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REVIEW OF LITERATURE
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Production, purification and properties of lipase from *Streptococcus faecalis*
REVIEW OF LITERATURE

I. TYPES OF MICROBIAL LIPASES

Many microorganisms have been known to elaborate extra- and intracellular lipases. The nature of these lipases, however, varies in different species and strains.

Nelson and Peters (1952) showed that lipase isolated from Mycotorula lipolytica was primarily extracellular in nature. Similarly, Nashif and Nelson (1953a) found Pseudomonas fragi lipase was extracellular. It was also shown that about 90% of activity of Pseudomonas fragi lipase was confined to the supernatant fluid, while no measurable activity was detected in the cells. The true extracellular nature of Pseudomonas lipase was subsequently confirmed by several workers (Lawrence et al., 1967a; Mencher and Alford, 1967) and Driessen and Stadhouders, 1974). Production of extracellular lipases has also been reported in several other microorganisms such as Achromobacter (Khan et al., 1967 and Driessen and Stadhouders, 1974), lactic acid bacteria such as Streptococcus lactis, S. diacetilactis, S. cremoria, Lactobacillus casei, L. plantarum, L. brevis and Leuconostoc mesenteroides (Fryer et al.; 1967), Staphylococcus aureus (Mates and Sudaevitz, 1973), Alcaligenes (Driessen and Stadhouders, 1974), Penicillium
roqueforti (Eitenmiller et al., 1968), Humicola lanuginosa (Arima et al., 1972 and Liu et al., 1973b), Geostrichum candidum (Jensen, 1974) and Torulopsis (Motai et al., 1966).

Rottem and Razin (1964) reported that Mycoplasma gallisepticum lipase was not bound to the cell membrane but appeared to be intracellular. Umemoto et al. (1968) and Carini et al. (1972) isolated intracellular lipases from several lactic streptococci and lactobacilli. Oi et al. (1969) showed that lipases isolated from Rhizopus species were intracellular. A similar finding about the intracellular nature of lipase has recently been recorded by Chander et al. (1973) in a strain of Lactobacillus brevis.

Some microorganisms produce both intra- and extracellular lipases. Morris and Jezeski (1963) suggested that Penicillium roqueforti produced more than one type of lipase. Subsequently, Imamura and Kataoka (1966) reported that out of the two lipases, one type could be recovered from the culture medium by 40% (NH₄)₂SO₄ saturation at pH 7.0, while the other was recoverable from an extract obtained by 50% (NH₄)₂SO₄ saturation at pH 4.0. Chandan et al. (1962) had also observed that Penicillium roqueforti and Aspergillus niger elaborated intra- as well as extracellular lipases. Nakanishi et al. (1964) found intra- and extracellular lipase activity in many Achromobacter strains. Singh et al. (1973) reported
both types of lipases from non lactic organisms. Hosono et al. (1973) isolated both intra- and extracellular lipases from the yeast, *Candida muscorum*.

Hobson and Summers (1966) showed that a gram negative vibrio shaped organism produced two enzymes, one an esterase associated mainly with cells and the other, a lipase mainly secreted into the culture medium. Oterholm et al. (1968) noted that glycerol ester hydrolase activity of lactic acid bacteria was associated with two intracellular enzymes, a lipase and an esterase. The latter appeared to be more strongly associated with the cell particulate matter than the former. Somkuti et al. (1969) reported the presence of lipase and an esterase from a strain of *Mucor pusillus*.

II. METHODS OF DETERMINING LIPASE ACTIVITY

Numerous procedures are in vogue for determination of lipase activity among microorganisms. Estimation of free fatty acids liberated from triglycerides, is the underlying principle in the assay of microbial lipases.

The general procedure for the estimation of lipase activity involves acidification of culture to pH 6.0 and subsequent extraction with organic solvents like chloroform, ether etc. The liberated free fatty acids are then titrated against standard alkali (Ramakrishnan and Banerjee, 1952;
Frankel and Tarassuk, 1956; Alford and Pierce, 1963; Lu and Liska, 1969; Somkuti et al., 1969; Nadkarni, 1971 and Ahmed et al., 1974). Umemoto et al. (1968) examined several cultures of lactic acid bacteria comprising *S. diacetylactis*, *Lactobacillus casei*, *L. helveticus* and *L. plantarum* for lipase activity by titration of the liberated free fatty acids against standard alkali. A similar procedure has been adopted by Mates and Sudakevitz (1973) for the assay of lipase activity of *Staphylococcus aureus*.

Zollinkoffer and Fuchs (1949) used fractional distillation techniques for recovering free fatty acids from a variety of microorganisms such as *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Achromobacter*, *Sarcina*, *Acrobacter*, *Escherichia*, *Bacillus* and *Micrococcus*.

Harper and coworkers (1966) have recommended a silica gel extraction procedure without prior extraction of fat for the estimation of lipase activity. The above procedure which resulted in quantitative recovery of free fatty acids, has been used by Khan et al. (1967) for estimation of lipase activity in *Achromobacter lipolyticum*. Stadhouders et al. (1967) made a comparative evaluation of three different methods for determination of lipolysis in milk. The first method involved elution of milk fat
on silica gel column. In the second procedure, fat was removed from milk by centrifugation, followed by treatment with a hot solution of Triton X-100 and sodium tetraphosphate. In the third method, butter was churned from cream, melted and centrifuged to separate the fat layer. The first two methods were more suitable for estimation of low lipolytic activity in milk samples.

Marchis-Mouren et al. (1959) developed a continuous automatic titration procedure known as the pH-stat method which has been subsequently used by Shahani et al. (1964) for measurement of lipase activity. The above method involves lipolysis of an emulsion of oil or fat at a constant but optimum pH. The liberated free fatty acids are continuously titrated against standard alkali. The titre value per unit time is considered to be a direct measure of the concentration of lipase. The above method which has been claimed to be more sensitive than the silica gel method, was employed by several workers for estimation of lipase activity of milk (Parry et al., 1965; Wallander and Swanson, 1967; Homer and Virtanen, 1968) and of *Pseudomonas fragi* (Mencher and Alford, 1967), lactic acid bacteria (Oterholm et al., 1968), *Microbacterium lacticum* (Thompson-Collins and Witter, 1971) and *Brevibacterium linens* (Sorhaug and Ordal, 1974).
Gas chromatographic techniques have recently been employed by many workers for the detection and identification of individual components of free fatty acids liberated during hydrolysis of fat. Using the above technique, Robertson et al. (1966) detected 11 identifiable and 8 unknown free fatty acids in saponified and hydrolysed milk fat, while Hemingway et al. (1970) found butyric acid to be the main component during lipolysis of fat fractions of tainted and untainted milk samples.

Ayazbekova and Repina (1970) extracted the methyl esters of free fatty acids from ewe's milk cheese using hexane in an acid medium. GLC profiles showed that free fatty acids from cheese were predominantly palmitic, butyric, caproic, caprylic and capric acids. Similarly, Kevei-Fichler and Nonnssas (1970) confirmed the presence of capric, lauric, myristic, palmitic, stearic, oleic and linoleic acids in cheese samples made from ewe's milk.

Umemoto et al. (1968) identified some C_{10} to C_{18} free fatty acids in cell free extracts of Lactobacillus casei and L. plantarum using GLC technique.

A thin layer agar diffusion technique based on measurement of diameter of clearance zones, has been adopted by Lawrence et al. (1967b) for routine estimation of lipase. The above assay depends not only on the
solubility of di- and monoglycerides, but also on the solubility of calcium salts of fatty acids produced by hydrolysis of triglycerides containing fatty acids of twelve or fewer carbon atoms. The advantages of the above method are detection of low levels of lipase activity as also requirement of smaller samples (0.004 ml), apart from the simplicity in carrying out the assay.

Colorimetric methods have been described involving the use of a special substrate which gives after hydrolysis, a coloured end product. The principle of the method is that after hydrolysis of a simple esterlike nitrophenyl butyrate, the liberated p-nitrophenol, is measured directly (Byrde and Fielding, 1955). Hobson and Summers (1966) used the above colorimetric method for hydrolysis of naphthyl esters of acetic, lauric and stearic acids to liberate naphthol. Mackenzie et al. (1967) also used a colorimeter method for determining free fatty acids after lipolysis. This procedure involved formation of coloured complexes with free fatty acids, with the use of uranyl acetate and the basic dye, Rhodamine B. The coloured complexes were unstable in presence of light, while a small amount of aqueous uranyl acetate stabilized the coloured compounds sufficiently enough for determination of lipase activity.
Legakis and Papavassiliou (1974) used thin layer chromatographic technique for estimation of lipase in Pseudomonas aeruginosa, Staphylococcus aureus and Staphylococcus epidermidis.

Baillie and Norris (1963) developed a staining technique in which the gel slice recovered after starch gel electrophoresis, was flooded with the developing solution consisting of tris-maleate buffer (0.1 M, pH 6.4), 50 ml, 1% \( \beta \)-naphthyl acetate in 50% acetone, 2 ml, and fast blue B, 50 mg. The enzyme bands were coloured red and reached maximum intensity within about 2 hr at room temperature.

III. FACTORS INFLUENCING PRODUCTION OF MICROBIAL LIPASES

Production of lipase can be considered as the most important characteristic among lipolytic organisms, although the extent of enzyme production is known to vary among different strains. Factors such as incubation temperature, pH, aeration and media ingredients are known to affect growth as well as lipase production.

1. Incubation temperature

Incubation temperature plays an important role in lipase production. The optimum temperature for lipase production varies with different species, although it is
commonly believed that 21°C is the most suitable incubation temperature for growth of majority of lipolytic microorganisms.

Nashif and Nelson (1953b) reported maximum production of extracellular lipase by *Pseudomonas fragi*, when it was grown at 15°C or below. No lipase was produced at 30°C. The above findings were confirmed by Lawrence *et al.* (1967a). In contrast to the above reports, Alford and Elliott (1960) showed that 40°C was the optimum temperature for production of lipase by *P. fluorescens* and higher temperatures of incubation inhibited enzyme production. It was further noted that at lower temperatures, lipolytic activity was higher than proteolytic activity. Lawrence *et al.* (1967a) reported that *Pseudomonas* produced an extracellular proteinase also and the activity of this enzyme was correlated with the destruction of lipase initially produced. Khan *et al.* (1967) found that another psychrophilic organism, *Achromobacter lipolyticum* produced maximum amount of lipase at 21°C.

Vadehra and Harman (1967) have recommended 37°C as optimum temperature for lipase production by *Staphylococcus aureus*, while according to Mates and Sudakowitz (1973), appreciable amount of lipase was produced by the above organism at 37°C in 48 hr. Jonsson and Snygg (1974) showed that 25°C was optimum for lipase production by
Staphylococcus aureus, while Bacillus licheniformis produced maximum amount of the enzyme at 45°C. Lawrence et al. (1967a) have recorded 30°C as optimum temperature for lipase production by Micrococcus freudenreichii. On the other hand, 40°C was optimum temperature for production of lipase by Micrococcus caseolyticus (Jonsson and Snygg, 1974).

Peters and Nelson (1948) observed that Mycotorula lipolytica produced lipase at an optimum temperature of 30°C. Variations in optimum temperature for lipase production have been reported in Penicillium roqueforti. Imamura and Kataoka (1963a) found that the above organism produced maximum amount of lipase at 7°C, whereas Eitenmiller and coworkers (1977) observed that the above organism required an optimum temperature of 27°C for lipase production.

Somkuti and Babel (1968) reported maximum lipase synthesis in Mucor pusillus at 35°C. A higher temperature of 45°C has been recorded for optimum production of lipase by Humincola lanuginosa (Liu et al., 1972).

Recently Shen et al. (1975) showed that Eremothecium ashbyii Du-32 elaborated appreciable amounts of lipase at 28°C.

2. pH

pH is known to play an important role in lipase production and each lipolytic organism exhibits an optimum pH for the production of enzyme.
Nashif and Nelson (1963c) showed that pH above 7.0 was most favourable for production of lipase by *Alcaligenes viscosus*, *Serratia marcesens* and *Flavobacterium*. The most optimum pH range for production of lipase by *Pseudomonas fragi* was between 6.5 to 7.0 (Nashif and Nelson, 1963b). Similarly, pH 7.0 has been found suitable for maximum lipase yield in *Pseudomonas fluorescens* (Alford and Elliott, 1960). Recently, desirability of a still higher pH of 8.75 has been reported in literature for lipase production by the same organism (Driessen and Stadhouders, 1974). Lawrence et al. (1967a) obtained higher yields of lipase in *Micrococcus* species when the organism was grown in media adjusted between pH 7.0 to 8.0. In contrast to the above observation, Scheibner (1973) found that the above organism showed increased yield of lipase at pH 5.0 to 7.0. Similar results were obtained in case of *Bacillus* and *Proteus* species.

Many workers have reported different pH values like 5.0 to 7.0, 7.5, 8.0 and 8.5 for lipase production by *Staphylococcus aureus* (Scheibner, 1973; Mates and Sudakevitz, 1973; Vadehra, 1974 and Jonsson and Snygg, 1974).

Nelson (1953) noted maximum lipase production by *Geotrichum candidum* in a buffered basal medium adjusted to
pH between 5.35 and 5.85 and in glycerol medium showing pH between 6.7 and 6.85. Niki et al. (1966) demonstrated that Penicillium roqueforti produced lipase in whey medium at an optimum pH $> 7.0$. On the contrary, Dolezalek and Minarik (1969) found pH 6.7 as most suitable for lipase production by Penicillium camemberti and Penicillium nalgiovensis. Grasas (1973) showed that the optimum pH for lipase production by G. olivarum was 7.0, while Hosono et al. (1973) noted that pH 6.0 was favourable for production of both intra- and extracellular lipases by Candida muscorum.

3. Aeration

The necessity of aeration for lipase production has been stressed by Nashif and Nelson (1953b), although the degree of aeration varied with different species. For instance, in Pseudomonas fragi, lipase production increased in shake cultures, in contrast to shallow cultures (Nashif and Nelson, 1953b). Higher yields of lipase by Staphyloccocus aureus was obtained in shorter time, when the culture was aerated (Alford and Smith, 1965; Vadehra and Harmon, 1965b and Mates and Sudakevitz, 1973). Lawrence et al. (1967a) showed that in Pseudomonas fragi, increased cell growth and lipase production were obtained in shake cultures, but the enzyme activity
decreased rapidly on continued shaking. In Micrococcus freudenreichii, however, lipase production decreased on aeration of the culture.

Alford and Smith (1965) demonstrated that when the surface volume ratio of a static culture of Geotrichum candidum was reduced, there was a sharp drop in the yield of lipase. With a slowly agitated culture, the enzyme yield remained essentially unchanged as the volume increased. Bitenmiller et al. (1968) observed that Penicillium roqueforti grown on a selective nutrient medium in shake culture, produced maximum amount of lipase.

4. Media and media ingredients

Cutchins et al. (1952) demonstrated the stimulatory action of thiamine-HCl on lipase production by Alcaligenes viscosus, Pseudomonas aeruginosa and Pseudomonas fluorescens, while Nelson (1963) found that water soluble vitamins did not markedly affect production of lipase by Geotrichum candidum.

Alford and Pierce (1963) compared growth and lipase production by Pseudomonas fragi using 41 different dehydrated growth media. Although good cell growth was obtained in buffered ammonium sulphate – glucose medium, lipase production was poor in the above medium.
Several workers have recorded that addition of peptone in a nutrient medium enhanced lipase production by *Pseudomonas fragi* (Nashif and Nelson, 1963; Alford and Pierce, 1963 and Lawrence *et al.*, 1967a). Umemoto (1969) developed a special nutrient medium for maximum lipase production by lactic acid bacteria. The ingredients in the above medium were peptone, yeast extract, sodium chloride, tween 80 and tomato juice. Petricca and Harper (1970) incorporated thioglycollate and milk protein hydrolysate into nutrient media for ensuring maximum lipase production by *Achromobacter lipolyticum* and *Pseudomonas fragi*.

Khan *et al.* (1967) found that *Achromobacter lipolyticum* produced appreciable amounts of lipase in casitone broth medium. Casitone has also been used by Hitenmiller *et al.* (1968) for enhancing lipase production by *Penicillium roqueforti*.

Imamura and Kataoka (1963a) observed that *Penicillium roqueforti* elaborated maximum lipase when an organic nitrogen source such as peptone was used in place of inorganic nitrogen sources like NaNO₃ or (NH₄)₂SO₄. Hosono and Tokita (1970) found that lipase production by *Candida mycoderma* and *Debaryomyces kloeckeri* was highest in Czapek's medium which contained 0.3% peptone, although addition of other ingredients like soluble starch, urea, magnesium
sulphate and potassium-hydrogen phosphate to the medium also helped in production of enzyme.

Somkuti and Babel (1968) observed that use of 4% wheat bran medium resulted in maximum lipase production by Mucor pusillus.

Addition of selected amino acids to a synthetic medium has been found to increase production of lipase by some microorganisms. Guthins et al. (1952) noted that incorporation of aspartic acid into buffered ammonium sulphate-glucose medium resulted in enhanced lipase production by Pseudomonas fluorescens.

Considerable increase in the production of lipase by Pseudomonas fragi was also noted when the organism was cultivated in a medium supplemented with amino acids such as leucine, isoleucine and valine, although the yield of the enzyme was comparatively lower than in peptone medium (Nashif and Nelson, 1953b). Similarly, increased yield of the enzyme approaching that obtained in peptone media, was also achieved in media supplemented with arginine, lysine, aspartic acid and glutamic acid (Alford and Pierce, 1963). In Pseudomonas aeruginosa, addition of alanine to the medium has been shown to promote lipase production (Sierra, 1967). Nadkarni (1971) obtained maximum yield
of lipase in the same organism with the use of amino acids like glutamic acid, tryptophane and arginine. The requirement of amino acids for the stimulation of lipase production by *Aspergillus niger* has also been demonstrated (Ramakrishnan, 1957).

5. **Adaptive nature of microbial lipase**

Many bacterial enzymes are known to be adaptive, since they can be induced in the presence of substrates.

In the year 1929, Barber reported stimulation in production of lipase in a strain of *Penicillium*, after the organism had synthesised lipids from a carbohydrate source. Goldman and Rayman (1952) noted that a *Pseudomonas* species effectively produced lipase when butter oil was incorporated into the medium. Subsequently, Nashif and Nelson (1953b) showed that addition of small amounts of synthetic substrates like tricaproin as also fatty acids such as caprylic and capric acids to a vitamin free casein or peptone media, resulted in increased lipase yield. Kingma Boltjes (1955) showed that a few strains of *Bacillus*, which did not show any lipase activity, acquired the property when grown for six weeks in the presence of fats and tweens.

While examining several microorganisms for lipolytic activity using lard as a substrate, Sierra (1957) demons-
treated that none of these cultures exhibited any lipolytic activity; but, addition of small quantities of tween 40 stimulated enzyme production. Similarly, Khan et al. (1967) reported that addition of 1% olive oil, corn oil or milk fat to a nutrient medium stimulated lipase production by Achromobacter lipolyticum by 86, 68 and 25%, respectively. Vadehra and Harmon (1965a) observed increased lipase synthesis in Staphylococcus aureus, when it was grown in milk as compared to its growth in broth and suggested that an inducible enzyme may be involved.

Oterholm et al. (1968) obtained maximum amount of lipase in lactic acid bacteria when the organisms were cultivated in MRS broth containing 0.1% cream. Bertoldini et al. (1970) found that incorporation of 1.2% milk fat into nutrient media resulted in maximum lipase production by Streptococcus faecalis, Streptococcus agalactiae and Micrococcus species. Umemo (1969) grew lactic acid bacteria in a medium containing tween 80 and noted appreciable lipase production. Angelis and Marth (1971) obtained increased lipase production by both streptococci and lactobacilli when cultivated in MRS broth fortified with 2% soya bean oil. Incorporation of 3% lard or butterfat into a nutrient medium has been shown to enhance

Some reports indicate reduction in lipase production when certain substances were incorporated into nutrient media. According to Hitenmiller *et al.* (1970), lipase production by *Penicillium roqueforti* was inhibited by addition of butter oil, corn oil or olive oil to the growth medium. Similarly, Mates and Sudakevitz (1973) found that lipase production by *Staphylococcus aureus* decreased when tween 80 was incorporated into nutrient medium. Addition of triolein, oleic acid or tributyrin had very little influence on lipase production by *Brevibacterium linens* (Sorhaug, 1974).

Lipase production by yeast and mold cultures has been studied by several workers.

Imamura and Kataoka (1963a) showed that lipase production by *Penicillium roqueforti* was significantly increased by addition of butterfat or tributyrin to the medium. Dolezalek and Hoza (1971) cultivated *Penicillium roqueforti* in a medium containing 10% milk fat for production of lipase. Similarly, Liu *et al.* (1972) observed that soya bean oil enhanced lipase production by *Humicola lanuginosa*. Recently, Shen *et al.* (1975) reported that lipids were required for the induction of lipase production by *Eremothecium ashbyii* (Du-32). In a
recent report, Lobyreva (1975) found that when *Candida utilis* 296t was grown in a synthetic medium without substrate, lipolytic activity occurred in cells only but when grown in presence of olive oil, lipase activity was observed in the supernatant fluid as well.

IV. PURIFICATION OF MICROBIAL LIPASES

Microbial lipases have been purified making use of several elegant biochemical techniques.

Rottem and Razin (1964) partially purified lipase from *Mycoplasma gallisepticum* by ammonium sulphate fractionation and anion exchange chromatography.

O'Leary and Weld (1964) achieved 225 fold purification of *Staphylococcus aureus* lipase by ammonium sulphate precipitation, gel filtration and DEAE cellulose chromatography, while Vadehra and Harmon (1965b) obtained 500 fold purification of the lipase from the same organism by using ethanol precipitation followed by Sephadex G-200 and Biogel-300 filtration. In subsequent studies with *S. aureus*, Vadehra (1974) claimed 350-450 fold purification of lipase by passing through Sephadex G-200 and Biogel-300.

Mencher and Alford (1967) obtained 103 fold purification of lipase from *Pseudomonas fragi* with 18% recovery.
using techniques such as ultrafiltration, lyophilization, ammonium sulphate fractionation and DEAE Sephadex A-50 column chromatography, while Lu and Liska (1969) achieved 100 fold purification of the enzyme from the same organism by ammonium sulphate fractionation followed by acetone precipitation, Sephadex G-200 filtration and DEAE cellulose chromatography.

Using a combination of techniques like ammonium sulphate precipitation, gel filtration and DEAE cellulose chromatography, Oterholm et al. (1970) purified lipase from Propionibacterium shermanii, while in subsequent studies, the same workers (1972) purified L. plantarum esterase, by employing ammonium sulphate precipitation, acetone fractionation and DEAE Sephadex A-50 column chromatography techniques. Fulton Jr. (1973) purified lipase from Corynebacterium acnes by ultrafiltration, Sephadex G-100 filtration followed by chromatography on CM cellulose.

Sugiura et al. (1974) obtained 23 fold purification with 2.8% recovery of Chromobacterium lipase, using a combination of techniques involving Amberlite CG-50, Sephadex G-75 filtration followed by re-chromatography on Sephadex G-75.

Recently, Pablo et al. (1974) reported purification of lipases from culture broths of Corynebacterium acnes and Staphylococcus epidermidis. Ultrafiltration or precipitation followed by gel filtration on Sephadex G-100 and chromatography with a cellulose ion exchanger were used for the purpose.

Some studies have been reported on the purification of lipases from yeasts and molds.
Fukumoto et al. (1963) obtained *Aspergillus niger* lipase in a crystalline form after ammonium sulphate fractionation, acetone and acid treatment, acrnilol precipitation and finally, by ion exchange chromatography. In a subsequent paper, Fukumoto et al. (1964) reported extraction of lipase from the shake culture of another organism, *Rhizopus delemar*. The enzyme was purified by ammonium sulphate fractionation, duolite A2 resin treatment, SE Sephadex G-50 column chromatography, acetone fraction and gel filtration technique. According to a recent report of Iwai and Tsujisaka (1974), *Rhizopus delemar* produced three types of lipases which were purified by ammonium sulphate fractionation followed by treatment with duolite A-2, column chromatography on SE Sephadex G-50 and gel filtration on Sephadex G-200 column.

According to Notai et al. (1966), *Torulopsis* lipase was purified to 230 fold by ammonium sulphate fractionation, DEAE cellulose chromatography and Sephadex G-100 gel filtration.

In 1967, Oi et al. employed DEAE cellulose column chromatography for fractionating *Penicillium crustosum* lipase into three fractions. The two fractions were further purified by ammonium sulphate precipitation and finally obtained in a crystalline form. The homogeneity of lipase was confirmed by electrophoresis and by ultracentrifugation. In a subsequent report, Oi et al. (1969) have recorded fractionation of
Rhizopus lipase into two components I and II by DEAE Sephadex A-50 column chromatography. The two fractions were further purified by precipitation with ammonium sulphate, passage, through CM Sephadex C-50. Finally, the enzyme was passed through Sephadex G-100 column.

Ota et al. (1970) reported 132 fold purification of Candida paralipolytica lipase with 32% recovery, using ammonium sulphate precipitation, chromatography on CM Sephadex C-50, DEAE Sephadex A-50 and rechromatography on CM Sephadex C-50.

Somkuti et al. (1969) tried several procedures for the purification of lipase from Mucor pusillus and these included ammonium sulphate precipitation, gel filtration on Sephadex G-75 and DEAE-Sephadex A-50 column chromatography, while Nagaoka and Yamada (1973) fractionated Mucor lipases into two fractions using CM Sephadex C-50 column chromatography.

Similarly, purification of Geotrichum candidum lipase has been achieved by ammonium sulphate fractionation, DEAE Sephadex column chromatography, gel filtration on Sephadex G-100 and G-200 (Tsujisaka et al., 1973). Jensen (1974) also succeeded in purifying G. candidum lipase to 81 fold by acetone precipitation, gel filtration on Sephadex G-25 and G-200.

V. FACTORS AFFECTING ACTIVITY OF MICROBIAL LIPASES

1. Substrate specificity

Lipases attack both natural and synthetic triglycerides and liberate free fatty acids at different rates. The hydro-
(Ota et al., 1972), while the preferential hydrolysis of triolein by *Achromobacter lipolyticum* lipase has been recorded by Khan et al. (1967).

Many lipases break down butter fat more easily, as compared to other natural fats. The specificity of lipases for butter fats has also been demonstrated in many microorganisms like *Lactobacillus casei* (Wolf, 1941), *Bacillus* species, *Micrococcus* species, *Serratia marcescens*, *Pseudomonas fluorescens* and *Penicillium* species (Brandl and Ziger, 1973).

A few microbial lipases have been known to hydrolyse natural triglycerides and examples of those which preferentially attack olive oil include *Geotrichum candidum* (Wilcox et al., 1955), *Serratia marcescens* (Hugo and Beveridge, 1962), *Aspergillus niger* and *Rhizopus delemar* (Fukumoto et al., 1963 and 1964), *Penicillium crustosum* and *Rhizopus* species (Oi et al., 1967 and 1969).

Wilcox et al. (1955) showed that *Geotrichum candidum* lipase preferentially hydrolysed long chain fatty acid esters as compared to short chain esters. Iwai et al. (1964a) confirmed the above observation with *Aspergillus niger* lipase and showed that this enzyme hydrolysed vegetable oil triglycerides of long chain fatty acids. However, the above workers found that the hydrolytic activity of the enzyme depended upon the chain length of fatty acids, since
maximum activity was observed in case of fatty acids with 8 and 16 carbon atoms.

The positional specificity of various microbial lipases has also been confirmed by gas liquid chromatographic analysis of the free fatty acids liberated by breakdown of mixed triglycerides of known composition.

Alford et al. (1961) examined the position of fatty acids in the triglyceride moiety using *Pseudomonas fragi* lipase. This enzyme was similar to pancreatic lipase in its properties and attacked primarily 1,3 position of the triglyceride molecule. Subsequent studies with *Staphylococcus aureus* and *Aspergillus flavus* lipases (Alford et al., 1964) proved that these enzymes attacked the first as well as the second position of triglyceride moiety. Mencher and Alford (1967) and Cooke (1973) independently confirmed the above findings using *Pseudomonas fragi* lipase which exhibited 1,3 position specificity of the triglyceride moiety. According to Cooke (1973), *Staphylococcus aureus* lipase showed a completely random hydrolysis of triglyceride. While Liu et al. (1973a) found that *Humicola lanuginosa* lipase had no positional specificity and split the ester bonds on all the positions of triolein at similar rates. On the contrary, Vadehra (1974) found that *S. aureus* lipase hydrolysed synthetic triglycerides in 1,2 and 3 positions, although there appeared to be slight preference.
for the first and third positions. Pablo et al. (1974) claimed that *S. epidermidis* lipase cleaved \( \beta \) position of the triglyceride more rapidly than \( \alpha \) position, while *Corynebacterium acnes* lipase hydrolysed both the positions with equal facility.

Franzke et al. (1973) observed that *Geotrichum candidum* lipase had a marked specificity for cis-9 unsaturated fatty acids, the activity against oleic acid being the greatest, followed in order by palmitoleic, linoleic and linolenic acids. Jensen (1974) reported that *Geotrichum candidum* lipase hydrolysed cis 9-16:1, cis, trans 9, 12-18:2, trans, cis 1, 12-18:2 of palmitoyl oleate and cholesteryl oleate. Recently, Rayond and Crisan (1975) have shown the substrate specificity of several lipolytic isolates on marine and non-marine substrates. Out of 170 isolates that were examined, 14 were active only on menhaden oil; 11 hydrolysed menhaden oil and tween 80 or tributyrin. One hundred and forty-five isolates hydrolysed non-marine lipids.

2. pH

Most of the bacterial lipases have an optimum pH range for enzyme activity, either at neutrality or towards the alkaline side. In case of fungal lipases, the optimum pH for enzyme activity appears to be on the acid side of neutrality, although exceptions are known.
Mencher and Alford (1967) found that the purified *Pseudomonas fragi* lipase was most active at pH of 8.6 - 8.7.

Cserhati and Hollo (1972) noted that lipase extracted from a *Bacillus* species showed maximum activity at pH 6.0.

Lipases from lactic acid bacteria exhibit an optimum pH range between 6.0 to 8.0 for activity on various substrates (Umemoto et al., 1968 and Carini, 1969). Chander et al. (1973) also found that *L. brevis* lipase was most active at pH 6.5, while Oterholm et al. (1970) observed that *Propionibacterium shermanii* lipase exhibited maximum activity at pH 7.2.

Some microorganisms show differences in lipase activity at specific pH ranges depending upon the type of the substrate that is hydrolysed. For instance, in the case of *Staphylococcus aureus* lipase, an optimum pH range of 6.5 to 7.5 was necessary for hydrolysis of ethyl laurate and olive oil (Hugo and Beveridge, 1962). When tributyrin was used as the substrate, the same lipase was most active at pH 7.8 and 7.5, respectively (Shah and Wilson, 1965 and Troller and Bozeman, 1970). A still higher pH range of 8.0 - 8.5 is said to be required for the hydrolysis of olive oil emulsion by the above enzyme (Vadehra and Harmon, 1965b and Vadehra, 1974). According to Hugo and Beveridge
(1962), *B. subtilis* lipase showed maximum activity at pH levels, between 8.5 and 9.5, with olive oil, ethyl butyrate and ethyl laureate as substrates, but when tributyrin was used as substrate, a pH range of 6.5 to 7.5 was required for maximum enzyme activity.

Microbial lipases which have their pH optimum between 8.0 to 8.5 for the hydrolysis of triglycerides include those obtained from *Serratia marcescens* (Tammisto, 1933 and Gorbach *et al.*, 1955), *Micrococcus* and *Pseudomonas* (Lawrence *et al.*, 1967a).

There are only few reports which deal with variations in pH optima of microbial lipases depending upon the extra- or intracellular nature of the enzyme.

Khan *et al.* (1967) found that extracellular lipase from *Achromobacter lipolyticum* showed maximum activity at pH 7.0. But, the same enzyme when intracellular was most active at pH 9.0 (Shahani *et al.*, 1964).

Considerable variations in pH optima for lipase activity have also been recorded among fungi. Thibodeau and Macy (1942) observed that *Penicillium roqueforti* lipase had a broad pH range between 5.3 - 7.5, while Fodor and Chari (1949) found that the above lipase required pH 5.0 for its maximum activity. According to Imamura and Kataoka (1963b), *Penicillium roqueforti* produced two types
of lipases, one having an optimum pH of 6.5 for its activity, while the other was most active at pH 7.5. In contrast to the above report, Niki et al. (1966) found that the pH optima for activity of the above two types of lipases were 6.0 and 7.5, respectively. Bitenmiller et al. (1970) claimed still higher pH optima of 8.5 for activity of above lipase.

Fodor and Chari (1949) showed that Aspergillus niger lipase was most active at pH 5.0, but according to Fukumoto et al. (1963 and 1964), both Aspergillus niger and Rhizopus lipases delemar exhibited maximum activity at pH 5.6. Iwai and Tsujisaka (1974) also showed that all three types of lipases from Rhizopus delemar were most active at pH 5.6. Although lipase A was more active at pH 5.5, there was no significant difference between pH activity curves of B and C lipases. Similar pH values ranging between 5.0 and 5.5 have been recorded by Somkuti et al. (1969) for maximum activity of Mucor pusillus lipase. Oi et al. (1969) observed the lowest pH optima of 3.5 for the activity of Rhizopus I and II lipases.

Some fungal lipases are most active at higher pH range of 8.0 to 9.0. Oi et al. (1967) isolated three types of lipases from Penicillium crustosum and designated them as I, II and III and all the three types showed maximum activity at pH 9.0. Similar results have been recorded by Lamberet
and Lemoir (1972) in case of *Penicillium caseicolum* lipase which was most active at pH 8.5. Liu *et al.* (1972) observed that the optimum pH for the activity of crude enzyme from *Hamicola lanuginosa* was at 8.0. Nagaoka and Yamada (1973) showed that two fractions of *Mucor* lipase designated as F-3A and F-3B were most active at pH 9.0 and 8.0, respectively.

Jensen (1974) found that the optimum pH for appreciable activity of *Geotrichum candidum* lipase was 8.2, while in an earlier report, Tsujisaka *et al.* (1973) had reported a lesser pH optimum ranging from 5.6 to 7.0 for activity of above lipase. Recently, Shen *et al.* (1975) have demonstrated highest activity of *Fremothecium ashbyi* lipase at pH 8.0.

Yeast lipases are known to possess lower pH optima for maximum activity. Motai *et al.* (1966) observed that *Torulopsis* lipase was most active at pH 6.5, while both *Candida mycoderma* and *Dharronvres kloeckeri* lipases exhibited maximum activity at pH 4.5 (Hosono and Tokita, 1970).

According to Hosono *et al.* (1973), both intra- and extracellular lipases of *Candida muscorum* were most active at pH 6.5.

Ota *et al.* (1970) noted that purified lipase preparation from *Candida paralipolytica* exhibited maximum activity
at pH 8.0, while a lower pH optima of 7.0 was required for the activity of the same enzyme preparation which had been modified after dialysis.

3. Temperature

Microbial lipases have an optimum temperature between 30 to 40°C, at which greater hydrolysis is brought about. Exceptions have, however, been noted in case of Aspergillus niger (Fukumoto et al., 1963) and Torulopsis lipases (Motai et al., 1966), which exhibited optimum temperatures of 25 and 45°C, respectively for maximum enzyme activity.

Tammisto (1933) showed that Serratia marcesens lipase required temperature optima ranging from 33 to 37°C for hydrolysis of butter fat and other substrates, while a higher optimum temperature of 60°C for lipolysis of butter fat has also been recorded for the above organism (Gorbach et al., 1955).

Alford and Pierce (1961) found considerable lipase activity in Pseudomonas fragi, Staphylococcus aureus, Geotrichum candidum, Penicillium species and Candida lipolytica, when the reaction mixtures containing the respective enzymes were stored at -7, -18 and -29°C for 2 days to 3 weeks. The substrates used in the above studies were emulsions of corn, coconut and lard oils.
Khan et al. (1967) demonstrated a definite disparity between optimum temperature for growth and lipolytic activity in *Achromobacter lipolyticum*. The lipase isolated from this organism hydrolysed natural and synthetic triglycerides at 37°C. Umemoto et al. (1968) reported that when tributyrin was used as substrate, lactic acid bacteria showed maximum lipase activity at temperatures between 30 to 40°C. Carini (1969) showed, however, that majority of lactic lipases were most active at temperatures ranging from 15 to 30°C. Chander et al. (1973) observed that 30°C was the optimum temperature for the activity of *Lactobacillus brevis* lipase. On the other hand, Oterholm et al. (1970) showed that purified *Propionibacterium shermanii* lipase exhibited an optimum temperature of 47°C for its activity.

Troller and Bozeman (1970) and Vadehra (1974) independently observed that *Staphylococcus aureus* lipase exhibited maximum activity between 32 - 37°C.

Fodor and Chari (1949) claimed that the optimum temperature for lipolytic activity of fungal mycelial extracts was 38°C. Shipe (1951) showed that 30°C was the optimum temperature for lipase activity of *Penicillium roqueforti* and *Aspergillus niger*. While according to Morris and Jezeski (1953), similar mycelial preparations hydrolysed tributyrin and butter fat at 30 - 32°C. On
the other hand, Bietenmiller et al. (1970) found that maximum activity of partially purified *Penicillium roqueforti* lipase occurred at 37°C, while maximum activity of *Penicillium caseicolum* lipase was noted at temperatures ranging from 20 to 35°C (Lamberet, 1970).

Nelson (1963) observed that the optimum temperature for the activity of *Geotrichum candidum* lipase was 30°C, while according to Tsujisaka et al. (1973), the above organism exhibited maximum enzyme activity at 40°C. Similarly, in case of *Bremothecium ashbyii*, Shen et al. (1975) found 40°C as the optimum temperature for lipase activity.

Fukumoto et al. (1964) showed that lipase extracted from *Rhizopus delemar* was most active at 35°C. But, Iwai and Tsujisaka (1974) observed that among three types of lipases produced by the above organism, lipase A was active at 30°C, while lipases B and C exhibited maximum activity at 35°C.

Hosono and Tokita (1970) reported that 35 and 30°C were optimum temperatures for the activity of *Candida mycoderma* and *Debaryomyces kloeckeri* lipases, respectively.

According to Liu et al. (1972), *Humicola lanuginosa* lipase exhibited maximum activity at a higher temperature of 60°C.
4. Activity of microbial lipases on storage

Microbial lipases are known to retain their activity for long periods, when stored at refrigeration temperatures.

According to Nashif and Nelson (1953a), *Pseudomonas fragi* lipase when stored at 3 - 5°C for 7 days, exhibited negligible loss in activity, although storage of the enzyme at 15°C for 24 hr, resulted in appreciable inactivation of enzyme.

Renshaw and Sanclemente (1966) observed no loss in the activity of *Staphylococcus aureus* lipase when stored for 2 hr at -20°C, while Troller and Bozeman (1970) found that the above lipase remained active under frozen condition for 4 weeks without denaturation. A reduction in enzyme activity to the extent of 40% was noted in 5 days at a storage temperature of 4°C, while 20% of activity was lost during storage of the enzyme for 4 hr at room temperature. Vadehra (1974) reported that purified *S. aureus* lipase was slightly less stable, since loss in activity was less than 10% at 21 days of storage at -23°C.

Eitenmiller et al. (1968) have shown that *Penicillium roqueforti* lipase was stable for three months in the frozen state. Somkuti et al. (1969) showed that the activity of *Mucor pusillus* lipase was unaffected during storage at -10°C for 30 days.
Recently, Jensen (1974) has reported that lyophilized microbial lipase preparation of Geotrichum candidum was extremely stable and retained its activity for at least 8 years when the enzyme stored at -20°C.

5. Heat stability

There is an optimum temperature up to which microbial lipases remain stable and any further rise in temperature inactivated the enzymes. Many reports deal with the heat stability of microbial lipases.

Nashif and Nelson (1963a) noted appreciable enzyme activity with Pseudomonas fragi lipase when it was heated at 61.6°C and 71.6°C for 30 min, but the enzyme was completely inactivated at 99°C for 20 min. The activity of the enzyme was retained to the extent of 50%, when it was exposed to 35°C for 10 min, although heat treatment at 40°C for 10 min, completely inactivated it (Mencher and Alford, 1967). Studies by Singh et al. (1973) showed that when Pseudomonas fragi lipase was subjected to pasteurisation temperature (63°C for 30 min), 49.4 - 68.1% of exocellular and 82.3 - 89.9% of endocellular lipase activities were inactivated.

Csiszar and Romlehner-Bakos (1966) investigated the activity of lipase preparations obtained from different
strains of *Pseudomonas fluorescens*. Exposure of the enzyme preparations for 10 min at 75, 85 and 95°C resulted in 68, 73 and 75% inactivation, respectively.

Khan *et al.* (1967) found that *Achromobacter lipolyticum* lipase was fairly heat stable, since heat treatment of the enzyme at 71°C for 180 min destroyed only 47% of the activity. The enzyme was, however, completely inactivated at 99°C for 40 min or after autoclaving at 121°C for 15 min.

Troller and Bozeman (1970) showed that *Staphylococcus aureus* lipase was inactivated completely when it was heated to a temperature of 70°C for 4 min, while complete inhibition occurred on exposure of the enzyme to 80°C for 3 min. Vadehra (1974) reported that purified *Staphylococcus aureus* lipase lost only 6% of the activity when exposed to heat treatment of 50°C for 30 min, although complete destruction of enzyme activity was observed when heated to 70°C for 30 min.

Oteholm *et al.* (1970) noted that purified *Propionibacterium shermanii* lipase lost 75% of its activity when heated for 10 min at 45°C.

Driessen and Stadhouders (1971) showed that *Alcaligenes viscolactis* lipase retained 8% of its activity on heating at
$94^\circ C$ for 10 sec, although the enzyme was completely inactivated in 10 sec at $90^\circ C$.

While studying the heat stability of *Lactobacillus brevis* lipase, Chander *et al.* (1973) found that the enzyme was completely inactivated at $62.8^\circ C$ for 30 min and also at $71.7^\circ C$ for 16 sec.

Different time-temperature combinations have been indicated for inactivation of fungal lipases.

Fukumoto *et al.* (1963 and 1964) reported that *Aspergillus niger* and *Rhizopus delemar* lipases were inactivated at 55 and $50^\circ C$, respectively and in this respect, the above enzymes resembled milk and pancreatic lipases. But according to Iwai and Tsujisaka (1974), purified B and C lipases of *Rhizopus delemar* were stable below $45^\circ C$, while lipase A was stable at $60^\circ C$. The thermostability of *Penicillium crustosum* lipase below $47^\circ C/15$ min has been demonstrated by Oi *et al.* (1967) and the enzyme was only inactivated at $80^\circ C$ in 15 min. Similarly, Eitenmiller *et al.* (1968) observed that *P. roqueforti* lipase was inactivated in 10 min at $50^\circ C$. Somkuti and Babel (1968) reported that heat treatment of *Mucor pusillus* lipase at $58^\circ C$ for 10, 25 and 45 min, resulted in 10, 27 and 50% loss in activity, respectively.
Liu et al. (1972) noted that *Humicola lanuginosa* lipase regained all of its activity after heat treatment at 60°C for 2 hr, while only 35% of the enzyme activity could be retained when the enzyme was heated at 70°C for 20 min. Shen et al. (1975) have recently shown that when *Fremothecium ashbyii* lipase was inactivated when exposed to various temperature-time combinations like 55°C for 10 min, 50°C for 20 min, 40°C for 4 hr and 28°C for 44 hr.

6. **pH stability**

Most of the microbial lipases are stable up to a particular pH range at a specific temperature and incubation period. Any further variation in pH resulted in retardation in enzyme activity.

Mencher and Alford (1967) observed that *Pseudomonas fragi* lipase was stable for 1 hr in the pH range of 6.6 - 7.8 at 2°C. Oterholm et al. (1970) reported that *Propionibacterium shermanii* lipase was stable for 5 min in the pH range between 5.5 - 8.0 at 35°C. Pablo et al. (1974) observed that *Corynebacterium acnes* lipase was stable in the acidic pH range of 5.8, while *Staphylococcus epidermidis* lipase exhibited its stability in alkaline pH range of 7.0 - 10.0.
01 et al. (1967) found that *Penicillium crustosum* lipase was stable for 24 hr at pH values ranging from 6.0 to 9.0 at 30°C. In a later paper, the above workers (1969) have reported that *Rhizopus* lipase exhibited stability for 20 hr at pH 4.0 to 7.0 at 30°C. Iwai and Tsujisaka (1974) also showed that B and C lipases of *Rhizopus delemar* were stable for 15 hr in the pH range of 4.0 to 7.0 at 30°C, while lipase A was stable in the pH range of 3.0 to 8.0. Tsujisaka et al. (1973) demonstrated that purified *Geotrichum candidum* lipase was stable for 24 hr in the pH range of 4.2 to 9.8 at 30°C. Shen et al. (1975) reported that *Fremothecium ashbyii* lipase exhibited pH stability at pH ranging from 5.0 to 8.0.

Motai et al. (1966) observed that *Tornilopsis* lipase was quite stable for 1 hr at pH ranging from 3.0 to 8.0 at 37°C. Ota et al. (1970) showed that the purified lipase obtained from *Candida paralipolytica* was stable for 22 hr at pH values ranging from 3.5 to 9.0 at 50°C.

7. **Effect of activators and inhibitors**

Metallic ions either stimulate or inhibit the rate of hydrolysis of microbial lipases.

Kirsh (1935) demonstrated that calcium ions stimulated the activity of fungal lipases. Several other reports also deal with the stimulatory action of calcium.
on both bacterial and fungal lipases, *Aspergillus niger* (Shipe, 1961 and Iwai *et al.*, 1964b); *Staphylococcus aureus* (O'Leary and Weld, 1964 and Troller and Bozeman, 1970); *Leptospira* species (Patel *et al.*, 1964); *Rhizopus* species (Ota *et al.*, 1969) and *Lactobacillus brevis* (Chander *et al.*, 1973).

According to Desmuelle *et al.* (1960), calcium ions play a double role in activating lipase activity as well as in the removal of liberated fatty acids.

Shah and Wilson (1965) found that *Staphylococcus aureus* lipase had an absolute requirement for a fatty acid acceptor like calcium, when the acyl moiety of triglycerides was insoluble in water. The substrates used in this study were coconut and olive oils. Ota and Yamada (1967) reported that lipolysis by *Candida paralipolytica* was enhanced by the addition of calcium and bile salts. The above salts promoted alignment of the enzyme to the substrate molecule and its subsequent trans-conformation at the interface.

The inability of calcium ions to stimulate the activity of microbial lipases has also been reported by a few workers. Khan *et al.* (1967), Eitenmiller *et al.* (1970) and Shen *et al.* (1975) have independently shown that calcium was not very helpful in stimulating the activity of lipases
from Achromobacter lipolyticum, Penicillium roqueforti and 
Eremothecium ashbyii.

Apart from calcium ions, several other metallic 
salts have also been known to significantly affect the 
activity of microbial lipases.

Oi et al. (1969) observed that two lipase fractions 
from Rhizopus were activated by metallic ions like Fe++, 
Mg++ and Na+. The stimulatory action of Mg++ and Mn++ 
has also been reported by Bitenmiller et al. (1970) in 
case of Penicillium roqueforti lipase, where Na+ had no 
appreciable effect. Similar results on stimulation by 
Mg++ ions on lipase activity have been recorded by Chander 
et al. (1973) in L. brevis.

In a detailed study on leptospiral lipase, Patel 
et al. (1964) showed that the enzyme activity was not 
affected by Hg+, Cu++, Co++ and Fe++. The inhibitory 
action of Co++ has been demonstrated in Aspergillus niger 
(Iwai et al., 1964b) and Rhizopus lipases (Oi et al., 
1969), while lipases from other organisms like Propionibacterium 
shermanii (Oterholm et al., 1970); Penicillium roqueforti 
(Bitenniller et al., 1970) and L. brevis (Chander et al., 
1973) are said to be inhibited by Hg+ and Ag+. The inhibi-
itory action of Ni++ has been recorded in Aspergillus 
niger lipase (Iwai et al., 1964b).
Motai et al. (1966) showed that Torulopsis lipase was inhibited by Hg⁺, Cu⁺⁺, Cd⁺⁺, Ag⁺, Fe⁺⁺ and Fe⁺⁺⁺. The enzyme activity was also inhibited by cyanogen bromide, N-bromosuccinimide, iodoacetic acid, bromoacetic acid, diazobenzene sulphonic acid and sodium lauryl sulphate. Cu⁺⁺ also inhibited the activity of Rhizopus lipase (Oj et al., 1969). The inhibitory action of Fe⁺⁺⁺ ion has also been demonstrated for lipases obtained from Rhizopus species (Oj et al., 1969) and Aspergillus niger (Iwai et al., 1970).

Lawrence et al. (1966) reported that oxidation of fatty acids by Penicillium roqueforti lipase was considerably retarded by sodium azide, 2-4 dinitrophenol and some divalent metallic ions.

Vadehra (1974) showed that Staphylococcus aureus lipase was inhibited by formaldehyde, mercaptoethanol, cysteine and glutathione, while hydrogen peroxide stimulated enzyme activity.

There appears to be some divergence of opinion in regard to the action of sulphydryl blocking agents on microbial lipases. Fiore and Nord (1950) observed that SH-blocking agents like iodoacetate and potassium ferricyanide did not affect activity of fungal lipases. Similarly, several
workers, notably, Patel et al. (1964), Somkuti et al. (1969) and Osterholm et al. (1970) showed that sulfhydryl blocking agents had little or no inhibitory effect on lipases produced by Leptospira, Mucor pusillus and Propionibacterium shermanii. Contrary to the above observations, Oi et al. (1969) showed that Penicillium crustosum lipase was sensitive to the SH-blocking agent, PCMB.

The group binding reagent like diisopropyl fluorophosphate inhibited the activity of Mucor pusillus (Somkuti et al., 1969) and Corynebacterium acnes lipases (Fulton Jr. 1973), but low concentrations of DFP were without any significant effect on the activity of Pseudomonas and Micrococcus lipases (Lawrence et al., 1967a). Recently, Pablo et al. (1974) has shown that Corynebacterium acnes lipase was very effectively inhibited by DFP, while Staphylococcus epidermidis lipase was not at all affected by the above inhibitor.

Chandan et al. (1961) reported that antibiotics like pimarcin, mycostatin and penicillin inhibited both intra- and extracellular lipases obtained from Aspergillus niger, Penicillium roqueforti, Achromobacter lipolyticum and Geotrichum candidum. The inhibitory as well as stimulatory action of some antibiotics have been demonstrated in Staphylococcus aureus lipase. Vadehra (1974)
showed that streptomycin and chlorotetracycline stimulated the activity of above lipase, while penicillin and oxytetracycline were inhibitory.