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Effect of Temperature and pH on Lipase Production Activity of Fungi

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Abstract: ***Fungal lipases remain preferable than lipases obtained from other sources due to their low cost of extraction as well as thermal and pH stability. Best incubation temperature for lipase enzyme activity in Penicillium chryogenum and Aspergillus oryzae it remain as 40ºC while in Fusarium solani it remain as 30ºC. All three tested fungal species showed different peaks with regard to impact of pH on lipase activity. In Pencicillium chrysogenum and Aspergillus oryzae optimum enzyme activity has been observed from pH 5.0 to 7.0 and 6.0 to 8.0 respectively. Whereas in Fusarium solani it has been observed from pH 4.0 to 9.0 It was observed during this research study that, besides temperature, pH also has a great influence on the characterization of enzyme. The optimum initial pH for lipase activity was maintained as 7.0 in all fungal strains tested.***

Keywords: Temperature, pH, Lipase production, Fungi

1. Introduction

Lipase enzyme catalyses hydrolytic reactions as well as esterification, transesterification, acidolysis, alcoholysis and interesterification. Lipase enzymes are produced by Bacteria, Fungi, Animals and Plants. But fungal lipases are most significant for commercial use due to low production cost and high stability. The rate of production of lipase enzyme by fungal members greatly influenced by several physico - chemical factors such as as pH, dissolved oxygen concentrtion, temperature, as well as nitrogen and carbon sources. Fungal members produces extracellular lipases that can be simply separeted from the fermentation media. Lipase enzymes are valubale biocatalysts with diverse application in different industries. Production of lipase enzymes by serveral microorgnisms such as beacteria and fungi have been reported by several authors. Dalmau *et al.* (2000), Mehta *et al*. (2017) and other authors reported *Aspergillus, Candida, Fusarium, Geotrichum, Humicola, Mucor, Penicillium, Pichia, Rhizopus and Yarrowia* as Lipase producers. Sharma *et al.* (2001) reported that most of the lipase producing fungal member naturally inhabitat lipid rich industrial and domestic waste.

Enzymes have recently gained more attention due to their ease of application and capacity to overcome the limitations. Lipase producing fungi play a key role in the enzymological remediation of polluted soil. Lipases produced by fungal strains are typically extracellular and therefore relatively easy to recover after the fermentation. Many genera of fungi have been reported as producers of lipase with desirable properties. Application of lipase enzyme in several industries have stimulated interest in isolation of new lipases from novel sources. Jaeger and Reetz (1998) stated that the reasons for the enormous biotechnological potential of microbial lipases are: their stability in organic solvents, they do not require cofactors, exhibit a high enantio - selectivity and possess broad substrate specificity. Due to these reasons lipases are currently given much attention with the rapid development of enzyme technology. In above mentioned perspective, present research work was conducted.

2. Materials and Method

Soil samples were collected from different oil and detergent rich sites of Chapra (Bihar) and mixed with each other. Experimental pots were prepared by additing water and milk in the soil of pots for isolation of fungi having degradation activities. The experimental pots were irrigated and turned over regularly. After a week, the soil samples were collected for experimentation by digging the soil of pots at 6 inch below the surface, cleaned and dried. Separate unused polythene bags were used for sampling of each soil sample. The polythene bags were sterilized using detergent followed by alcohol wash. The bags were properly sealed with heavy duty rubber bands and tagged. These bags were stored at 4ºC and used as a source of lipolytic fungal strains for further experimentation.

Primary selection of lipase producing fungal species was done by preliminary lipolytic screening. For this purpose, the isolated and identified fungal members were separately spread on the tributyrin agar plates contaning 1% Tributyrin and 2% Agar. The formation of clear zone around the fungal colony is indication of the lipolytic activity. Thus tributyrin agar medium was used for determination of lipase producing ability of specific fungal species according to the tributyrin cleaning zone technique. On the basis of the zone of hydrolysis around the colonies, positive strains for this study were selected. Selected strains were identified as *Penicillium chrysogenum*, *Fusarium solani* and *Aspergillus oryzae* and used for optimization studies. It was observed that all three fungal species mentioned above showed lipase producing activity by producing clear zone around the colony.

The lipase production was monitored at 10, 20, 30, 40, 50, 60, 70 and 80ºC temperature separately. The effect of initial temperature of the fermentation on enzyme production activity was determined by varying incubation temperature and then lipase production activity was measured at different temperatures. Innoculum concentration of 10% was dispensed aseptically in culture medium containing 250 ml Erlenmeyer flasks and sterilized for 15 minutes at 121ºC and

then 120 rpm shaking speed was maintained. Initial pH as 7.0 was maintained.

The impact of pH value ranging from 3.0 to 11.0 with one unit interval was observed separately by using 0.1 N HCl and 0.1 N NaOH buffer. The lipase production was monitored at different intial pH levels of the culture medium for all three fungal strains separately. Optimum temperature observed during previous experiment was maintained. Lipase producing brooths were prepared in 250 ml Erlenmeyer's flark with use of tribuyrin as sole carbon source and sterilized for 15 minutes at 121ºC. The fungal strains were innoculated. pH of broths were adjusted at 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with use of above described buffer. Cultures were maintained for 6 hours separately at 120 rpm shaking speed. After incubation period the reaction was stopped and absorption was measured spectrophotometrically at 410 nm. Thus optimal initial pH level was determined for maximum lipase production by all three fungal strains separately and fixed for subsequent experiments.

3. Result and Discussion

All selected fungal strains remained able to grow at pH range 3.0 to 11.0 but showed good enzyme activity at pH range of 4.0 to 9.0. *Fusarium solani* showed highest lipase activity among all the three tested fungal members during this investigative research study. Temperature ranging from 30ºC to 50ºC showed good lipase activity in *Penicillium chyrsogenum* and *Aspergillus oryzae.* But a temperature range of 20ºC to 60ºC affected positively the lipase activity in *Fusarium solani.*

Table 1: Effect of different temperature on lipase production activity (U/ml) at neutral pH (7.0)

Sl. No.	Lipase producing Fungi	Temperature							
		10ºC	20ºC	30ºC	40ºC	50ºC	60ºC	70ºC	80ºC
1.	*Penicillium chrysogenum*	5.3	9.6	18.4	19.8	17.2	9.1	6.3	3.7
2.	*Fusarium solani*	9.5	20.3	37.5	31.3	30.3	28.2	15.1	4.3
3.	*Aspergillus oryzae*	7.2	10.4	18.1	18.6	13.1	7.3	4.2	3.3

The lipase activity of *Penicillium crysogenum, Fusarium solani* and *Aspergillus oryzae* at 10ºC, 20ºC, 30ºC, 40ºC, 50ºC, 60ºC, 70ºC and 80ºC temperature at constant pH of 7 was separately observed to identify the effect of different temperature on lipase activity of these fungal members. It was observed that at 10ºC temperature 5.3, 9.5 and 7.2 U/ml lipase activity were showed by *Penicillium chrysogenum, Fusarium solani* and *Aspergillus oryzae* respectively. *Penicillium chrysogenum* exhibited increasing trend of lipase activity by increase of temperature upto 40ºC then decrease of enzyme activity by further increase of temperature as observed upto 80ºC. *Fusarium solani* showed increasing trend of lipase activity upto 30ºC temperature and by further increase of temperature reduced ability of lipase activity of this fungi was observed. *Aspergillus oryzae* showed increasing trend of lipase enzyme activity upto 40ºC temperature and further increase of temperature gradually decreased enzyme activity upto 80ºC temperature. The highest temperature of 80ºC affected adversely on enzyme activity of all three fungal species under study and minimum enzyme activity was observed at this temperature.

Table 2: Effect of different pH on lipase production activity (U/ml) at 40ºC temperature

S. No.	Lipase producing Fungi	pH								
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0
1.	*Penicillium chrysogenum*	7.1	10.3	15.4	17.8	19.9	11.2	9.0	6.2	5.4
2.	*Fusarium solani*	11.8	24.3	28.1	36.7	40.3	32.8	29.3	17.3	8.2
3.	*Aspergillus oryzae*	5.5	7.3	10.8	16.5	18.7	14.3	11.2	8.0	6.1

Effect of different pH on constant 40ºC temperature on lipase activity exhibited by *Penicillium chrysogenum, Fusarium solani* and *Aspergillus oryzae* was observed and data obtained were presented in above given table. It was observed that optimum lipase activity showed by all three fungal members undertaken during present study at neutral pH of 7. The enzyme activity by *Penicillium chrysogenum* showed increasing trend from 3 to 7 pH then enzyme activity decreased from 8 to 11 pH. This trend was also observed with respect of *Fusarium solani* and *Aspergillus oryzae.* Optimum enzyme activity was exhibited as 40.3 U/ml at pH 7 by *Fusarium solani.* Minimum enzyme activity as 5.4 U/ml was exhibited by *Penicillium chrysogenum at* pH 11.

Thus it can be said that the increase in temperature increases the number of effective collision between the enzyme and substrate to form the activated complex and thus the rate of reaction increases. However, there is a limit to the increase in enzyme activity with the increase in temperature. When the rate of enzyme catalysed reactions is measured at several temperatures, the reaction rate decreases sharply mainly due to the denaturation of enzyme by heat. It has been reported that the drop in the percentage of residual activity at high temperatures results in some conformational changes in the tertiary structure, and then almost complete inactivation of the enzyme. Temperature of the culture medium is also an important factor which affects the enzyme yield by fungi. Rifaat *et al*. (2010) reported that 28ºC temperature remain suitable for lipase production by *Fusarium oxysporum* and Dimitries *et al*. (1992) reported that 30ºC temperature remain suitable for optimum lipase production by *Rhizopus glutinis.*22ºC, 25ºC and 26ºC temperature remain suitable for lipase production by *Penicillium citrinum, Colletotrichum gloeosporioides* and *Rhizopus arrhizus* respectively as reported by Balaji and Ebenezer (2008). Thus it became clear from the above mentioned results obtained by several researchers that production of lipase is affected by physico - chemical parameters such as pH, and temperature.

Dina Helny *et al*. (2017) observed during their experimental research that *Curvularia* sp. produced maximum extracellular lipase at 30ºC temperature. Mukhtar *et al*. (2015) conducted a study to understand the suitable temperature for maximum lipase production by *Aspergillus niger*. They applied inculcation temperature from 25ºC to 55ºC and observed maximum lipase production by this fungal strain at 30ºC followed by 25ºC, 35ºC, 40ºC, 45ºC, 50ºC and 55ºC. Gwen *et al*. (2006) observed during their study that lipase obtained from *Aspergillus niger* remain optimally active within a temperature range of 40 to 60ºC and Namboodri *et al*. (2002) also reported that lipase from this fungal species remain active between 40 to 55ºC temperature.

Stability of lipase under different pH conditions is important for their further utilization. Therefore, the effect of pH stability of lipase was evaluated during present study in varying pHs ranging from 3.0 to 11.0 for 10 minutes and further incubated for 45 minutes at 40ºC.

Begum and Munjam (2020) observed maximum enzyme production by *A. niger* and *A. flavus* on optimal temperature between 30ºC and 35ºC and optimal pH 6.0 and 5.0, respectively. Fungal members are well known source for the production of extracellular enzymes. Temperature remain directely related to the metabolic activities of fungi. Enzyme producing fungal strain has its own optimal temperature at which it grows fastly and produces maximum amount of enzyme. Thus it is observed during present research study that maintenance of optimal temperature is must for any fungal strain. Temperature of culture medium have very good influence on enzyme production by them. Optimal temperature is that temperature at which maximum velocity of the enzymatic reaction takes place. Above optimal temperature rate of enzymatic reaction decreases due to thermal inactivation. The very slight changes in the growth temperature, may affect lipase production. Maximum enzyme activity at optimum temperature and pH may be due to increase in protein content leading to extracellular enzyme production in culture supernatant.

4. Conclusion

On the basis of results obtained during present study it is concluded that the fungal lipase production is greatly influenced by the initial pH and temperature of culture media. The initial pH of culture medium can influence fungal growth and product formation due to its effect on the solubility of nutrients and ionization of the substrate. Filamentous fungal strains easily develops over a wide intital pH range but each fungal species has a unique optimal initial pH for its development and activity.

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