Production of lipase by *Aspergillus sydowii* using solid state fermentation

PROJECT REPORT

Submitted in partial fulfillment of the requirements for the award of the degree of

BACHELOR OF TECHNOLOGY
IN
DEPARTMENT OF BIOTECHNOLOGY

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MAY 2020

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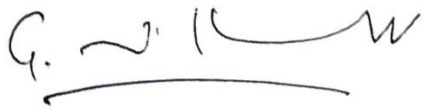
SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled ***"Production of lipase by Aspergillus sydowii using solid state fermentation"*,** submitted by Rachit Thakur (161801), Mansi Sharma (161808) at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of his original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.



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Date:15/07/20

DECLARATION

We hereby declare that the work reported in the B. Tech. thesis entitled "***Production of lipase by Aspergillus sydowii using solid state fermentation***" submitted at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of our work carried out under the supervision of Dr. Garlapati Vijay Kumar, Dept. of Biotechnology and Bioinformatics, JUIT, Waknaghat, HP-173234, India. We have not submitted this work elsewhere for any other degree or diploma**.**

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Date: 15/07/20

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Rachit Thakur(161801) Mansi Sharma(161808)

LIST OF SYMBOLS AND ACRONYMS

°C	Degree Celsius
µg/ml	Microgram per milliliter
mg/ml	Milligram per milliliter
mm	Millimeter
µl	micro-liter
nm	Nanometre
gm	grams
mg	milligram
OD	Optical Density
SSF	Solidstatefermentation
SmF	submerged fermentation
PHA	polyhydroxyalkanoates
PCL	polycaprolactone
W/V	weight by volume
V/V	volume by volume
pNPP	para-Nitrophenylphosphate

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ABSTRACT

Enzymes are vastly used as a catalyst to reinforce the varied biochemical reactions which are an integral part of the bio based industries and plants. They are also referred as macromolecular biocatalysts that accelerate any chemical reaction. The molecules upon which they typically act are called substrates which are converted into the final products with help of enzymes. Enzymes are also known to have catalysed more than 5000 biochemical reaction types.

SSF technique has been the most foremost accepted method to supply lipase due to its economical properties and safe handling. Solid state maturation uses culture substrates with decreased water levels (diminished water movement), which is especially right for shape creation. The procedures need to develop filamentous organisms utilizing strong state aging permit the least complex generation in their regular habitat. The way of life is being immersed with water however little of it is free-streaming. The SSF technique comprises of both the substrate and in this way the strong help on which the maturation happens. The substrate is especislly made out of vegetal side-effects like beet mash or wheat bran.[At the beginning of the development procedure, the substrates and strong culture mixes are non-solvent mixes made out of exceptionally enormous, biochemically complex particles that the parasite will stop to ask basic C and N supplements. To build up its normal substrate, the contagious living being presents its whole hereditary potential to gracefully the metabolites important for its development. The creation of the development medium coordinates the microorganism's digestion towards the get together of chemicals that discharge bio-accessible single atoms like sugars or amino acids via cutting out macromolecules. Along these lines, while choosing the parts of extension medium its conceivable to control the phones towards the gathering of the predetermined metabolite(s), fundamentally compounds that change polymers (cellulose, hemicelluloses, gelatins, proteins) into single moieties during an exceptionally productive and savvy way

Keywords: Crude lipases, solid state fermentation, fruit pulp, fungus *Aspergillus sydowii*.

CHAPTER 1

INTRODUCTION

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Lipases is a triacylglycerol acylhydrolases are a class of hydrolase which is an enzymethat catalyze the hydrolysis of triglycerides to glycerol and free unsaturated fats over an oil–water interface. In addition, lipases mobilizes the hydrolysis and transesterification of other esters as well as the synthesis of esters and exhibit enantioselective properties. The capacity of lipases to perform quite certain concoction change (biotransformation) has make them progressively mainstream in the food, cleanser, corrective, natural union, and pharmaceutical enterprises.It plays key .jobs in absorption, transport and preparing of dietary lipids in most living beings. Lipases have developed as one of the main biocatalysts with demonstrated potential for adding to the multibillion-dollar underexploited lipid innovation bio-industry and have been utilized in-situ lipid digestion and ex situ multifaceted modern applications. Lipases are created by creatures, plants, and microorganisms. Microbial lipases have increased exceptional mechanical consideration because of their strength, selectivity, and expansive substrate explicitness. Numerous living beings are known as expected makers of extracellular lipases, including microscopic organisms, yeast, and growths. Contagious species are ideally developed in Solid State Fermentation (SSF), while microorganisms and yeast are developed in lowered aging. Most economically significant lipase-delivering growths are perceived as having a place with the genera Rhizopus sp., Aspergillus sp., Penicillium sp., Geotrichum sp., Mucor sp. and so forth. Lipase creation by organisms differs as indicated by the strain, the sythesis of the development medium, development conditions, pH, temperature, and the sort of carbon and nitrogen sources. The quantity of properties accessible lipases has expanded since the 1980s and utilized as modern biocatalysts due to their properties like biodegradability, high particularity and high synergist proficiency. Some interesting properties of lipase, for example, their particularity, temperature, pH reliance, action in natural solvents and nontoxic nature prompts their significant commitment in the food preparing businesses. The most wanted attributes of the lipase are its capacity to use all mono-, di-, and tri-glycerides just as the free unsaturated fats in

transesterification, low item hindrance, high action/yield in non-fluid media, low response time, protection from adjusted temperature, pH, liquor and reusability of immobilized catalyst. Furthermore, lipases can complete responses under gentle states of pH and temperature and this decreases vitality needs to coordinate responses at uncommon temperatures and pressures3. Utilizations of Lipases structure an indispensable piece of the enterprises running from food, dairy, pharmaceuticals, agrochemical and cleansers to oleo-synthetics, tea businesses, makeup, cowhide and in a few bioremediation forms. Due to the tremendous applications, more up to date microorganisms are to be screened for creation of lipases having attractive properties. The comprehension of structure function relations will empower analysts to tailor new lipases for biotechnological applications. For the creation or development of growths the strong state aging is progressively favored on the grounds that it is having better return of optional metabolites or proteins and showed uncommon showed when develops in strong culture and fundamental atomic components. There are two kind of aging procedure that as of late utilized for catalyst creation are either SSF or SmF. Through this aging, a few catalysts can be produce contingent upon the creature utilized. Growths and microbes are the most life forms contemplated that fit for creating chemical. As per Krishna (2005), filamentous growths are favored in either SSF or SmF for cellulose creation. Furthermore, lowered aging (SmF) is a procedure of development of microorganism in a fluid medium. Strong state aging (SSF) is an important procedure for use of agro-modern side-effects to create esteem included product(s) of business intrigue. SSF is characterized as any aging procedure performed on a non-solvent material that demonstrations both as physical help just as wellspring of supplements without free streaming fluid. The method includes vaccination and development of microorganisms on permeable particulate strong substrate keeping up low dampness content. The water substance and supplements present in the substrate bolster the development of microorganisms and the living beings emit valuable compounds while developing on strong substrate. Lipases a fascinating class of acyl hydrolases which has been in the middle phase of bio-synergist responses as they are normally blessed with the possibility to hold bio-reactant movement in both watery just as natural media. Lipases are pervasive in nature and are delivered by different creatures and the majority of the microorganisms. Lipases of microbial inception, for the most part bacterial and parasitic speak to the most broadly utilized class of proteins in biotechnological applications and natural science. Countless lipases have been screened for their utilization as food added substances (flavor

adjusting catalysts), modern reagents (glyceride hydrolyzing protein), stain removers (cleanser added substances), stomach related medications, analytic compounds in clinical applications, neutraceuticals, surfactants and added substances in beautifying agents. The regular responses performed by lipases in fluid media are frequently alluded as hydrolysis; arrival of liquor and relating greasy acid(s) particles during enzymatic activity on substrates, for example, glycerol or comparable esters.

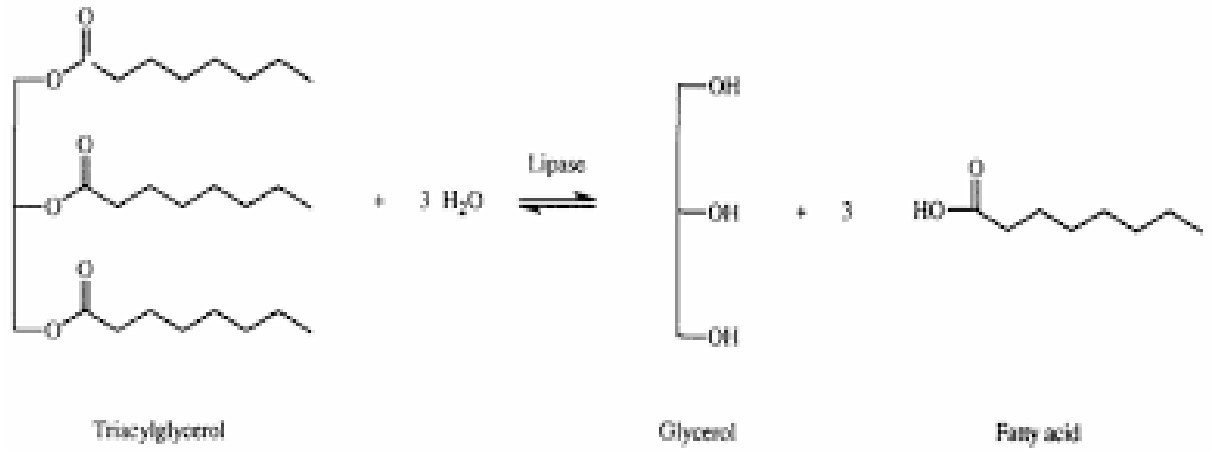

Fig (A): lipase catalyzed hydrolytic reaction.

These responses are irreplaceable for the bioconversion of lipids (triacylglycerol) and as a rule continue with higher regio-as well as enantio-selectivity. Notwithstanding, the converse response alluded as esterification that could be effectively accomplished in natural media/water limited conditions (natural solvents or non-ionic liquids) includes the development of an ester alongside water particles as result of such responses. Microbial lipases show a wide range of mechanical applications because of their more noteworthy security, substrate explicitness and lower creation cost when contrasted with different sources. Furthermore, a colossal biodiversity of microorganisms improves elective biotechnological forms and legitimizes the quest for new lipases. Filamentous organisms are regularly perceived as the best lipase makers and are at present the favored sources since they produce extracellular lipases. Besides, organisms are considered as the most effective wellspring of lipases and different proteins including the procedure of SSF. The utilization of coordinated development can be exceptionally useful to advance existing lipases as for wanted properties. SSF has additionally been utilized to deliver lipases from different results of agribusiness and mechanical source that have minimal business esteem.

1.1 Brief of SSF

SSF can be characterized as the development of microorganisms on soggy, water-insoluble strong substrates in the nonattendance or close nonappearance of free fluid, thusly being shut to the regular habitat to which microorganism adjusted (Pandey et al., 2000 and Gabiatti et al., 2006). Or on the other hand it very well may be generally characterized as those procedures where microbial development and items arrangement happen on the surfaces of strong substrates in the close to nonattendance of free water. Because of this low measure of water accessible in strong state bioprocessing, the class of microorganisms that are most generally utilized is organisms agro-modern waste and side-effects, for example, orange bagasse (Martins et al, 2002), sugar stick bagasse (Silva et al., 2002) wheat grain (Cavalitto et al., 1996) and other food handling waste (Zheng and Shetty,2000) or natural product mash are viable substrates for depolymerizing chemical creation by strong state aging. As of late, an enormous number of microorganisms, secluded from various materials, have been screened for their capacity to debase p-nitrophenyl palmitate to p-nitrophenol present in substrate (organic product mash) biomass in nearness of lipase protein on strong state culture. Notwithstanding the previously mentioned benefits for parasites, SSF has a few possible favorable circumstances over SmF, for example, lowcapital cost, low vitality consumption, more affordable downstream preparing, low waste water yield and likely higher volumetric efficiency, higher aging profitability, higher endconcentration of items and soundness, lower catabolic suppression, development of microorganism explicitly for insoluble substrate. Eco-accommodating procedure that stays away from condition contamination and horticulture squander or side-effect aggregation. Financial and low capital speculation Equipment particulars and space necessity Manual work cost and compensation time Increased thermo-strength of lipases.

CHAPTER 2

REVIEW OF LITERATURE

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2.1 Importance and uses of enzymes

Enzymes are basically catalysts for different kinds of chemical reactions and are produced by all living cells.They have a very important role in the food sector industries for quite a long time.Before they were known to the people or their studies came up they were still used in many processes for example tenderization of meat etc . Enzymes can be extracted from any source like humans, plants ,microbes and from any living organisms. Majority of the enzymes that are used in industries belong to the microbial source . In the food sector industries these enzymes have been widely used to enhance all the required properties of food. Enzymes produce dfrom the microbes are always preffered over the other sources of enzymes because they can be manufactured from them in a precise and controlled manner.Another is that the enzymes produced from microbes processes less harm as compared to the other two.We will be dealing more with the microbial enzymes that serve their use in many industries.

2.2Increasing popularity of lipase

Lipases cause the hydrolysis of triglycerides into diglycerides, monoglycerides, glycerol and fatty acids. Their is observed a vast jump in the utilization and demand for these enzymes in the past few decades as they comprise of many diverse applications for medicinal purposes ,additives in food, testing reagents.Besides these applications lipases is also used in the conversion of plastics to a environment friendly formand Recent applications, such as the designing of racemic mixtures to produce visionally active compounds, must also arise from the stereospecific acting properties of some lipases[8.] Lipase enzymeis also responsible for the bioconversion reactions via.,interinterifaction, esterifaction, alcoholysis, acidolysis and aminolysis[9]. Besides ,these enzymes also serve their use in many food sector and flavoring sector industries. Lipases are also used in the production of chemicals with main focus on on

pharmaceutical ,coesmetic sector etc. These enzymes also play a vital role in the management of waste and improving tanning techniques. Various microrganisms like bacteria ,yeast and fungi are most commonly used for the production of this enzyme beacuse they produce lipases of high stability . A new method for the separation of lipolytic fungi was analysed from soil on an oil miner medium that was laid on silica gel plates. Lipases are integrated in the presence of inducers. The material that induces or act like inducers s are wheat bran, rice bran, dextrins, sugar cane bagasse, coconut cake, olive oil cake, and gingley oil. Lipases are also produced by solid-state fermentation or submerged fermentation. The technique of SSF has been most widely used because of their simplicity and they are particularly economic then submerged fermentation. Some scientists have analysed and stated that the lipases can be produced from an *Alkalophlic form of yeast*by SSF using rice and wheat bran as substrates.

2.3 Structural features of lipase

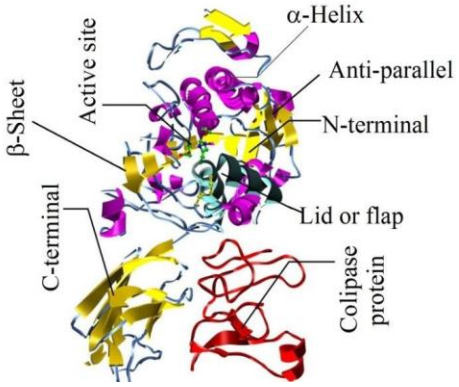

Fig 2.1 Pancreatic lipase

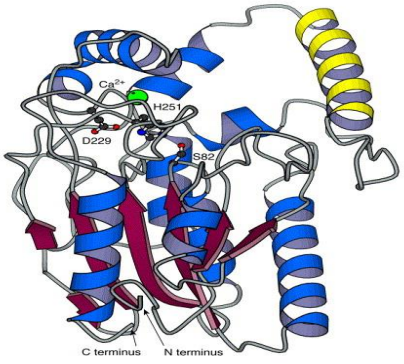

Fig 2.2 Microbial lipase

Auxiliary highlights assumes an indispensable job in the building and planning of the lipases proteins. Yet many structures are yet to be resolved and designed.

2.4Characterization of lipases

Lipase characterisation can be done through various parameters like -:

1. **Temperature analysis on lipase acitvity** - lipase activity can be calculated using spectrophotmetric analysis , optimal temperature can be analysed using spectrophotometric analysis using a particular substrate for eg (p-NPP) observed at different temperature ranges.
2. .**pH analysis on lipase activity** - buffer solutions are prepared at different ph ranges using the substrate and are analysed using spectrophotometric analysis.
3. **Analysis of substrate concentrations onlipase activity**- lipases enzyme activity graph can calculated at 410nm using a spectrophotometer . values of Kmax and V max are obtained and specific activity is obtained increasing the amount of the substrate.

2.5 Catalytic mechanism of lipase

The point by point steps of lipase component of activity in hydrolysis response are summed up beneath:

Stage 1: The procedure of hydrolysis begins by authoritative of the lipid particle and the enactment of nucleophilic buildup next to present histidine, to which a proton from the serine hydroxyl bunch is conveyed. Proton move is advanced by the existence of the reactant corrosive, which precisely aligns the imidazole ring of histidine and to some degree kills the charge. Initiation is trailed by an assault by the oxygen particle (O-) of the serine hydroxyl bunch on the enacted carbonyl carbon of the powerless lipid ester bond (Jaeger et al., 1999).

Stage 2: A transient tetrahedral middle of the road is framed, which is seen by a negative charge on the carbonyl oxygen particle of the scissile ester bond and four molecules clung to the carbonyl carbon iota orchestrated as a tetrahedron. The middle of the road is balanced out by the

macrodipole of helix C, and by hydrogen bonds between the contrarily charged carbonyl oxygen particle (oxyanion) and in any event two fundamental chain NH gatherings (oxyanion gap).

Stage 3: The extra proton of histidine is laterly given to the ester oxygen of the vulnerable bond, which is in this manner partitioned. At this stage, the corrosive segment of the substrate is esterified to the nucleophilic serine (the covalent middle of the road), while the liquor part diffuses away .

Stage 4: The histidine buildup gives the extra proton to the oxygen iota of the dynamic serine buildup, that breaks the ester security among serine and the acyl part, and frees the acyl item. After dissemination of the acyl item, the catalyst is prepared for additional rounds of catalysis .

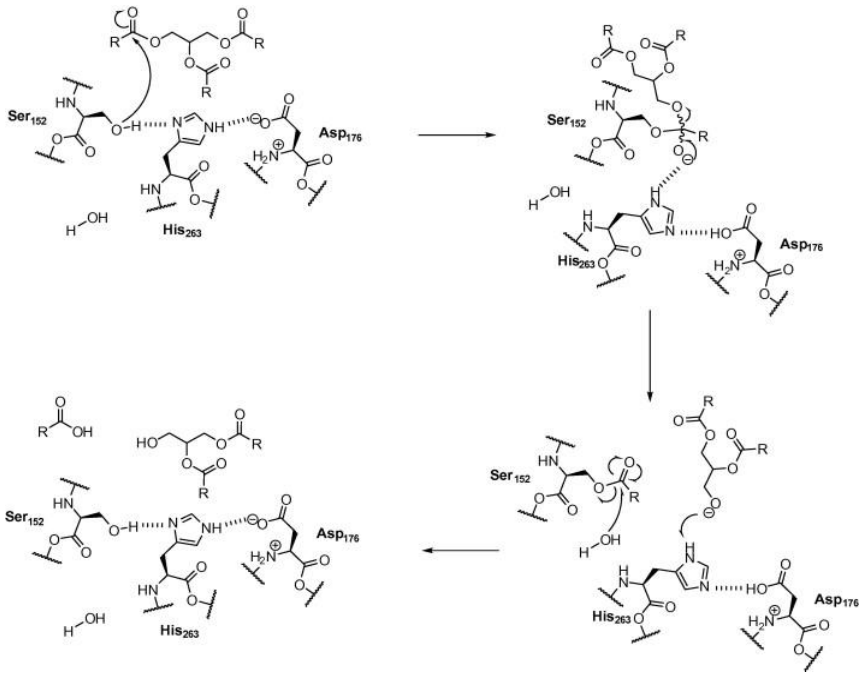

Fig 2.3: Mechanism of action of lipase in hydrolytic reaction

CHAPTER 3

MATERIALS AND METHODS

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3.1Culturing.

Fungal cultures samples of *Aspergillus sydowii* was collected from the previous samples in the biotechnology labs. Its selection was done considering the availability and suitability for the production of the enzyme.

3.2Culture maintenance

The fungal culture of *Aspergillus sydowii* was collected and streaked over the PDB media which was 2.4gm of PDB in 2.5gm of agar-agar which was kept in slanting position for better growth of fungal stain in test tube and then was incubated at 30degree for 4-5 days and was stored at 4degree for further usage.

3.3Subculturing of media

The periodically or within 4-5 weeks the sub culturing of fungal strain was done for avoiding any kind of contamination in the fungal strains which was previously streaked. Mainly sub culturing was done by taking small quantity or colonies from previously streaked strain and streaked to another test tube which is having same amount of PDB(2.4gm)+agar-agar(2.5gm) in solidified slant position and then again kept in incubator for 4-5 days and replaced the previously streaked slants.

3.4 Inoculum composition

The spore inoculum was utilized in the current investigation. Spores from 3 to 5-days-old inclination culture were utilized for current inoculation.

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3.5 Substrate selection

Dried fruit pulp of fruits like orange, carrot and pine apple was used upon which the enzyme will act. There are different types of parameters which are being used like different amount of substrate concentration, incubation time difference, incubation temperature difference, inoculum amount difference etc for good extraction of enzyme through solid surface fermentation.

3.6 Sampling and Enzyme extraction

The fungal strain was added to the fruit pulp varying in concentration i.e. 2gm, 4gm etc with some autoclaved distilled water in different flasks and incubated for 5 days. The mixture was separated into liquid and solid using muslin cloth in order to separate the solid fruit pulp and to obtain the liquid containing the respective enzyme.

3.7 Flowcharts representation of culturing of *Aspergillus sidowii* strain

3.7.1For reviving the *A. sydowii* strain

2.4gm PDB in 2 flasks (100 ml)

⇩

Autoclave

⇩

1.5ml of PDB added to cultured flask for reviving that previously used strain

3.7.2For growing the *aspergillus sydowii* strain

2.4gm PDB in 2.5gm agar-agar in 100ml distilled water

⇩

Autoclave

⇩

Streak the strain from revived culture by taking some colonies with the help of loop

⇩

Incubated at 30 ^{o}C for 4-5days

⇩

stored at 4 ^{o}C

3.7.3 For culturing the fungal strain onto SSF

prepare Czepadox media (W/V)

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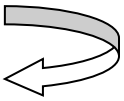
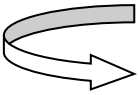
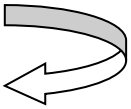
Add 30 ml of czepadox media into a beaker and add 5.00%(w/v) - glucose



add 5ml-5ml to 5 different flasks having substrate



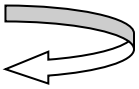
add 10.00 %(v/v) coconut oil to each flasks



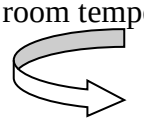
close the top of all flacks with cotton plugs and cover with catalog paper and autoclave all the flask

1ml of inoculum is added to all flasks and incubated at 35 ^{o}C for 5days

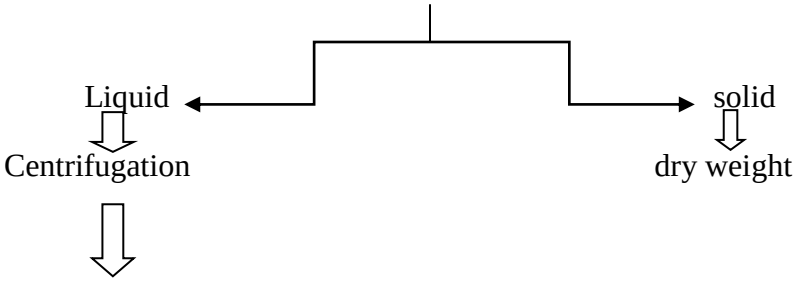
16ml autoclave distilled water is added to each flask



2hr. incubation at room temperature



After 2hrs squeeze culture with muslin Cloth



Crude lipase

(In supernatant)

3.7.4 For lipase assay

Material required:

Sol. A :- 40mg of pNPP in 12ml of isopropanol

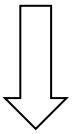
Sol. B :- 0.1gm of gum arabic+0.4gm of triton-100 was added in distilled water (90ml)

Protocol:

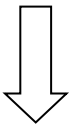
Prepare substrate solution: Adding solution A dropwise to Solution B (9.5ml) with intense stirring

Prepare assay mixture: 0.9ml substrate solution was taken + Tris-HCL buffer (0.1ml) (50Mm concentration and ph 8.0) + 0.025ml

Of diluted enzyme(crude lipase) into different test tubes



incubation was done at room temperature for 10 minutes



optical density was calculated at 410nm using spectrometer

CHAPTER 4

RESULT AND DISCUSSION

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Fig4.1: **Morphological examination of *A. sydowii***

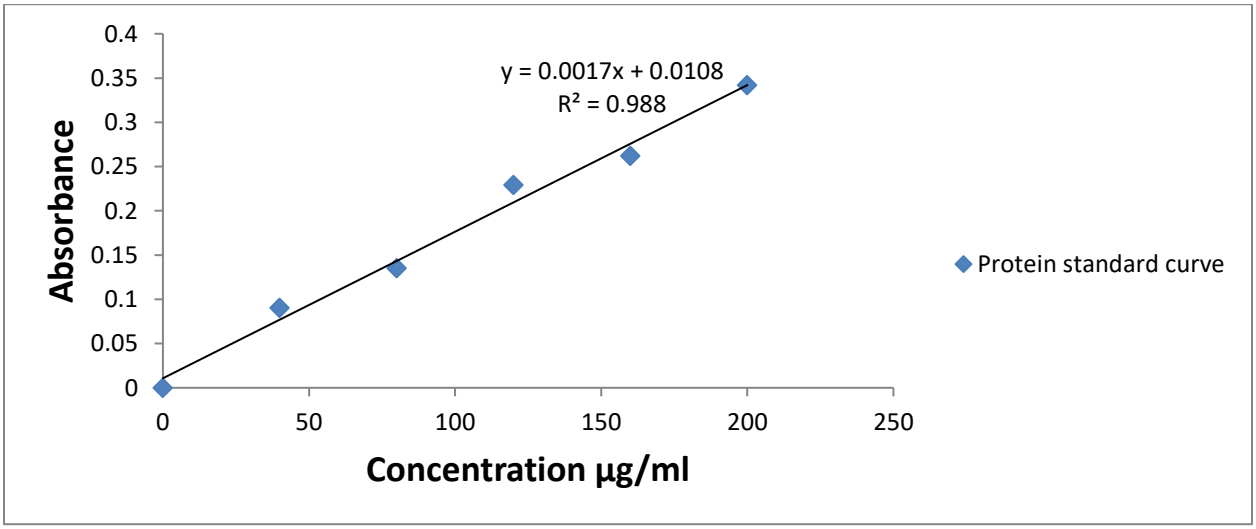

Fig.4.2 PNPP standard curve

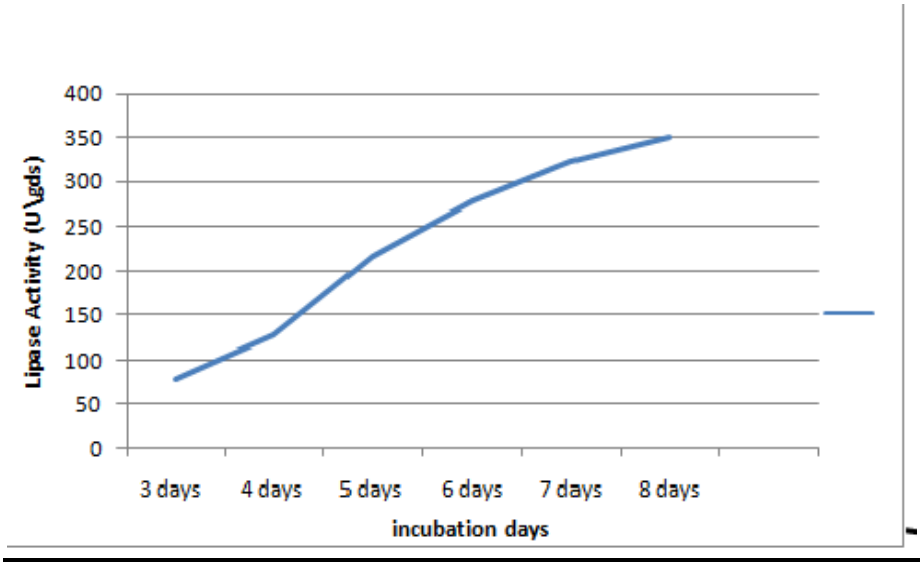

Fig. 4.3 Enzyme activity graph at varying incubation days (mixed fruit pulp as substrate)

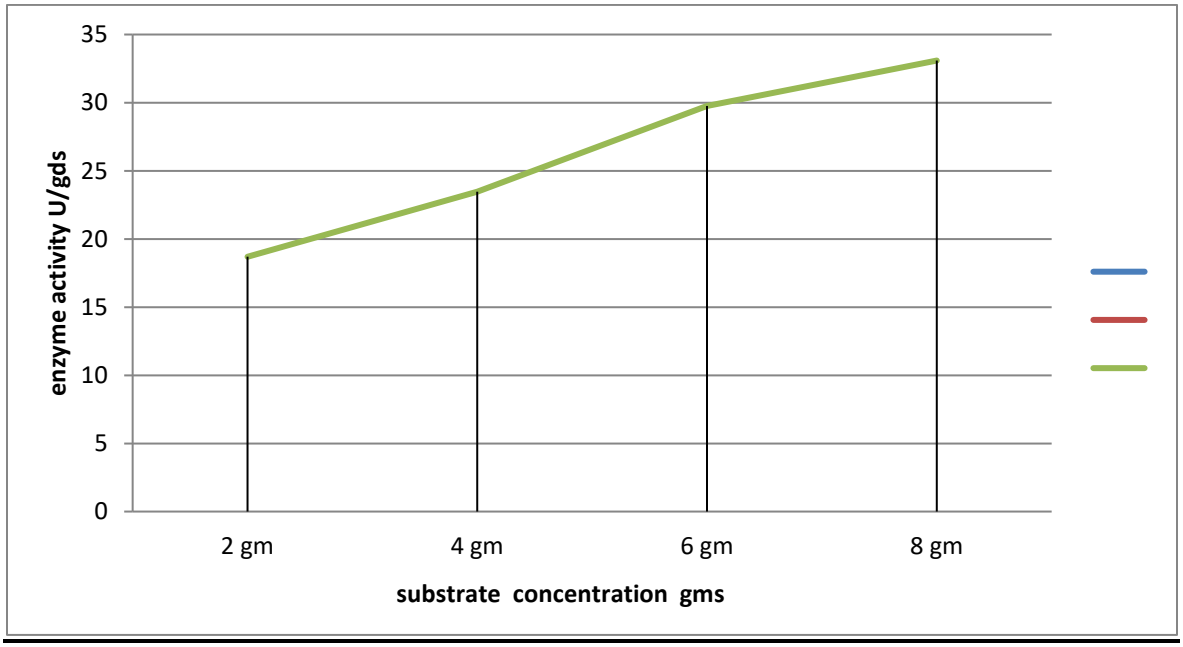

Fig 4.4 Enzyme activity at varied substrate concentration (pineapple pomace as substrate)

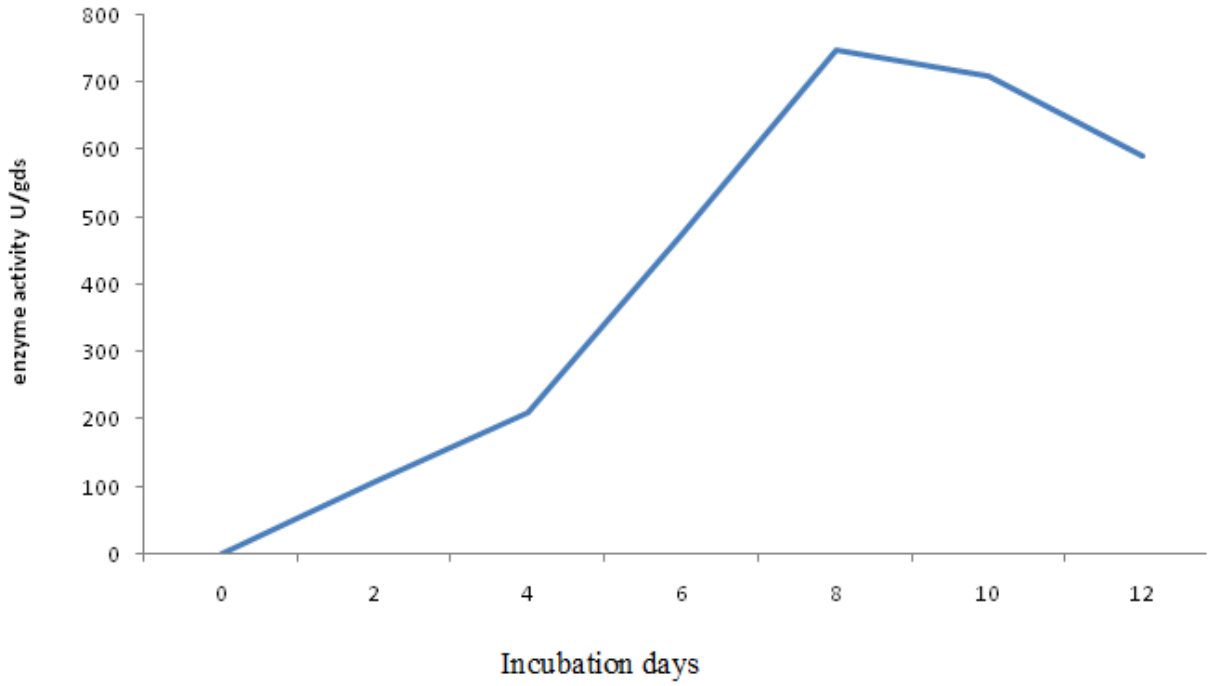

Fig. 4.5 Enzyme activity at varied incubation days

CHAPTER 5

CONCLUSIONAND FUTURE PROSPECTS

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5.1 Conclusions:

- This report highlights the production of lipases by the techniques of SSF and fungal species .
- Using fungal species as a substrate is particularly proffered because fungus has desired characteristics in obtaining high enzyme activity levels that are observed at different parameters.
- The main role of the SSF technique is to enhance the yield of the enzyme,optimal use of the substrate in a brief period of time .It was observed that lipase produced by the technique of SSF was observed to be more stable
- The varying parameters like substrate concentration , different temperature and ph conditions etc had a huge impact on the activity of the enzyme.
- Thus, we can judge which particular parameter is optimal for the production of the lipase enzyme.
- Exploiting waste materials to produce valuable and commercial enzymes like lipase can prove beneficial in the developing countries.

5.2 Future prospects:

- Since the fungal species has been found more reliable for the production of lipases and it can be found or grown easily it opens the door for researchers to start of their research from any place around the globe.
- This research proves to be beneficial to the developing countries having diary ,pharma etc sectors .

REFERENCES

1) Sugiura M. Bacterial lipases. In: Borgstro¨m B, Brockman HL, editors. Lipases. Amsterdam: Elsevier, 1984:505–23.
2) Jaeger K-E, Steinbu¨ chel A, Jendrossek D. Substrate specificities of bacterial polyhydroxyalkanoate depolymerases and lipases: bacterial lipases hydrolyze poly(v-hydroxyalkanoates). Appl Environ Microbiol 1995;61:3113–8.
3) Mochizuki M, Hirano M, Kanmuri Y, Kudo K, Tokiwa Y.Hydrolysis of polycaprolactone by lipase: effects of draw ratio on enzymatic degradation. J Appl Polymer Sci 1995;55:289–96.
4) Kim GJ, Choi GS, Kim JY, Lee JB, Jo DH, et al. (2002) Screening, production and properties of a stereo specific esterase from Pseudomonas sp. S34 with high selectivity to (S)-ketoprofen ethyl ester. J Mol Catal B-Enzym 17:19-38.
5) Ohnishi K, Yoshida Y, Sekiguchi J (1994) Lipase production of Aspergillus oryzae. J Ferment Bioeng 77: 490- 495.
6) Falch EA, Falch EA (1991) Industrial enzymes developments in production and application. Biotechnol Adv; 9:643-658.
7) Fuji T, Tatara T, Minagawa M (1986) Studies on application of lipolytic enzyme in detergent industries. J Am Oil Chem Soc 63:796-799
8) Sharma R, Christi Y, Banerjee UC (2001) Production, purification, characterization and application of lipases. Biotech Adv 19:627-662.
9) Linko YY, Lamsa M, Wu X, Uosukainen E, Seppala JM, et al. (1998) Biodegradable products by lipase biocatalysis. J Biotechnol 66: 41-50.
10) Hasan F, Shah AA, Hameed A (2006) Industrial applications of microbial lipases. Enzyme Microbial Tech 39: 235-251.
11) Eugene WS (1974) Industrial application of microbial lipases: A review. J Am Oil Chem Soc 51: 12-16.
12) Bajpai P (1999) Application of enzymes in the pulp and paper industry. Biotechnol Progr 15:147-157.
13) Noureddini H, Gao X, Philkana RS (2005) Immobilized Pseudomonas cepacia lipase for biodiesel fuel production from soybean oil. Bioresour Technol 96: 769-777.

14) Nakamura K, Nasu T (1990) Enzyme containing bleaching composition. Japanese Patent 2: 208-400.

15) Higaki S, Morohashi M (2003) Propionibacterium acnes lipase in seborrheic dermatitis and other skin diseases and Unsei-in Drugs. Exp Clin Res 29:157- 159

16) Kamini NR, Mala JGS, Puvanakrishnan R (1998) Lipase production from Aspergillus niger by solid-state fermentation using gingelly oil cake. Process Biochem; 33: 505-511.

17) Kumar S, Kikon K, Upadhyay A, Kanwar SS, Gupta R (2005) Production, purification and characterization of lipase from thermophilic and alkaliphilic Bacillus coagulans BTS-3. Protein Expr Purif 41: 38-44.

18) Nahas E (1988) Control of lipase production by Rhizopus oligosporus under various growth conditions J General Microbiol 134:227-233

19) Odibo FJC, Okereke UO, Oyeka CA (1995) Influence of culture conditions on the production of lipase of Hendersonula toruloidea Bioresource Technol 54:81-83

20) Park EY, Sato M, Kojima S (2006) Fatty acid methyl ester production using lipaseimmobilizing silica particles with different particle sizes and different specific surface areas.Enzyme Microb Technol 39: 889–896 Paques FW, Pio TF, Carvalho PO, Macedo GA (2008) Braz. J Food Technol 11: 20–27.

21) Paques FW, Macedo GA, Quim Nova (2006) 29: 93–99 Raghukumar C, Raghukumar S, Sheelu G, Gupta SM, Nath BN, Rao BR (2004) Buried in time: culturable fungi in a deep-sea sediment core from the Chagos Trench, Indian Ocean. Deep- Sea Research Part-I 51: 1759–1768

22) Raghukumar C, Raghukumar S (1998) Barotolerance of fungiisolated from deep-sea sediments of the Indian Ocean. Aquat.microbiol. Ecol. 15: 153–163

23) Raghukumar C, Raghukumar S, Sharma S, Chandramohan D (1992) Endolithic fungi from deep-sea calcareous substrata: isolation and laboratory studies. In: Desai, B.N. (Ed.) Oceanography of the Indian Ocean. Oxford IBH Publication, New Delhi

24) Rao PV, Jayaranam K, Lakshmanan LM (1993) Production of lipase by Candida rugosa in solid state fermentation2:Medium optimization and effect of aeration. Process Biochem 28:391-395

25) Reshma MV, Saritha SS, Balachandran C, Arumughan C (2008)Lipase catalyzed interesterification of palm stearin and rice bran oil blends for preparation of zero trans shortening with bioactive phytochemicals. Bioresour Technol 99 (11):5011–5019

26) Roth F J, Orpurt P A, Ahearn DG (1964) Occurrence and distribution of fungi in a subtropical marine environment. Can J. Bot 42:375–383

27) Salah RB, Ghamghui H, Miled N, Mejdoub H, Gargouri Y (2007)Production of butyl acetate ester by lipase from novel strain of Rhizopus oryzae. J. Biosci Bioeng 103 (4):368–372

28) Saxena RK, Davidson WS, Sheron A, Giri B (2003) Process Biochem. 39:239–247. Schmidt RD ,Verger R, Angew (1998) Chem. Int. Ed. Engl. 37:1608–1633

29) Takami H, Inoue A, Fuji F, Horikoshi K (1997) Microbial flora in the deepest sea mud of the Mariana Trench. FEMS Microbiology Letters 152:279–285

30) Tan T, Zhang M, Xu J, Zhang J (2004) Optimization of culture conditions and properties of lipase from Pencillium camembertii Thom PG-3. Process Biochem.39:1495-1502.

31) Thomas A, Mathew M, Valsa AK, Mohan S, Manjula R (2003) Optimzation of growth conditions for the production of extracellular lipase by Bacillus mycoides. Ind. J. Microbiol 43:67-69

32) Toske S G, Jensen PR, Kauffman CA, Fenical W (1998) Aspergillamides A and B: modified cytotoxic tripeptides produced by a marine fungus of the genus Aspergillus. Tetrahed 54: 13459–13466

33) Wang PY, Chen YJ, Wu AC, Lin YS, Kao MF, Chen JR, Ciou JF, Tsai SW(2009) Adv. Synth. Catal. 351:2333–2341

34) Zaunstock B, Molitoris HP (1995) Germination of fungal spores under deep-sea conditions. Abstr. VI International

35) Pandey A, Benjamin S, Soccol CR, Nigam P, Krieger N, Soccol VT. The realm of microbial lipases in biotechnology. Biotechnol Appl Biochem 1999;29:119–31.

36) Benjamin S, Pandey A. Coconut cake: a potent substrate for the production of lipase by *Candida rugosa* in solid-state fermentation. Acta Biotechnol 1997;17:241–51.

37) Bhushan B, Dosanjh NS, Hoondal GS. Lipase production from an alkalophilic yeast sp. by solid-state fermentation. Biotechnol Lett 1994;16:841–2.

38) Lin SF, Lee JC, Chiou CM. Purifiation and characterization of a lipase from *Neurospora* sp. Tt-241. J Am Chem Soc 1996;73:739–45.

39) Cordova J, Nemmaoui M, Ismailialaoui M, Morin A, Roussos S, Raimbault M, Benjilali B. Lipase production by solid-state fermentation of olive cake and sugar cane *baggase*. J Mol Catal B Enzyme 1998;5:75–8.

40) Christen P, Auria R, Vega C, Villegas E, Revah S. Growth of *Candida utilis* in solid-state fermentation. Biotechnol Adv 1993;11:549–57.

41) Kamini NR, Mala JGS, Puvanakrishnan R. Lipase production from *Aspergillus niger* by solid state fermentation using Gingelly oil cake. Process Biochem 1998;33:505–11.

42) Hunag J, Sui Q, Zheng Y, Wu S. Alkaline mesophilic lipases. II. Production of lipase from *Penicillium expansum* PF 868. J Gongye Weish Engwn 1995;25:10–4.

43) Chandrasekaran M. Industrial enzymes from marine microorganism. The Indian scenario. J Mar Biotechnol 1997;5:86–9.

44) Rajoka MI, Shahid R. Kinetics of xylanase and B-Xylosidaes by *Aspergillus niger*. Biologia 1998;40(1 and 2):275–89.

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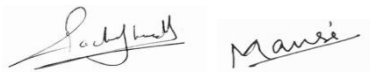

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