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Effect of pH on lipase specific activities of thermophilic fungi from city waste of Bareilly

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ABSTRACT

The lipase of the *Sporotrichum thermophile, Aspergillus fumigatus* are important and versatile enzymes that are mainly used in fat and oil modification due to their strong 1, 3- regiospecificty. In the present study, five samples were collected randomly from different sites of Bareilly city, Transport Nagar, Nakatiya, Barabazar, University campus, Railway junction. Bareilly city were primarily screened by sprinkling method and serial dilution techniques for isolation of lipase producing species of fungi from Bareilly city. Out of five samples collected two isolates were primarily identified as excellent producers of lipase in modified lipase assay media. Further it was found out that among five, two isolates of fungi were producing lipase extracellulary and their activity was tested using titrimetric assays. The two fungi *Aspergillus fumigatus and Sporotrichum thermophile* were found to be the excellent producers of lipase. These isolated fungi have been tested for their pH-activity relationships and pH range is 4-8.5. Excellent lipase specific activity was observed from pH 6-7.

Key words: *Sporotrichum thermophile, Aspergillus fumigatus*, Lipase.

INTRODUCTION

Thermophillic fungi mainly are responsible for compost maturation (Miller 1996). Fungi have been isolated from sewage, more or less polluted soil and water by different workers (Cooke 1952, 1954, 1957, 1959; Backer Snaw 1955; Niebla and Aquero, 1982; Rebox et al. 1984). Fungi are important enzyme producers since their enzymes are produced externally (Borgestrom and Brackman 1984; Venkat Eshawarlu and Reddy, 1993; Hang and Woodams 1990; Ferreira Costa and Pralta, 1999). Lipase (E.C.3.1.1.3) occur extensively in nature and catalyses the hydrolysis of triacylglycerols in aqueous media. In this study, the synthesis of extracellular lipases by two lipolytic thermophilic species *sporotrichum thermophile and Aspergillus fumigatus* and the conditions which determine their activities were examined. Therefore the present investigation has been under taken for the thermophilic fungi isolated from different sites of Bareilly.

MATERIAL AND METHODS

Samples collection

Samples were collected from the sites contaminated with lipids, oil and decaying organic matter from five different sites. Samples were collected as undecomposed, semidecomposed and totally decomposed.

Isolation of lipolytic molds

Selective isolation of fungi will be made using enriched (YPS's) medium by using recent technique (Warcup 1950). PH is 7.0 and temperature is 45°C.

Identification of fungi

Identification of fungi is done with the help of camera lucida.

Extraction of Lipase

Lipase was extracted by the method of Yamada and Machida (1962). Isolated fungus were grown on modified Czapek's dox medium. Composition is oil cake 30 gm, $MgSO4.7H_2O$ 0.5 gm, KCl 0.5 gm, $NaNO_3$ 2.0 gm , K_2HPO_4 1.0 gm, $FeSO_4$ 0.01 gm.

Lipase specific activity

Lipase specific activity was measured by universal titrimetric method using automatic titrator from Radiometer (Copenhagen Denmark) .

Following reagents were prepared for enzyme assay –

1. Coconut oil - 1% O/W emulsion as substrate
2. Calcium Chloride - 0.1 M solution
3. Sodium Hydroxide - 0.05 N solution
4. Phosphate Buffer - 0.1 M solution
5. Stock solution A - 0.1 M K_2HPO_4
6. Stock solution B - 0.1 M KH_2PO_4
7. Phenolphthalein indicator

Calculation

Lipase specific activity was calculated using the formula given by Peled. N, Krenz (1981).

$$U/ml = (V_S – V_b) \times 0.05 \times 10^3 \times D \times 18.5$$

18.5 - Total assay media.
V_S - Titration value.
V_b - Blank titration value.
0.05 - Molar concentration of NaOH used for neutralization.
10^3 - factor for µ mole.
D - Dilution.

One unit of lipase specific activity was defined as the amount which liberated one µ mole of acid per minute at 45°C. (somkuti and Babel , 1967) .

Protein estimation

The protein contents of the filtrates were determined using the method of Lowry *et al* (1951) with crystalline bovine albumin as standard.

Effect of pH on lipase specific activity

The effect of pH on lipase specific activity was determined by using 0.1 M phosphate buffer pH range from 4 to 8.5.

RESULTS AND DISCUSSIONS

It has been found that lipase specific activity of different isolates vary at different pH. *Aspergillus fumigatus* showed maximum lipase specific activity 3.870±0.0026 U/mg protein at pH 6.0 on 5th day

Table 1: Effect of pH on the activity of lipase by *Aspergillus fumigatus* from city waste of Bareilly

pH	Days		
	3rd	5th	7th
4.0	-	1.283±0.0029	1.006±0.0370
4.5	1.545±0.0015	0.561±0.0026	0.459±0.0026
5.0	0.826±0.0038	1.045±0.0020	0.998±0.0029
5.5	0.728±0.0029	1.878±0.0017	1.261±0.0023
6.0	2.250±0.0031	3.870±0.0026	4.213±0.0020
6.5	2.867±0.0032	2.981±0.0023	3.015±0.0040
7.0	0.831±0.0028	1.918±0.0032	1.936±0.0032
7.5	0.825±0.0017	0.828±0.0026	0.830±0.0031
8.0	0.337±0.0015	0.344±0.0020	0.371±0.0020
8.5	0.452±0.0020	0.444±0.0034	0.503±0.0033

Specific activity (U/mg Protein)

Table 2: Effect of pH on the activity of lipase by *Sporotrichum thermophile* from city waste of Bareilly

pH	Days		
	3rd	5th	7th
4.5	0.858±0.0147	0.667±0.0126	0.625±0.0095
5.0	0.976±0.0130	0.723±0.0096	0.785±0.0162
5.5	0.921±0.0080	0.785±0.0084	1.079±0.0206
6.0	0.858±0.0170	0.728±0.0187	0.780±0.0403
6.5	1.196±0.0198	1.235±0.0345	0.846±0.0691
7.0	1.987±0.0094	1.847±0.0254	1.043±0.0067
7.5	0.665±0.5070	0.445±0.254	0.576±0.0159
8.0	0.960±0.0093	0.914±0.0066	0.940±0.0132
8.5	0.529±0.0147	0.702±0.0042	0.676±0.0159

Specific activity (U/mg Protein)

(table 1), while *Sporotrichum thermophile* showed maximum lipase specific activity at 1.847±0.0254 U/mg protein at pH 7.0 on 5th day (Table 2). *Aspergillus fumigatus* showed lowest lipase specific activity 0.337±0.0015 U/mg protein at pH 8.0 on 3rd day (Table 1), while *Sporotrichum thermophile* showed lowest lipase specific activity at pH 0.445±0.254 U/mg protein on 5th day (Table 2). Optimum range of pH for lipase specific activity was 6-7.

CONCLUSION

pH 6.0 was optimal for lipases of *Aspergillus fumigatus* while pH 7 was best for *Sporotrichum thermophile.* Similar peaks were recorded for thermophilic fungi by Somkuti and Babel (1968).

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