et al. [23] reported that highest lipase production by *A. niger* was done at 30 ºc which is close to that mentioned for *A. flavipes* (28°c)

The influence of incubation time on production of lipase by different microorganisms has been studied by many workers. Maia et al. [24] reported the optimum activity of lipase by Fusarium solani at 25 ºC after 3day incubation. The highest increase in 5 days cultures of *A. niger* and *A. terreus* have been reported by Abdel Fattah and Hammad [21].

Carbon source has always been recognized as the main factor for the expression of lipase activity due to inducible nature of lipases. Generally lipase production is stimulated by lipids [25] but addition of different carbon source can affect the lipase productivity. Maltose increase the lipase production of *A. flavipes* to 232.14U/gds (1.6 fold increase). In the same manner, Mahanta *et al.* [26] reported that Jatropha seed cake without supplementation showed a lipase activity of 625 U/gds with *P. aeruginosa* When supplemented with maltose, the activity extended to 976 U/gds and this emphasis our results on maltose. On the other hand, Bindiya and Ramana [27] found that highest lipase activity was achieved with sucrose (1% w/v) and lowest activity with fructose (1% w/v). The suitable nitrogen source that showed the highest enzyme activity was Yeast extract similar to that reported for *A. aculeatus* [28] and *P. citrinum* [29].

Conclusion

Based on the above results, *A. flavipes* was reported for the first time as a lipolytic fungi. The optimization studies were carried to identify culture conditions that would improve lipase production by *A. flavipes.* The carbon and nitrogen sources and physiological factors such as pH, temperature and incubation time were optimized. The highest activity of lipase by *A. Flavipes* occurred at pH 7.2 after 6 days of incubation at 28 ºC using maltose (10%, w/w) as a carbon source and yeast extract (10%, w/w) as an nitrogen source.

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