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COMPARTIVE STUDY OF LIPASE AND PROTEASE ENZYMES IN TWO DIFFERENT SOURCES

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ABSTRACT:

The production of extracellular lipase (triacylglycerol hydrolase) is a widely distributed phenomenon among eukaryotic and prokaryotic microorganisms.Commercial microbial lipase have been used in dairy and other food processes, and lipase produced in situ by Microorganisms are important in making food palatable and acceptable. Microbial lipases have been in detergents, pharmaceuticals, cosmetic, leather processing, production of aliphatic acid, and in the treatment of domestic and industrial wastes. The treatement of lipolytic enzyme in powder from free of other enzyme, and sometime they are microencapsulated for specialized applications.

In the present study, when we compared to Microbial Lipase and protease with Vegetable seeds. Protease activity more in Mustard seeds 6188 HUT /gm and least in Thil seeds 2917 HUT /gm it is due to microbes serve as a preferred source of these industrial enzymes, 75% are hydrolytic. Proteases from plant, animal and microbial sources account for about 60% of total enzyme sales. Most of the commercial proteases are of bacterial origin and have been extensively studied.

Whereas Lipase activity more in Thil seeds 50 LU/gm to least in Sunflower seeds 10 LU/gm was observed since, oil as carbon source, peptone as nitrogen source, at pH 7.0 and temperature at 37°C. Lipase is synthesized in the cytosol and gets associated with oil body membrane during seed germination. Lipase activity has also been shown to be initially localized at its storage sites in the protein storage vacuoles (PSVs) prior to seed germination, and its transfer to oil body surface during early phase of seed germination in sunflower.

The microbial Lipase from Bacillus sps, and Pseudomonas putida and protease in Bacillus subtilis, the protease activity increased as the increase in enzyme concentration; optimum substrate concentration (gelatin) was 0.5% (w/v); an optimum incubation temperature was 35 ºC. Purified protease enzyme had a maximum activity at pH 7.0 of phosphate buffer, and the optimum incubation time was 24 h. Lipase enzyme producing organism was isolated and Bacillus sp and Pseudomonas sp. was identified as highly active strain in the present study.The present study emphasized the possibility of the production and purification microbial protease and lipase enzymes are ideal for application in industrial scale.

Keywords: Lipase, Proteases, Bacillus sps, Mustard seeds, Thill seeds, Sunflower seeds.

[1]. INTRODUCTION

Lipases (Triacylglycerol lipases, EC 3.1.1.3) are water-soluble enzymes, which have the ability to hydrolyse triacylglycerols to release free fatty acids and glycerol. Lipases constitute a major group of biocatalysts that have immense biotechnology applications. Lipases have been isolated and purified from fungi, yeast, bacteria,

plant and animal sources[1]. All these bacterial lipases are more economical and stable[2]. Bacterial lipases are used extensively in food and dairy industry for the hydrolysis of milk fat, cheese ripening, flavour enhancement and lipolysis of butterfat and cream[3]. Lipases are also used in detergent industry as additive in washing powder[4], textile industry to increase fabric absorbency[5], for synthesis of biodegradable polymers or compounds[6]and different transesterification reactions[7]. In addition, the enzyme is used as a catalyst for production of different products used in cosmetic industry[8], in pulp and paper industry[9], in synthesis of biodiesel[10], degreasing of leather[11] and in pharmaceutical industry[12].

Lipase are versatile enzyme that catalyze the hydrolysis of linkage ,primarily in neutral lipids such as triglycerides .They hydrolyze that acyl chains either at primary or secondary positions. However, a few lipases do not show any positional specificity. Lipolytic bacteria were isolated from oil contaminated soils and grown on tributyrin media containing 1% (w/v) olive oil. The isolate showing maximum activity was identified by following Berger's manual. Different media parameters were optimized for maximal enzyme production. Peak lipase activity was observed for palm oil as carbon source, peptone as nitrogen source, at pH 7.0 and temperature at 37°C.

Out of lipase isolated from different sources bacterial lipase are more economical and stable. Lipases are also used in detergent industry as additive in washing powder, textile industry increase fabric absorbency, for synthesis of biodegradable polymers or compounds and different transesterification reactions. In addition, the enzyme is used as a catalyst for production of different products used in cosmetic industry, in pulp and paper industry in synthesis of biodiesel, degreasing of leather and in pharmaceutical industry.

Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms, and are essential for cell growth and differentiation. The extracellular proteases are commercial value and find multiple applications in various industrial sectors. Although there are many microbial sources available for producing proteases, only a few are recognized as commercial producers[13]. Of these, strains of Bacillus sp. dominate the industrial sector[14]. Early in 1977, It was, reported that, the gram-positive, spore forming bacterium Bacillus subtilis produces and secretes proteases, esterases, and other kinds of exoenzymes at the end of the exponential phase of growth[15].

In addition to that, several workers investigated the production of protease and alkaline protease from Bacillus subtilis[16-20]. Microorganisms produce a large variety of enzymes, most of which are made in only small amounts and are involved in cellular proteases[21]. Proteolytic enzymes from microorganisms may be located within the cell (intracellular), cell wall associated (periplasmic), or excreted into the media[22] (extracelluar). The chief producers of commercial lipases are *Aspergillus niger*, *Candida cylindracea*, *Humicola lanuginosa*, *Mucor miehei*, *Rhizopus arrhizus*, Protease inhibitiors can be isolated from Tomato fruits.

[2]. MATERIALS AND METHODS

2.1. Production medium used for lipase production

The production media used for lipase production contained w/v of Peptone-3%, Yeast extract-1%, 0.5% Sodium chloride- 1% Olive oil-at pH.7

2.2. Lipase assay

Lipase activity was measured by titrimetric method using olive oil as a substrate. Olive oil (10% v/v) was emulsified with gum Arabic (5% w/v) in 100mM potassium phosphate buffer pH 7.0. 100 µl of enzyme was added to the emulsion and incubated for 15 minutes at 37°C.

The reaction was stopped and fatty acids were extracted by addition of 1.0ml of acetone: ethanol solution (1:1). The amounts of fatty acids liberated were estimated by titrating with 0.05M NaOH until pH 10.5 using a phenophathelin indicator[23].

2.3. Optimization of fermentation media

For media optimization the organisms were incubated in the production media at different incubation periods (24, 48 and 72 hours), pH (5, 7, 9), temperature 27^{o}C, 37^{o}C, 47^{o}C, substrates such as Tween 20, Tween 40, Tween 60 and chicken intestine.

2.4. Preparation of crude enzyme

After 24 hours of incubation, cells were harvested by centrifugation at 10,000 rpm and the supernatant was collected. To this phosphate buffer having pH7 was added.Culture was assayed for extracellular lipase using titrimetric method.Phosphate Buffer (pH7)

2.5 Spectrophotometric determination of Lipase and protease

Selective oil seeds of Mustard, Sunflower, Groundnut, Soya bean, and Thill Microbes selected were *Aspergillus niger, Pseudomonas, and Bacillus* sp.

2.6 Microorganism and inoculum preparation

A culture of *Bacillus subtilis* previously isolated from water and identified by standard method for bacterial identification. Stock cultures were maintained in nutrient broth medium (Difco) with 70% glycerol, cultures were preserved at -20 ºC.

A Loopful of bacterial strain (*Bacillus subtilis*) were transferred to a tube of sterile nutrient broth and allowed to grow overnight at 37 ºC before being used to inoculation. A stock suspension was prepared and adjusted to 7 x 103 cell/ml^{-1}.

2.7 Fermentation procedure:

Protease crude enzyme was produced by fermentation of the (50 ml-1/flask). The nutrient broth {production medium (PM)} was supplemented with gelatin (10 g) and then autoclaved at 120 ºC for 20 min before inoculation. The contents of the flasks were mixed thoroughly and then incubated for 24 h at 37 ºC) before enzyme assay.

2.8 Extraction of Protease:

The whole contents of fermented containing protease were filtered through Whitman No. 1 filter paper to obtain the extracted volume then preserved in the refrigerator at 4 ºC as a crude protease filtrate [24]

2.9 Enzyme purification

The protease purification steps were followed[25]. This includes the following steps:

Step: 1. Enzyme production and preparation of cell free filtrate Bacillus subtilis was grown under optimized conditions. The filtrate broth (crude protease) was collected and centrifuged at 4000 rpm for 15 min at 4ºC in order to obtain a cell free filtrate (CFF). After performing a test for sterility, 200 ml of the cell free filtrate (CFF) containing protease were collected and their proteolytic activities and protein content were determined.

Step: 2. Ammonium sulfate fractionation: 200 ml-1 of the crude protease enzyme were first brought to 20% (w/v) saturation with solid ammonium sulfate (enzyme grade) according to the chart[26, 27]. The precipitated proteins were regimented by centrifugation for 15 min at 500 min-1. The resulted pellet was dissolved in 5 ml of phosphate buffer at (pH 7.0). The left supernatant was applied again with ammonium sulfate to achieve 20, 40, 60, 80, and 100% (w/v) saturation. Both enzyme activity and protein content were determined for each separate fraction.

Step:3. Dialysis against distilled water and buffer: The obtained ammonium sulfate precipitate (in solution) was introduced into special plastic bag for dialysis against distilled water for 3 h, followed by dialysis against phosphate buffer at pH 7.0. The obtained protease enzyme preparation was concentrated against crystals of sucrose and kept in the refrigerator at 5ºC for further purification.

Step: 4. Application on column chromatographic technique: Preparation of the gel column and the fractionation procedures was determined as previously mentioned[28].

Proteolytic activity and protein content were carried out for each individual fraction. Sharp peaks of fractions obtained after applying Sephadex G 200 column were collected and

investigated for the properties of the partially purified protease enzyme.

Step 5: Protein determination: The protein content of protease enzyme was determined by the method of Biuret[29].

Step 6: Determination of the specific activity of protease *enzyme:* The specific activity of the protease enzyme protein was expressed in terms of units/mg protein/ml-1 according the following equation: Specific activity = enzyme activity / protein content (mg/ml)

[3]. RESULTS

3.1. These studies revealed that, protease enhanced its maximum productivity 2344.0 units/ml^{-1} when both beef extract and NaCl were added into the production medium.

The *Bacillus subtilis* culture supernatant containing an initial protease activity (234.5units/ml^{-1}).

The optimum ammonium sulfate fractionation was (40% (w/v) saturation) showed the specific activity 36.5 (units/mg, protein/ml) compared to the unconcentrated supernatant. Protease enzyme was purified by ammonium sulfate precipitation and Sephadex G200 filtration.

Table-1

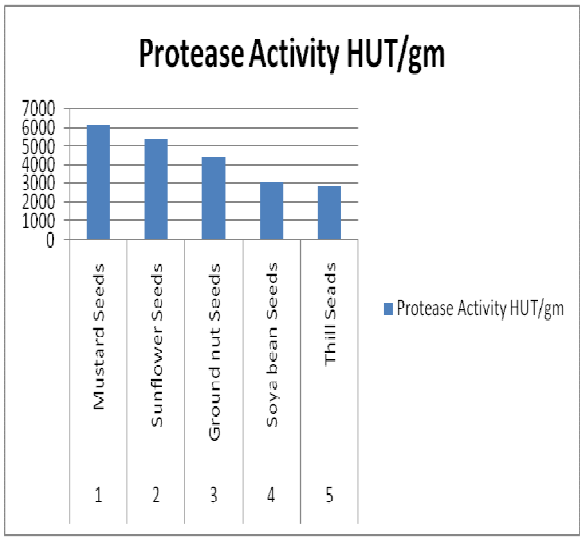
Sl. no	SAMPLE	PROTEASE ACTIVITY HUT /gm	LIPASE ACTIVITY LU/gm
1	**MUSTARD SEEDS**	**6188**	**30**
2	**SUNFLOWER SEEDS**	**5436**	**10**
3	**Groundnut seeds**	**4508**	**20**
4	**Soya bean seeds**	**3094**	**33.33**
5	**Thill seeds**	**2917**	**50**

Table: 1. Biochemical: enzyme assays

Substrate, Test	Result
Gram Staining	Positive
Morphology	Cocci
Citrate	Negative
Catalase	Positive
Gelatin liquefaction	Negative
Nitrate reduction	Weakly positive
Oxidase	Negative
Fermentation of gas and acid Glucose	Positive
Sucrose	Positive
Lactose	Positive

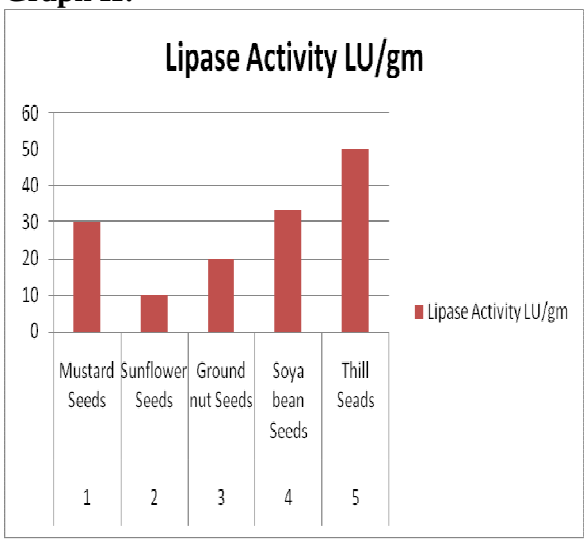
Table 2: Morphological and biochemical characterisation of Lipase in microorganism *Bacillus sps*

Graph I:



Graph I: Protease Activity HUT/gm

Graph II:



Graph II: Lipase Activity LU/gm

[4] DISCUSSION

In the present study, when we compared to Microbial Lipase and protease with Vegetable seeds. Protease activity more in Mustard seeds 6188 HUT /gm and least in Thill seeds 2917 HUT /gm it is due to microbes serve as a preferred source of these enzymes. Proteases from plant, animal and microbial sources account for about 60% of total enzyme sales. Most of the commercial proteases are of bacterial origin and have been extensively studied.

Where as Lipase activity more in Thil seeds 50 LU/gm to least in Sunflower seeds 10 LU/gm was observed since, oil as carbon source, peptone as nitrogen source, at pH 7.0 and temperature at 37°C. The microbial Lipase from *Bacillus sps*, and *Pseudomonas putida* and protease in *Bacillus subtilis*, the protease activity increased as the increase in enzyme concentration; optimum substrate concentration (gelatin) was 0.5% (w/v); an optimum incubation temperature was 35 ºC. Purified protease enzyme had a maximum activity at pH 7.0 of phosphate buffer, and the optimum incubation time was 24 h.

In one of the study peptone, sodium chloride, yeast extract and olive oil were found to have more significance on lipase production on *Bacillus* sp.

Lipases are not involved in any anabolic processes. Since this enzyme acts at the oil–water interface, it can be used as a catalyst for the preparation of industrially important compounds. Lipases catalyse the hydrolysis of triglycerides into diglycerides, monoglycerides, glycerol and fatty acids, and under certain conditions the reverse reaction leads to esterification and formation of glycerides from glycerol and fatty acids. Research has been carried out on plant lipases, animal lipases, and microbial lipases, particularly bacterial and fungal. Although pancreatic lipases have been traditionally used for various purposes, it is now well established that microbial lipases are preferred for commercial applications due to their multifold properties, easy extraction procedures, and unlimited supply.

Production of an extracellular microbial lipase possessing pronounced stereospecificity in the hydrolysis of triacylglycerols would be of considerable commercial interest. Most lipases attack triglycerides as readily as partially esterified glycerides, but an enzyme from a specific *P. cyclopium* strain has been shown to attack monoglycerides most rapidly followed by di- and triglycerides, respectively, and it has been described as a partial glycerol ester hydrolase.

Several kinds of microbial lipases commercially exploited for their potential to catalyse a large number of hydrolytic and synthetic reactions in both aqueous and organic media. A relatively smaller number of bacterial lipases have been well studied compared to plant and fungal lipases. Bacterial lipases are glycoproteins, but some extracellular bacterial lipases are lipoproteins. It had been reported that enzyme production in most of the bacteria is affected by certain polysaccharides[30]. Most of the bacterial lipases reported so far are constitutive and are nonspecific in their substrate specificity, and a few bacterial lipases are thermostable.

Staphylococcal lipases are lipoprotein in nature. Lipases purified from *S. aureus* and *S. hyicus* show molecular weights ranging between 34–46 kDa, They are stimulated by Ca^{++} and inhibited by EDTA. The optimum pH varies between 7.5 and 9.0. The lipase genes from *S. hyicus* and *S. aureus* have been cloned, sequenced, and compared with other lipases. This revealed two conserved domains separated by 100 amino acids which are likely to form active site. Putative active site residues around His 269 and Ser 369 of the *S. hyicus* lipase are highly conserved in the two *S. aureus* lipases and in several eukaryotic lipases.

Lipases from different species of *Psuedonomas* were purified by acidification of the culture supernatant, ammonium sulphate precipitation, sepharose CL-6B chromatography, and isoelectric focussing using CHAPS. The purified lipase of *P. fragi, P. fluorescens*, and *P. aeruginosa* were monomeric with molecular weight of 33 kDa, 45 kDa, and 29 kDa, respectively. The lipase was inhibited by Zn^{++}, Fe^{++}, and Al^{+++} and activated by Ca^{++}. The lipase gene of *P. fragi* has been cloned and sequenced.

Fungal lipases have been studied since 1950s, and presented comprehensive reviews [31, 32]. These lipases are being exploited due to their low cost of extraction, thermal and pH stability, substrate specificity, and activity in organic solvents.

Fungal lipases which degrade lipids from palm oil were investigated by Turner. The thermophilic *M. pusillus* is well known as a producer of thermostable extracellular lipase. From a lipase-producing strain of *M. miehei*, two isoenzymes with slightly different isoelectric points but a high degree of antigenic identity could be isolated. A lipase of *M. miehei*, immobilized on a resin (Lipozyme ^{TM}) has been commercialized by Novo Industries.

Lipids are insoluble in water and need to be broken down extracellularly into their more polar components to facilitate absorption if they are to function as nutrients for the cell. Therefore majority of the lipases are secreted extracellularly. A 65-kDa protease, active at pH 7.5-9.5, was zymographically detected in the total soluble protein. Its activity increased along with in vivo accumulation of the protease-protected fragment during seed germination and accompanying lipid mobilization.

Protease-treated oil bodies were more susceptible to maize lipase action. Differential proteolytic sensitivity of different oleosins in the oil body membranes could be a determinant of oil body longevity during seed germination.

In the cotyledon extracts of seedlings of many oil seeds, including soybean, sunflower, cucumber, and peanut, the in vitro lipase activity was too low to account for the observed in vivo lipolysis. The low in vitro lipase activity was due to the presence of lipase inhibitors in the extracts. The inhibitors from soybean were characterized based on their effects on the hydrolysis of trilinolein by corn, pancreatic, and Rhizopus lipases.

The inhibitors were not dialyzable and unaltered by RNase and beta-galactosidase treatment. However, they were sensitive to heating and protease digestion. Most of the inhibitors were recovered in the soluble fraction in subcellular fractionation. They were present in the 2-4S and not in the 7S, and 11S (storage proteins) protein fraction. There was a gradual decrease of the inhibitors in the cotyledons in the postgerminative growth.

The inhibitors can be proteins which bind to the surface of the substrate micelles. The binding prevents the normal functioning of lipase which acts on the interfacial area between the aqueous medium and the micelle surface.

The optimum temperature for protease productivity by *Bacillus subtilis* was 40 ºC. A *Pseudomonas* sp. produced an extracellular thermostable protease Growth of the organism and the production of protease was optimum at 30 ºC[33]. Moreover, it had already been reported that alkaline protease secreted by *Bacillus clausii* of industrial significance at optimum temperature of 60 º C[34]. Similarly, optimum temperature for protease activity was 70 ºC[35] produced by thermo alkaliphilic *Bacillus* sp. JB-99.

They found that protease production was greatly enhanced by the addition of lactose or arbinose into the medium and that 1% (w/v) arabinose was the most effective substrate and concentration for protease production. Their results were revealed that sucrose gave maximum protease activity. Moreover, protease production reaches to the maximum when added D-glucose to the medium especially when used at low concentrations (40g/l) [21].

Three proteinase inhibitor genes have been identified in the rapeseed (Brassica napus) genome. They are highly homologous to other genes of the mustard inhibitor (MSI) family of proteinase inhibitors characteristic of Cruciferae. In germinating seeds, only the transcript of one gene, coding for a trypsin inhibitor, is detectable by Northern analysis. The other two genes are transcribed at basal levels detectable only by reverse transcription PCR. One of the other two genes (rti-2) encodes a polypeptide with a glutamic residue in the P1 position, characteristic of glutamyl proteinase inhibitors. The recombinant RTI-2 protein strongly inhibits (Ki=44 nM) a glutamyl proteinase from Streptomyces griseus.

Lunasin is a novel and promising cancer preventive peptide from soybean. The Bowman-Birk protease inhibitor (BBI) and isoflavones are well-studied substances from soy. The levels and bioactivities of these three compounds as affected by stages of seed development and sprouting under light and dark conditions. Lunasin and BBI suppress foci formation more than the isoflavones. Sprouting decreases lunasin and BBI contents but increases isoflavones. Protein extracts from early soaking times inhibit foci formation more and suppress cell viability less than those from later soaking times. Light and dark conditions have no influence on the bioactivities of protein extracts. These data are useful in the preparation of soy fractions enriched in lunasin, BBI, and isoflavones and in making dietary recommendations.

Lipases are known to hydrolyse triglycerides and give rise to free fatty acids and glycerol. Therefore, the assay methods involve spectrophotometry or titrimetry, radiolabelling assay, fluorimetry, surface tension method, and estimation of free fatty acids by high performance liquid chromatography (HPLC). Tributyrin plate assay and titrimetry are the most commonly used methods for screening of lipase producers and estimation of lipase activity, respectively

Lipases have been purified from animal, plant, fungal and bacterial sources by different methods involving ammonium sulphate precipitation, gel filtration, and ion exchange chromatography. In recent years, affinity chromatographic techniques have come into use as this technique decreases the number of steps necessary for lipase purification as well as increases specificity. Cofactors are not required for the expression of lipase activity. Divalent cations, such as calcium, generally stimulate the activity.

Lipases have applications as industrial catalysts for the resolution of racemic alcohols in the preparation of some prostaglandins, steroids, and carbocyclic nucleoside analogues. Regioselective modification of polyfunctional organic compounds is yet another rapidly expanding area of lipase application, particularly in the field of AIDS treatment. Lipases from *A. carneus* and *A. terreus* show chemo- and regiospecificity in the hydrolysis of peracetates of pharmaceutically important polyphenolic compounds

Lipases are also useful in the synthesis of the artificial sweetner sucralose by regioselective hydrolysis of octa-acetylsucrose. Owing to the industrial applications of lipases, the Department of Biotechnology, New Delhi, promoted research activities in this important area and consequently the momentum of research on lipases picked up in India. Extracellular microbial lipases had been utilized for transesterification reactions for producing valuable transformed edible oils which cannot be obtained by chemical interesterification methods[33]. Chand[36] *et al* carried out fat splitting using castor-bean lipase. Lipases from *H. lanuginosa* and *Y. lipolytica* have also been reported for the synthesis of geranyl esters, an extracellular lipase were isolated and characterized from the conidia of *N. crassa*, with an apparent molecular weight of 54 kDa and 27 kDa, determined by gel filtration and SDS-PAGE, suggesting thereby the presence of two identical subunits.

In addition to triglycerides, lipases are also known to degrade tween and water soluble and insoluble esters. There are reports of a few animals and microbial lipases that hydrolyze phospholipids. Enzymatic activity of many lipases has been shown to be modulated by calcium, ricinoleic acid and bile salt.

In plant, the regulation in some cases the location and the exact physiological roles of lipases are not very clear. Oilseed lipases that are the best described has been to be localized in oil bodies or glyoxysomes .They are known to play an essential role in the mobilization of seed storage lipids to support germination and post germinative embryonal growth.

Lipases are generally considered to be absent in most dry seed and are probably synthesized denovo after the germination; a triacylglycerol

lipase has been purified from ungerminted dry seed of vernonia galamensis.

The determination of Lipase and its three-dimensional structure has thrown light into their unique structure–function relationship. Among lipases of plant, both animal and microbial origin, it is the microbial lipases that find immense application. This is because microbes can be easily cultivated and their lipases can catalyst a wide variety of hydrolytic and synthetic reactions.

Lipases find use in a variety of biotechnological fields such as food and dairy (cheese ripening, flavour development, EMC technology), detergent, pharmaceutical (naproxen, ibuprofen), agrochemical (insecticide, pesticide) and oleo chemical (fat and oil hydrolysis, biosurfactant synthesis) industries.

It is in the last decade that lipases have gained importance to a certain extent over proteases and amylases, especially in the area of organic synthesis. The enantioselective and regioselective nature of lipases have been utilized for the resolution of chiral drugs, fat modification, synthesis of cocoa butter substituents, biofuels, and for synthesis of personal care products and flavour enhancers Thus, lipases are today the enzymes of choice for organic chemists, pharmacists, biophysicists, biochemical and process engineers, biotechnologists, microbiologists and biochemists.

Lipases (triacylglycerol acylhydrolases) belong to the class of serine hydrolyses and therefore do not require any cofactor. Furthermore, comparison of some of the lipases produced by microorganisms indigenously is at par or even better than the well-known commercially available imported lipases. Thus, utilizing these lipases will greatly boost many biotechnology-based industries with the ushering of the 21st century.

Protease inhibitors are indispensable in protein purification procedures to prevent undesired proteolysis during heterologous expression or protein extraction. They are also valuable tools for simple and effective purification of proteases, using affinity chromatography. Because there are such a large number and diversity of proteases in prokaryotes, yeasts, filamentous fungi and mushrooms, we can expect them to be a rich source of protease inhibitors as well[37].

To detect extracellular proteases produced by both fungi as well as bacteria and can be easily performed for screening of large number of microbial cultures. This is the first report on the use of tannic acid for the detection of microbial proteases[38].

The protease activity reached to the maximum with optimum substrate (gelatin) concentration 0.5 % (w/v) with enzyme activity 59.56 units/ml-1. Increase or decrease of substrate concentration gave the decrease in protease activity. Moreover, it was reported that, a novel protease, was purified from the culture supernatant of *Yersinia ruckeri* [39]. (fish pathogen) Exoprotease, it was more active in the range of 25 to 42 ºC and had an optimum activity at 37 ºC.

In addition to that, the optimum pH for extracellular proteinase (PSCP) produced by *Pseudomonas cepacia* was 6[40]. On the other hand, the protease enzyme had an optimum pH of around 8[39]. Moreover, it had already been reported that, the optimum pH of purified protease was pH 8[41].

[5]: CONCLUSION

Lipases are amongst the most important biocatalysts that carry out novel reactions in both aqueous and nonaqueous media. Lipases have the remarkable ability to carry out a wide variety of chemo-, regio- and enantioselective transformations.

Bacterial lipases are one of the enzymes having huge market demand. The isolated *Staphylococus sps* isolate have shown the production of extracellular lipases. Optimization studies on media parameters for maximum lipase activity were done on isolated lipolytic bacteria. The isolate has shown a broad range of pH and temperature. The extracellular lipase enzyme can

be further purified and used in different industrial applications.

Protease enzyme were obtained by *Bacillus subtilis* by Sephafdex G-200 column purification in this study become maximum, when compared to other oil seeds Mustard seeds possess more protease enzyme is due to may be the presence of a highly homologous to other genes of the mustard inhibitor (MSI) family of proteinase inhibitors were present.

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