The Journal of Animal & Plant Sciences, 24(5): 2014, Page: 1430-1437
ISSN: 1018-7081

POTENTIAL USE OF AGRICULTURAL WASTES FOR THE PRODUCTION OF LIPASE BY *ASPERGILLUS MELLEUS* UNDER SOLID STATE FERMENTATION

M. Amin, H. N. Bhatti[†], M. Zuber[*], I. A. Bhatti[1] and M. Asgher[**]

Department of Chemistry, University of Agriculture, Faisalabad-38040, Pakistan
[*]Department of Applied Chemistry, Government College University, Faisalabad-38030, Pakistan
[**]Department of Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan
[†]Corresponding Author Email: hnbhatti2005@yahoo.com; haq_nawaz@uaf.edu.pk

ABSTRACT

Lipases are well known among the extensively used biocatalysts, because they have the ability to catalyze several remarkable reactions in aqueous and non-aqueous media like esterification and transesterification. The present study explores the production of lipase by *Aspergillus melleus* under solid-state fermentation (SSF). Screening of different agricultural wastes such as rice bran, wheat bran, canola seed oil cake, sunflower hulls and peanut shells was carried out and canola seed oil cake was selected as best substrate due to highest lipase activity. Various process parameters like initial pH of basal media, moisture content, incubation time, inoculum size, incubation temperature and substrate levels were optimized for lipase production. Effect of various carbon and nitrogen sources was also studied. Maximum lipase activity (684.02 U/gds) was observed after 96 h of reaction in the fermentation medium with initial pH 4.0; canola seed oil cake 10 g; moisture contents 60 % and olive oil 3% as inducer using at 30 °C. The characteristics of isolated lipase (alkaline) indicated that this enzyme could be used in soaps and detergents manufacturing industries.

Key words: Lipase, *Aspergillus melleus*; Fermentation; Optimization; Agricultural wastes.

INTRODUCTION

Utilization and recycling of renewable resources that pose threat to the environment can be systematically carried out to bring about resource productivity needed to make human activity sustainable. Pakistan is an agro-based country that produces large quantities of agro-industrial residues which are rich in nutrients like carbon, nitrogen, minerals, and biomass residues. These agricultural wastes can be used as substrate for enzyme production owing to economical feasibility, as it can help in solving pollution problems which may be caused by their disposal (de Azeredo *et al*., 2007; Arvanitoyannis and Varzakas, 2008). Oil cakes of various agro-industrial residues obtained from extraction of oils have been utilized for fermentative production of lipases and other industrial enzymes because their residual oil contents act as inducers for lipase production.

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) member of hydrolase family, constitute a very important group of enzymes due to their excellent activity and stability in both aqueous and non-aqueous media. This property makes possible for them to execute a number of catalytic reactions, such as alcoholysis, acidolysis, aminolysis, esterification and transesterification (Coradi *et al*., 2012; Bueno *et al*., 2014; Prasad, 2014). Owing to these properties, lipases have a broad spectrum of industrial applications such as synthesis of biopolymers and biodiesel, synthesis of fine chemicals, and are also important in detergents and in paper manufacturing (Masomian *et al*., 2013). The enantioselective and regioselective properties of lipases make their utilization necessary for fat modifications and synthesis of cocoa butter substituents, emulsifiers, moisturizing agents, body care products and flavour enhancers (Brooks and Asamudo, 2011**).** Each application requires unique properties with respect to stability, temperature and pH dependence or ability to catalyze reactions in organic solvents which is fulfilled remarkably by lipases (Kumar *et al*., 2005; Romero *et al*., 2012).

Enzymes of industrial interest have traditionally been obtained from submerged fermentation (SmF) due to easy handling and control. In recent years, solid state fermentation (SSF) has received renewed interest since SSF can produce higher yields or better product characteristics than SmF. SSF has been recently reassessed, especially because of the possibility of using low-cost agro-industrial residues as raw materials (Pérez-Guerra *et al*., 2003; Malilas *et al*., 2013).

Lipases occur naturally in plants, animals, and microorganisms. Among these sources, microbial lipases are most favorable due to their industrial applications because their production can be carried out at a very high rate and they do not require cofactors for their action (Dey *et al*., 2014). Therefore, most lipases used for biotechnological purposes have been isolated from bacteria and fungi (Lin *et al*., 2006). Fungi are mostly preferable, because they generally produce extracellular enzymes which facilitate recovery of the lipase from the fermentation broth. Meanwhile fungi have several other

advantages over unicellular microorganisms in the colonization of solid substrates and their good tolerance to low water activity, high osmotic pressure conditions etc. (Yu *et al*., 2007). Filamentous fungi like *Aspergillus, Penicillium* etc. are the most ideal and best adapted microorganisms for SSF.

The accessibility of lipases holding the suitable characteristics for a particular application is still a limiting factor. The remarkable potential of lipases in biotechnological applications shows the need to develop novel cost-effective technologies for increased production of this versatile enzyme. The industrial demand for new sources of lipases with different catalytic characteristics stimulates the isolation and selection of new strains. In this regard, the present research paper reports the production of lipases from a new fungal strain, *Aspergillus melleus* using agro-industrial residues under SSF.

MATERIALS AND METHODS

Microorganism and inoculum preparation: Fungal strain used in this study was obtained from the Fungal Bank, University of The Punjab, Lahore. Spores of *A. melleus* were maintained on PDA slants. The slants were inoculated with the spores of the fungus under aseptic conditions in laminar air flow and were incubated at 30 ºC till sporulation and stored at 4 ºC for further use. Kirk basal medium was used to prepare the inoculum. For sterilization purpose, medium was autoclaved at 121 ºC for 15 minutes. Inoculum was prepared by inoculating a loop full of three days old culture from the slants to the autoclaved medium under aseptic conditions, followed by incubation at 30 °C and agitation at 120 rpm. The 72 h old culture with spore count of 1×10^8 spores/mL was used as inoculum.

Lipase production in SSF: Five different agro-industrial wastes (canola oilseed cake, peanut shells, wheat bran, rice bran and sunflower hulls) were collected from the local market of Faisalabad Pakistan and used as substrates. All the substrates before use were dried, ground in an electric mill and then sieved. Ten grams of each substrate was transferred in a series of 250 mL Erlenmeyer flasks, moistened with water (50 %), and sterilized at 15 lbs/in at 121 °C for 15 minutes. Afterwards the flasks were inoculated with 2 mL of spore suspension and incubated for fermentation at 30 °C for 72 hours.

Optimization studies: The fundamental factors influencing the lipases production studied were moisture content (%), pH, incubation time (h), amount of substrate (g), inoculum size (mL), incubation temperature (°C) and olive oil concentration (%). Effect of supplementary carbon and nitrogen sources was also investigated. At the end of fermentation, crude enzyme was extracted by mixing the fermented substrate with 100 mL of phosphate buffer (0.1 M; pH 7) and then shaking the mixture in an orbital shaker at 100 rpm. The obtained extract was filtered and the supernatants were used for lipase assay.

Enzyme assay: Lipase activity was determined by following the method of Yagiz*et al.* (2007), using para-nitrophenylpalmitate (PNPP) as substrate. Crude enzyme extract (100 µL) was mixed with 0.9 mL (900 µL) of solution which contains 3 mg PNPP substrate dissolved in 1 mL of propane-2-ol diluted in 9 mL of the 50 mM Tris-HCl, pH 8, having 40 mg triton X-100 and 10 mg of Gum Arabic. This mixture was incubated at 37 ºC for 30 minutes and liberated para-nitrophenol was recorded at 410 nm.

Activity units were determined by the following formula

$$Lipase\ Activity\ (U/mL) = \frac{Absorbance\ of\ sample \times Standard\ factor}{Time\ of\ reaction \times Enzyme\ extract\ (mL)}$$

One unit of lipase activity is defined as the amount of enzyme liberating 1µmol of para-nitrophenol per mL per minute under standard assay conditions.

Protein estimation: Total protein was estimated by using Bradford method (Bradford, 1976). Bovine serum albumin (BSA) was used as standard.

Statistical analysis: The data represents the mean of three independent trials. The results are presented as mean ± SD values.

RESULTS AND DISCUSSION

Screening and selection of substrate: Solid substrate in SSF has a key role in the enzyme production. Most of the lipases of microbial origin are extracellular and excreted through the cell membrane into the culture medium consisting of a suitable solid substrate. Among all the wastes tested for lipase production, canola oilseed cake holds the best capacity for lipase production by *A. melleus* (Fig. 1, a), which indicates that it contains all the basic nutrients for the fungal growth and lipase production. At moisture level 50 %, pH 5.5 and 72 h of incubation period, canola oilseed cake produced 373.88 U/gds lipase by *A. melleus*. Canola oilseed cake proves to be a good lipase producer because it is rich in lipid contents than any other source used in the present study. Other four wastes are mainly composed of carbohydrates with little lipid contents and are the rich source of insoluble fibre. Therefore, canola oilseed cake was used as a substrate in the subsequent study.

Effect of moisture content: The substrate used in SSF should have enough moisture content to meet the microorganism's requirements for the liberation of value-added products such as enzymes (Vaseghi *et al*., 2013).

The water which exists as such or is absorbed within the solid substrate is important for fungal growth on the substrate due to efficient oxygen transport process. To examine the effect of moisture content on lipase production by *A. melleus*, solid substrate (canola oil seed cake) was moistened with distilled water from 30-80 % (V/W). Figure (1, b) depicts that lipase activity reached to its maximum value (402.08 U/gds) at 60 % moisture level and further increase in moisture content caused a gradual decrease in lipase production. The minimum lipase activity (228 U/gds) was shown with 80 % moisture level.

It has been reported that higher moisture contents lead to decrease porosity, promote development of stickiness and increase the chances of contamination, while lower moisture contents reduce the nutrients solubility in the substrate (Mahanta *et al*., 2008). So the low enzyme production by *A. melleus* at very high or low moisture levels during this study might be related to the low diffusion of nutrients and metabolites in lower moisture contents whereas compaction of substrate at higher moisture contents. Our findings are in agreement with Kamini *et al*. (1998) who reported the requirement of 60 % initial moisture content for maximum lipase production by *Aspergillus niger* with gingelly oilseed cake.

Effect of incubation time: The optimum incubation time was monitored by assessing the lipase activity and fungal biomass till 146 h after every 24 h time interval. After first interval of 24 h, significant production of lipase was commenced but maximum lipase activity (447.44 U/gds) was obtained after 96 h. Further incubation after 96 h did not lead to an increase in lipase activity, but a steep decrease in lipase activity reached up to 155.68 U/gds after 146 h (Fig. 2, a).

It is suggested that decline in lipase yield after prolonged incubation times might be due to production of proteases leading to inactivation of enzyme (Sanchez *et al*., 1999). It might also be possible that in the beginning, micro-organism was adapting to the environmental conditions. Lipase activity was at its maximum during the exponential growth phase, gradually decreased at the late logarithm phase of growth due to the production of citric acid in the medium (Kamzolova *et al*., 2005). Similar results were documented by Hiol *et al*. (2000) who observed maximum lipase activity after 4 days of incubation by *Rhizopus oryzae*. Hamdy and Abo-Tahon (2012) and Sethi *et al*. (2013) also reported maximum lipase production after 4 days of fermentation at 30 °C.

Effect of pH: The initial pH of the culture medium is one of the most important environmental factors affecting the fungal growth and enzyme production. Optimum pH was examined for lipase production conducting the experiment at different pH values ranging from 3.0-8.0. Figure 2 (b) revealed that maximum lipase production (494.02 U/gds) was obtained at pH 4.0. It is clearly indicated from the figure that the organism had the ability to grow well in a pH range of 3.0-8.0. Lipase activity was declined significantly at the alkaline pH which represented that lipase produced by *A. melleus* was more active in acidic range of pH.

Our results are in agreement with Coradi *et al*. (2012) who observed that lipase activity was highest at pH 6.0, but it was also significant at pH 4.0 and 5.0. But other researchers had reported optimum pH values higher than optimal value for *A. melleus:* pH 9 for *Penicillium candidum* (Ruiz *et al*., 2001); pH 8 for *Bacillus sp. FH5* (Hasan*et al*., 2006); pH 9 for *Pseudomonas sp. 7323* (Zhang and Zeng, 2008).

Effect of substrate level: Maintaining the pH and moisture contents of culture broth at their optimum level, amount of substrate was standardized for lipase production by *A. melleus*. It was carried out by varying the level of substrate i.e. canola oil seed cake from 5 to 25 g. Maximum lipase (556.54 U/gds) was synthesized by *A. melleus* with 5 g of substrate (Fig. 3, a). Lipase activity with 25 g of canola oilseed cake was 195.65U/gds. Results revealed that lipase activity per gram dry substrate was declined by increasing the amount of substrate. This might be due to easy diffusion of mycelial mass in small amount of substrate which yielded more lipase owing to high growth rate with 5 g canola oilseed cake as a substrate. Similar results were obtained by Singh *et al*. (2010) and Amin and Bhatti (2014).

Effect of inoculum size: In SSF, inoculum size must be distributed in sufficient amount for the micro-organism to grow well. The fungal spores firstly attach on the outer surface of the substrate particles. They slowly grow, multiply and then penetrate into the substrate for their action. Therefore, a suitable inoculum size is required to attain the reasonable lipase production. Different inoculum levels i.e. 1 to 6 mL were used to describe their effect on lipase activity. Figure 3 (b) revealed that lipase production was increased by increasing the inoculum size and attained the maximum lipase activity (567.57 U/gds) with 3 mL of inoculum after 96 h of fermentation. Higher inoculum sizes affected the lipase production negatively.

It has been recommended that lower inoculum size is not able to proliferate quickly the microbial biomass. Thus, with lower inoculum size, the degradation of the substrates is slow that influences the production of metabolites. However, higher inoculum sizes are inhibitory in nature. It may be correlated to the fact that amount of available oxygen and nutrients are depleted in the culture medium due to higher inoculum size (Ramachandran *et al*., 2004).

In various studies, different levels of inoculums have been used for lipase production employing different microorganisms. Our results are in agreement with previous reports of Kumar *et al*. (2010) and Sethi *et al*.

(2013) who also observed that higher inoculum size had produced too much biomass and depleted the nutrients that necessary for enzyme production.

Effect of incubation temperature: Incubation temperature significantly affects the fermentation process. To optimize the incubation temperature, experiments were conducted at different temperatures ranging from 25 to 45 °C with the increment of 5 °C keeping other parameters constant (Fig. 4, a). Lipase production and mycelial growth were gradually increased with the increase in temperature up to 30 °C and significantly decreased thereafter. This negative effect of high temperature on the lipase production might be due to the enhanced production of proteases at higher temperatures. It has been suggested that higher temperatures may cause enzyme deactivation leading to lower lipase activity. It might be due to the reason that higher temperature alters the cell membrane composition and stimulates protein catabolism, thus causes cell death.

Our results correlate with the findings of Rajesh *et al*. (2010) who reported maximum lipase production by *Trichoderma reesei* at 30 °C. Similarly Kashmiri *et al.* (2006) and Zhang and Zeng (2008) also reported optimum temperature, 30 °C for the production of lipase by *Trichoderma viride* and *Pseudomonas sp*. 7323 respectively.

Effect of carbon source: The culture medium was supplemented with nutrients to observe their effect on lipase production. Although carbon sources are required for microbial growth and production of biochemical materials, their efficiencies for production of any target material can differ among microbial species and under different fermentation conditions. The fungi are usually able to produce lipases using the carbohydrates as carbon sources. Simple and complex carbon sources (glucose, sucrose, fructose, maltose, lactose, trisodium citrate and sodium acetate) were added at 1 % (w/w) to consider their effects on lipase production. For comparison, experiment was also conducted without carbon source saying as control, and the results are presented in figure (4, b). Results revealed that all the carbon sources enhanced the lipase production but the addition of lactose resulted in the highest activity (639.89 U/gds). Similar results were obtained by Sethi *et al*. (2013) who had reported the enhanced lipase activity in the presence of lactose as a carbon source.

Effect of olive oil concentration: Lipases are generally produced in the presence of a lipid such as oil or any other inducer, such as fatty acids, triglycerols, hydrolysable esters, bile salts, tweens, and glycerol. Although few authors have reported good yields in the absence of fats and oils but Fadiloglu and Erkmen (1999); Rohit *et al*. (2001); Gupta *et al*. (2004); Mohan *et al.* (2008) and Anbu (2014) reported that high level of lipase activity had been obtained when olive oil was used as the substrate. That's why in present study the concentration of olive oil was optimized for the enhanced production of lipase by supplementing the growth medium with 1-5 % olive oil. Control experiments were also conducted to evaluate the influence of olive oil clearly. Olive oil was found to have a significant effect on the lipase production leading to optimum concentration at 3 % of olive oil, boosting up the lipase activity up to 684.02 U/gds (Fig. 5, a). It was observed that further increase in olive oil concentration had no positive effect on the lipase activity. It might be due to poor oxygen transfer at higher level which could modify the microbial metabolism leading to less lipase production.

Effect of nitrogen source: The choice of nitrogen source is of crucial importance for the reduction of catabolite repression and induction of lipase production. Both organic and inorganic nitrogen sources have been traditionally used for enhanced lipase production. The basal medium consisting of canola oilseed cake was supplemented with different nitrogen sources (ammonium sulfate, urea, peptone, ammonium nitrate, yeast extract, sodium nitrate and diammoniumtartarate) to determine the effect of nitrogen on lipase production. All of these nitrogen sources promoted the lipase production with the exception of yeast extract (Fig. 5, b). Diammoniumtartarate was found to be an excellent nitrogen source giving lipase activity as 679.65 U/gds. Similar results were reported by Sun and Xu (2008).

Protein estimation: Lipase activity with optimized growth conditions by *A. melleus* led to 1.83 folds enhancement that means from 373.88 U/gds to 684.02 U/gds. Total lipase units and total protein contents calculated in crude extract were 38614.27 U and 171.788 mg respectively. Specific activity of crude enzyme extract was found 224.779 U/mg.

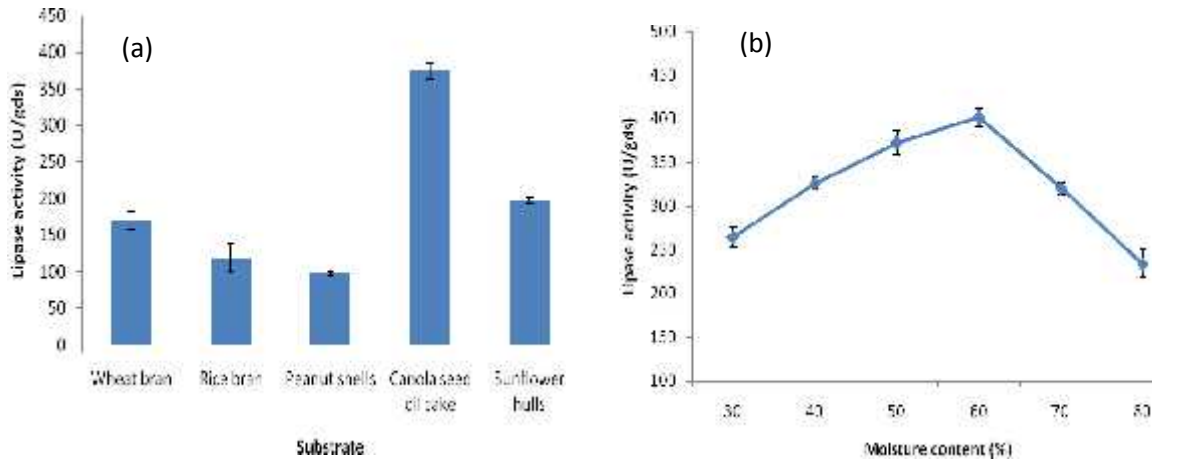

Fig.1. Lipase production by *A. melleus*: (a) Screening of substrates and (b) Effect of moisture content. Reaction time 72 h, T = 30 °C, substrate amount 10 g, inoculum size 2 mL and pH 5.5.

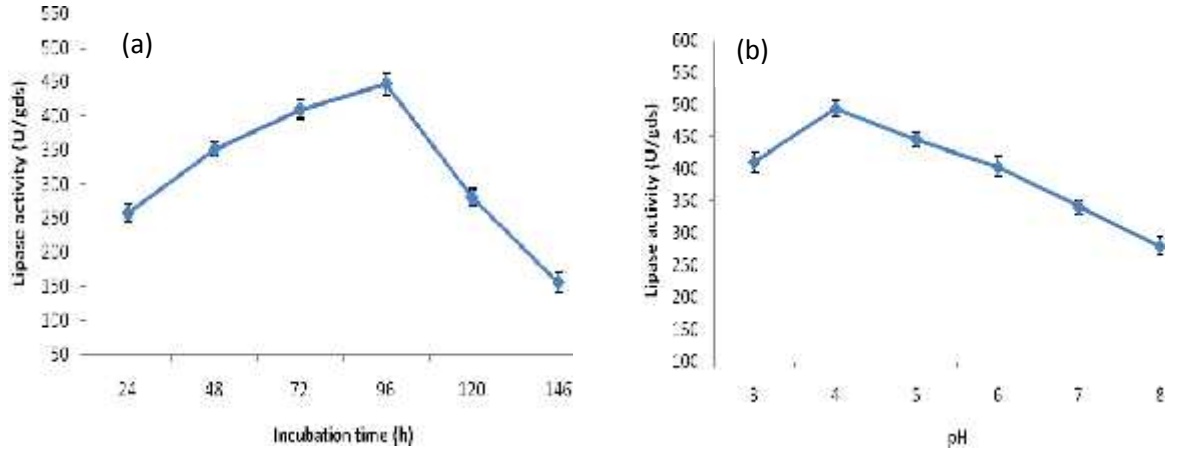

Fig. 2. Lipase production by *A. melleus*: (a) Effect of incubation time and (b) Effect of pH. Moisture content 60 %, T 30 °C, substrate amount 10 g, inoculum size 2 mL.

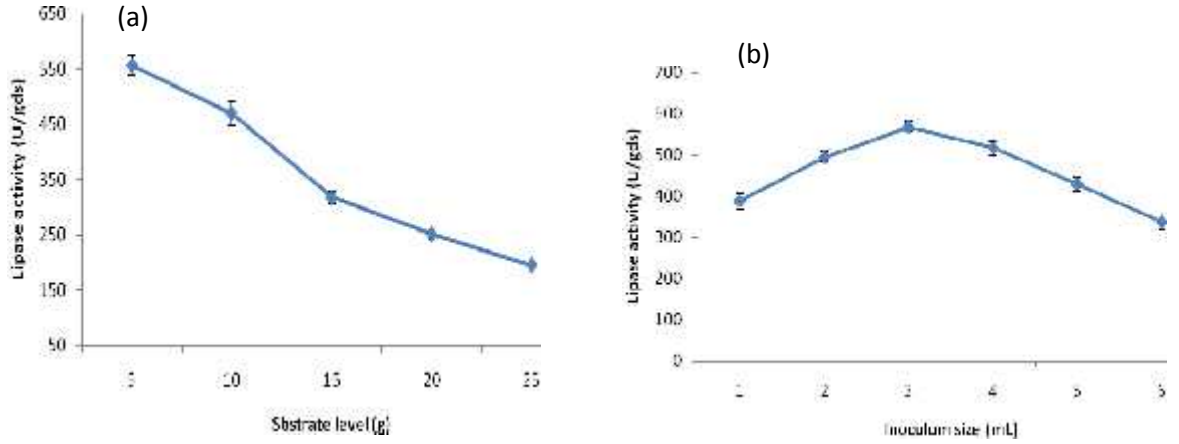

Fig. 3. Lipase production by *A. melleus*: (a) Effect of substrate level and (b) Effect of inoculum size. Moisture content 60 %, reaction time 96 h, T 30 °C, and pH 4.0.

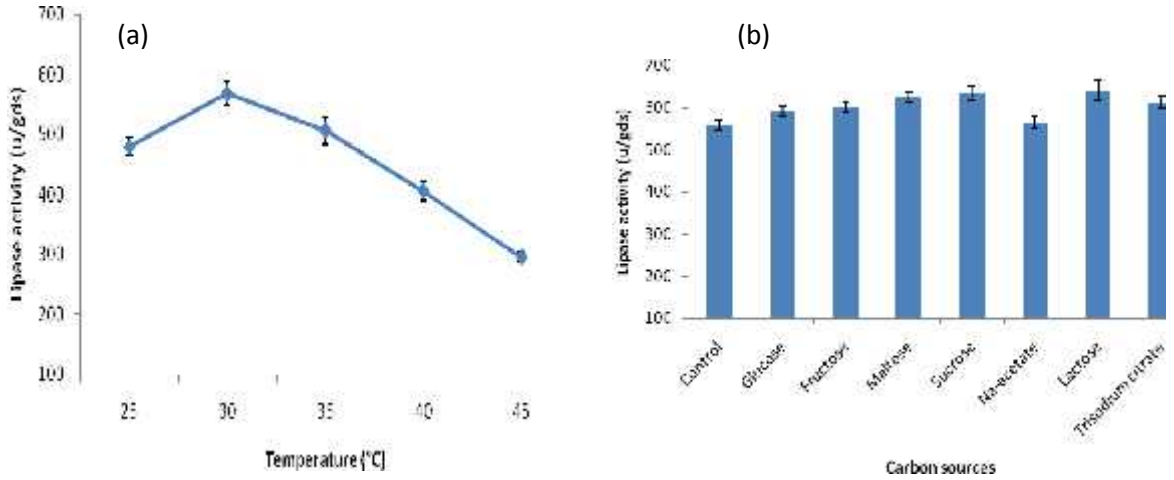

Fig.4. Lipase production by *A. melleus*: (a) Effect of temperature and (b) Effect of additional carbon sources. Moisture content 60 %, reaction time 96 h, substrate amount 10 g, inoculum size 3 mL and pH 4.0).

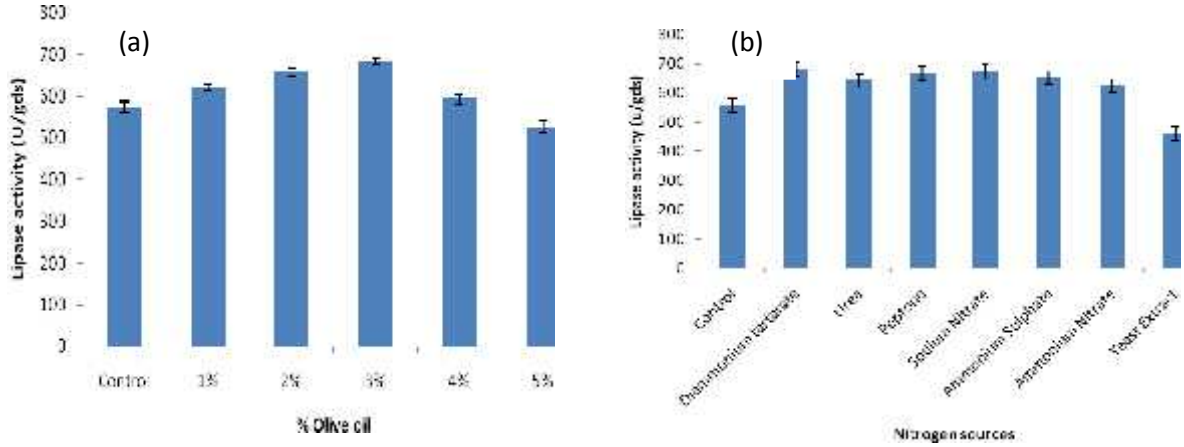

Fig. 5. Lipase production by *A. melleus*: (a) Effect of olive oil concentration and (b) Effect of nitrogen sources (right). Moisture content 60 %, reaction time 96 h, T = 30 °C, substrate amount 10 g, inoculum size 3 mL and pH 4.0).

Conclusion: The present study explores an agro-industrial residue (canola oil seed cake) which has the potential to produce lipase in an economically feasible process by a new promising fungal strain of *A. melleus*. Such a high level of lipase activity (684.02 U/gds) was achieved in 96 h of solid state fermentation at 30 °C when supplemented the basal medium with 3 % olive oil as an inducer. Lipase activity with optimized growth conditions by *A. melleus* led to 1.83 folds enhancement in lipase biosynthesis. It is concluded from the results that lipase produced by *A. melleus* was stable in acidic pH and even at higher temperatures. In conclusion, this work will be of great value for production of lipase commercially by *A. melleus* with high enzyme activity.

Acknowledgement: This work is a part of PhD thesis of Miss Misbah Amin. The authors are grateful to the Higher EducationCommission (HEC) of Pakistan for the financial support to achieve this goal.

REFERENCES

Amin, M. and H. N. Bhatti (2014). Effect of physicochemical parameters on lipase production by *Penicillium fellutanum* using canola seed oil cake as substrate. Intl. J. Agri. Biol. 16: 118-124.

Anbu, P. (2014). Characterization of an extracellular lipase by *Pseudomonas koreensis* BK-L07 isolated from soil. Prep. Biochem. Biotechnol. 44: 66-280.

Arvanitoyannis, I.S. and T.H. Varzakas (2008). Vegetable waste treatment: Comparison and

critical presentation of methodologies. Crit. Rev. Food Sci. Nut. 48: 205-247.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248-254.

Brooks, A.A. and N.U. Asamudo (2011). Lipase production by strains of *Aspergillus* species isolated from contaminated body creams. J. Toxicol. Environ. Health Sci. 3: 311-316.

Bueno, P.R.M., T.F. de Oliveira, M. Caliari, G.L. Castiglioni and S.S. Junior (2014). Selection and optimization of extracellular lipase production using agro-industrial waste. Afr. J. Biotechnol. 13**:** 566-573.

Coradi, G.V., V.L. da Visitação, E.A. de Lima, L.Y.T. Saito, D.A. Palmieri, M.A. Takita, P.O. Neto and V.M.G. de Lima (2012). Comparing submerged and solid-state fermentation of agro-industrial residues for the production and characterization of lipase by *Trichoderma harzianum*. Annals Microbiol. DOI 10.1007/s13213-012-0500-1.

de Azeredo, L.A.I., P.M. Gomes, G.L. Sant'Anna, L. R. Castilho and D.M.G. Freire (2007). Production and regulation of lipase activity from *Penicillium restrictum* in submerged and solid-state fermentations. Current Microbiol. 54:361–365.

Dey, A., A. Chattopadhyay, P. Saha, S. Mukhopadhyay, T.K. Maiti, S. Chatterjee, and P. Roy (2014). An Approach to the identification and characterisation of a psychrotrophic lipase producing *Pseudomonas* sp ADT3 from Arctic region. Adva, Biosci. Biotechnol. 5: 322-332.

Fadiloglu, S. and O. Erkmen (1999). Lipase production by *Rhizopus oryzae* growing on different carbon and nitrogen sources. J. Sci. Food Agric. 79: 1936-1938.

Gupta, R., N. Gupta and P. Rathi (2004). Bacterial lipases: An overview of production, purification and biochemical properties. Appl. Microbiol. Biotechnol. 64: 763-781.

Hamdy, H.S. and M.A. Abo-Tahon (2012). Extracellular lipase of *Aspergillus terreus* var. africanus (CBS 130.55): production, purification and characterization. Annals Microbiol. 62: 1723-1736.

Hasan, F., A.A. Shah, and A. Abul-Hameed (2006). Influence of culture conditions on lipase production by *Bacillus* sp.FH5. Annals Microbiol. 56: 247-252.

Hiol, A., M.D. Jonzo, N. Rugani, D. Druet, L. Sarda and L.C. Comeau (2000). Purification and characterization of an extracellular lipase from a thermophilic *Rhizopus oryzae* strain isolated from palm fruit. Enzyme Microb. Technol. 26: 421-430.

Kamini, N.R., T. Fujii, T. Kurosu and H. Iefuji (1998). Lipase production from *Aspergillus niger, by* solid-state fermentation using gingelly oil cake. Process Biochem. 33: 505-511.

Kamzolova, S.V., I.G. Morgunov, A. Aurich, O.A. Perevoznikova, N.V. Shishkanova, U. Stottmeister and T.V. Finogenova (2005). Lipase secretion and citric acid production in *Y. lipolytica*. Food Technol. Biotechnol. 43: 113-122.

Kashmiri, M.A., A. Adnan, and B.W. Butt (2006). Production, purification and partial characterization of lipase from *Trichoderma viride.* Afr. J. Biotechnol. 5: 878-882.

Kumar, S., K. Kikon, A. Upadhyay, S.S. Kanwar and R. Gupta (2005). Production, purification and characterization of lipase from thermophilic and alkalophilic *Bacillus coagulans* BTS-3. Protein Exp. Purif. 41: 38-44.

Kumar, R., S. Balaji, T.S. Uma, A.B. Mandal, and A.B. Sehgal (2010). Optimization of influential parameters for extracellular keratinase production by *Bacillus subtilis* (MTCC9102) in solid state fermentation using horn meal-a biowaste management. Appl. Biochem. Biotechnol. 160: 30-39.

Lin, E.S., C.C. Wang and S.C. Sung (2006). Cultivating conditions influence lipase production by the edible basidiomycete *Antrodia cinnamomea* in submerged culture. Enzyme Microb. Technol. 39: 98-102.

Mahanta N., A. Gupta and S.K. Khare (2008). Production of protease and lipase by solvent tolerant *Pseudomonas aeruginosa* PseA in solid-state fermentation using *Jatropha curcas* seed cake as substrate. Bioresour. Technol. 99: 1729-1735.

Malilas, W., S.W. Kang, S.B. Kim, H.Y. Yoo, W. Chulalaksananukul and S.W. Kim (2013). Lipase from *Penicillium camembertii* KCCM 11268: Optimization of solid state fermentation and application to biodiesel production. Korean J. Chem. Eng. 30: 405-412.

Masomian, M., R.N. Zaliha, R.A. Rahman, A.B. Salleh, and M. Basri (2013). A new thermostable and organic solvent-tolerant lipase from *Aneurinibacillus thermoaerophilus* strain HZ. Process Biochem. 48: 169-175.

Mohan, S.T., A. Palavesam and G. Immanvel (2008). Isolation and characterization of lipase-producing *Bacillus* strains from oil mill waste. Afr. J. Biotechnol. 7: 2728-2735.

Pérez-Guerra, N., A. Torrado-Agrasar, C. López-Macias and L. Pastrana (2003). Main characteristics and applications of solid substrate fermentation.

Electronic J. Environ. Agric. Food Chem. 2: 343-350.

Prasad, M.P. (2014). Production of lipase enzyme from *Pseudomonas aeruginosa* isolated from lipid rich soil. Inter. J. Pure Appl. Biosci. 2: 77-81.

Rajesh, E.M., R. Arthe, R. Rajendran, C. Balakumar, N. Pradeepa and S. Anitha (2010). Investigation of lipase production by *Trichoderma reesei* and optimization of production parameters. Electronic J. Environ. Agric. Food Chem. 9: 1177-1189.

Ramachandran, S., A.K. Patel, K.M. Nampoothiri, F. Francis, V. Nagy, G. Szackacs and A. Pandey (2004). Coconut oil cake-a potential material for production of α-amylase. Bioresour. Technol. 93: 169-174.

Rohit, S., C. Yusuf and B. Ullamchand (2001). Production, purification, characterization and application of lipases. Biotechnol. Adv. 19: 627-662.

Romero, C.M., L.M. Pera, F. Loto, C. Vallejos, G. Castro and M.D. Baigori (2012). Purification of an organic solvent-tolerant lipase from *Aspergillus niger* MYA 135 and its application in ester synthesis. Biocat. Agri. Biotechnol. 1: 25-31.

Ruiz, B. A.. E. Farres, F. Langley, Masso and S. Sanchez (2001). Purification and characterization of an extracellular lipase from *Penicillium candidum*. *Lipids*, 36: 283-289.

Sanchez, A., P. Ferrer, A. Serrano, F. Valero, C. Sola, M. Pemas, M. L. Rua, R. Femandez-Lafuente, J.M. Guisan, R.M. de la Casa, J. V. Sinisterra and J.M. Sinchez-Montero (1999). A controlled fed batch cultivation for the production of new crude lipases from *Candida rugosa* with improved properties in fine chemistry. J. Biotechnol. 69: 169–182.

Sethi, B.K., J. R. Rout, R. Das, P. K. Nanda and S. L. Sahoo (2013). Lipase production by *Aspergillus terreus* using mustard seed oil cake as a carbon source. Annals Microbiol. 63: 241-252.

Singh, M., K. Saurav, N. Srivastava and K. Kannabiran (2010). Lipase Production by *Bacillus subtilis* OCR-4 in Solid State Fermentation Using Ground Nut Oil Cakes as Substrate. Current Res. J. Biol. Sci. 2: 241-245.

Sun, S.Y. and Y. Xu (2008). Solid-state for whole-cell synthetic lipase production from *Rhizopus chinesis* and identification of the functional enzyme. Process Biochem. 43: 219-224.

Vaseghi, Z., G.D. Najafpour, S. Mohseni and S. Mahjoub (2013). Production of active lipase by *Rhizopusoryzae* from sugarcane bagasse: solid state fermentation in a tray bioreactor. Inter. J. Food Sci. Technol. 48: 283-289.

Yagiz, F., D. Kazan and A.N. Akin (2007). Biodiesel production from waste oils by using lipase immobilized on hydrotalcite and zeolites. Chem. Eng. J. 134: 262-267.

Yu, M., S. Qin and T. Tan (2007). Purification and characterization of the extracellular lipase Lip2 from *Yarrowiali polytica*. Process Biochem. 42: 384-391.

Zhang, J.W. and R.Y. Zeng (2008). Molecular cloning and expression of a cold-adapted lipase gene from an Antarctic deep sea psychrotrophic bacterium *Pseudomonas* sp. 7323. Mar. Biotechnol. 10: 612-621.