



Microbial lipases and their applications – a review

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Abstract

This review focuses on the key aspects of lipases. Lipases (EC 3.1.1.3) are triacylglycerol acylhydrolases that act on carboxylic ester bonds. They breakdown triacylglycerides into glycerides (diglycerides or monoglycerides), fatty acids and glycerol. Their mass ranges from 19 kDa for *B. stratosphericus* to 92 kDa for *P. gessardii*. Their optimum temperature and pH ranges from 15 °C to 80 °C for *Acinetobacter* sp. and *Janibacter* sp. and 5 to 11 for *P. gessardii* and *E. faecium* respectively. Lipases chemo-, regio-, and enantio- specific features make them first choice of enzymes in research. Their kinetics for substrate hydrolysis depends on different esters. Mostly lipases are extracellular. Type 1 secretory system (T1SS) and Type 2 secretory system (T2SS) are involved in secreting lipases to external medium. They are found in eukaryotes and prokaryotes including animals, plants and microorganisms. Moreover, bacterial and fungal enzymes have diverse industrial applications in food, health, pharmaceutical, medical, textile, detergent, cosmetic and paper industries. Genetic engineering is employed to improve the properties of lipases. Their increasing demand in market has made them a hot topic in scientific research. Scientists are trying to discover novel lipase producing microorganisms due to their expanding commercial value.

Keywords: Lipases, esterification, transesterification, biochemical and physicochemical properties, recombinant DNA technology

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Abbreviations:

T1SS, Type 1 secretory system; T2SS, Type 2 secretory system; HSL, Homoserine sensitive lipase; p-NP, para-nitrophenyl; SDS-PAGE, Polyacrylamide gel electrophoresis; EDTA, Ethylenediaminetetraacetic acid; kDa, kilodalton; V_{max}, maximum velocity; DEAE, diethylaminoethanol; PUFA, polyunsaturated fatty acids; DNA, Deoxyribonucleic acid

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1. ENZYMES

1.1 Enzymes and their significance

Enzymes are catalytic agents that speed up the chemical reactions. The concept of enzyme is as old as the formation of yogurt or cheese in human history. Mankind is using variety of enzymes since centuries. In this decade, nearly 4000 enzymes are reported out of which only 200 enzymes are in commercial use ¹. According to previous literature, nearly 75 % of industrial enzymes possess hydrolytic properties and they are of microbial origin ^{1,2,3,4}. Microbial enzymes are biological catalysts which are used as a catalyst in any reaction. They are preferred over chemical catalysts as they are highly specific, economic, easier to produce and eco-friendly ¹. The significance can be estimated by their diverse industrial applications including industrial, research, therapeutics, cosmetics, dairy industries etc. ⁵. The aims and objectives of this review included overview of microbial lipases, their biochemical, physicochemical and kinetic properties, cellular location, classification, production, screening, modification, mode of action and applications in different industries.

1.2 Benefits of using microorganisms as a biocatalyst

The properties like specificity, stability, ease of genetic manipulation and enhanced production in shorter period of time, growth in inexpensive growth medium and growth at any time in year make microbial enzymes preferable over animal or plant enzymes ⁶. Following are the benefits of using microbial enzymes as catalyst ^{1,7}.

1. Microbial enzymes work at mild physical conditions including temperature, pH, and oxygen availability.
2. As they are eco-friendly and consumed up during a chemical reaction, they are preferred over synthetic chemicals or compounds which may get transformed to more toxic form at the end of reactions.
3. They are highly specific in nature. By employing microbial enzymes at work, expensive downstream processes can be avoided.
4. Due to immobilization of enzymes, they can be reused several times.
5. They are also involved in treating waste composed of harmful solvents etc.

6. They are biodegradable.

2. MICROBIAL ENZYMES

Enzymes from bacteria, fungi, yeasts are previously reported^{8,9}. Of all naturally occurring microorganisms, only 2 % have been checked for enzyme production. To date, bacteria share more market as compared to fungi or yeast^{1,10}. As compared to fungal enzymes, bacterial enzymes are usually thermostable and neutral or basic in nature. Moreover, due to simple nutritional requirements, short generation time and screening procedures these microscopic creatures are preferred over higher organisms. By the help of recombinant technology, genetic manipulation in bacteria helped to generate enzymes that can perform biotransformation over wide range of temperature or pH and increase their yield many times as compared to the wild type strain¹.

Types of microbial enzymes

Microorganisms produce variety of enzymes. For example, amylase, cellulases, proteases, phytases, xylanases, phosphatases, lipases, etc.⁸.

3. LIPASES

In 1848, Claude Bernard observed that pancreas produced a substance that could emulsify and saponify neutral fats ("*ferment emulsify et saponifiant*")¹¹. This substance was later identified as pancreatic lipase enzyme. For long time, animal lipases served the market. The increased demand and supply shortage lead to discover microbial lipases¹².

Carboxylesterases is a class of enzymes comprised of esterases and lipases which are differentiated on the basis of substrate specificity. According to previous knowledge, esterase was active on short acyl chain esters in aqueous solutions whereas lipase was active on water insoluble triacylglycerols¹³. Based on amino acid sequence homology, another classification was proposed. According to it, carboxylesterases are divided into three families (i) LPL family which includes hepatic and pancreatic lipases, lipoprotein lipase (ii) EST family (cholinesterase and lipases from *Geotrichum candidum* and *Candida rugosa*) and (iii) HSL (homoserine sensitive lipase) which includes mammalian and bacterial lipases¹⁴.

Lipases are enzymes that are responsible for breaking the fats into fatty acids and glycerol^{2, 16} (Fig. 1). They are triacyl glycerol acylhydrolases (EC 3.1.1.3). They are class of enzymes consisting of carboxyl-esterases capable of catalyzing hydrolysis as well as synthesis of long chain triglycerides¹¹. The term long chain usually means 10 carbon atoms with triglyceride being the standard substrate¹. They are serine hydrolases due to which they do not require any cofactor and because their active site (Ser-Asp (Glu)-His) is similar to serine proteases^{1,17,18}. The natural substrate of lipases is triacyl glycerol which is very less soluble in water. They carry out reactions at interface of aqueous and non-aqueous media because of their tolerance to high temperatures, pH, solvents and ability to utilize many substrates¹. The molecular weights of lipases range from 30-50 kDa on SDS-PAGE. Inducers of lipases include Ca^{2+} , whereas EDTA acts as inhibitor².

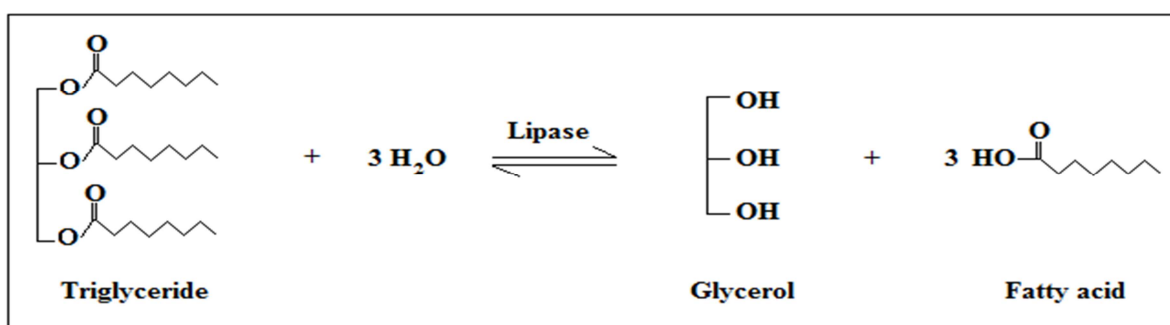


Fig. 1: General reaction showing lipase activity on triglycerol yielding glycerol and fatty acid¹⁵.

3.1 Biochemistry and physicochemical properties of lipases

The biochemical and physicochemical properties of lipases are affected by various factors like pH, temperature, metal ions, solvents and substrates, etc.¹⁹. The mass of bacterial lipase ranges from 19 kDa to 92 kDa for *B. stratosphericus*²⁰ to *P. gessardii*²¹. The optimum pH ranges from 5 for *P. gessardii* to 11 *E. faecium*²². The optimum temperature ranges from 15 °C for *Acinetobacter* sp. XMZ-26²³ to 80 °C for *Janibacter* sp. R02²⁴. Generally lipases are stable in organic solvents. The cofactors are not required for lipase activity but divalent cations as calcium often enhance its activity. Based on substrate activity lipases can be non-specific, region-specific and fatty-acid specific¹⁹. A detail account about biochemical¹⁹ and physicochemical²⁵ properties of bacterial lipases are well reported previously^{19,25}.

3.2 Kinetic properties of lipases

Bacterial lipases are responsible for hydrolysis of para-nitrophenyl (p-NP) esters having C2 to C16 (p-NP acetate to p-NP palmitate) in their fatty acid chain. Kinetics of lipases for substrate hydrolysis depends on different esters. It is determined by Michaelis constant (Km) that is substrate concentration at which the rate of reaction is half of the maximum rate (Vmax). Vmax is the maximum rate when an enzyme is fully saturated with substrate concentration²⁶. A detail account of kinetic properties of bacterial lipases was reported previously²⁵.

3.3 Cellular location of lipases

According to Sangeetha et al.¹² lipases can be intracellular, extracellular or membrane bound. Some bacteria produce only intracellular lipase e.g. *B. clusii*²⁷. Such bacterial species can grow only on simple lipids or glycerol but not on long chain triglycerides. *Bacillus* sp. could produce both intracellular and extracellular lipases²⁸. The extracellular lipase was reported by Boekema et al.²⁹. Two types of secretory systems are involved in secreting lipase to external medium; Type 1 secretory system (T1SS) and Type 2 secretory system (T2SS). T1SS is made up of three proteins subunits which constitute energy driven exporter complex. T2SS is made up of two components Sec-dependent pathway (general protein secretion) and Tat-dependent (twin-arginine translocation) pathway. It is previously reported that bacterial lipases are secreted in periplasmic spaces in unfolded form by Sec-dependent pathway. The folding takes place in periplasmic space by the help of chaperone known as lipase-specific foldase (Lif). Finally the folded form of lipases are transported to outside the periplasmic space in the extracellular growth medium by Tat-dependent pathway^{12,30,31}.

3.4 Sources of lipases

Animals: They can be extracted from animal's pancreatic gland¹.

Plants: Papaya latex, oat seed, castor seed are plant's lipase sources^{1,32}.

Microorganisms: Microorganisms including bacteria (both Gram positive and Gram negative bacteria), fungi and yeasts are reported to be lipase producers^{1,8}.

3.5 Classification of lipases

Lipases can be classified on the basis of site of production, temperature and pH.

(a) Site of production

On the basis of site of production, lipases can be extracellular or intracellular.

Extracellular lipases: It includes lipase which microbial culture cells excrete out of their cells. Mostly they are extracellular. They can be produced by submerged or solid state fermentation. As extracellularly excreted form is also known as crude form, it can be purified by employing various techniques^{33,34,35}. However, the purification techniques are expensive from economic point of view. The well-known extracellular lipases available in market include Novozyme 435, Lipozyme TLIM and Lipozyme RMIM which are obtained from *Candida antarctica*, *Thermomyces lanuginosus* and *Rhizomucor miehei*, respectively^{1,33}.

Intracellular lipases: Due to high cost of purification, the use of whole cells for extracting lipases is considered more economical for the production of polyesters, biodiesel etc.³⁶. The only demand in the production of intracellular lipases is the choice of solid support to immobilize the cells^{1,37}.

(b) Temperature

On the basis of temperature, lipases can be classified as psychrophilic, mesophilic or thermophilic.

Psychrophilic lipases: Generally, they are recognized as cold adapted lipases. They are present in microorganisms which exist at low temperatures *i.e.* 5°C or below³⁸. Such microorganisms include *Aeromonas hydrophila*³⁹, *Photobacterium lipolyticum*⁴⁰. They have diverse applications in biotechnology and industries¹.

Mesophilic lipases: They include lipases that function optimally at 37 °C. They are reported from *Bacillus* sp.⁹. Mostly mesophilic lipases are stable and work at high temperatures as well.

Thermophilic lipases: They function optimally at higher temperatures. They are reported from *Pyrococcus furiosus* and *Thermotoga* sp.⁴¹, *Bacillus* sp.⁴². Their application in industry is growing rapidly.

(c) pH

On the basis of pH, lipases can be acidophilic, neutral or alkaliphilic.

Acidophilic lipases: These enzyme functions at acidic pH. Such lipases were previously reported from *Acinetobacter radioresistens*⁴³ and *Aspergillus* sp.⁴⁴

Neutral lipases: They work optimally at pH 7. They are reported from *Bacillus* sp.^{9,45}.

Alkaliphilic lipases: These lipases function at alkaline pH. They were isolated from *Serratia rubidaea*⁴⁶, *Bacillus subtilis*⁴⁷, etc. They have major applications in detergent and leather industries⁸.

3.6 Types of microbial lipases

(a) Bacterial lipases

Bacterial lipases can be classified as intracellular or extracellular. The intracellular are called glycoproteins and extracellular are called as lipoproteins. Physical factors that affect intracellular production include temperature, pH, oxygen, nitrogen, carbon, presence of lipids, inorganic salts, etc. The effect of polysaccharides was first reported in 1979⁴⁸. The nature of bacterial lipases is constitutive. They can be inhibited by serine hydrolase inhibitors. Non-specific in substrate specificity and few are thermostable^{2,49}. The famous lipases producers are *Streptomyces* sp., *Alcaligenes* sp., *Arthrobacter*, *Pseudomonas* sp., *Chromobacterium*, and *Achromobacter* sp. Among all these, *Staphylococcus* sp. and *Pseudomonas* sp. are commercially used². The Gram positive and Gram negative lipase producers are given in Tables 1-2.

(b) Fungal lipases

Fungal lipases were in study since 1950s^{2,86,87}. Fungal lipases possess the following properties temperature and pH stability, substrate specificity and activity in organic solvents. The use of batch fermentation and low cost extraction methods make them preferable over bacterial lipases². Famous fungal lipase producers include *Aspergillus* sp., *Candida* sp., *Humicola* sp., *Pichia* sp., *Rhizopus* sp., *Mucor* sp., *Saccharomyces* sp., *Geotrichum* sp., *Penicillium* sp. Among these, *Rhizopus* sp. is well known for the conversion of triglycerides to monoglycerides and inter-esterification reaction of fats and oils. *Mucor* sp. is famous for thermostable extracellular lipase. *Penicillium* sp. is well known for the development of blue cheese flavor². Fungal lipases are given in Table 3.

3.7 Production of lipases

Bacterial lipase can be produced by solid state¹⁰³ or submerged¹⁰⁴ fermentation. Fermentation requires carbon and nitrogen sources. In case of lipase production, lipid is added as carbon source because simple

sugars are not desired carbon source in this case ^{105,106}. Lipase substrates including vegetable oils ¹⁰⁷, Tween 20/80 ¹⁰⁸, hexadecane ²⁹, synthetic glyceride tributyrin, tripalmitin ¹⁰⁹ etc. are considered inducers for lipase production ¹¹⁰. Inducers should be added in optimal amount otherwise lipase repression occurs ¹². Nitrogen sources including peptone etc. also affect lipase production ¹¹¹ but repression is not reported yet ¹². However, addition of surfactant enhances the lipase activity. Because it decreases the surface tension between organic and aqueous phase present in the reaction mixture and increase the rate of emulsification ¹¹². Commonly used surfactants include Triton X-100, Tween 20/80 ¹¹³.

Table 1. Lipase producing Gram positive bacterial strains

Bacterial strains	References
<i>Bacillus cereus</i>	El-Shafei and Rezkallah ⁴²
<i>Bacillus coagulans</i>	El-Shafei and Rezkallah ⁴²
<i>Bacillus stearothermophilus</i>	Kim et al. ⁵⁰
<i>Bacillus</i> sp.	Wang et al. ⁵¹ ; Kambourova et al. ⁵² ; Handelsmann et al. ⁵³ ; Sugihara et al. ⁵⁴ ; Imamura and Kitaura ⁵⁵
<i>Bacillus subtilis</i>	Ruiz et al. ⁵⁶ ; Eggert et al. ⁵⁷
<i>Bacillus thermoleovorans</i>	Rua et al. ⁵⁸
<i>Bacillus thermocatenuatus</i>	Lee et al. ⁵⁹
<i>Staphylococcus aureus</i>	Gotz et al. ⁶⁰ ; Simons et al. ⁶¹
<i>Staphylococcus haemolyticus</i>	Oh et al. ⁶²
<i>Staphylococcus warneri</i>	Talon et al. ⁶³
<i>Staphylococcus xylosus</i>	Mosbah et al. ⁶⁴

Table 2. Lipase producing Gram negative bacterial strains

Bacterial strains	References
<i>Acinetobacter baylyi</i>	Uttatree et al. ⁶⁵ , Uttatree and Charoenpanich ⁶⁶
<i>Acinetobacter radioresistens</i>	Chen et al. ⁶⁷
<i>Acinetobacter junii</i>	Anbu et al. ⁶⁸
<i>Aeromonas hydrophila</i>	Pemberton et al. ⁶⁹
<i>Aeromonas sobria</i>	Lotrakul and Dharmsthiti ⁷⁰
<i>Aeromonas</i> sp.	Lee et al. ³⁹
<i>Burkholderia</i> sp.	Yuan et al. ⁷¹
<i>Enterococcus faecalis</i>	Kar et al. ⁷²
<i>Lactobacillus plantarm</i>	Lopes et al. ⁷³
<i>Photobacterium lipolyticum</i>	Ryu et al. ⁴⁰
<i>Pseudoalteromonas</i> sp.	Zeng et al. ⁷⁴
<i>Pseudomonas aeruginosa</i>	Sharon et al. ⁷⁵ , Mobarak-Qamsari et al. ⁷⁶
<i>Pseudomonas alcaligenes</i>	Chen et al. ⁶⁷
<i>Pseudomonas cepacia</i>	Kaieda et al. ⁷⁷
<i>Pseudomonas fragi</i>	Nishio et al. ⁷⁸
<i>Pseudomonas mendocina</i>	Chen et al. ⁶⁷
<i>Pseudomonas</i> sp.	Buisman et al. ⁷⁹ , Rajmohan et al. ⁸⁰ , Feller and Gerday ⁸¹
<i>Pseudomonas fluorescens</i>	Kojima et al. ⁸² , Rajmohan et al. ⁸⁰
<i>Psychrobacter</i> sp.	Zeng et al. ⁷⁴
<i>Pyrococcus furiosus</i>	Adams et al. ⁸³ , Fischer et al. ⁸⁴
<i>Thermotoga</i> sp.	Adams et al. ⁸³ , Fischer et al. ⁸⁴
<i>Yersinia enterocolitica</i>	Glogauer et al. ⁸⁵

Screening of lipase production

Lipase can be screened by various methods including plate detection, calorimetric or spectrophotometric methods¹². In plate detection method results are depicted either by observing clear halo/ zones^{110,114} or change in color of medium due to pH variation^{109,115}. Lipase break down lipids into fatty acids which decreases the pH of the medium thus causes change in color of the medium. Chromogenic substrates include Rhodamine B¹¹⁶, phenol red, Victoria blue^{109,115} etc. Calorimetric methods measure complex formation between released free fatty acids and a divalent metal ion most commonly copper¹⁰⁹. Other commonly used methods include chromatographic, titrimetric, fluorimetric, turbidimetric, immunological, radioactive assays, etc.¹¹⁷. The spectrophotometric methods detect p-nitrophenyl esters of fatty acids. The presence of lipase releases p-nitrophenol which is detected at specific wavelength¹¹⁸.

Table 3. Fungal lipases

Fungal strains	References
<i>Aspergillus niger</i>	Namboodiri and Chattopadhaya ⁸⁸
<i>Aspergillus oryzae</i>	Toida et al. ⁸⁹
<i>Botrytis cinerea</i>	Commenil et al. ⁹⁰
<i>Candida antartica</i>	Robles-Medina et al. ⁹¹
<i>Candida cylindracea</i>	Muralidhar et al. ⁹²
<i>Candida rugosa</i>	Jaeger and Reetz ⁹³
<i>Fusarium solani</i>	Knight et al. ⁹⁴
<i>Geotrichum candidum</i>	Buisman et al. ⁷⁹
<i>Geotrichum sp.</i>	Lotrakul and Dharmsthiti ⁷⁰
<i>Humicola lanuginose</i>	Buisman et al. ⁷⁹ , Chen et al. ⁶⁷
<i>Penicillium roqueforti</i>	Alford and Pierce ³⁸
<i>Penicillium cyclopium</i>	Chahinian et al. ⁹⁵ , Ibrik et al. ⁹⁶
<i>Penicillium simplicissimum</i>	Sztajer et al. ⁹⁷
<i>Pichia burtonii</i>	Sugihara et al. ⁹⁸
<i>Rhizomucor miehei</i>	Robles-Medina et al. ⁹¹ , Pabai et al. ⁹⁹
<i>Streptomyces flavogriseus</i>	Mostafa and Ali ¹⁰⁰
<i>Thermomyces lanuginosus</i>	Chen et al. ⁶⁷ , Robles-Medina et al. ⁹¹
<i>Trisporon asteroides</i>	Dharmasthiti and Ammaraanond ¹⁰¹
<i>Trisporon laibacchi</i>	Liu et al. ¹⁰²

Purification

In order to obtain a pure product, downstream processing of fermentation process is very important¹¹⁹. As bacterial lipases are mostly extracellular, its purification is easy but expensive. A detail account was published by Sangeetha et al.¹². The purification involves combination of physical and biochemical methods. Physical method involves ammonium sulphate precipitation¹¹⁶, ultrafiltration¹¹⁷, precipitation using ice cold organic solvents¹⁰⁴. Biochemical methods involve combination of chromatography for obtaining high purity enzyme^{120,121}. Commonly employed chromatographic techniques include ion exchange and column chromatography using Sephadex, DEAE etc.¹².

3.8 Modification of bacterial lipases

Lipase tailoring for desired properties is always a hot topic for genetic engineers. Most commonly used methods for lipase engineering include site directed mutagenesis, UV and gamma rays irradiation, chemical modifications, amino acid tailoring and immobilization, temperature tolerance, surface hydrophobicity, protein activity, stability in organic solvents and enhanced production²⁵. The application of recombinant

DNA technology is possible after having detailed knowledge about structure and function of lipases, choice of expression system and genetics modifications including cloning and sequencing of lipase genes¹².

3.9 Mode of action of lipases

Lipases perform only catabolic activities; they are not involved in any anabolic process *in vivo*². In the presence of water, they catalyze hydrolysis of ester bonds at interphase between insoluble substrate phase and aqueous phase where enzyme remains in dissolved form. It mostly occurs naturally. It is the basis of many industrial applications and kinetic analysis of the reaction⁴⁹. In the absence of water, they result in esterification and formation of glycerides from fatty acids. Esterification occurs in laboratory conditions².

4. APPLICATIONS OF LIPASES

They have gain popularity due to their diverse applications which ranges from oleo-chemistry to nutrition. Due to their increasing demand, they have been isolated from variety of source including animals, plant and microorganisms^{49,122}. Their vast applications (Fig. 2) on industrial level have made them standing in the front line². Lipases are considered special types of esterases with vast application in industry. Their mode of reaction is they act on fats or oil, and break down into glycerides and fatty acids and finally into glycerol and fatty acids. Lipases can be generated from various sources, on this basis their enzyme specificity vary. They have the ability to break short-chain fatty acids to long-chains, saturated to unsaturated fatty acids. Short chain fatty acids include C2, C4 to C10. Unsaturated fatty acids include oleic, linoleic and linoleic fatty acids. Saturated fatty acids include triglycerides. For triglycerides, lipases show positional specificity and attack fatty acids of position 1 or 3, not 2. The only exception is lipase of *Goetrichum* sp. In case of breakdown of fatty acid at oil-water interface, the amount of oil available at interface determines the activity of the enzyme. It can be enhanced by altering the physical conditions as agitation, addition of emulsifiers, etc.^{2,123,124,125}. The industrial applications of lipases fat breaking, transesterification, development of different flavors, detergents^{49,126}. Lipases originated from plant¹²⁷, animals^{128,129} and microorganisms^{123,124,125,126} are well documented. Microbial lipases are usually preferred because of properties like easy extraction procedure and unlimited supply². Lipases have wide range of applications including production of chemicals, pharmaceuticals, food industry, production of polymers, etc.^{2,130,131}. Their vast applications owe to their enantio-selective and regio- selective and chemo-specific nature^{1,94}. These properties like regio-, enantio-, and chemo-specific features have made them on the top scientific research list^{1,8,132}.

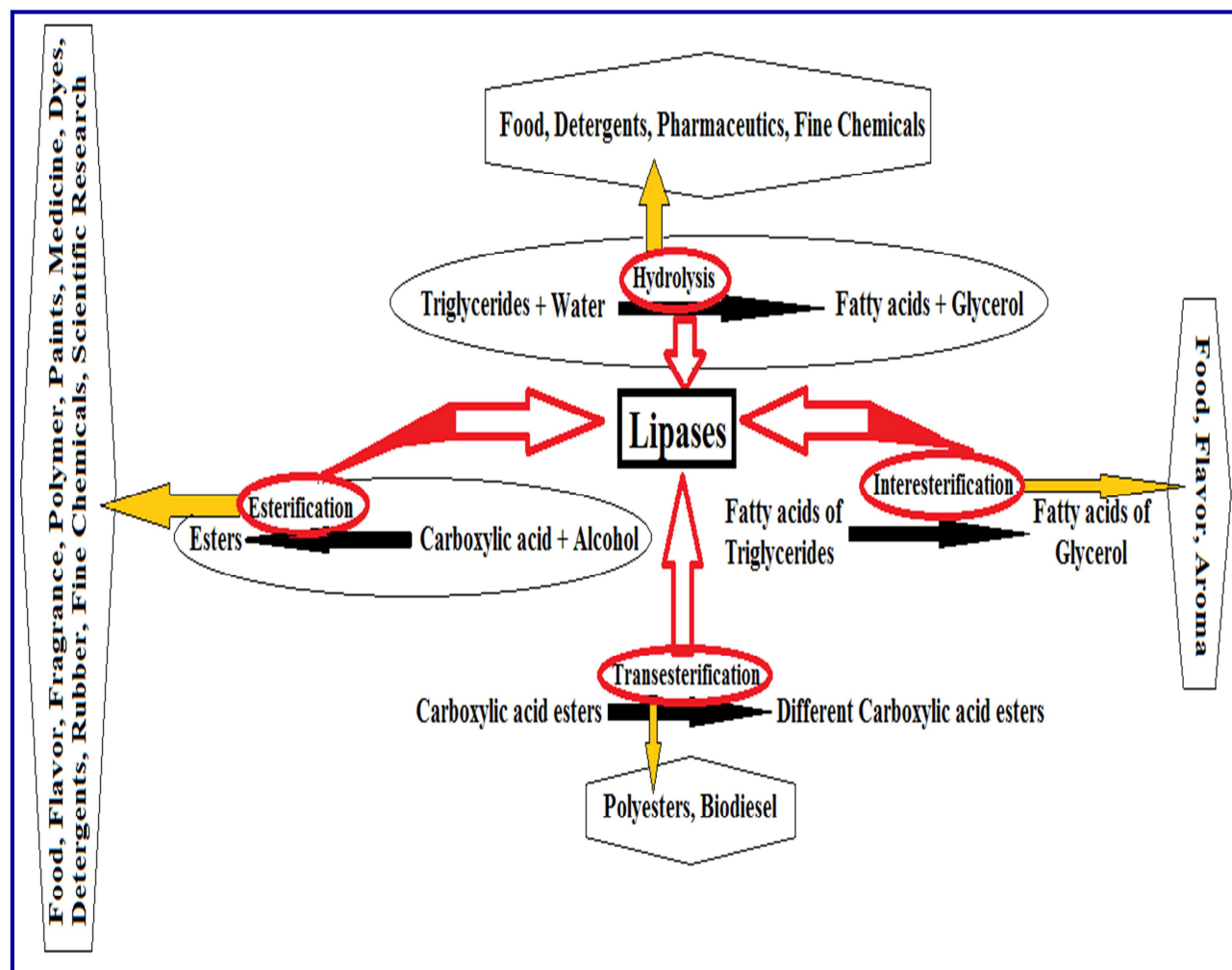


Fig. 2: Overview of applications of lipases

4.1 Food industry

(a) Baking

Lipases are used in baking industry to obtain better crust, increase loaf volume, increase shelf life of breads and controlled non-enzymatic browning. All these features are claimed by Bio-Cat Inc., Enzyme Industry, Troy, VA. in commercial lipase they have introduced in baking industry. On the other hand, another Italian company Millbo S.P.A marketed M300LF lipases which offer characteristic features to improve the quality of breads. Microbial sources of lipase for baking industry are reported from *C. cylindracea*, *A. niger* and *R. oryzae*⁸.

(b) Tea

For preparation of tea, leaves of tea are subjected to fermentation after drying and breaking mechanism, which releases volatile compounds, that gives characteristic aroma and flavor of tea. The basic principle is the breakdown of membrane lipids that are present in the tea leaves. For tea processing, lipase obtained from *Rhizomucor meihei* is in use^{8,133}.

(c) Oil and fat industry

This industry is considered a thrilling one, as production of new kinds of oils and fats are desired products from consumer point of view¹. For example, cocoa butter fat remained high in demand as it is used in the manufacture of chocolate and different varieties of chocolates ranging from soft to hard chocolates. The

significance of fat in industry is obvious by the fact that supply of cocoa butter does not meet its required demand. The basic principle is lipase catalysed transesterification of cheaper oils. Vegetable oils are used for producing substitutes for cocoa butter¹³⁴, human milk fat, polyunsaturated fatty acids (PUFA), production of biodiesels, etc.¹³⁵. The significance of PUFA can be observed with the facts that it holds increasing demand in pharmaceuticals, nutraceuticals and food additives¹. *Rhizopus miehei* lipase is immobilized for transesterification reactions that replace stearic acid with palmitic acid during palm oil processing. Pabai et al.⁹⁹ discussed lipase based interesterification of butter fat, vegetable oils including sunflower oil, corn oil, peanut oil, olive oil, soyabean oil containing omega-3 polyunsaturated fatty acids. Animal and plant lipids like tuna oil, borage oil, menhaden oil etc. are processed by microbial lipases to obtain PUFA¹.

(d) Flavor development

Cheese industry: Lipolysis is lipid breakdown of butter fat and cream into cheese like products. A term is used for cheese manufactured by using lipases called Enzyme Modified Cheese (EMC). EMC is a cheese in which milk is incubated with enzyme at elevated temperature and is used for preparing sauces, dips, snacks, soups etc.². Cheese tailoring to improve taste, aroma and overall quality has been in research even before the knowledge of science. Commercially available cultures belong to *Mucor meihei* (Piccnate, Gist-Brocades: Palatase M, Novo Nordisk), *A. niger*, *A. oryzae* (Palatase M, Novo Nordisk; Lipase AP, Amano; Flavour AGE, Chr. Hansen)⁸. Blue cheese flavor improvement is due to lipases of *Pecillium roqueforti*¹³⁶.

Dairy industry: The major purpose of using lipase in dairy industry is to hydrolyze fat milk. The applications of lipase in this industry include cheese ripening and cheese flavoring. Synthesis of esters of short chain fatty acids and alcohols are responsible for improving flavor and fragrance of food^{8,137}. Improvement in milk whiteners is done to produce creamy flavor and buttery textures of toffees and caramel¹³⁸. Better flavored alcoholic beverage can be obtained by employing *Candida utilis*¹³⁹. Hydrolysis of triglycerides to get free fatty acids has practical application in enhancing the flavor of dairy products like butter, cheese, margarine, bakery products, alcoholic beverages, milk chocolates and sweets⁹⁴. Other important areas includes sausage manufacture⁸, refining rice flavor, modification of soya-bean milk and improve in aroma of apple wine^{1,8,140}. According to Buisman et al.⁷⁹, lipases from *Geotrichum candidum*, *Pseudomonas* sp., *H. lanuhinosa*, *C. antartica* (CAL-B), *C. cylindracea* AY30 are immobilized for esterification of functional phenolics for synthesis of lipophilic antioxidants in sunflower oil¹. Bio-lipolysis is the removal of fat from fish meat to produce leaner meat. It is used widely in markets⁸.

4.2 Health industry

(a) Pharmaceutical and medicinal industry

Lipases play a very significant role in pharmaceutical and medicine industries. Lipase originated reactions including interesterification, transesterification, enantioselective, etc. greatly helped in selective acetylation and deacetylation^{1,141} which is the core of pharmaceutical industry for producing drugs and their derivatives. The production of digestive aid using lipases is reported earlier^{1,131}. Pharmaceutical industries also demand the production of emulsifiers in form of monoglycerides which is required as precursor for many reactions¹⁴². Other major applications of lipases in pharmaceutical industry include synthesis of cardiovascular drug diltiazem^{143,144}, β -blockers, steroids, prostaglandins, production of artificial sweetener sucralose¹⁴⁵ and polyfunctional organic compounds for AIDS treatment². A drug used to lower the cholesterol level named as lovastatin is obtained from *Candida rugosa* is already marketed^{1,144}. The lipase of *C. antartica* has been used in the production of anti-Alzheimer drug⁸. Baclofen, which is pain and muscle relaxant, is obtained from *C. cylindracea*⁹³. Similarly, antitumor and immunosuppressive agent 15-deoxyspergualin is synthesized by using lipase. An anti-cholesterol drug (BMS-188494) is synthesized by employing lipase of *G. candidum*⁸.

(b) Cosmetic industry

Lipases as emollient in the skin care products as skin cream, sun tan creams and bath oils has already been launched by Unichem International ². For emollient production, *Rhizomucor miehei* lipase is widely used as a biocatalyst. This lipase has substituted acid catalyst lowering the cost of downstream processing. Wax esters in skin care industry are being prepared by *C. cylindracea* in batch fermentation. Another important cosmetic product is cocoa butter which is a triglyceride mixture. It is also obtained by employing 1,3-specific lipases. It is commercialized by Unilever and Fuji Oil ². The silhouette appearance is dream of every lady which is claimed to be achieved by using anti-obese cream or anti-obese food supplement ¹⁴⁶. The work in this context was already started in 1970s ¹⁴⁷. Hair waving industry also uses lipase ^{1,8,148}.

(c) Diagnostic tool

Lipases have another important application in the detection of presence of infections that may lead to disease. For example, detection of lipases in blood serum for diagnosing medical conditions linked acute pancreatitis, pancreatic injury, etc. ¹⁴⁹. Although conventional medical detection methods are still in use but kit method to determine serum lipase and amylase are used ¹⁵⁰. The application of microbial lipases obtained from *Propionibacterium acnes*¹⁵¹, *Corynebacterium acnes* and *Staphylococcus aureus*¹⁵² for detection of skin diseases is well known ⁸.

4.3 Environmental applications

(a) Biodiesel

It is the production of fuels by employing microbial lipase. The basic principle of biodiesel production is transesterification of fats with short chain alcohols in the presence of catalyst (lipase) ¹⁵³. Transesterification is similar to hydrolysis with exception of displacement of alcohol for water. Here alcohol is replaced with another alcohol ¹⁵⁴. Short chain alcohols include ethanol, methanol, etc. To be more precise, methanol is used due to its low cost and physical chemical properties. In 1980s, optimization of biodiesel production was started. For this, scientists tried to optimize the reaction of triglycerides to alcohol, catalysts, reaction temperature, time, contents of free fatty acids and water in oils and fats ¹⁵⁵. The shortcomings in this area include removal of catalyst, wastewater treatment, methanol evaporation, removal of saponified products, neutralization and concentration, glycerol recovery, difficulty in purifying glycerol, etc. All these problems were overcome by using microbial lipase although cost is major hindrance. It can be overcome by using whole cell as biocatalyst and using immobilization technique ¹⁵⁶. For effective biodiesel production, 90-95 % high methyl ester content is required. For biodiesel production using microbial lipase, following microorganisms were studied; *Pseudomonas cepacia*⁷⁸ and *Candida antarctica*¹⁵⁷. These microorganisms exhibit high methyl esters of more than 95 %. However, research is carried out in this field. Biodiesel production using microbial lipases is not yet commercialized ¹.

(b) Biodegradable compounds production

Due to pollution, production of biodegradable compounds is desired. Lipases play an important role in this area. Trimethylpropane esters were synthesized as biodegradable lubricants. Hasan et al. ⁸ reported that 1-butyl oleate synthesis involved direct esterification to decrease the viscosity of biodiesel in winter use. Synthesis of biodegradable polyesters is based on production of esters using transesterification reactions in organic solvent systems. Aromatic polyesters by employing lipases were reported earlier ^{1,158,159}.

(c) Biosensors

For preparation of biosensor, lipase catalysed degradation of biodegradable polymer film *i.e.* poly(trimethylene) succinate was developed. The major application of this biosensor was detection of concentration of lipase enzyme, and disposable immunosensors ¹⁶⁰. Another application was detection of complementary nucleic acid based on hybridization by using radiolabeled polynucleotide probes. Recently radiolabeled isotopes are being avoided due to environmental exposure problem and enzyme labeled probes was introduced. Among various sources of lipases, fungus wins its name in this market ¹⁶¹. Another

way of constructing biosensors was to immobilize the lipase on pH/ oxygen electrodes along with glucose oxidase which later on termed as lipid biosensors¹⁶². They have practical applications in detecting blood cholesterol level¹⁶³ and triglycerides levels¹⁶⁴. Lipase activity can also be used as indicator of fresh degradation of oils and hydrocarbons^{8,165}.

(d) Biodegradation/ Bioremediation

The significance of lipases in degradation of alkanes and hydrocarbons is well reported. Following bacterial species are involved in the degradation of n-alkanes; *P. putida* GPo1 alkB, *Acinetobacter* sp. alkM, *Rhodococcus* sp. alkB1, alkB2; *P. putida* xylE (aromatic hydrocarbons); *P. putida* ndoB, *Mycobacterium* sp. strain PYR-1 nidA (polycyclic aromatic hydrocarbons)¹⁶⁵. The bacteria responsible for bio-augmentation is previously reported¹⁶⁶. Fungal species involved in oil degradation can be used for oil bioremediation^{8,167}.

(e) Waste water treatment

C. rugosa has practical application in cleaning the environment by hydrolyzing the fats components of wastewater in septic tanks, sewage treatment, grease traps, etc.¹⁵⁸. The industries like poultry waste, leather, food processing, oil processing, etc. discharge fats and oils in their effluents. For this purpose, lipase of *P. aeruginosa* LP602 was found effective treatment of such effluents¹⁶⁸. Immobilized lipase is found suitable for wastewater treatment plants¹⁶⁹. Oasis Environmental Ltd. WW07P formulated and claimed a product for biological treatment of wastewater⁸.

4.4 Miscellaneous applications

(a) Oleochemical industry

The application of lipases in soap industry can be estimated from the fact that 60 million tons per annum is produced and marketed. Out of it, 2 million tons per annum consumes high energy processes including hydrolysis, glycerolysis and acidolysis. The chemical industries used the advancement in knowledge of chemistry and biology to improve the quality of soaps⁸. It is well known that *C. cylindracea* is used to obtain cheap and high thermostable enzymes. A Japanese company Miyoshi Oil and Fat Co., has commercialized bio-soaps^{2,170} containing lipase of *C. cylindracea*. They claimed to produce a better soap than already produced by Colgate-Emery process⁸.

(b) Emulsifiers

Emulsifiers are used in food formulations as mayonnaises, ice creams, sauces, low fat spreads, etc. Lipases are employed in the production of emulsifiers and surfactants^{2,171,172}.

(c) Agro-based chemicals

The chemical properties of lipases including enantio-, regio- and stereo-selectivity and activity at hydrophilic-lipophilic interface have made them first choice of enzymes to be used in the manufacture of pesticides, insecticides or related -cidal products¹. Herbicide production is based on selective esterification of *S*-isomers in the presence of lipases². The synthesis of enantiopure compounds has previously reported^{173,174}. According to Hasan et al.⁸, *Pseudomonas* sp. plays an important role in the production of chiral compounds which are considered precursors for the production of pesticides, and insecticides. Chemie Linz Co. (Austria) has obtained a license from Massachusetts Institute of Technology, USA to market a phenoxypropionate based herbicide which works on the principle of selective esterification of (*S*)-isomers with butanol catalysed by porcine pancreatic lipase in anhydrous hexane. The *S* or *R* derivative of phenoxypropionic acid can be obtained by (*trans*) esterification or hydrolysis of corresponding esters. This method is adopted by many companies globally for obtaining multi-kilograms of the required product⁸.

(d) Polymer industry

The polymers have wide range of usage as sorbents. Lipases are employed for synthesis of optically active polymer due to property of stereo-selectivity^{2,175}.

(e) Detergent industry

Detergents have wide applications in home, restaurants, hotels, industries, etc. It holds the single biggest market for industrial enzymes. According to Godfrey and West³, about 1000 tons of lipases are consumed only in detergent industry. As lipases breakdown triglycerides into more hydrophilic substance which can be removed easily¹⁷⁶. Although, all detergents contain similar formulation but cleaning power of the detergents can be improved by making blend of lipases, protease, amylase and cellulase¹⁷⁷. Lipase intended to be used in the detergent industries must have following features (i) can withstand harsh conditions of temperature and pH *i.e.*, 30-60 °C and pH 10-11. (ii) able to tolerate other surfactants or enzymes like linear alkyl benzene sulfonates, proteases etc. (iii) low substrate specificity *i.e.*, it can hydrolyze fats of different compositions.

Novo Nordisk's Lipolase was obtained from *Humicola* lipase expressed in *Aspergillus oryzae*². It was not marketed in 1988 as lipase quantity was very less. After application of genetic engineering, enough quantities of lipase won the detergent industry. Lipolase obtained from *T. lanuginosus* was also expressed in *A. oryzae*. The bacterial sources for lipases include *Pseudomonas* sp. more specifically *P. alcaligenes* and *P. mendocina*. The lipase of *Acinetobacter radioresistens* is strongly alkaline as it is stable over wide range of pH *i.e.* 6-10⁷⁷. In 1995, Genencor International, Au-KBC Research Center, Life Sciences, Anna University, Chennai, India introduced Lumafast and Lipomax from *P. mendocina* and *P. alcaligenes* respectively⁸. Other microorganisms that contributed their lipases for detergent industry included *Chromobacterium*¹⁷⁸ and *Candida*¹⁷⁹. Lipases functional in alkaline medium are preferred for detergent uses^{180,181,182}.

According to Feller and Gerday⁸², the market of cold active lipases is demanding due to property of cold washing which is meant to reduce energy consumption and wear and tear of textile fibers. Nakamura and Nasu,¹⁸³ reported about formulation of cold active enzyme in bleaching composition and liquid leather cleaner. During 1990s, scientists reported about lipase applications in toilet cleaners¹⁸⁴, contact lens cleaning¹⁸⁵ and in dry-cleaning solvents¹⁸⁶, drain opener¹⁶⁴. Due to high demand, screening for new lipases with diverse properties and modification of existing lipase through recombinant DNA technology and protein engineering is in process since last three decades^{51,187,188,189}. In order to remove oil from the fabrics, the lipase was immobilized on fabric surface. Such lipase from *Pseudomonas* sp. was patented (Patent No. 6,265,191 issued on July 24, 2001). GatewayProClean Inc. USA marketed solid and liquid forms of detergents to remove fat or similar stains from fabrics^{1,8}.

(f) Fine chemicals industry

For the preparation of fine chemicals; gentle and efficient approach is preferred. It can be achieved by using microbial lipases. They promise satisfactory specificity and great rapidity in mild conditions¹³¹. The application of cold active lipase obtained from *C. antarctica* is in use by various industries like food, pharmaceutical and chemical industries¹.

(g) Textile industry

The importance of lipases in textile industry cannot be ignored. Lipases confer following properties to fabric when used in textile industry; desizing, lubricants and cracks removal and long lasting dyeing properties. According to Rowe *et al.*¹⁹⁰, the combination of amylases and lipases help commercially in denim and cotton fabrics desizing. Polyester is core of heart of textile industries due to softness, stretch resistance, strength, stain resistance, wrinkle resistance, abrasion resistance and washing machine abilities. Polyesterase is a closely related to lipase enzyme that is broadly used in textile industries to modify polyester. Treatment with lipase make polyesterase more susceptible to post modification including improve uptake of chemical compounds like fabric finishing formulations, dyes, cationic compounds, anti-staining compounds, anti-static compounds, anti-microbial compounds, anti-perspirant and anti-deodorant compounds. In addition to polyester fabric, synthetic fibers are also modified by lipases for production of textiles, fabrics, rugs, yarns, and other related items^{1,8,190}.

Commercially speaking, Rakuto Kasei Ltd. is producing enzymes for textiles industry for desizing, stone washing of denim and jeans, enzymatic wash, bio-polishing of knitted goods, improving quality and durability of jeans⁸. Bayer AG (PCT WO 97/43014) also reported the formulation consisting of lipase,

esterase and protease for treatment of polyesteramide improvement. Genencor International Inc. PCT Publication No. 97/33001 patented *Pseudomonas* sp. lipase which can be used to modify absorbance and wetness ability of polyester. Amano Pharmaceutical KK Publication No. JP 5344897 filed a patent about formulation, which modifies the fabric without affecting its strength⁸.

(h) Leather industry

In our routine, leather products are widely used ranging from hats, belts, purses, jackets to shoes. Leather products are prepared from hides and skins of animals. If hides/ skin are treated with conventional chemicals, they release harmful by-products in the environment which is toxic for aquatic and other life forms. On the other hand, lipases offer eco-friendly way of processing the hides/ skin into leather products⁸. First of all, dirt, blood, debris etc. present on hides/skin are removed in water, followed by treatment with proteases which not only remove proteins but also emulsify fats by breaking the cell wall of fat cells. At this stage, they are treated with lipases, which specifically degrade fats and do not damage the leather itself. In this way, fat is removed with minimum environmental impact. For sheep-skins, although chemicals are being used, lipases can also be used. Lipases can also be used to treat bovine hides¹. Maps (India) have commercialized lipases which are working over wide range of pHs. Palkodegrease AL, Lipase offers lipases which are working in acidic conditions. Acidic and alkaline stable lipases are used defatting of animal skin/hides⁸. Degreasing, deliming, bating (removal of hair and fat protein debris associated with hide)^{191,192} are major steps performed by lipases during treatment of skins and hides. Acid active lipases are used to treat skins that were stored in pickled state. Lipases can have following effects on hide etc. cleaner appearance, uniform color, production of waterproof leather, leather for car seats to reduce fogging issue^{1,8}.

Novozyme (Denmark) sells NovoCor ABL and NovoCor ADL which are combinations of an acid lipase and acid protease, having application for acid bating of fur and wool. NovoLime a protease/lipase is mixture of enzyme assisted liming of hides and skins. NovoCor AD is an acid lipase for degreasing hides and skin⁸. *R. nodosus* is used to obtain lipase which has applications in degreasing suede clothing leathers from woolen sheep skins^{1,8,193}.

(i) Paper and pulp industry

Wood is processed to obtain paper and pulp. During this, pitch is produced which is a hydrophobic mixture of triglycerides and waxes. This pitch is undesired as it jams the machine and damages the quality of paper by producing holes and spots⁹³. Here lipase plays an important role by breaking down 90 % triglycerides present in the pitch, into monoglycerides and fatty acids which are less sticky and easily washed with water¹⁹⁴. A Japan-based company, Nippon Paper Industries, uses *C. rugosa* lipase to control pitch. Other benefits that lipases confer to this industry includes deinking properties of paper which generally includes decrease chemical usage, increase whiteness, prolong equipment life, increase pulping rate of pulp, increase whiteness intensity, save energy, reduce pollution and composite cost. Fukuda et al.¹⁹⁵ reported *Pseudomonas* KWI-56 lipase in deinking composition for ethylene oxide-propylene oxide adduct stearate improved whiteness of paper and reduce residual ink spots^{1,8}.

5. CONCLUSIONS

Lipases perform hydrolysis, esterification, transesterification and interesterification which results in fatty acids, glycerol and carboxylic acids esters that are involved in almost every industry ranging from food, flavor, aroma to detergent, rubber, fine chemicals, polymers, paints etc. Metagenomics has opened new era for biotechnologists. The direct cloning of metagenomic DNA can lead to screening and identification of new microbial species. However, development of new techniques for: isolation of desired microbial species and enzymes, cost effective purification and downstream processing, development of time-saving methods and techniques are the need of hour. Recombinant DNA technology, protein engineering and molecular enzymology are the key players for bringing explorations in the existing world of enzymes.

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CONFLICT OF INTEREST

All authors declare no conflict of interest regarding this article.

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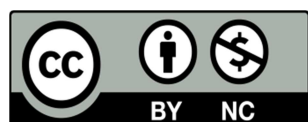
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