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Review Article

Production of Microbial Lipase Enzyme – Review

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Abstract

Lipases are widely distributed among yeast, fungi and bacteria. Short chain triglycerides are also very good substrates for lipases. Enzymes such as proteases and amylases have dominated the world market owing to their hydrolytic reactions for proteins and carbohydrates. However with the realization of the biocatalytic potential of microbial lipases in both aqueous and non-aqueous media in the last one and a half decades, industrial fronts have shifted towards utilizing these enzymes for a variety for reactions of immense importance. 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 regioselective nature of lipases have been utilized for the resolution of chiral drugs, fat modification, synthesize of cocoa butter constituents, biofuels and for synthesize of personal care products and flavor enhancers. Thus lipases are today the enzyme choice for organic chemist, pharmacists, biophysicists, biochemical and process engineers, biotechnologist, microbiologist and biochemist.

Keywords: Lipases;- microbial lipases ; biofuels ; flavor enhancers; synthesize of cocoa butter.

Introduction

Lipases – definition and history

Lipases (triacylglycerol acyl hydrolases) belong to the class of serine hydrolases and therefore do not require any cofactor. The natural substrates of lipases were triacylglycerols, having very low solubility in water. Under natural conditions, they catalyze the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase in which the enzyme is dissolved. Under certain natural conditions, such as in the absence of water, they are capable of reversing their reactions. The reverse reactions leads to esterification and formation of glecerides form fatty acids and glycerol. The occurrence of the lipase reaction at an interface between the substrate and aqueous phase causes difficulties in the assay and kinetic analysis of the reaction (Macrae and Hammond 1985).

The usual industrial lipases are special classes of esterase enzyme that act on fats and oils, and hydrolysis them initially in to the substituted glycerides and fatty acids, and finally on total hydrolysis and fatty acids (Ghosh et al., 1996, Bjorkling *et al*., 1991).

Lipases as enzyme group

Multi faceted microbial lipases (glycerol ester hydrolases (EC 3.1.1.3) have an unsurpassed role in swiftly growing modern biotechnology. Lipases are indispensable for the bioconversion of lipids (Triacylglycerols) from one organism to another and within in the organism.

Lipases possess the unique feature of acting at an interface between the aqueous and non – aqueous (i.e. organic) phase; this feature distinguishes them fromesterases. The concept of lipase interfacial activation arises from the fact that their catalytic activity generally depends on the aggregation state f the substrates. It is believed that activation involves the unmasking and structuring of the enzymes active site through conformational changes requiring the presence of oil- in water droplets. (Ashok Pandy *et al*., 1999).

Classification of Lipases

Lipases have been classified in to three types based on their presence. Lipases have been classified into three types, there are,

1. Microbial lipases
2. Plant lipases
3. Animal lipases

Microbial lipases

Lipases are widely distributed among bacteria, yeast and fungi. Short chain triglycerides are also very good substrates for lipases. The lipases of the following microorganisms have been recently isolated in an apparently homogenous form and some of them have been crystallized. They include *Aspergillus niger, Candida cylindracea, Mucor javaniscus , Pseudomonas fragi* and *Rhizopus arrhizus*. Several purified microbial lipases have shown to be glycoprotein.

Plant lipases

Few studies have been made so far on the distribution of lipases in whole plants except in seeds and germinating seeds of fruits have an especially high lipolytic activity. However, the best –characterized enzyme of this group caster bean lipase is also present in dormant seeds. Lipase activity has been reported in wheat oats, corn and palm fruits (Martin and Peers, 1953; Gillis, 1988).

Animal lipases

In animals, mostly mammalian lipases have received attention in recent years. Among the digestive lipases, the enzyme synthesized by the acinar cells of the pancreas should be considered first. Despite its low-level when compared to that of many other pancreatic enzymes (1.2% of many other pancreatic juices, 2.5% in pig and 3.4% in rat), this lipases gained importance due to its essential role during the intraluminar digestion of dietary triglycerides because of its unusual high molecular weight. Lipases have been identified in milk; fish and invertebrates are also presumed to contain lipase (Volhard, 1901; Hull and Keaton, 1917; Moreau *et al*., 1988; Winkler *et al.,* 1990, Carriere *et al*., 1991; Winkler and Gubernator, 1994).

Taxonomic distribution of fungal lipases

Turner (cited by Lazer and schroder, (1992), and listed by Sztajar and Zbinska, (1988) investigated fungal lipases, which degrade lipids from palm oil. Among Mucorales, the lipolytic enzymes of the moulds *Mucor hiemalis, Mucor miehei, Mucor lipolyticus, Mucor pusillus, Rhizopus japonicus , R.arrhizus, R.delear, R.nigricans, R. microsporus* and *R. chinesis* have been studied (Lazar and Schroder, 1992).

Lipase properties

pH optima.
Temperature optima and thermal stability.
Activation and inactivation f the enzyme.
Substrate specificity

pH optima

The Lipase that has been studied show profound stability around pH 6.0-7.5 with considerable

stability at acidic pH up to 4 and at an alkaline pH up to 8. Extracellular lipase of *A.niger, Chromobacterium viscosum* and *Rhizopus* species are active at acidic pH (Laboureur and Labrousse, 1966; Yamaguchi *et al*., 1973). An alkaline lipase active at pH 11.0 has been isolated from *P.nitroreducens* (Watanabe *et al*., 1977).

Temperature optima and thermal stability

The pancreatic lipase loose activity on storage at temperatures above 40˚C but some microbial lipases was more resistant to heat inactivation. While lipases of *A.niger*, (Fukumota *et al*., 1963, *A. japonicus*, (Aisaka and Terada, 1980) and *C.viscosum* (Yamaguchi *et al*., 1973) are stable at 50˚C, lipase of thermo tolerant *H. lanuginose* is stable at 60 ˚C and 70˚C (Liu *et al*., 1973). In our laboratory, we observed that purified lipase from *Aspergillus tereus* (Yadav *et al*., 1998) retained 100% of its activity at 60˚C after 24 hours. Thermophilic bacterial lipases obtained from Icelandic hot spring showed higher lipase activity at 40˚C to 60˚C (Sigurgisladottir *et al*., 1993).

Activation and inactivation of the enzyme

Cofactors are not required for the expression of lipase activity. Divalent cations, such as calcium, generally stimulate the activity. It has been postulated that this is based on the formulation of calcium salts of long chain fatty acids, (Macrae and Hammond 1985, Godfredson, 1990). The lipase activity is inhibited drastically by Ca^{++}, Mg^{++}, $Ni^{++,}$ Hg^{++} and Sn^{++} and is slightly inhibited by Zn^{++}, Mg^{++}, EDTA and SDS (Patkar and Bjorkling, 1994). In *H.lanugionosa* S-38, sulphydryl reducing agents like dithiothreitol, did not alter the enzyme activity but did render it ore susceptible to heat inactivation. Inactivation is accelerated by the addition of Urea. Reducing compounds (Cysteine, 2-mercaptoethanol), chelating agents, (EDTA, 0-Phenanthroline), and thiol group inhibitors (P-chloro mercuric benzoate, monoiodoacteate) did not show a detectable effect on lipase in *M.pullius*, suggesting that lipase is not a metallo-enzyme and it does not require either free-SH group or intact S_S bridge for its activity. Spontaneous and cyclic AMP- induced lipase *formation* is greatly enhanced in *Serratia marcescens* SM-6 on exposure to glycogen, hyaluronate, pectin B and gum Arabic (Nishio et al., 1987).

Substrate specificity

Specificity of lipases is controlled by the molecular properties of the enzyme, structure of the substrate and factors affecting binding of the enzyme to the substrate. Substrate specificity of lipases is often crucial to their application for analytical and industrial purposes. Specificity is shown both with respect to either fatty acyl or alcohol parts of their substrates (Jenson et al., 1983). Many microbes produce two or more extra cellular lipases with different fatty acid specificities. Tributyrin is hydrolyzed slowly by some microbial lipases (Patkar and Bjorkling 1994; Sugur and Isobe, 1975). Lipases show both region – and stereo specificity with respect to the alcohol moiety of their substrates.

Current status of lipase research in India

Researches on microbial lipases in India date back to late seventies when a few reports on screening and production of lipase from a few fungi and bacteria appeared. The initial emphasis on screening exercises was followed by process optimization for maximum lipase production. Scientists at the National Dairy Research Institute, Karnal investigated physical – chemical condition of lipase produced by *M.racemosus, A.wentii and P.chrosogenum.* In 1981, one group highlighted the lipolytic activity of thermophilic fungi of paddy straw compost (Satyanarayana, 1981). Systematic screening strategies were employed by (Bhaduria, 1989). This study reported *Aspergillus niger, Aspergillsus flavus, A.fumigatus* and *Penicillium glaucum* as the potential lipase producers isolated form the kernels of Chironji and Walnut.

Owing to the industrial application 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. Large – scale process

optimization for lipase production using *Aspergillus terreus, A.carneus and Bacillus stearothermophilus* have been carried out (Yadav *et al*., 1998).

Chakrabarthy *et al.,* (1987), utilized extracellular microbial lipases for transesterification reactions for producing valuable transformed edible oils, which cannot be obtained by chemical interesterification methods. (Chakraborthy 1987; Chand *et al*., 1974) carried out fat splitting using castor - bean lipase. Lipases from *H. lanuginose and Y.lipolytica* have also been reported for the synthesize of genaryl esters (Chand *et al*., 1998; Kundu *et al.,* 1987).

Since 1988, extensive work on various aspects of lipase research, starting from production and purification to characterization and industrial application has been carried out on various fungi and bacteria in south campus Delhi University Parmar *et al.,* (1998). Novel thermostable and alkaline lipases from *Aspergillus terreus* and *A.carneus* are being developed for the production of biosurfactants, glycerides and pharmaceutically important compounds. These lipases show regio and chemo selective cleavage of polyphenolic compounds in a novel manner. Lipase from a strain of *B.stearothermophilus* shows remarkable activity even at 100° C.

The ability of lipases to show increased stability and selectivity in organic solvents has been exploited by various researchers. Biotransformations on polyacetoxy aceylmethyketones, benzylphenylketone per acetates, esters of polyacetoxyaromatic acids and peracetylated benzopyranones, using commercial lipases have been carried out by Paramr etal., (1997). Gupta and his group have investigated enzyme activity in organic solvents. They noted enhancement of enzyme activity in aqueous-organic solvent mixtures. Scientists at the central Leather Research Institute, Madras and at the Central Food Technology Research Institute, Mysore is using microbial lipases in the treatment of leather, and the production of flavour esters, respectively.

The work being carried out in Indian Laboratories has made considerable progress. Novel lipases with properties of chemo-regio and enantio selectivity have been isolated, which may be eligible for exploitation at the commercial level for industrial applications in course of time.

Lipase production

Lipids are insoluble in water and need to be broken down extracellularly into their more polar compounds to facilitate absorption if they are to function as nutrients for the cell. Therefore majority of lipases are secreted extracellularly. Kamini *et al*., (1997) investigated the production and characterization of an extra cellular lipase from *Aspergillus niger*. Consecutive optimization of nitrogen and carbon sources and salts enhanced the lipase activity.

Prabhakar *et al.,* (2002) reported the effect of cultural conditions on the production of lipase by Fungi. They found that selected organism *A.niger, A.flavus, A.japonicus* and a fungi isolated from the contaminated ghee belonging to the genus, *Aspergillus* sps were tested for the production of lipase on four different media by submerged fermentation technique. Benjamin and Pandey, (1996) studied on the efficiency of copra waste (coconut oil cake) extract (CWE) to be used as substrate for the production of lipase using *Candida rugosa* and found that the supplemented CWE further improved the lipase yield.

Ellaiah *et al.,* (2002) attempted to strain improvement of *Aspergillus niger* for the production of lipase. They found that indigenous strain of *Aspergillus niger* Au15 which produced lipase constitutively was used to produce mutants using ultraviolet and N-methyl-N-nitroguanidine. A mutant strain with lipase productivity of 2-fold higher was obtained. Prabhdeep sidhu et al., (1998) isolated a thermophilic *Bacillus* sp Rs-12 from soil which grew optimally at 50 °C.

Ogundero (1980) reported the production of extra cellular lipases by thermophilic fungi associated with mouldy Nigerian groundnuts and the conditions influencing the activities of the enzymes were determined.

Anshu Gupta *et al*., (2002) studied an extra cellular alkaline lipase producing strain of *Enterobacter aerogenes* was isolated form the soil near an oil extraction unit. The lipase possessed the properties desirable for application in detergent formulations. Roberts et al., (1987) reported the extra cellular lipase production by fungi from sunflower seed. They found that two hundred and twenty three lyophilized isolates of fungi representing all flower from sunflower seed were revived and grown in lipase medium with and without emulsified sunflower oil. Lipase production and activity in culture filtrates was assayed by tributyrin agar diffusion and by sunflower oil –gas chromatographic technique. One hundred and thirty-two isolated strains have been produced extra cellular lipase in liquid media. Culture filtrates of both field and storage fungi produced extra cellular lipases, which were active against sunflower oil, but generally more activity was observed in storage fungus filtrates than in fungus filtrates.

Christiane Brahimi – Hom *et al*., (1989) studied a lipase from *Candida cylindracea* was shown to hydrolyze several synthetic lipid esters, including p-nitrophenyl esters and wax esters. The dispersion of the wax ester in an organic solvent increased the efficiency of hydrolysis particularly when incubations were carried out under conditions of pH and temperature which were found to be non-optimal for aqueous hydrolysis.

Achamma Thomas *et al.,* (2003) reported the production of extra cellular lipase was isolated from coconut oil cake and identified as *Bacillus mycoides* by morphological and biochemical characteristics. Growth of the organism and lipase production was maximum after 72hr of incubation.

Mazneeru *et al*., (2001) studied the extra cellular lipase produced by *Micrococcus* Sp.*Mc.1* for its applicability in the commonly used detergents for the removal of oily strains from various types of fabrics. The crude lipase was used in combination with various commonly used detergents. The alkaline pH optima of the lipase and its ability to enhance the stain removing capacity of different detergents suggest its use in laundry.

Alkaline *et al*., (1980) reported the influence on lipase induction in *Mucor hiemalis* of different types of triglycerides containing mainly oleic acid (olive oil), erucic acid (mustard oil), or saturated fatty acids of 18to 16 carbons (coconut oil).

Dong-won Choo *et al*., (1988) studied a psychotropic bacterium producing a cold-adapted lipase upon growth at low temperature was isolated from Alaskan soil and identified as *Pseudomonas* strain. The lipase gene was cloned from the strain and sequenced. Pol and Sawant (1995) reported the changes in lipase activity during metamorphosis of *Chrymyia rufifacies* have been studied.

Sangiliyandi and Gunasekaran (1996) described and extra cellular lipase producing bacterium was isolated from an oil refinery effluent, which was identified as *Bacillus licheniformis.* Rohit Sharma *et al.,* (2002) found that production of extra cellular alkaline lipase from *Bacillus* sps RsJ1 and its application in ester hydrolysis.

The pattern of extra cellular alkaline lipase production was found to be growth associated and its secretion started as soon as the organism entered the logarithmic phase with the maximum release in the late exponential phase. Production of lipase was substantially enhanced when the type, concentration and sources of carbon, nitrogen and surfactant were consecutively optimized.

Omar *et al*., (1987) studied the thermo stable lipase production by *Humicola lamuginosa* grown on Sorbitol- Corn steep liquor medium. The enzyme was able to hydrolyze almost all forms of natural fats tested, coconut oil being the most rapidly hydrolyzed. Almeidaran et al., (1998) had undertaken a study to select lipolytic lipase production in order to determine the best lipase producing strain screening was carried out with several fungal strains belonging to their collection.

Yadav *et al*., (1987) purified and characterized of a regiospecific lipase from *Aspergillus tereus.* The purified enzyme showed excelled temperature tolerance and was highly thermo stable. The enzyme showed good pH tolerance. Ionic detergents inhibited enzyme activity where as non-ionic

detergents stimulated enzyme activity. The lipase was highly active on pig fat and groundnut oil. Nitayananda ray *et al.,* (1999) isolated a bacterial strain from spoiled coconut and identified, as *Corynebacterium species* was found capable of producing alkaline thermo stable extra cellular lipase.

Optimization of growth parameters

A number of reports exist on influences of various environmental factors such as temperature, pH, nitrogen, carbon, salt, lipid sources and phosphate concentration on lipase production. Optimization of growth parameter studied by various workers is summarized below,

Carbon sources

Benjamin and Pandey, (1996) reported the lipase production by *Candida rugosa* on copra waste extract. They found that the effect addition sugars, via, glucose, fructose, maltose, lactose and sucrose at 1-6% concentrations on lipase production by the yeast culture. Apparently, disaccharides were more promising than monosaccharides. Sucrose ranked first (1.7 $Ug^{-1}g^{-1}ds$) among others to reckon as best carbon source. Prabhakar *et al.,* (2002) investigated the effect of cultural conditions on the production of lipase by fungi. They found that the various carbon sources studied the maximum production of enzyme was obtained with glucose followed by dextrin, maltose and fructose. After established the best carbon source for maximum production, attempts were made to determine the optimum concentration of glucose.

Kamini *et al.,* (1997) observed the extra cellular lipase from *Aspergillus niger.* They showed effect of lipase different carbon compounds on lipase production. The maximum lipase production was observed with sucrose. Ibrahim Cheomar et al., (1987) showed that the production of a thermo stable lipase by *Humicola lanuginose.* They found that the sorbitol was found to be a better alternative to olive oil compared to the other sugars. Prabhdeep sidhu et al., (1998) reported lipase from *Bacillus species Rs-12* was found to be inducible in nature and its yield was significantly affected by the type of carbon source used. Tween80 appeared to be the best inducers for producing extra cellular lipase.

Ellaiah *et al.,* (2002) studied the strain improvement of *Aspergillus niger* for the production of lipase. They found that the carbon sources examined, dextrose gave the highest enzyme activity.

Petrovic *et al*., (1990) reported maximum lipase production when glucose was incorporated in the production medium using *Penicillium roquefortii.* Rohit Sharma *et al*., (2002) reported the induction of lipase in Bacillus species RsJ1 took place in early log phase in the presence of Tween-80 and olive oil and the maximum yield was obtained at late exponential phase.

Nitrogen sources

Benjamin and Pandy (1996) studied the effect of supplementation of copra waste extract and peptone was added at 1-5% concentration to Copra waste extract. While peptone showed best results giving as high as 19.1$Ug^{-1}ds$ activity of seventy two hours. Kamini *et al.,* (1997) reported that the inorganic nitrogen sources tested, urea was found to be very effective for the production of lipase. Prabhakar *et al.,* (2001) observed the effect of various nitrogen sources on the production of enzyme, different nitrogen sources were studied at 2% level incorporating in the production medium. Corn steep liquor was found to be the best nitrogen source.

Achamma Thomas *et al.,* (2003) studied the production of extra cellular lipase by *Bacillus mycoides.* They found that the 3% beef extract was found to be the best source in lipase enzyme production. Ellaiah *et al*., (2002) studied the strain improvement of *Aspergillus niger* for the production of lipase. They found that the different nitrogen sources, corn steep liquor and peptone showed the high enzyme activity. Petrovic *et al*., (1990) reported maximum lipase production when peptone was incorporated in the production medium using *Penicillium roquefortii.* Rohit Sharma *et al.,* (2002) studied the alkaline lipase from *Bacillus*

species RsJ1. They found that various organic nitrogen sources, a combination of yeast extract and peptone produced highest lipase.

Inducers

Kamini *et al.,* (1997) reported the various types of various types of inducers, Olive oils were found to be the most effective in inducing the lipase of *Aspergillus niger*. Sunflower oil and gingili oil was also observed to be the equal effective for induction, Tween-80 and paraffin oil enzyme production. Akhtar *et al*., (1980) reported the lipase enzyme production *from Mucor heimalis.* They found that the olive oil was used as the best induces in lipase enzyme production. Prabhakar *et al.*, (2002) reported that the coconut oil was best in inducers in lipase production from fungi.

Raman *et al*., (2002) studied the substrate specificity of *Aspergillus aculeatus* lipase towards various natural lipids was tested. It is evident that this lipase is highly active on the substrate neem oil and least observed in mustard oil. Ibrahim Cheomar *et al*., (1987) reported the effect of the concentration of fatty substances such as caster oil, tung oil triacetin, cholesterol and lecithin, (from soybean), in the lipase formation was investigated (0.1, 0.3 and 0.5%). As a result, 0.3% addition was found to be the most effective, being slightly better than 0.1% addition, while 0.5% addition was the least effective for lipase formation. Prabhdeep Sidhu *et al.,* (1998) observed that the groundnut oil was best inducers in lipase enzyme production from *Bacillus species* rs-12.

Trace elements (salts)

Kamini *et al*., (1997) showed that the K^+ and Mg^+ at a concentration of 0.05% (w/v) were essential for lipase production using *Aspergillus niger*. Achamma Thomas *et al.,* (*2003*) studied various concentration of KCl, NaCl and $MgCl_2$ were added to the medium and incubated optimum concentration was found to be 0.05% in all the three cases stimulation of lipase was more by Na^+ followed by K^+ with Mg^{++}, only 0.6 units of lipase was produced. Rohit Sharma et al., (2002) reported, that maximum lipase yield from *Bacillus species* RsJ1 was observed when calcium chloride (1mM) was added to the production medium. Most other metal ion salts were inhibitory to lipase production. Petrovic *et al.,* (1990) studied that maximum lipase production with Mg^{++} and stimulation with Ca^{++} in *Penicillium roqueforti*.

Omar *et al*., (1987) found that the presence Ca^{2+} in the cultivation medium was shown to stimulate lipase activity. Various minerals (0.01% w/v) were tested in comparison to Ca^{2+}. As a result, $CaCl_2.2H2O$ was expectedly found to be most effective, enhancing the enzyme production.

Pradeep Sidhu *et al*., (1998) studied the lipolytic activity was promoted in the presence of Ca^{2+}, Na^+ and Ba^{2+} and was unaffected by K^+, Mn^{2+} and Mg^{2+} while most other metal ions such as Cr^{3+}, Co^{2+}, $Fe3^+$, Cu^{2+}, Ag^+, $Pb2^+$, Zn^{2+} and Hg^{2+} exhibited strong inhibitory effect. Kamini *et al*., (1997) studied a combination of $0.1\%KH_2PO_4$ with 0.3% Na_2HPO_4 was found to be essential for lipase production and the activity was found to be 32.2 Uml^{-1} at 48 hours.

Temperature and pH

Prabhakar *et al*., (2002) carried out fermentation at different temperatures 26ºC is favorable for getting good lipase activity. There is a decline in activity above 26ºC. Kamini *et al.,* (1997) reported the lipase production from *Aspergillus niger*. They found that the enzyme was stable between 40 and 45ºC and retained 77.9% of its activity at 50ºC. Raman *et al*., (2002) studied production of lipase from *Aspergillus aculeatus.* They found that the microbial lipases are more active within 30-40ºC. The enzyme functioned optimally at pH7.0 and was stable in pH range of 4 to 9. Lipases from fungi have been reported to show variation in Ph optima depending on species and strain optimum pH around 7 has been reported for lipases.

Anshu Gupta *et al*., (2002) reported that the lipase was found to have pH optima at the pH range of 8-11. Raman (2002) studied that *Aspergillus aculeatus* lipase tolerate a broad range of pH 5-10

with maximum activity at pH 8.0. It undergoes inactivation at pH 10.0. Achamma Thomas et al., (2003) reported that the pH 8.0 were found to be most conductive for the production of lipase from *Bacillus mycoides.* Pradhdeep sidhu *et al*., (1998) studied the growth and the lipase production by *Bacillus species* RS-12 was also affected by initial pH of the production medium. The organism was not able to grow at pH 4.0. The growth and the lipase production started at pH 5.0, increased gradually with increase in initial pH exhibiting the highest growth and lipase yield at pH 8.0.

A number of reports exist on influences of various environmental factors such as temperature, pH, nitrogen, carbon and lipid sources, agitation and dissolved oxygen concentration on lipase production (Watanabe et al., 1997). Lipase production is generally stimulated lipids (Omar et al., 1987). The lipase activity steadily increases to a peak and declines. Lipase production is usually coordinated with and dependent on the availability of triglycerides. Besides this, free fatty acids, hydrolysable esters, bile salts and glycerol also stimulate lipase production. A strain of *Penicillium roqueforti* produces maximum amount of lipase when grown in 0.5% casitone (Eitenmiller *et al*., 1970). Growth and lipase production by *Micrococcus* sp were unaffected by peptone of 0.5% to 2% but lipase production by Pseudomonas sp, *A. wentii, M. hiemalis, R. nigricans* and *M. racemosus* were stimulated by peptone (akhtar *et al*., 1980). Soybean meal extract in *Rhizopus oligosporus* culture medium supported good growth and lipase production (Nahas, 1988). Physiological regulation of lipase activity by thermo tolerant strain of *P. aeruginosa* EF2 under various conditions in batch, fed batch and continuous culture support the production of lipase (Gilbert *et al*., 1991).

Milk is a good medium for growth of psychotropic bacteria and lipase production, which was found to be susceptible to catabolite repression by glucose (Akhtar *et al*., 1980; Gilbert *et al*., 1991). While glucose in essential for the production of lipase by *P.fragi, A.wentii, M.hiemalis, R.nigricans and M. racemosus. P. aeruginosa* EF2 showed no such requirement (Nadkarni, 1971). Lipase activity per milligram dry weight of mycelium was higher on Lactose, Mannose, Xylose, Fructose, Dextrin, Maltose, Raffinose or Ribose produced less amount of lipase caused decreased growth with corresponding reduction in lipase activity in *M.racenosus*. Polysaccharides such as glycogen, hyaluronate, laminarin, gum Arabic and pectin stimulated production of lipase in *Serratia marcescens and Saccharomycopsis lipolytica*. This might probably be due to the detachment from the soil surface. A similar mechanism may explain the stimulating effect of lecithin on lipase production as investigated in *R. japonicus* (Aisaka and terada., 1980). Triglyceride is important for lipase production as it can act both as an inducer and inhibitor. Among the triglycerides, olive oil was observed to be effective in inducing lipae (Akhtar *et al*., 1980).

Salts of saturated fatty acids inhibited lipase production by *P. fragi,* where as tributyrin had no effect on lipase production by *P. fragi* and M. freudenreichii. Butter oil, Corn oil or Olive oil inhibited lipase production by *P. roquefortii, Saccharomycopsis sp, B. licheniformis, M. caseolyticus and Staphylococcus sp.* Triglycerides such as olive oil, ground nut oil and cotton seed oil and fatty acids such as oleic acid, linoleic acid stimulated lipase production by *P.mephitica*. Lipids are considered not to be true inducers (Nashiff and Nelson, 1953; Lawerence *et al.,* 1967). Emulsification of culture media containing oil by gum acacia supported good growth and lipase production in *R. oligosporus* (Nahas, 1988). Olive oil, tributyrin and oleic acid butyl ester were able to induce lipase in immobilized protoplasts, where as tween 80 enhanced lipase activity (Johri *et al*., 1990).

The initial pH of the growth medium is also important for lipase production. Maximum activity was observed at pH 7.0 for *P. fragi* and pH 9.0 for *P. aeruginosa* where in development of acidity in media reduced lipase activity (Nashif and Nelson, 1953). In contrast maximum growth at acidic pH (4.0-7.0) was reported for *S. lipolytica,*

M.caeseolyticus, B.licheniformais, A. wentii, M. hiemalis, R. migricans, Mucor racemosus, R. oligosporus and P. aeruginosa EF2 (Gilbert *et al*., 1991).

It has been observed that increasing the temperature above 80º C results in a depressing effect on lipase production by *P. fluorescens and Pseudomonas fragi.* Oso *et al*., (1978) determined 45º C to be the best temperature for lipase production by T. emersonii. Temperatures in the range of 22C-35 C were however observed to be optimum for maximum lipase production for *A. wentii, M. hiemalis, R. migricans, Mucor racemosus, R. oligosporus and P. aeruginosa.*

Aeration has variable effect on lipase production by different organisms. The degree of aeration appears to be critical in some cases shallow layer culture (moderate aeration) produced much more lipase than shake culture (high aeration) (Nashif and Nelson, 1953). Vigorous aeration greatly reduced lipase production by *R. oligosporus and P. aeruginosa, P. frag and M. racemosus* resulted in increased lipase production in static culture conditions. However, high aeration was needed for high lipase activity by *P. mephitica var. lipolytica, A. wentii and M. hiemalis.* Oso reported that stationary conditions in *T. emersonii* favoured maximum lipase production. Roe and Gilmour observed that lipase synthesis, by two strains of *P. fluorescens* (psychotroph), stimulated in milk medium at 70º C, was immediately preceded by a decrease in O_2 tension, which resulted in earlier production of lipase.

Lipase assay

Lipases are known to hydrolyze triglycerides and give rise to free fatty acid and glycerol. Therefore, the assay methods involve spectrophotometry or titrimetry, Jenson (1983), Nachlas and Blachburn, (1958). Rogel *et al*., (1989), radiolabelling assay, Huang *et al*., (1989), surface tension method, Verger. , (1989), Benjamin and Pandey, (1996) reported that lipase production by *Candida rugosa* on copra waste extract (CWE). CWE contained about 11% total solids, which include 2.2% soluble sugars. They found that the effect of supplementation of gum Arabic acid at different concentrations in the presence of 10% olive oil. The different concentration of gumarabic, 0.4% was the best. The combined effect of olive oil and gum Arabic could raise yields to 24.6Ug^{-1}ds as against 14.4 Ug^{-1}ds with copra waste extract (CWE).

When fermentation was carried out in medium constituted with the optimum concentrations of all the supplements such as CWE, peptone, sucrose, olive oil, caprylic acid and gum Arabic. The results showed highly encouraging trends. Around 95% of the soluble sugars were consumed in 60 and 72 hrs of yeast cultivation in this medium when the maximum growth (16.5mg-1ds glucosamine) and lipase yields (31.4Ug-1ds) were recorded.

Growth curve

Anshu Gupta *et al*., (2002) studied the lipase from *Enterobacter aerogens*. They found that the secretion of extra cellular lipase increased along with the growth and reached maximum in 48 hrs. Rohit Sharma *et al*., (2002) found that the induction in Bacillus species RsJ1 took place in early log phase in the presence of Tween-80 and olive oil and the maximum yield was obtained at late exponential growth. The fall in lipase yield observed in stationary and decline phase might be due to the increase in free fatty acids.

Screening of lipase producing organisms:

Kamini *et al.,* (1997) studied the fungal strain was isolated from curd. The fungal strain was identified as *Aspergillus niger.* Tributyrin was the substrate for examining lipase production on agar plates. A holozone of 9mm diameter around a colony in the tributyrin agar plate clearly indicated the production of lipase. The initial lipase activity was 8U ml^{-1} at 72 hrs in the culture supernatant of the basal medium, which indicated the extra cellular nature of the lipase.

Sangiliyandi and Gunasekaran (1996) studied that the lipase producing *Bacillus licheniformis* from an oil mill refinery effluent. They found that the

tributyrin agar, approximately 25 colonies showing clear zones were considered as lipolytic, out of which 10 colonies showing large clearing zones wee picked up and further examined for their ability to form clearing zones. Achamma Thomas *et al*., (2003) isolate were identified as *Bacillus mycoides* based on morphological and biochemical characteristics. It showed that lipase *production* by the formation of halozone around the colony on tributyrin agar.

Anshu Gupta *et al.,* (2002) studied that lipase from *Enterobacter aerogens*. They found that the lipase producers among purified colonies were detected by plating on tributyrin agar. The microbes showing clear zone was selected as potent producer of the lipolytic enzyme.

Enzyme characterization

Thermostability and pH stability

Pradeep Sidhu *et al*., (1998) studied the stability profiles of lipase produced by Bacillus sp R-12 at different temperatures and pH. The enzyme was stable at 50ºC for 30 min and retained 99% and 97% activities after two hours at these respective temperatures. It had a half-life of 60, 18 and 15 min at 65ºC, 70ºC and 75ºC respectively. The enzyme was fairly stable in pH range of 7.0 to 9.0 with residual activity of 100%, 98.5% and 84.6% at pH 7.0, 8.0 and 9.0 respectively.

Dong – Won cho *et al*., (1988) studied the enzyme was incubated at various temperatures (30, 40, 50, 60 and 70ºC) for 30 min, and then the residual activity was measure at 25ºC (100, 88, 66, 25 and 0% respectively). Remaining activities after 60 minutes incubations at same temperature was 92, 85, 47, 0 and 0%.

Omar *et al*., (1987) observed the production of lipase from *Humicola anginosa.* They found that the thermal stability was investigated by determining the residual activity after leaving the enzyme at various temperatures, between 30 to 100ºC, for 10 min. The enzyme was stable up to 70ºC, i.e., it retained 100% activity, however the activity decreased rapidly at 25% at 100ºC. Another thermal stability test was carried out at 60, 65 and 70ºC, for a longer incubation time. The enzyme retained 100%activity at 60ºC for 20 hr and at 65ºC for 70 min, and more than 75% of the activity was still observed at 70ºC after 90 minutes.

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