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Isolation, Production and Partial Characterization of Extracellular Lipase from *Trichoderma* Species

Sonika Pandey, Mukesh Srivastava, Mohammad Shahid, Vipul Kumar, Anuradha Singh, Shubha Trivedi, Ved Ratan, Manoj Maurya and Y.K. Srivastava

Chandra Shekhar Azad University of Agriculture & Technology, Kanpur - 208 002, India.

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Lipases are very important from the industrial point of view, they are frequently used in food materials, detergents, medicines, cosmetics, leather, perfumes etc. This is the first report which characterizes lipase enzymes extracted from eight different species of *Trichoderma*. All species were isolated from the different locations of Uttar Pradesh. The best carbon source that induces the maximum lipase production was Tween-80. Maximum extracellular lipase activity and biomass production was achieved with *Trichoderma harzianum* on media supplemented with Tween- 80. The optimum temperature and pH for extracellular lipase activity were 7.0 and 40°C.

Keywords: *Trichoderma*, Extracellular lipase, Enzyme activity.

Lipases are ubiquitous enzymes present in animals, plants, fungi and bacteria. In human diet lipases are present as triacylglycerols. Lipases are biocatalysts, they act in an oil water interphase. Lipases (triacylglycerol acylhydrolase; EC3.1.1.3) catalyze the hydrolysis of triacylglycerols to release free fatty acids and glycerol. Today researchers are looking towards the discovery of lipases of microbial origin as lipases are frequently used in various areas like food, dairy, cosmetic, tanning, pharmaceutical, medical, waste treatments etc. Lipases are produced by animals, plants, and microorganisms, but lipases of microbial origin are frequently used in biotechnological purpose. Filamentous fungi are found to be the promising source of lipase production. Most common genera that are efficient producer of lipases are *Aspergillus, Rhizopus, Mucor, Penicillium* and *Geotrichium.* Besides possesing lipolytic activity lipase is estereolytic also, thus they have a wide substrate range. After cellulase and amylase lipase are on the third position according to market sales, their market value is up to billions of dollars, this property of lipases make them industrially important. Lipases are widely used in organic synthesis and more than 20% biotransformation is performed with lipases (Gitlesen et al., 1997). *Trichoderma* is a well known biocontrol agent, it has the potential of secreting cellulase, chitinase, glucanase and amylases enzymes. Topal et al in 2004 first time reported the production of lipases from *Trichoderma harzianum* isolated from turkey. Lipases are produced during the late logarithmic or stationary phase (Ghosh *et al* 1996). *Trichoderma* a member of family *Hypocreaceae,* which is well known for the production of cellulose, chitinase, glucanase. The manuscript here describes the lipase production capacity of this genus (Kashmiri *et al*., 2004)

* To whom all correspondence should be addressed.
E-mail: sonica.dey@gmail.com

MATERIALS AND METHODS

Isolation and identification of *Trichoderma*

The soil samples for the identification of *Trichoderma* species were collected from the different regions of Uttar Pradesh. Isolate of *Trichoderma* species was isolated and identified in potato dextrose agar (PDA) with low sugar medium[11]. The identification of *Trichoderma* isolates were confirmed both by morphological and molecular characters (ITS) and deposited in Indian Type Culture Collection (ITCC), IARI, Pusa, New Delhi. After taking ITCC Acc. No, sequences were submitted in NCBI database.

Name of Bioagent	Culture No.	Source/ District	ITCC Acc. No.	Gen Bank NCBI No.
T. harzianum	*Th* azad	CSA Kanpur Nagar	6796	KC800922
T. viride	01PP	Hardoi	8315	JX119211
T. asperellum	T_{asp}/CSAU	CSA Kanpur Nagar	8940	KC800921
T. koningii	T_K (CSAU)	CSA Kanpur Nagar	5201	KC800923
T. atroviride	71 L	Hardoi	7445	KC008065
T. longibrachiatum	21 PP	Kaushambi	7437	JX978542
T. virens	T_{vi} (CSAU)	CSA Kanpur Nagar	4177	KC800924
T. reesei	Tr(CSAU)	CSA Kanpur Nagar	7284	KM999966

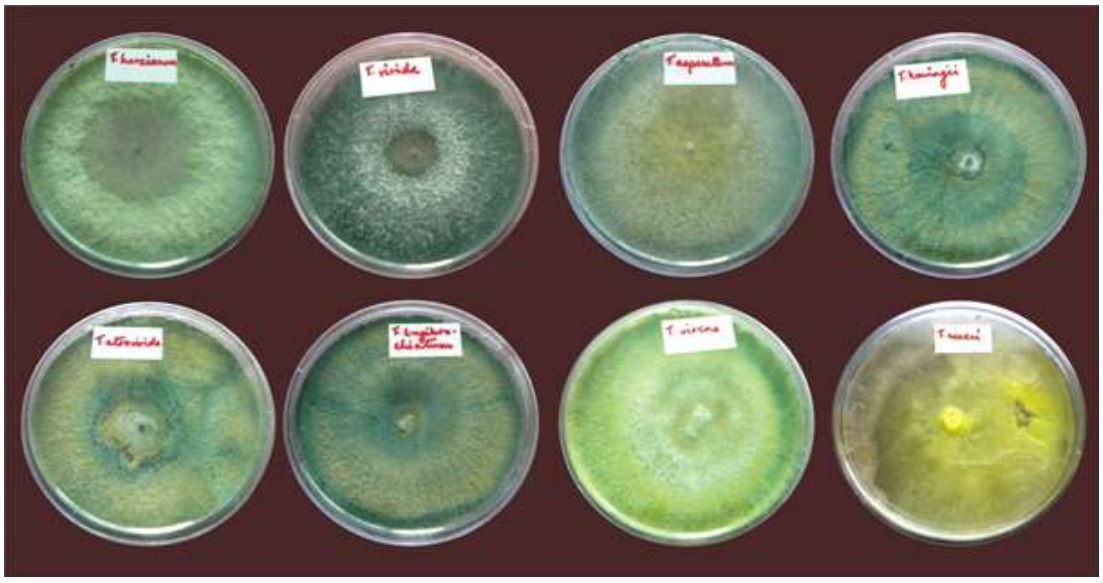

Fig. 1. Plate culture of different species of *Trichoderma* isolated from U P

Culture conditions and optimization

The organism was cultured in 100 mL of basal mineral medium (g/L). The chemical composition of lipase medium is as follows glucose 10 g, Dipotassium hydrogen phosphate 2g, Sodium Nitrate 0.5 g, Magnesium sulphate 0.5 g, carbon source 1% and DW 1000 ML. The initial pH of the media was adjusted to 7.0. 50 ml of the media is poured into a 250 mL conical flask shaken at 150 rpm at 30°C for 7 days. The effects of various carbon sources (Tweeen 80, coconut and soybean oil) were estimated in relation to enzyme yield.

Biomass dry weight

The *Trichoderma* biomass was determined according to its dry weight. The growth media was filtered through pre-weighted filter paper (Whatman No.1) to extract the biomass, which was subsequently dried in an incubator at 30 °C for 24 h (Cihangir et al., 2004)

Crude lipase preparation

In order to remove fungus cells and spores, the culture was filtered through filter paper

(Whatman No.1) and then the filtrate was centrifuged at 10,000 rpm at 4°C for 15 min. The supernatant was collected to perform lipolytic activity assays (Pimentel et al., 1994). The crude extract was stored at –20°C until used.

Determination of lipase activity

Lipase activity in the synthetic media was determined titrimatrically on the basis of olive oil hydrolysis. One ml of culture supernatant was added to assay substrate, containing 10 ml of 10% (v/v) homogenized olive oil in 10% (w/v) gum acacia, 2.0 ml of 0.6% CaCl2 solution and 5 ml of phosphate buffer (pH 7.0). The enzyme substrate mixture was incubated on rotary shaker with 150 rpm at 30°C for one hour. 20 ml of alcohol: acetone (1:1) mixture was added to the reaction mixture. Liberated fatty acids were titrated with 0.1N NaOH using phenolphthalein as an indicator (Kempka *et al.,* 2008). The end point was light pink in colour. One unit of lipase is defined as "the amount of enzyme which releases one micro mole fatty acid per minute under specified assay conditions".

Lipase unit calculation

$$Lipase\ Activity = \frac{\Delta V \times N}{V\ sample} \times \frac{1000}{60}$$

ΔV= V2 – V1

V1= Volume of NaOH used against control flask

V2= Volume of NaOH used against experimental flask

N= Normality of NaOH

V (Sample) = Volume of enzyme extract

Units of extracellular lipase activity were units per ml (U $mL^{-1)}$

Protein quantification

The protein quantity of the crude enzyme extract was determined by the Lowry method using bovine serum albumin as standard (Lowry et al., 1951.).

Effect of pH on the activity and stability of lipase

To detect the effect of pH on the activity of lipase enzyme, crude enzyme extract obtained by using the best lipase enzyme inducer carbon source were incubated over a temperature range from 4 to 9 for 30 min and enzyme activity was determined.

Effect of temperature on the activity and stability of lipase

To detect the effect of temperature on the activity of lipase enzyme, crude enzyme extract obtained by using the best lipase enzyme inducer carbon source was incubated over a range of temperature between 30°C to 70°C for 20 min., after incubation enzyme activity was determined.

RESULTS AND DISCUSSION

The production medium supplemented with different carbon sources like sunflower oil, coconut oil and tween-80 was estimated for lipase activity and the results are depicted in Fig. 2. All strains shows maximum lipase activity in media supplemented with Tween 80. Minimum lipase activity occurs in Coconut oil supplemented media. Among all the tested strains *T.harzianum* shows maximum lipase activity on media supplemented with tween-80. The maximum biomass and lipase enzyme production was achieved with Tween- 80 supplemented media (Fig. 2 and 3).

Ohnishi *et al*., 1994 reported that lipase activity tended to increase as the glucose concentration increased from 0% to 4%. In another study Iftikhar *et al*., 2002, found that there was no difference between glucose and olive oil for lipase production. Moreover, the lipase activities of many other fungi, such as *Aspergillus wentii* (Chander *et al* 1980) *Mucor hiemalis* (Akhtar *et al*., 1980) (Rathi *et al* 2001) are also stimulated by the addition of glucose to the production medium.

Effect of pH on the activity and stability of lipase

For the optimization of Initial ph of the lipase production medium for lipase production, fermentation was carried out at different pH range from 3 to 9 (figure 4). A linear increase in lipase activity occurs from pH 4 to 9. Highest lipase activity occurs at pH 7. Further increase in pH shows gradual decrease in lipase activity. Most of the microbial lipases have their optimum activity between pH ranges 7-9. For *Aspergilus hiemaliss* the optimum pH range reported was 7.0 by Alhir et al., 1990, for *Penicillium* the best pH range was found to be 9.0 by Saxena et al 2003.

Effect of temperature on the activity and stability of lipase

Temperature plays an important role in metabolic activities of microorganism. Figure 5 shows the effect of temperature on lipase activity. Lipase activity increases with temperature. Highest lipase activity occurs at 40°C. The lipase activity start to decrease from 40°C and minimum lipase

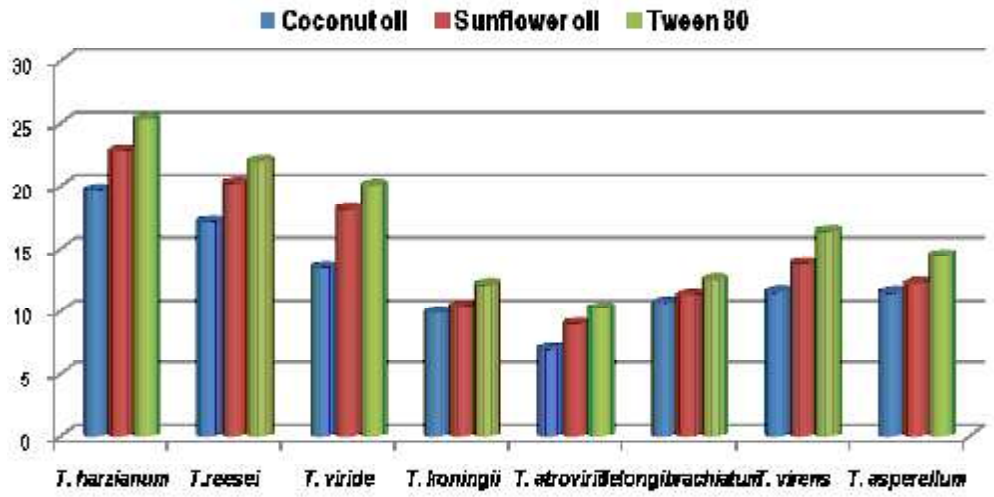

Fig. 2. Lipase activity of *Trichoderma* strains on media supplemented with different carbon source

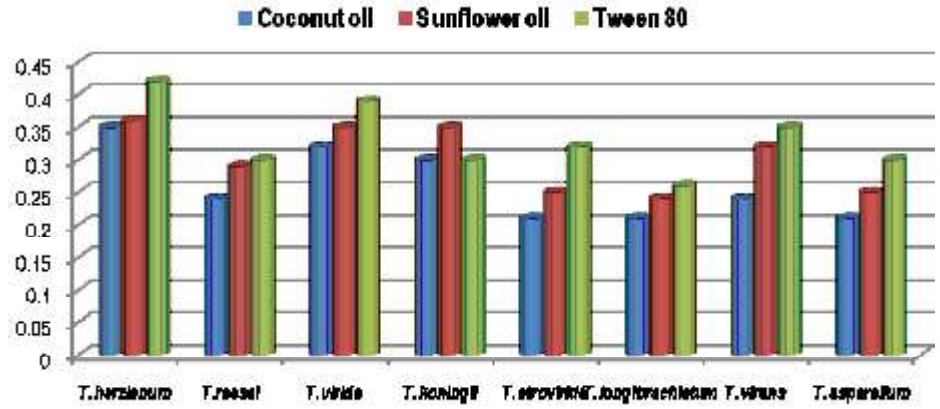

Fig. 3. Biomass of *Trichoderma* strains on lipase production media supplemented with different carbon source

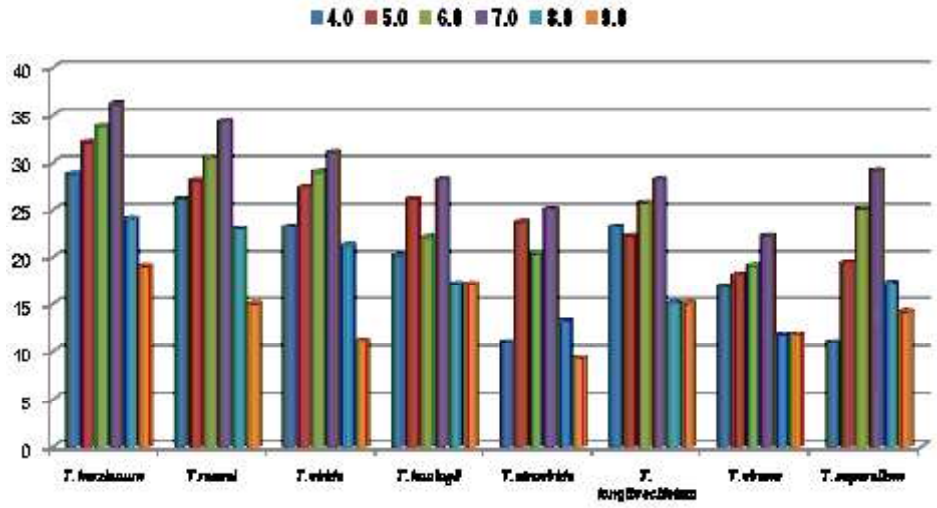

Fig. 4. Effect of pH on the lipase activity of *Trichoderma* species

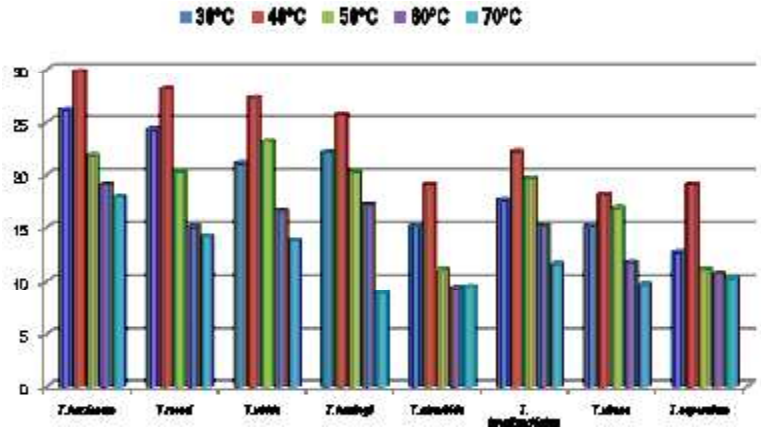

Fig. 5. Effect of temperature on the lipase activity of *Trichoderma* species

activity occurs at 70°C .Any change either decrease or increase in temperature results in gradual decrease in protein production (Ikram -ul-Haq et al., 2006). Higher temperature has the ability to alter cell membrane composition and induces protein catabolism, thus results in death.

Similar results were reported to be 40°C for *Mucor hiemalis f. hiemelis* by Hiol et al. and for *Fusarium solani* lipase Castilho et al., 2000 found that the optimum temp range is 40°C. Akram Kashmiri et al 2006 reported the production of lipase by *Trichoderma viride* in a complex medium containing olive oil and reported maximum lipase activity at 30°C. For *Aspergillus niger* the optimum temperature range reported is 24°C (Ohnishi et al., 1994).

CONCLUSION

In this study we screened eight different species of *Trichoderma* for the lipase production. This report presents lipase extraction and its characterization from eight different species of *Trichoderma*. Lipases from other different genus have already been characterized but in regard of *Trichoderma* there is not much information available. The lipases isolated from *Trichoderma* species shows that they are highly active at neutral pH and at temp 40°C. Considering the overall properties of the studied lipase, we can say that it may be used on different industries such as organic solvent synthesis, detergent formulations etc.

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