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HARNESSING LIPOLYTIC BIOCATALYSTS FROM FUNGAL ORIGIN: AN UNCONVENTIONAL APPROACH TO APPLY BIO-CATALYSIS IN INDUSTRIES

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Abstract: The current scenario worldwide demands a more reliable, sustainable, environment–safe and economically viable ways to deal with the ever-increasing problems of ecological issues. Therefore, the main aim of this work is to opt for such ideas and technologies which include cleaner, safer and greener procedures for reusing and valorizing trash materials thereby obtaining value added products. The leather goods spoiled by excessive fungal growth on them are otherwise thrown away as waste. In order to reuse waste to derive valuable products in form of enzymes is my focus of work. The fungal colonies if isolated from leather goods and cultured in laboratory to ferment it and produce fungal enzymes (biocatalysts), then these fermented end- products can be employed in several industries for carrying out various chemical reactions and other operations. Conventional use of chemicals can hence be reduced to a great extent by replacing them with fungal enzymes in the leather industries itself to use it in various pre-tanning operations. So, in this work, we first targeted to isolate fungal colonies from waste leather items, cultured in laboratory and fermented them under various conditions to extract fungal enzyme i.e. lipase . Lipases are highly versatile and industrially important enzymes. Deriving the lipases from waste areas is the main attraction of this work and is a venture strategizing the "waste to value" approach.

Keywords: ecological issues, valorizing, fungal colonies, oil rich source, trash to treasure

I. INTRODUCTION

Due to the ever-increasing pollution rate of environment, there has been a global drive to promote ideas supporting green technology. Major advances have been made for encrypting protein structure and function for using it in biocatalytic applications. Scientific breakthrough in enzyme directed biocatalytic transformation has become an important tool for rational designing of sustainable ecofriendly industrial process development [1]. Engineering of enzyme properties such as stability, activity, selectivity and substrate specificity modify the rate of biocatalytic process [2]. Fungal lipases are preferable over bacterial lipases because of their easier extraction and purification processes, given to the fact that fungal enzymes are extracellular in nature. Lipolytic fungal species compete efficiently with other forms of life for survival by some important control mechanisms. Lipid sources seem to be generally essential for obtaining a high lipase producing fungi. Discarded leather products spoiled by fungal growth are used as main source of fungal colonies. Lipases produced from microbial origin has huge industrial requirement [3]. The global market demands for industrial lipases application scope for development of new industrial process including leather industries. Indigenous microbial populations clean up biologically these organic components by utilizing them as their nutritional requirements. A great deal of scientific research has been carried out to explore the consequences of these biological cleaning machineries, including lipase. Lipases have many applications and benefits in the pre tanning operations of leather processing [4,5]. Lipases have the ability to bring about hydrolytic and synthetic reactions in both aqueous and non-aqueous media, hence have multifold applications in other post tanning operation in leather processing as well [6,7]. The most traditional application of lipases has been found in the degreasing operation [8] which might be extended to a variety of other operation such as fat liquoring. Conventional fat liquoring entails huge drawbacks. Uneven diffusion of fat molecules inside the hide and skin is responsible for uneven dyeing and finishing, waxy patches which demerits the product quality. Accordingly, one strategy to overcome this difficulty is by utilizing fungal lipase for hydrolysis of fat liquor.

II. OBJECTIVE

The objective of this study was to identify extracellular fungal lipase sources, which can be used as a basis for further basic and applied researches in leather processing. In addition, this work is aimed to examine enzyme production by lipase producer isolates

on various environmental conditions. The goals included the isolation of lipase producer and characterization of lipase. For this purpose, the following specific objectives have been formulated:

- Isolation of fungal colonies from leather samples
- Screening of extracellular lipase producing fungi using tributyrin contained agar plates
- Investigation of lipase production of selected strains under various culture conditions

III. MATERIALS AND METHODS

Potato dextrose agar (PDA) medium PDA medium was used for storage of fungal cultures or fresh seeding for preparation of liquid cultures. Tributyrin Agar (TBA) medium was used for selective isolation of lipophilic fungi. Tributyrin was replaced by mustard oil in general composition of TBA medium. All other compositions were same. Other chemicals Lactophenol cotton blue was used for fungal staining. Tween 80, $CaCl_2$, KH_2PO_4, K_2HPO_4, HCl, NaOH, Ninhydrin, BSA were also required. For the present study samples were collected from soil and oil contaminated soil from Diamond Harbor, District-South 24 PGS, West Bengal, India. One pure strain is obtained from discarded leather samples were used for the study i.e., *Aspergillus niger*.

MEASUREMENT OF GROWTH CURVE

The growth pattern of the pure isolate was characterized in liquid media. 150 µl of PDB and TBB were inoculated with 25µl PDB seed culture. The microorganisms were then placed in an incubated orbital shaker (MODEL ZHWY100B) at the speed of 120 rpm and temperature of 37°C. Growth of the cells was monitored by checking its optical density at 600 nm at an interval of every 10mins with UVvisible spectrophotometer (Model HOLMARC SPECTRA HO-SP1911). Each of them is analyzed in triplicate. The values recorded were then used to prepare growth curves for each of the microorganisms. Optimization of media parameters was carried out for profound enzyme activity. Fungal growth and lipase enzyme accumulation were studied in the present investigation in flask batch cultures under different growth conditions [9,10,11]. These conditions include incubation time, incubation temperature, pH, different carbon and lipid sources, and surfactant. The aim of these experiments is the optimization of lipase enzyme production by the strain under investigation. Lipase production is influenced by these physiologically important growth parameters i.e., temperature, pH, lipid, carbon source, presence of surfactants and time [12]. Each experiment was repeated three times. 25 µL of mother culture were added into the 200 ml tributyrin broth from 1 to 3 hours at 20, 37, and 45°C. The lipase production was then evaluated over a wide range of pH 4, pH 7 and pH 10. Further the changes of lipase production in response to the following lipid source (1% w/v) were evaluated, mustard oil, castor oil and sunflower oil.

Effect of temperature: Temperature is one of the most important parameters regulating the activity of microorganisms in natural environments. Generally, there is an optimal temperature for the enzymatic activity produced by different microorganisms which is responsible for the biosynthesis or degradation of compounds. This optimal temperature may be similar or different from the optimal temperature of the microbial growth and the growth curve was studied by varying the temperature at 20, 37 and 45°C to select the optimum temperature for maximum enzyme production by keeping the remaining parameters constant. To determine the effect of incubation temperature on mycelia growth which in turn influences enzyme production.

Effect of substrate on biomass yield: Substrate specificity affects the mycelial growth and enzyme production. To evaluate the effect of various substrates on lipase activity, 1% mustard oil, sunflower oil and castor oil was added to the culture media, separately. Remaining culture conditions were maintained constant

Effect of pH: By varying the pH to acidic, neutral and basic conditions and keeping other variables constant, the effect was studied to optimize the growth rate curve of the pure isolate and to examine at what specific pH condition, maximum growth rate can be obtained.

IV. RESULT AND DISCUSSION

The only organisms isolated were fungi as bacteria did not grow on the isolation medium. This may be due to differential growth conditions, especially composition. Due to the oil rich environments of the substrates, special attention was given to screening of lipolytic enzymes [13]. The enzyme activity was associated with growth of the cell and favorable environmental conditions. The recorded optical density with an interval of 10 min is representative of increased cell biomass with different time interval. Graphs were plotted with time versus optical density. Growth and multiplication of microorganisms on any substrates is often considered as the first step toward its bioconversion. Activity of lipase is directed by the biomass yield. This biomass yield is controlled by a variety of factors such as type of substrate, pH, temperature. These include factors that alter the binding of the enzyme to the substrate, the molecular properties of the enzyme, and structure of the substrate [14]. Therefore, in the work reported here, it was vital to institute an experimental design to test the effects of all the factors on the growth pattern of sample could be distinguished based on OD changes. The first phase was lag phase where no change in OD was observed. Rapid increase in OD indicates log phase. The next phase was the stationary phase, where there were no changes in OD. Last phase was death phase where negative slopes of the growth curve were observed.

Optimization of culture condition

Nutrient medium is a major factor that influences on fungal growth. All the media supported the growth of filamentous fungi to various degrees. An optimal nutrient medium should provide adequate growth and best possible growth, allowing molds to grow without restrictions. The growth of filamentous fungi is characterized by smoother curve and long transition periods although it is dependent of medium and species. The stages of fungal growth differ depending on the growth of microbes in the different media. Prolonged log phase is observed in case of colonies growing in TBB media as compared to PDB media. This could be due to the fact that the media nutrients are depleted more readily in PDB, so the isolates tend to enter in the death phase sooner. Tributyrin agar is a differential medium for lipophilic fungal growth.

Optimization of culture condition for temperature

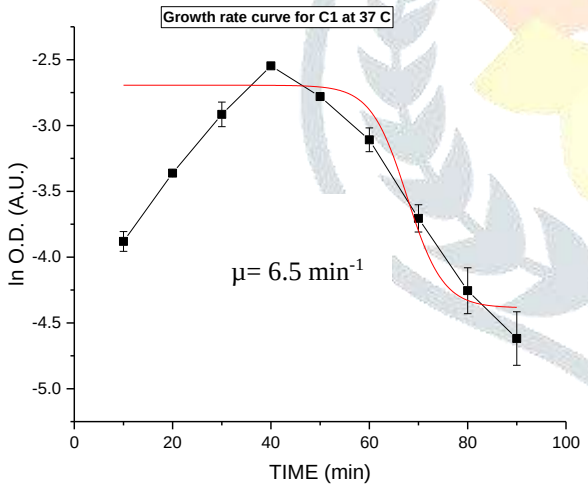
A detailed characterization of temperature-dependent growth of three isolates demonstrates interisolate variation in growth performance. It demonstrates that environmental conditions, specifically temperature, exert a strong influence on growth performance of the fungal isolate. Maximum growth rate is observed at 25°C which might also be the optimum temperature for enzyme production in further work. pH is one of the most important factors affecting the fungal growth and development and their relationships have been investigated. At acidic pH, it showed maximum growth rate and beyond that level, the growth rate kept decreasing.

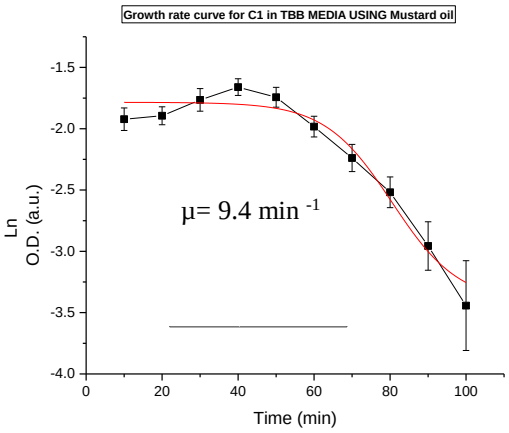
Optimization of culture condition for inducer

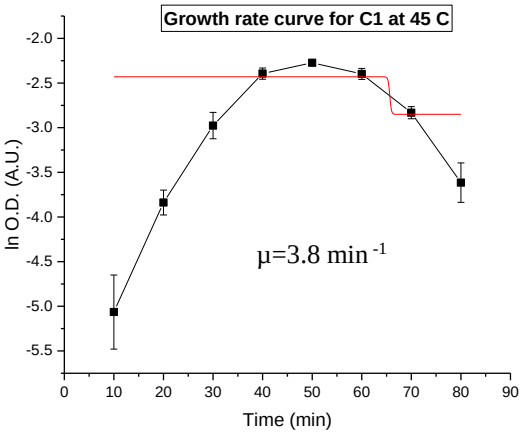
The compound, the inducer (oil), is one of the major factors for biomass yields. It is able to "turn on" production in cells in such a way that the enzymes are produced only when needed. It has shown that presence of lipid (especially natural oils) stimulates lipase production [15].

V. CONCLUSION

In present investigation, an effort has been made to study the lipase production by pure strain of fungi under various optimizing conditions. These observations provided interesting perspectives, demonstrating that fungi isolated from leather wastes represent a source of several enzymes potentially exploitable for biotechnological purposes. The use of fungal lipases for catalyzing esterification reaction became considerable interest because lipase mediated hydrolysis is an energy saving process. The great advantages of fungal lipases are that they are easily amenable to extraction due to their extracellular nature, which will significantly reduce the cost. Microbial lipases have gained special industrial attention due to their ability to remain active under extremes of temperature, pH and organic solvents, and chemo-, regio and enantioselectivity [16]. Lipase is frequently used to catalyze the hydrolysis of wide non-natural substrates in order to obtain enantio- and region selective substrates. Among those enzymes, lipase is predominantly used in several applications. These fat splitting enzymes are attractive because of their wide industrial applications. The reasons for the enormous biotechnological potential of microbial lipases are: their stability in organic solvents, they do not require cofactors, possess broad substrate specificity and exhibit a high enantioselectivity. The use of fungal lipase in leather industry is becoming increasingly important. Lipases have found application in the soaking, dehairing, bating, and degreasing operation in leather making. The great advantage of fungal lipases is that they are easily amenable to extraction due to their extracellular nature, which will significantly reduce the cost and makes these lipases more attractive than those bacteria. In nature, microorganisms can adapt to a changing ecological situation within a certain limit. Experimental results suggest that various media compositions influenced fungal growth. Optimization of growth parameters viz. temperature, pH, carbon source, had significant effects on the growth rate. After optimization under various parameters, maximum growth rate was observed. Further work has to be done by varying other physiological and chemical conditions to obtain the most favorable conditions under which lipase can be produced and hence can be applied on leather and other industries to carry out different operations and processes. This will ultimately lead to a shift from the dependency on harsh chemicals to safer, greener and more economic option of using enzymes or biocatalyst and will also have a huge effect on reducing the environmental pollution rate in the long run.







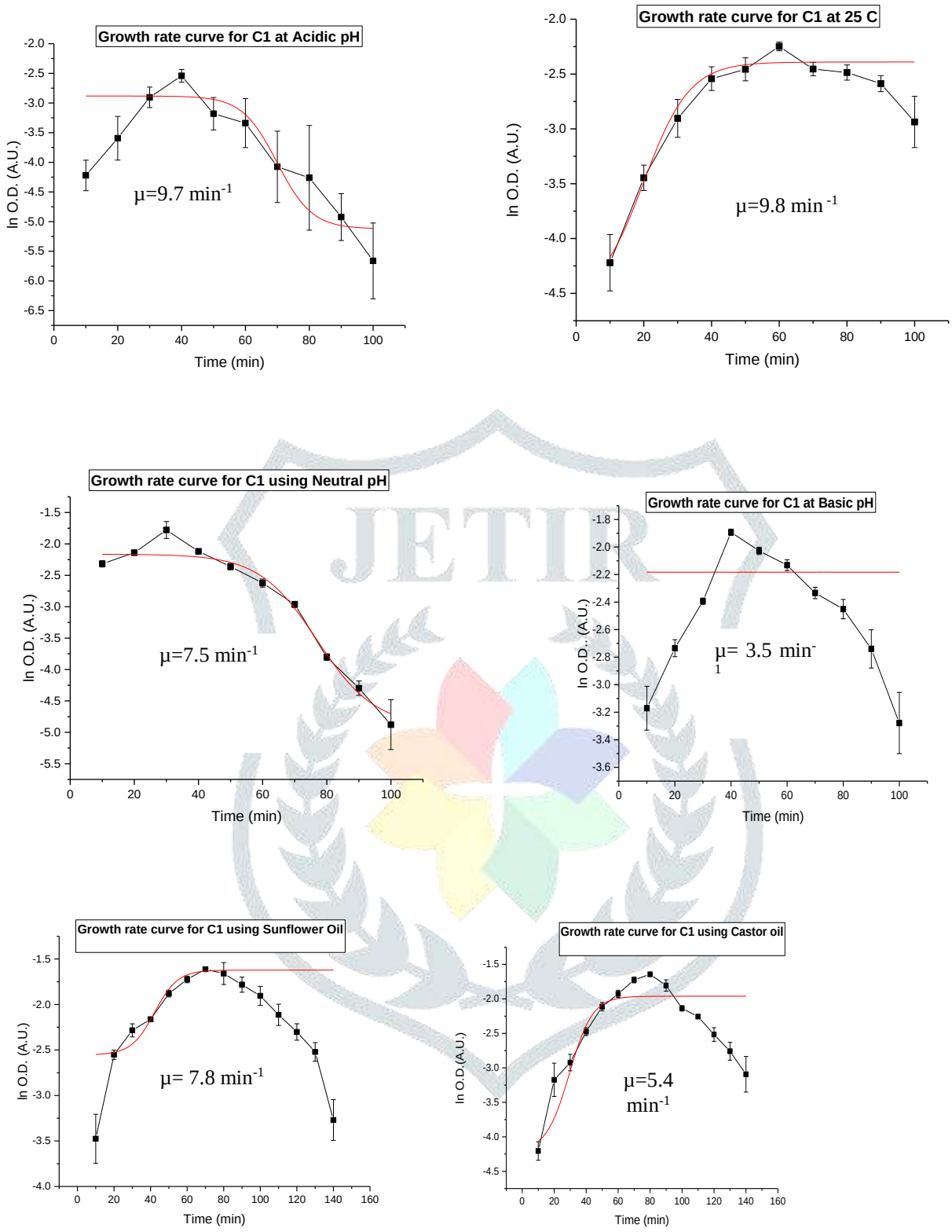


Fig 1: Graphs show the growth rate curve for the pure strain of fungal isolate (*Aspergillus niger*) under varying pH, Temperature and lipid source. The specific growth rate is denoted by μ in each case. greater the value of μ, better is the growth rate under that specific condition.

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