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Effect of Carbon and Nitrogen Sources on Lipase Production Activity of Fungi

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Abstract: ***Carbon sources of the culture medium support both growth and lipase production ability of fungi. Sucrose is observed to be the best sugar source for lipase activity in Fusarium solani. It became evident during this research study that sucrose enhances lipase activity in Fusarium solani but glucose in other two fungal species. Potassium chloride for Penicillum chrysogenum and Aspergillus oryzae and Potassium nitrate for Fusarium solani remain suitable nitrogen source for maximum lipase activity. It became evident that nitrogen content of the culture medium is one of the most important factors with regard to lipase production by fungi.***

Keywords: Carbon source, Nitrogen source, Lipase production, Activity of fungi

1. Introduction

Fungal lipases are preferable than lipases obtained from other source due to their low cost of extraction, thermal and pH stability, substrate specificity and activity in organic solvents. Lipase producer strains are widespread in the fungi kingdom. The main producers of fungal lipases are species of filamentous fungi such as *Aspergillus, Rhizopus, Penicillium, Mucor, Geotrichum, Humicola* and *Fusarium.* Lipases from *Candida, Yarrowia* and *Pichia* have also reported by Benjamin and Pandey (2001). They reported that the extracellular lipase production by fungi depends upon several culture conditions such as carbon, nitrogen, lipid inducer and presence of inorganic salts. Hasan *et al*. (2006) reported that microbial lipases find promising applications in organic chemical processing, detergent formulations, synthesis of biosurfactants, the oleochemical, diary and agrochemical industries, paper manufature, nutrition, cosmetics, perfumery and biocatalytic resolution of pharmaceuticals. As well as novel lipase applications have been successfully established as a solution to many evironmental problems.

Lipase enzyme have great potentiality to degrate lipid. Lipase enzyme can be used in bioremedian of lipid rich waste water. This can be an important contribution to eco - friendly environment. Thus, microbial lipase find application in detergent formulation also. In context of above mentioned facts, a research was conducted to find out suitable carbon and nitrogen sources for optimum lipase production by three fungal species.

2. Materials and Method

Soil samples were subjected for serial dilution.0.1 gm soil sample was suspended in 9.9 ml normal saline aseptically and subjected to five fold serial dilutions such as 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made. Different workers used media such as Potato Dextrose Agar (PDA), Czapek Dox Agar, Sabouraud Dextrose Agar or Martin's Rose Bengal Medium for isolation of fungi. Sharma *et al.* (2010) observed that PDA remain as best media for fungal mycelial growth. PDA remain as one of the most commonly used culture media due to its simple formulation and its ability to support mycelian growth of a wide range of fungi. They also stated that the fungal culture media may be supplemented with antibiotics such as streptomycin etc to suppress undesirable bacterial growth.

Thus 0.1 ml innoculum from desired dilution was spread on sterile and solified Potato Dextrose Agar (PDA) plates supplemented with streptomycin at a concentration of 250 mg/ml to inhibit the undesired bacterial growth. Plates were incubated at 28+2°C temperature. After three days, plates were observed and fungal colonies showing morphological differences were picked up individually by sterile innoculating needle and streaked on separate PDA plates and incubated at 28+2ºC temperature. Repeated restreaking of fresh media results in pure cultures, which were then transferred to PDA slants overlaid with liquid paraffin and preserved in refrigerater for identification.

Identification of fungal members under study was carried out with the help of compound microscope and mannual of soil fungi (Gilman, 1975) on the basis of colony characters as well as morphology and Fungal Taxonomy classification (Costa and Peralta 1999). Fungal species have been identified by observation of isolates from well developed large colonies with abundant aerial hyphae. The basis of identifcation remain based on Lactophenol cotton blue staining, mycelial branching, sporulation pattern, shape, size and arrangement of phalids, conidiophores, conidia and spores observed under compound microscope.

The effect of two carbon sources on lipase production by three fungal strains was observed separately. Each carbon source was used as sole carbon source in the culture medium. Carbohydrates like glucose and sucrose were separately used as the sole carbon source in basal medium and were assayed for their efficiency to support lipase production. Such as glucose and sucrose as well as ammonium chloride and potassium nitrate as nitrogen source were added separately in the brooth. Broth medium was dispensed in 250 ml Erlenmeyer's flask and sterilized for 15 minutes at 121ºC temperature. Innoculum concentration of 10% was dispensed aseptically in culture medium containing

Erlenmeyer's flask for all three fungal strains separately. Optimum initial temperature as 40ºC and intial pH as 7.0 were maintained to measure lipase production activity. Specific carbon and nitrogen source responsible for maximum lipase production was determined after incubation period of 96 hours. Shaking speed was maintained as 120 rpm throughout this experiment. After incubation period the reaction was stopped and absorption was measured spectrophotometrically at 410 nm. Most suitable carbon source observed during this experiment was used for further experiment.

3. Result and Discussion

The results of present study indicates that sucrose as sugar source stimulates lipase activity in both the fungal strains such as *Fusarium solani* and *Aspergillus oryzae* but glucose remained suitable for *Penicillium chrysogenum.* Potassium nitrate is observed as best nitrogen source for lipase activity in *Fusarium solani* but ammonium chloride was found most suitable nitrogen source for *Penicillium* sp. and *Aspergillus* sp.

Table 1: Effect of carbon source on lipase production activity (U/ml) at 40ºC temp. and pH 7

Sl. No.	Lipase producing Fungi	Sucrose	Glulose
1.	*Penicillium chrysogenum*	21.8	23.3
2.	*Fusarium solani*	40.4	38.2
3.	*Aspergillus oryzae*	21.7	19.3

The effect of different carbon sources taken in growth medium as sucrose and glucose at 40ºC temperature and pH7 on enzyme activity by *Pencillium chrysogenum, Fusarium solani* and *Aspergillus oryzae* was observed and the data obtained is presented in above mentioned table. *Penicillium chrysogenum* exhibited lower enzyme activity in sucrose as 21.8 U/ml as compared to glucose (23.3 U/ml). But *Fusarium solani* exhibited high level of enzyme activity in sucrose as 40.4 U/ml as compared to glucose in medium (38.2 U/ml). *Aspergillus oryzae* exhibited high lipase enzyme activity in sucrose as 21.7 U/ml than glucose taken as carbon source in medium (19.3 U/ml). Thus it became evident that *Aspergillus oryzae* exhibited lowest enzyme activity in glucose containing medium among all fungal members undertaken for observation during present research work.

Table 2: Effect of nitrogen source on lipase production activity (U/ml) at 40ºC temp. and pH 7

Sl. No.	Lipase producing Fungi	Ammonium chloride	Potassium nitrate
1.	*Penicillium chrysogenum*	20.2	17.8
2.	*Fusarium solani*	40.9	42.4
3.	*Aspergillus oryzae*	23.1	20.0

Data presented in above given table about effect of different nitrogen sources as Pottassium chloride and Pottassium nitrate at 40ºC temperature and pH 7 on lipase production activity of three fungal species such as *Penicillium chrysogenum, Fusarium solani* and *Aspergillus oryzae* indicates that maximum lipase producing activity was exhibited by *Fusarium solani* in both nitrogen sources containing media separately. *Fusarium solani* showed 40.9 and 42.4 U/ml enzyme producing ability in ammonium chloride and pottassium nitrate containg media respectivily. *Penicillium chrysogenum* exhibited lowest enzyme production activity among all tested fungi as 20.2 U/ml and 17.8 U/ml in ammonium chloride and potassium nitrate containing media respectively. *Aspergillus oryzae* exhibited 23.1 U/ml and 20.0 U/ml lipase enzyme producing activity in ammonium chloride and potassium nitrate containing media respectively. Thus it became clear that amonium chloride remain most suitable nitrogen source for lipase producing activity for all the three fungal species undertaken for analysis during present experimental research study.

Ghosh *et al*. (1996) observed that the requirement of sugar as a carbon source in addition to lipids varies with the microorganism. They also observed that media supplemented with glucose along with triglycerides stimulate the lipase production in *Rhizopus nigricans.* In *Penicillium citrinum* both olive oil and Tween - 80 increase the production of extracellular lipase at 0.1 and 0.7% (v/v), respectively. Both organic and inorganic nitrogen sources have been traditionally used for lipase production. Ghosh *et al*. (1996) investigated in their research that in *Aspergillus wentii, Mucor racemosus* and *Rhizopus nigricans,* lipase yield is stimulated by addition of peptone in the production medium at the concentration of 20 g/L. However, it was also reported that for lipase production by *Rhodotorula glutinis,* inorganic nitrogen sources, like ammonium phosphate is most suitable. It was also reported that peptone supplemented media stimulate maximum production of extracellular lipase in thermophilic fungi, *Rhizopus oryzae.* In case of *Rhizopus arrhizus,* both peptone and yeast extract enhance the lipase production. Falony *et al*. (2006) observed that a conbination of 2% olive oil and 2% glucose remained conducive for lipase production by *Aspergillus niger*. Murlidhar *et al*. (2001) observed optimal lipase production by *Candida cylindracea* as a yield of 17.30 U/ml using glucose as carbon source.

Nitrogenous sources present in culture medium also affects lipase production by fungi. But Montesinos *et al*. (1995) while working on lipase production by *Candida rugosa* and *Aspergillus niger* respectively observed no effect of nitrogen source on lipase production. During present study *Penicillium chrysogenum* and *Aspergillus oryzae*¸both the fungal strains have highest enzyme activity in presence of ammonium chloride but *Fusarium solani* in presence of potassium nitrate. Similar observation has also been found during investigations by other workers. The production of primary and secondary metabolites by fungi remain highly influenced by their growth, which depends on the nutrients provided. Nitrogen content of the culture medium is one of the most important factors. The lipase production potential of any fungal strain can be enhanced several times through the use of suitable physico chemical parameters during culture.

4. Conclusion

On the basis of overall findings of the present investigation, it may be concluded that the *Fusarium solani* is among all the three selected fungal strains which show maximum lipase activity in the broth. Sucrose enhances lipase activity in *Fusarium solani* but glucose in other two fungal strains.

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