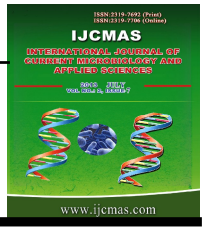
Int.J.Curr.Microbiol.App.Sci (2013) 2(7): 135-146

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 7 (2013) pp. 135-146**
http://www.ijcmas.com



Review Article

Microbial Esterases: An overview

Kulkarni Sayali[1], Patil Sadichha[2] and Satpute Surekha[1*]

Department of Microbiology, Sinhgad College of Science, Ambegaon Bk, Pune-411041, Maharashtra, India
***Corresponding author e-mail:** surekharose1@gmail.com*

A B S T R A C T

Esterases (EC 3.1.1.x.) are the enzymes of hydrolase class involved in catalysis of cleavage and formation of ester bonds. Esterases are involved in interesterification, intraesterification and transesterification reactions. Various strategies for screening and identification are employed for micro-organisms producing esterases. Different purification steps are followed for obtaining the maximum purity of esterase. The industrial applications of esterases provide an immense contribution to the eco-friendly approaches towards nature as also in the food, textile industries. Current review is focused on various aspects namely the screening process, purification protocols, for studying esterase enzyme. A highlight on its potential applications in diverse fields is also presented in the current review. We aim to provide a complete information on esterase enzyme to study in detail. It is important to note that, esterases are less explored enzymes having low literature survey as compared to lipases so the future scope of research is highly valuable.

Keywords

Esterase; Microbial Source; Purification; Potential industrial applications.

Introduction

Enzymes were known to mankind since the late 17th century where the digestion of meat in humans was thought to occur using stomach secretions. The term 'enzyme' [in Greek, enyzyme = in leaven] was used for first time by German physiologist Wilhelm Künhe. Further in 1897, Eduard Buchner, named the enzyme that bought about fermentation of sucrose as 'zymase'. Following Buchner's example, the enzymes are usually named according to reactions they carry out.

Esterases belong to hydrolases class of enzymes which catalyze the cleavage and formation of ester bonds. The enzyme commission number assigned to esterase is E.C 3.1.1.x where x depends on the substrate (Bornscheuer, 2002). Esterases can catalyze three types of reactions: esterification, interesterification and transesterification reactions with very good chemo-, regio- and/or enantioselectivity (Gupta *et al.,* 2012).

Esterases do not require cofactors and this property makes them attractive biocatalysts (Godinho *et al.,* 2011). The two major classes of hydrolases are esterases and lipases. Both these classes share certain common features but also have some unique properties. The 3-dimensional structure of both shows characteristic α/β hydrolase fold (Bornscheuer, 2002; Faiz *et al.,* 2007). Both the enzymes contain a catalytic triad of Ser-Asp-His (Bornscheuer, 2002; Khalameyzer *et al.,* 1999; Kim *et al.,* 2005).

The serine is mostly found embedded in the consensus sequence G-X-S-X-G (where X is any amino acid) at the active site while other reported motifs include GDSL, GDXD (Gupta *et al.,* 2012). Esterases hydrolyze short chain carboxylic acids (C≤12) while lipases hydrolyze insoluble long chain (C≥12) triglycerides and secondary alcohols (Faiz *et al.,* 2007; Kim *et al.,* 2005, Meghji *et al.,* 1990). Lipases can also be distinguished from esterases by a pH dependent electrostatic 'signature': the active site of lipases shows a negative potential at pH 8.0 while esterases show a similar pattern typically at pH 6.0 (Bornscheuer, 2002). Overall enzymatic activity is given the figure 1.

A study indicated that the enzymes have higher stability in ionic liquids relative to organic solvents. This is advantageous because the properties of ionic liquids such as negligible vapour pressure, high thermal and chemical stability will be useful in potential industrial applications (Persson and Bornscheuer, 2003). Reports also suggest that esterases might be directly involved in the process of bacterial sporulation (Higerd, 1977). Esterases can also be used in identification and nomenclature of bacterial strains. This was shown for identification and naming of *Propionibacterium freudenreichii* subsp. *Freudenreichii* because of their esterase patterns (Dupuis *et al.,* 1993). In last few years, there is an increase in the use of enzymes in industrial processes due to development of immobilization of whole cells. Certain advantages conferred by whole cell immobilization include reduction in cost required for enzyme purification, greater resistance to environmental changes as well as greater operational stability (Faiz *et al.,* 2007).

Source of Esterase

Esterases have been isolated from plants, animals and micro-organisms. Microbial enzymes are attractive because the cost to grow and maintain them is less and they are easy to manipulate. All classes of microorganisms like bacteria, fungi, actinomycetes produce esterases- either constitutively or it is inducible. Different microbial sources for esterase production are usually incurred from culture collections or they can be isolated. Organisms isolated from cheese surface (Gandolfi *et al.,* 2005), oil contaminated area of city garbage (Gupta *et al.,* 2012) or marine squid (Ranjitha *et al.,* 2009) have been used for esterase production. A newer approach for metagenome screening for esterase has been accepted and the source of esterase comes from metagenomic libraries in many cases (Fan *et al.,* 2012; Kim *et al.,* 2005; Li *et al.,* 2008) and more specific activated sludge metagenome (Liu *et al.,* 2009). General protocols followed for isolation and purification of microbial esterase enzyme are summerized in the table 1.

Screening Methods

Different screening methods are available and used for screening and selection of organisms producing esterase.

Table.1 Summary of the different properties of microbial esterases

S. No.	Microbial source	Type of Esterase	Optimal pH	Optimal Temp (°C)	Molecular Weight* (kDa)	Km (µM)	Vmax (µM/min/mg)	pI	Reference
1	*Micropolyspora faeni*	-	7.8-8.0	Upto 60	31	-	-	-	Bannerman and Nicolet,1976
2	*Monascus* sp.	-	3.5	35	-	-	-	-	Chen *et al*, 2011
3	*Bacillus pumilis*	Acetyl xylan esterase	8.0	55	40	1540	360	4.8	Degrassi *et al*, 1998
4	*Anoxybacillus gonensis* A4	-	5.5	60-80	62	176.5	800#	-	Faiz *et al*, 2007
5	Recombinant *Escherichia coli*	Pyrethroid-hydrolyzing enzyme	6.5	Upto 45	36.7	14.1	-	-	Fan *et al*, 2012
6	*Bacillus subtilis*	Acetyl esterase	-	Upto 65	31	-	-	6.4	Higerd and Spizizen, 1973
7	*Bacillus subtilis* (RRL 1789)	-	8.0	37	52	-	-	-	Kaiser *et al*, 2006
8	*Pseudomonas fluorescens*	-	7.5	43	44	-	-	-	Khalameyzer *et al*, 1999
9	*Escherichia coli* EPI300 TM-T1^{R}	-	7.0	25	38.3	1000	63.7#	4.8	Kim *et al*, 2005
10	*Aspergillus awamori*	Acetylesterase	7.0	Upto 40	31	-	-	-	Koseki *et al*, 1997
11	Metagenome	Pyrethroid-hydrolyzing	7.0	40	31	0.1-1.41	-	-	Li *et al*, 2008
12	*Halobacillus* sp. strain LY5	-	10.0	50	96	-	-	-	Li *et al*, 2012
13	*Bacillus subtilis* NRRL 365	Carboxyl esterase I	-	-	36	910	-	-	Meghji *et al*, 1990
14	*Bacillus subtilis* NRRL 365	Carboxyl esterase II	-	-	105	670	-	-	Meghji *et al*, 1990
15	*Rhodosporidium toruloides*	Cephalosporin esterase	6.0	25	80	51800	7.9	5.6	Politino *et al*, 1997
16	*Vibrio fischeri*	-	7.0	30	37	-	-	-	Ranjitha *et al*, 2009
17	*Aureobasidium pullulans*	Feruloyl esterase	6.7	60-65	210	-	-	6.5	Rumbold *et al*, 2003.
18	*Streptomyces lividans* 66	-	6.0-9.0	45-55	48	-	-	-	Schrempf and Haar, 1995
19	Thermoanaero- bacterium sp. Strain JW/SL-YS485	Acetyl xylan esterase I	7.0	80	195	-	-	-	Shao and Wiegel, 1995
20	Thermoanaero- bacterium sp. Strain JW/SL-YS485	Acetyl xylan esterase II	7.5	84	106	-	-	-	Shao and Wiegel, 1995

Figure.1 Representative diagram of mechanism of action of esterase catalysis. (Diagram constructed based on information from Bornscheuer (2002)

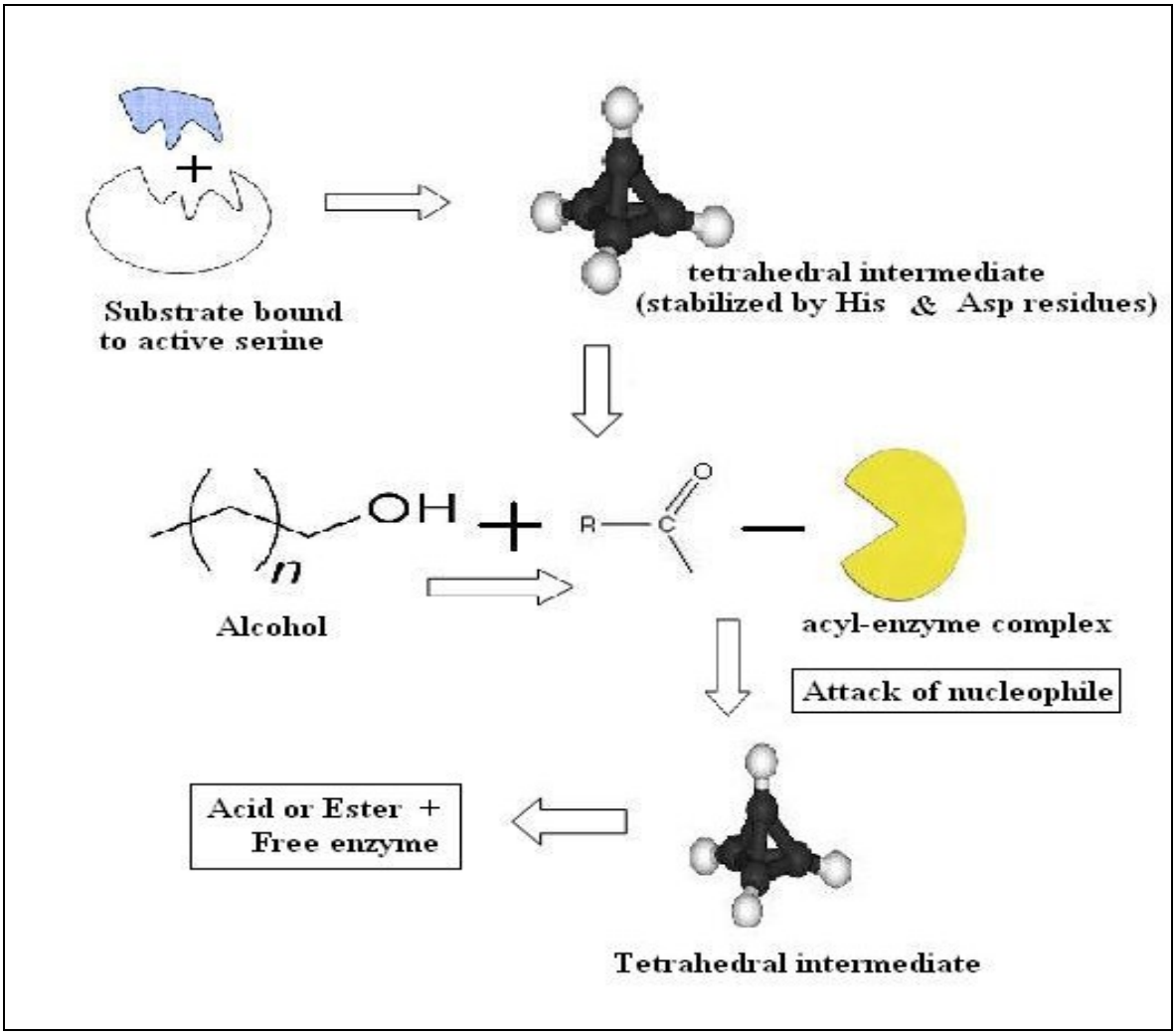


Plate assay method

This is the most commonly used assay where different substrates can be used in Luria-Bertani agar. Esterase producing organisms produce clear zones around the colony after 24-48hrs of incubation at 37°C. The different substrates used are ethyl acetate, sodium lactate, tween-20, tween-80, rhodamine olive oil, tributyrin, α- naphthyl acetate. Some additional chemicals can be used to detect the clearance zones. Fluorescent substances like rhodamine B which can be detected under UV radiations, or chemicals like Fast Blue R R can be used to produce a brown coloured product. Even after incubation, plates can be exposed to chemicals like Lugol's iodine solution which helps enhance the clear zones (Faiz *et al.*, 2007; Kim *et al.*, 2005; Gupta *et al.*, 2012).

UV fluorescence method

Method for screening esterase producing microbial colonies within a mixed culture was reported in 1971. The protocol included overlaying the culture with sterile fibre filter saturated with 8.0* 10^{-5}M of 4-MUB (ester of 7-hydroxy-4-methylcoumarin) and incubating it at room temperature for 3mins. After incubation, the glass fibre filter is placed in another Petri plate with same orientation. It is then photographed with UV light (360-365nm). The highly fluorescent 4-MU (7-hydroxy-4-umbelliferone) spots are obtained by colonies producing extracellular esterase (Pancholy and Lynd, 1971).

Preparation of cell fractions

To determine the location of enzyme (i.e whether the enzyme is extracellular, intracellular or membrane-bound) three cell fractions can be prepared. The extracellular fraction is the supernatant obtained after centrifuging the culture broth. The pellet obtained is resuspended in the culture medium and it is lysed using mechanical or chemical means. This is then centrifuged and the supernatant is kept as the intracellular fraction. The pellet obtained is further resuspended in the culture medium and considered as the membrane- bound fraction. The enzyme activity of each fraction is estimated. Highest enzyme activity will indicate the location of enzyme.

Fermentation

The enzyme production can be carried out by any of the three types of fermentations- Submerged fermentation, Solid state fermentation, Slurry state fermentation (Jacob and Prema, 2006; Gupta *et al.*, 2012). All the conditions for fermentation process are optimized and production of enzymes is carried out.

Purification of enzymes

The crude enzyme can be purified using different techniques to obtain maximum purity of the enzyme.

Ammonium sulfate precipitation

Ammonium sulfate precipitation is a method to purify enzymes based on the technique of salting out. Ammonium sulfate is commonly used because of its high solubility in water; it has no adverse effect on enzyme activity as well as it is cheap. The protocol includes addition of increased ammonium sulfate concentration in the protein to be purified. Precipitation is obtained after 24hrs of refrigeration. A better yield is obtained if it is provided with magnetic stirring.

Dialysis

This is a method of protein purification where semi permeable membranes are used. The pore size of these membranes is such that small molecules like salt ions can pass easily whereas the large molecules like proteins remain in the membrane itself. The molecular cut off of the dialysis membrane is an important consideration for purifying the protein of interest. The different membranes which can be used for dialysis are cellulose or cellophane.

Acid hydrolysis

This process includes treating the proteins with acid, usually at high temperature. Most common acid hydrolysis protocols use 6N HCl for 20-24hrs at 110°C. This is an optional step in protein purification and is not used by many researchers.

Chromatography

Preparative chromatography is used to purify sufficient quantities of a substance or further use, rather than analysis. For this physical technique separates complex mixtures in two phases- stationary phase and mobile phase; the separated components can be collected individually. Different chromatographic techniques which are used for protein purification are Column chromatography, Ion exchange chromatography, Hydrophobic interaction chromatography, Reversed phase chromatography and Affinity chromatography.

Effect of different parameters on enzyme activity

pH

Enzyme activity is affected by a change in pH. The optimal pH range is that in which the enzyme shows its maximum activity. Extreme high or low pH may result in complete loss of enzyme activity. The enzymes having their optimal pH in the alkaline or acidic range prove to be of great importance in processes requiring alkaline/acidic pH.

Temperature

The optimum temperature of an enzyme is the temperature at which maximum activity of enzyme is obtained. In most cases, the enzyme starts loosing its activity as the temperature increases. But many industrial processes operate at high temperatures. The thermo stable enzymes play a major role in such processes. The normal range of working temperature is room temperature to 40°C and they are stored below 4°C.

Inhibitors

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition. The various inhibitors of esterase include diisopro- pylphosphofluoride, phenyl-methylsulfonylfluoride, divalent ions like Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+}, Ag^{2+} (Bannerman *et al.,* 1976; Degrassi *et al*, 1998). The metal ions contribute in maintaining the enzyme in active and stable structure by binding to the amino acid residues with negative charge in specific sites (Faiz *et al.,* 2007).

Enzyme assays

The most commonly used method is the use of p-Nitrophenol esters (p- nitrophenyl acetate or p-nitrophenyl butyrate). The reaction mixture contains sodium phosphate buffer, p-NP ester, distilled water and enzyme. It is incubated for 30mins at 30°C followed by taking absorbance at 400nm. One unit of esterase activity is defined as the amount of enzyme producing 1μmol of p-nitrophenol per minute at 30°C (Faiz *et al.,* 2007; Higerd, 1977; Meghji *et al.,* 1990).

Titrimetry

Esterase enzyme can be quantitated using titrimetry method. 1% substrate is titrated against 10mM NaOH. Esterase activity can be calculated using the formula

Esterase activity = Volume of NaOH consumed (ml) * Molarity of NaOH Volume of enzyme (ml) * Rection time (min)

One unit of esterase activity can be defined as the amount of enzyme that liberates 1μmol of fatty acid per minute at 30°C at pH 7 under the assay conditions (Gupta *et al.,* 2012).

Spectrophotometric assay

A spectrophotometric assay for the quantitative determination of esterases was developed by Mastihuba and coworkers. They worked on estimation of feruloyl esterase where 4-nitrophenyl ferulate releases 4-nitrophenol. Advantages of using this method are that it is easy, rapid and accurate. Enzyme activity can be calculated from the slope of the plot absorbance v/s time (Mastihuba *et al.,* 2002).

Saponification

Esterifying power of the ester can be measured using saponification method (Chen *et al.,* 2011)

Enzyme stability

Half-life of enzyme can be calculated from the first order exponential decay of activity.

$A = A_0e^{-kt}$ (where A = residual enzyme activity; A_0 = initial enzyme activity; k = first order deactivation rate constant)

Properties

Physical properties

Isoelectric point

The isoelectric point of proteins is the pH at which the protein molecule has no electrical charge. The pI value can affect the solubility of a molecule at a given pH. The molecules have minimum solubility in water or salt solution and precipitate in solution when the pH corresponds to their pI. The pI of esterase enzyme was calculated using Ampholine Polyacrylamide Gel plate with pH value ranging from 3 to 10 (Degrassi *et al.,* 1998; Higerd and Spizizen, 1973; Kim *et al.,* 2005; Politino *et al.,* 1997; Rumbold *et al.,* 2003).

Circular dichroism

CD (circular dichroism) is the differential absorption of left and right circularly polarized light. UV CD is used to investigate the secondary structure of proteins. Far UV CD can be carried out for esterase structure analysis (Puchart *et al.,* 2006).

Chemical properties

The protein assays can be done using various simple techniques. The estimation of protein content is commonly carried out using the Folin-Lowry method (Bannerman *et al.,* 1976; Dupuis *et al.,* 1993; Faiz *et al.,* 2007; Higerd and Spizizen, 1973; Li *et al.,* 2012; Meghji *et al.,* 1990). Other methods of protein estimation included Bradford method (Persson and Bornscheuer, 2003; Kaiser *et al.,* 2006), Dye-binding method (Puchart *et al.,* 2006) and Spectrophotometric method (Niazi *et al.,* 2001). All these methods use BSA (bovine serum albumin) as standard.

Molecular properties

SDS-PAGE

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis is a technique widely used in determination of molecular weight of proteins. The proteins are separated according to their electrophoretic mobility.

SDS is an anionic detergent used to linearize the proteins as well as to impart a negative charge on the proteins. A molecular marker with known molecular weights is run along with test proteins in order to determine the molecular weight of unknown proteins by comparing the distance travelled by test protein with the known one. The molecular weight of esterase enzyme have been studied by various authors using 4-5% stacking gel and 10-15% resolving gel (Degrassi *et al.,* 1998; Faiz *et al.,* 2007; Kaiser *et al.,* 2006; Kim *et al.,* 2005; Koseki *et al.,* 1997; Li *et al.,* 2012; Meghji *et al.,* 1990; Puchart *et al.,* 2006; Rumbold *et al.,* 2003; Schrempf *et al.,* 1995; Schultheiss *et al.,* 2008). Some researchers use the Native-PAGE for molecular determination. The difference between these two PAGE procedures is the use of SDS. Native-PAGE does not make use of any denaturing agent. The molecular weight of whole protein is obtained in this procedure (Dupuis *et al.,* 1993; Niazi *et al.,* 2001; Politino *et al.,* 1997)

Zymography

It is an electrophoretic technique, based on SDS-PAGE, where a substrate is copolymerized with the polyacrylamide gel, for detection of enzyme activity. This technique has been used for many enzymes such as xylanases, lipases, and chitinases. Zymogram of esterase was illustrated by Reddy *et al.,* (1970).

Plasmid curing

Plasmids confer some unique properties onto the host. Curing of plasmid involves the elimination of plasmid. The process is carried out in order to check whether the esterase encoding gene was present on the plasmid. Niazi and coworkers reported that the gene encoding esterase from Bacillus species is present in the 60kb plasmid (Nazi *et al.,* 2001).

Sequencing

Sequencing of a protein is the determination of order of amino acids in the protein. Esterase sequencing was reported using Automated Edman degradation on pulsed liquid-phase protein sequencer (Degrassi *et al.,* 1998; Kaiser *et al.,* 2006; Koseki *et al.,* 1997; Politino *et al.,* 1997; Rumbold *et al.,* 2003).

Kinetic properties

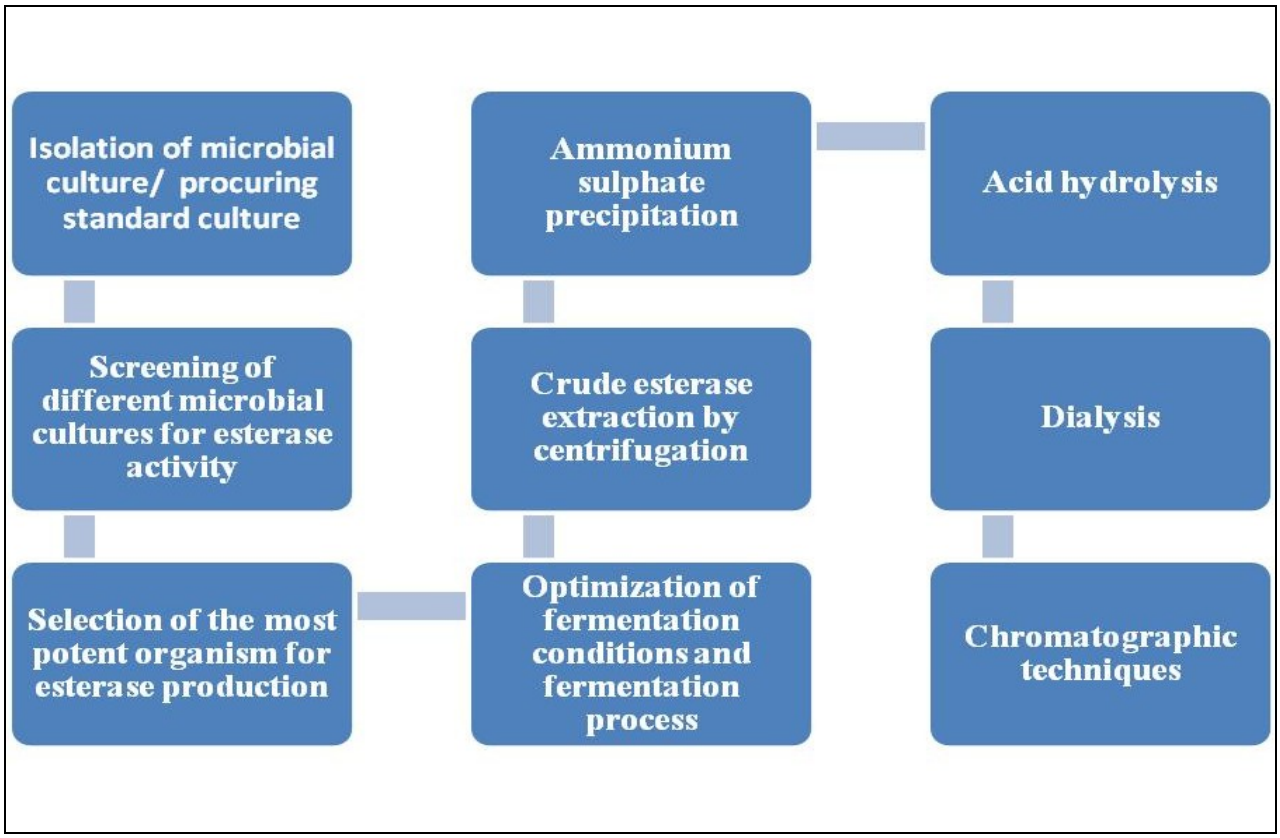
Enzyme kinetics is the measure of the reaction rate catalyzed by that enzyme and the effects of various conditions. The Michealis Menten constant (km), maximum velocity (Vmax), turnover number (kcat) using different subtrates were determined (Degrassi *et al.,* 1998; Faiz *et al.,* 2007; Khalameyzer *et al.,* 1999; Meghji *et al.,* 1990; Politino *et al.,* 1997).

Applications of esterase

Enzymatic degradation of plastic

Plastic degrading microbes belong to genera *Pseudomonas*, *Comamonas* and *Bacillus*. Mechanism of enzymatic degradation of plastic is a 3 step process. Firstly, the enzyme secreted by micro-organism attaches to the plastic surface and carries out hydrolytic cleavage of polyethylene. Depolymerization is the second step where complex polymers disintegrate into simple short monomers, dimers. This is followed by the mineralization which is a degradation process where CO_2, H_2O and CH_4 are the end products. Certain esterases have been investigated for plastic degradation.

Figure.2 General protocol followed for isolation and purification of esterase enzyme



Polyurethane esterase from *Comamonas acidovorans* degrades low molecular weight PLA and also ES-PU made up of poly (diethylene adipate) by cleaving the ester bond between polymers (Bhardwaj *et al.*, 2012).

Ester synthesis

Esterases are involved in formation of ester bonds. The enzymatic synthesis of esters is catalyzed by esterases. Gandolfi and workers carried out the ester synthesis using the esterases of non-starter bacteria which were isolated from cheese surface (Gandolfi *et al.*, 2000).

Preparation of optically active compounds

Optically active compounds are the compounds which have the ability to rotate plane-polarized light. Optically pure acid 2S-6 with high chemical yield was produced. The optical rotation was measured on a polarimeter using tetramethylsaline as standard. The enantiomeric excess (e.e) was determined by HPLC analysis (Tombo *et al.*, 1987).

Deinking

Deinking is the process of separation and removal of ink from the printed material. Enzymatic deinking is gaining more and

more importance because of its high efficiency and low effect on quality of the resulting paper. Different enzymes like cellulases, xylanases, esterases, lipases and lignolytic enzymes are used for enzymatic deinking process (Bolanča and Bolanča, 2004).

References

Bannerman, E. N., and Nicolet, J. 1976. Isolation and characterization of an enzyme with esterase activity from *Micropolyspora faeni.* Appl. Environ. Microbiol. 32(1):138.

Beate von der Haar., and Schrempf, H. 1995. Purification and characterization of a novel extracellular *Streptomyces lividans* 66 enzyme inactivating fusidic acid. J Bacteriol. 177(1):152-155.

Bhardwaj, H., R.Gupta and Tiwari, A. 2012 Microbial population associated with plastic degradation. Scientific reports. 1(5).

Bolanča, I., and Bolanča, Z. 2004. Chemical and enzymatic deinking flotation of digital prints. 4th International DAAAM conference "Industrial Engineering – Innovation as competitive edge for SME". 29 - 30th April, 2004, Tallinn, Estonia.pp. 173-176

Bornscheuer, U. T., 2002. Microbial carboxyl esterases: classification, properties and application in biocatalysis. FEMS Microbiol Rev. 26:73-81.

Chen, M. B., H. Liu, D. Zhen and Fang, S. 2011. Research on the esterification property of esterase produced by *Monascus* sp. Afr. J. Biotechnol. 10(18):5166-5172.

Degrassi, G., B.C. Okeke, C.V. Bruschi and Venturi, V. 1998. Purification and characterization of an acetyl xylan esterase from *Bacillus pumilis*. Appl. Environ. Microbiol. 64(2):789-792.

Dherbécourt, J., H. Falentin, S. Canaan and Thierry, A. 2008. A genomic search approach to identify esterases in *Propionibacterium freudenreichii* involved in the formation of flavour in emmental cheese. Microbial Cell Factories. 7(16).

Dupuis, C., C. Corre and Boyaval, P. 1993. Lipase and esterase activities of *Propionibacterium freudenreichii* subsp. *Freudenreichii.* Appl. Environ. Microbiol. 59(12):4004-4009.

Faiz, Ö., A. Colak, N. Saglam, S. Çanakçi and Beldüz, A. O. 2007. Determination and characterization of thermostable esterolytic activity from a novel thermophilic bacterium *Anoxybacillus gonensis* A4. J. Biochem. Mol. Biol. 40(4):588-594.

Fan, X., X. Liu, R. Huang and Liu, Y. 2012. Identification and characterization of a novel thermostable pyrethroid-hydrolyzing enzyme isolated through metagenomic approach. Micro. Cell Factor. 11(33).

Fazary, A. E., and Ju, Y. 2007. Feruloyl esterases as biotechnological tools: Current and future perspectives. Acta Biochim Biophys Sin. 39(11):811-828.

Gandolfi, R., F. Gaspari, L. Franzetti and Molinari, F. 2000. Hydrolytic and synthetic activities of esterases and lipases of non-starter bacteria isolated from cheese surface. Ann. Microbiol. 50:183-189.

Godinh, L. F., C.R. Reis, P.G. Tepper, G.J. Poelarends and Quax, W. J. 2011. Discovery of an *Escherichia coli* esterase with high activity and enantioselectivity towards 1, 2-O-Isopropylideneglycerol esters. Appl. Environ. Microbiol. 77(17):6094-6099.

Hespell, R. B., O'Bryan and Shah. P. J. 1988 Esterase activities in *Butyrivibrio*

fibrisolvens strains. Appl. Environ. Microbiol. 54(8):1917-1922.

Higerd, T. B., 1977. Isolation of acetyl esterase mutants of *Bacillus subtilis* 168. J Bacteriol. 129(2):973-977.

Higerd, T. B., and Spizizen, J. 1973. Isolation of two acetyl esterases from extracts of *Bacillus subtilis*. J Bacteriol. 114(3):1184-1192.

Jacob, N., and Prema, P. 2006. Influence of mode of fermentation on production of polygalacturonase by a novel strain of *Streptomyces lydicus*. Food Technol. Biotechnol. 44(2):263-267.

Kaiser, P., C. Raina, R. Parshad, S. Johri, V. Verma , K.I. Andrabi and Qazi, G. N. 2006. A novel esterase from *Bacillus subtilis* (RRL 1789): purification and characterization of the enzyme. Protein Expre. Purificat. 45:262-268.

Khalameyzer, V., I. Fischer, U.T. Bornscheuer and Altenbuchner, J. 1999. Screening, nucleotide sequence and biochemical characterization of an esterase from *Pseudomonas fluorescens* with high activity towards lactones. Appl. Environ. Microbiol. 65(2):477-482.

Kim, Y. J., G.S. Choi, S.B. Kim, G.S. Yoon, Y.S. Kim and Ryu, Y. W. 2005. Screening and characterization of a novel esterase from a metagenomic library. Protein Expre. Purificat.

Koseki, T., S. Furuse, K. Iwano, H. Sakai and Matsuzawa, H. 1997. An *Aspergillus awamori* acetylesterase: purification of the enzyme, and cloning and sequencing of the gene. Biochem J. 326:485-490.

Kumar, D., L. Kumar, S. Nagar, C. Raina, R. Parshad and Gupta, V. K. 2012. Isolation, production and application of lipase/esterase from *Bacillus* sp. strain DVL43. J. Microbiol. Biotech. Res. 2(4):521-528.

Kumar, D., L. Kumar, S. Nagar, C. Raina, R. Parshad and Gupta, V. K. 2012. Screening, isolation and production of lipase/esterase producing *Bacillus* sp. strain DVL2 and its potential evaluation in esterification and resolution reactions. J. Microbiol. Biotech. Res. 4(4):1763-1770.

Li, G., K. Wang and Liu, Y. H. 2008. Molecular cloning and characterization of a novel pyrethroid-hydrolyzing esterase originating from the metagenome. Microbial Cell Factories. 7(38).

Li, X., H.Y. Yu and Lin, Y. F. 2012. Purification and characterization of an extracellular esterase from a moderately halophilic bacterium, *Halobacillus* sp. strain LY5. Afr. J. Biotechnol. 11(23):6327-6334.

Mastihuba, V., L. Kremnicky, M. Mastihubová, J.L. Willett and Côté, 2002. A spectrophotometric assay for feruloyl esterases. Analytical Biochemistry. 309:96-101.

Meghji, K., O.P. Ward and Araujo, A. 1990. Production, purification and properties of extracellular carboxyl esterases from *Bacillus subtilis* NRRL 365. Appl. Environ. Microbiol. 56(12):3735-3740.

Niazi, J. H., D.T. Prasad and Karegoudar, T. B. 2001. Initial degradation of dimethylphthalate by esterases from *Bacillus* species. FEMS Microbiol. Lett. 196:201-205.

Pancholy, S. K., and Lynd, J. Q. 197.) Microbial esterase detection with ultraviolet fluorescence. Appl. Microbiol. 22(5):939-941.

Persson, M., and Bornscheuer, U. T. 2003 Increased stability of an esterase from *Bacillus stearothermophilus* in ionic liquids as compared to organic solvents. Journal of Molecular Catalysis B: Enzymatic. 22:21-27.

Politino, M., S.M. Tonzi, W.V. Burnett, G. Romancik and Usher J J (1997) Purification and characterization of a cephalosporin esterase from *Rhodosporidium toruloides*. Appl. Environ. Microbiol. 63(12):4807-4811.

Puchart, V., F. Geriépy, Shareck and Dupont, C. 2006. Identification of catalytically important amino acid residues of *Streptomyces lividans* acetylxylan esterase A from carbohydrate esterase family 4. Biochimica et Biophysica Acta. 1764:263-274.

Ranjitha, P., E.S. Karthy and Mohankumar, A. 2009. Purification and partial characterization of esterase from marine *Vibrio fischeri*. Mod. Appl. Sci. 3(6).

Reddy, M. C., R.C. Lindsay and Montgomery, M. W. 1970. Ester production by *Pseudomonas fragi*. Appl. Microbiol. 20(4):555-557.

Rumbold, K., P. Biely, M. Mastihubová, M. Gudelj, G. Gübitz, K.H. Robra and Prior, B. A. 2003. Purification and properties of a feruloyl esterase involved in lignocellulose degradation by *Aureobasidium pullulans*. Appl. Environ. Microbiol. 69(9):5622-5626.

Schultheiss, E., S. Weiss, E. Winterer, R. Maas, E. Heinzle and Jose, J. 2008. Esterase autodisplay: enzyme engineering and whole cell activity determination in microplates with pH sensors. Appl. Environ. Microbiol. 74(15):4782-4791.

Shao, W., and Wiegel, J. 1995. Purification and characterization of two thermostable acetyl xylan esterases from *Thermoanaerobacterium* sp. strain JW/SL-YS485. Appl. Environ. Microbiol. 61(2):729-733.

Szwajgier, D., J. Pielecki and Targoński, Z. 2005. The release of ferulic acid and feruloylated oligosaccharides during wort and beer production. J. Inst. Brew. 111(4):372-379.

Tombo, G. M. R., H.P. Schär and Ghisalba, O. 1987. Application of Microbes and microbial esterases to the preparation of optically active N-acetylindoline-2-carboxylic acid. Agric. Biol. Chem. 51(7):1833-1838.

Zhang, T., W.J. Han and Liu, Z. P. 2009. Gene cloning and characterization of a novel esterase from activated sludge metagenome. Micro. Cell. Facto. 8:67.