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Actinobacteria — A Biofactory of Novel Enzymes

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Abstract

Biocatalysis offers green and clean solutions to chemical processes and is emerging as an effective alternative to chemical technology. The chemical processes are now carried out by biocatalysts (enzymes) which are essential components of all biological systems. However, the utility of enzymes is not naive to us, as they have been a vital part of our lives from immemorial times. Their use in fermentation processes like wine and beer manufacture, vinegar production, and bread making has been practised for several decades. However, a commercial breakthrough happened during the middle of the 20th century with the first commercial protease production. Since then, due to the development of newer industries, the enzyme industry has not only seen a remarkable growth but has also matured with a technology-oriented perspective. Commercially available enzymes are derived from plants, animals, and microorganisms. However, a major fraction of enzymes are chiefly derived from microbes due to their ease of growth, nutritional requirements, and low-cost downstream processing. In addition, enzymes with new physical and physiological characteristics like high productivity, specificity, stability at extreme conditions, low cost of production, and tolerance to inhibitors are always the most sought after properties from an industrial standpoint. To meet the increasing demand of robust, high-turnover, economical, and easily available biocatalysts, research is always channelized for novelty in enzyme or its source or for improvement of existing enzymes by engineering at gene and protein levels. The novel actinobacteria and their industrially important enzymes will assist effective productivity and fulfill the requirements of industries.

Keywords: actinobacteria, extra-cellular enzymes, applications

1. Introduction

Among the microorganisms, actinobacteria are of special interest since they are known to produce chemically diverse compounds with a wide range of biological activities. Actinobac-

teria, the filamentous Gram positive bacteria are primarily saprophytes of the soils, where they contribute notably to the turnover of complex biopolymers such as lignocellulose, hemicellulose, pectin, keratin, and chitin. Undoubtedly, they are also well known as a rich source of antibiotics, enzymes, and other bioactive molecules, and are of considerable importance in pharmaceutical and other industries [1].

The ever-increasing requirement for enzymatic preparations is being met by such classical sources as animal and higher plant tissues, and that has stimulated the search for similar enzymes from the microbial world. The value of microorganisms, including actinobacteria, in the production of enzymes is enhanced by their relatively high yields, cost-efficiency, and susceptibility to genetic manipulation [2]. At present, enzymes of microbial origin are widely used in food processing, detergent manufacturing, the textile and pharmaceutical industries, medical therapy, bioorganic chemistry, and molecular biology. The wide use of enzymes reflects their characteristic specificity of action as biocatalysts. However, enzymes of identical substrate profile produced by different microorganisms may significantly vary in the optimal conditions of their productivity. For this reason, it is necessary to obtain microorganisms which produce enzymes with required substrate specificity, at particular temperature and pH ranges demanded by the production process. The biochemical heterogeneity, ecological diversity, and exceptional capacity of actinobacteria for secondary metabolites production make them an obvious target for enzymes displaying new activities and/or specificities. For many years, actinobacteria are best known as the source of majority of antibiotics. More recently, they have been found to be a promising source of a wide range of industrially important enzymes. Keeping this in mind and recognizing the significance of actinobacteria, especially *Streptomyces* species, as a source of novel enzymes, many studies have focused to understand the diversity of marine actinobacteria and to screen the enzyme-producing ability of actinobacteria occurring in the less explored hypersaline saltpan, mangrove, and marine sediments.

2. Significance of actinobacterial enzymes

Actinobacteria are one of the ubiquitous dominant groups of Gram positive bacteria. Actinobacteria have been commercially exploited for the production of pharmaceuticals, nutraceuticals, enzymes, antitumor agents, enzyme inhibitors, and so forth [3]. These bioactive compounds are of high commercial value, and hence actinobacteria are regularly screened for the production of novel bioactive compounds. A wide array of enzymes applied in biotechnological industries and biomedical fields have been reported from various genera of actinobacteria. Since there is vital information available due to the advent of genome and protein sequencing data, actinobacteria has been continuously screened for the production of proteases, cellulases, chitinases, amylases, xylanases, and other enzymes. The industrial applications of several actinobacterial enzymes are given in Table 1.

Use	Enzyme	Applications
Detergent (laundry and dish wash)	Protease	Protein stain removal
	Amylase	Starch stain removal
	Lipase	Lipid stain removal
	Cellulase	Cleaning, color clarification, anti-redeposition (cotton)
	Mannanase	Mannanan stain removal (reappearing stains)
Starch and fuel	Amylase	Starch liquefaction and saccharification
	Amyloglucosidase	Saccharification
	Pullulanase	Saccharification
	Glucose isomerase	Glucose to fructose conversion
	Cyclodextrin-glycosyltransferase	Cyclodextrin production
Xylanase	Viscosity reduction (fuel and starch)	
Food (including dairy)	Protease	Milk clotting, infant formulas (low allergenic), flavor
	Lipase	Cheese flavor
	Lactase	Lactose removal (milk)
	Pectin methyl esterase	Firming fruit-based products
	Pectinase	Fruit-based products
	Transglutaminase	Modify visco-elastic properties
Baking	Amylase	Bread softness and volume, flour adjustment dough conditioning
	Xylanase	Dough stability and conditioning (<i>in situ</i> emulsifier)
	Lipase	Dough stability and conditioning (<i>in situ</i> emulsifier)
	Phospholipase	Dough strengthening
	Glucose oxidase	Dough strengthening
	Lipoxygenase	Bread whitening
	Protease	Biscuits, cookies
	Transglutaminase	Laminated dough strengths
Animal feed	Phytase	Phytate digestibility – phosphorus release
	Xylanase	Digestibility
	β -Glucanase	Digestibility
	Pectinase	De-pectinization, mashing
Beverage	Amylase	Juice treatment, low calorie beer
	β -Glucanase	Mashing
	Acetolactate decarboxylase	Maturation (beer)
	Laccase	Clarification (juice), flavor (beer), cork stopper treatment
Textile	Cellulase	Denim finishing, cotton softening

Use	Enzyme	Applications
	Amylase	De-sizing
	Pectatelyase	Scouring
	Catalase	Bleach termination
	Laccase	Bleaching
	Peroxidase	Excess dye removal
Pulp and paper	Lipase	Pitch control, contaminant control
	Protease	Biofilm removal
	Amylase	Starch-coating, de-inking, drainage improvement
	Xylanase	Bleach boosting
	Cellulase	De-inking, drainage improvement, fiber modification
Fats and oils	Lipase	Transesterification
	Phospholipase	De-gumming, lyso-lecithin production
Organic synthesis	Lipase	Resolution of chiral alcohols and amides
	Acylase	Synthesis of semisynthetic penicillin
	Nitrilase	Synthesis of enantiopure carboxylic acids
Leather	Protease	Unhearing, bating
	Lipase	De-pickling
Personal care	Amyloglucosidase	Antimicrobial (combined with glucose oxidase)
	Glucose oxidase	Bleaching, antimicrobial
	Peroxidase	Antimicrobial
	L-Asparagine	Antitumor
	Neuraminidase	Antiviral agents
	Aminoacylase	Regulation of urea cycle

Source: Goodfellow (1988).

Table 1. Industrial applications of actinobacterial enzymes

3. Types of actinobacterial enzymes

3.1. Aminoacylase

Aminoacylase (N-acylamino-acid amidohydrolase) catalyzes the hydrolysis of acylated D- or L-amino acids to D- or L-amino acids and an appropriate carboxylic acid: N-acetyl-(D) or (L)-amino acid> carboxylic acid+(D)-or (L)-amino acid (Figure 1). Aminoacylases are interesting and ever-increasing enzymes due to the growing demand in the pharmaceutical industry for optically active amino acids. In enzymology, an aminoacylase is an enzyme that catalyzes the following chemical reaction:

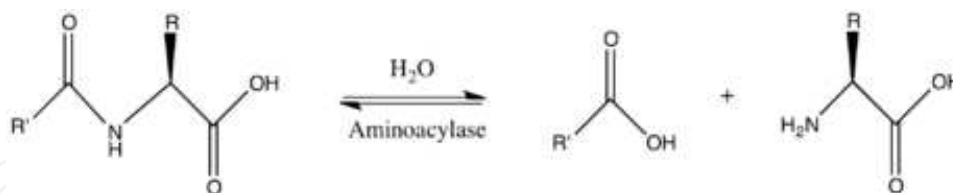


Figure 1. Catalytic reaction of aminoacylase

This enzyme belonged to the family of hydrolases, those acting on carbon-nitrogen bonds other than peptide bonds, specifically in linear amides. This enzyme mainly concerns D-amino acids, both natural and synthetic, such as D-phenylglycine and its derivatives which are used for the production of semisynthetic penicillins and cephalosporins. Phenylglycine obtained synthetically as a racemic mixture can be split into enantiomers by chemical or enzymatic reactions. The latter are usually applied because they are simpler and more efficient. Two methods have been proposed for the isolation of pure enantiomers of D-amino acids using enzymatic hydrolysis of racemic mixtures of their N-acetylated derivatives. In the first method, a stereospecific enzymatic hydrolysis of N-acetyl-DL-amino acids has been used to obtain a mixture of D-amino acid and unaffected N-acetyl-L-amino acid which has to be racemized before its reuse in the process, while in the second method enzymatic cleavage of N-acetyl-L-amino acid, a component of the racemic mixture, results in a mixture of L-amino acid and non-hydrolyzed N-acetyl-D-amino acid. D-amino acid is obtained from the latter as a result of chemical deacylation of the N-acetylated derivative. The second method of obtaining D-amino acids is the one applied in practice. D-aminoacylases are uncommon in microorganisms, though Szwajcer *et al.* [4] reported the occurrence of one such enzyme from *Micrococcus agilis*. Many examples of D-aminoacylases have been found in some species of actinobacteria; 427 strains of *Streptomyces* and 16 strains of *Streptoverticillium* were screened for D-aminoacylases and found only in four species of streptomycetes, namely *S. olivaceus*, *S. roseiscleroticus*, *S. sparsogenes*, and *S. tuius* [5]. All the species produced D-aminoacylase intracellularly when inducers such as D-leucine, D-phenylglycine, D-valine, and their N-acetylated derivatives were supplemented with the production medium [6]. The D-aminoacylases obtained from *S. olivaceus* and *S. tuius* were purified and characterized according to their substrate specificity. Both enzymes were active at pH 7.0 and both were hydrolyzed hydrophobic N-acetyl-D-amino acids rather than hydrophilic amino acids. Extracellular production of both L-aminoacylase and penicillin V amidase has been demonstrated in *Mycobacterium smegmatis* [7] and several strains belonging to the genus *Streptoverticillium* [8]. L-aminoacylase isolated from a *Streptoverticillium* sp. [9] displayed a high hydrolytic activity toward N-acetylated aliphatic and aromatic L-amino acids [10]. Additionally, N-acyl-L-amino acids cannot be used directly as building blocks for proteins and must first be converted to L-amino acids by aminoacylase. Again, the L-amino acid products can be used for biosynthesis or catabolized energy.

3.2. Amylase

Amylase comprises a group of industrial enzymes having approximately 25% of the global enzyme market. Specifically, an extracellular amylase with the ability to digest raw starch has found important applications in bioconversion of starches and starch-based substrates. The level of alpha amylase activity in various fluids of human body is of clinical importance, e.g., in diabetes, pancreatitis, and cancer research, while plant and microbial alpha amylases are used as industrial enzymes. Starch-degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, and textile to paper industries (Table_1). Although amylases can be derived from several sources, such as plants, animals, and microorganisms, the enzymes from microbial sources are generally used to meet industrial demands and have made significant contribution to the production of foods and beverages in the last three decades. The microbial amylases have almost completely replaced the starch hydrolyzing chemicals in starch processing industry.

Like most microorganisms, actinobacteria can also survive in both mesophilic and thermophilic conditions; they have the ability to degrade starch by hydrolysis [15]. The occurrence of amylases in actinobacteria has been a well-known phenomenon since it was established that several representatives of the genera *Nocardia* and *Streptomyces* display amylolytic activity when cultivated on media with maltose [16], although, amylolytic enzymes quite often occur in mesophilic actinobacteria. Unfortunately, only a few of them have been studied in detail, as their enzymes are similar to bacilli amylases which are relatively thermolabile, thus limiting their value in biotechnological processes. On the basis of the literature survey, the more promising amylase producers are the strains of *Streptomyces hygroscopicus* [17], *S. limosus* [18], and *S. praecox* [19] as a result of extensive screening program from among others. To date, amylases from *S. hygroscopicus* and *S. praecox* have been used for the commercial preparation of high-maltose syrups [20]. More attention has been paid to thermostable amylases produced by the thermophilic actinobacteria namely *Thermomonospora curvata* [21] and *T. vulgaris* [22] and by a *Thermoactinomyces* sp. [23; 24]. The amylases from *Thermomonospora* species and *T. vulgaris* are highly active and stable at 60°-70°C and act at slightly acidic and neutral pH values (Table 2) [25].

Enzyme producers	Enzyme	Temperature (°C)	pH	Reference
<i>T. fusca</i> NTU22	α-amylase	60	7.0	Chao-Hsun and Wen-Hsiung, 2007
<i>Streptomyces transformant</i> T3-1	Cellulase	50	6.5	Hung-Der and Kuo-Shu, 2003
<i>Thermoactinomyces</i> sp. HS682	Protease	70	11.0	Tsuchiya <i>et al.</i> , 1991
<i>Streptomyces rimosus</i> R6-554W	Lipase	50	9.0-10.0 (4-10)	Abrami <i>et al.</i> , 1999
<i>Thermomonospora fusca</i>	Xylanase	60-80	7.0 (6-8)	McCarthy <i>et al.</i> , 1995

Table 2. Industrially important enzymes from thermophilic actinobacteria

3.3. β -N-Acetyl-D-Glucosaminidase

β -N-acetyl-D-glucosaminidase (2-acetamido-2-deoxy- β -D-glucoside) is frequently encountered in microorganisms, higher plants, and mammalian tissues. This enzyme splits hydrolytically chitobiose, N,N'-diacetylchitobiose moieties of asparagines-linked oligosaccharides of various glycoprotein and hydrolyzes N-acetyl- β -D-galactosaminidases, yielding oligosaccharide chains from glycoproteins [30]. Thus, it has been found to be very useful for the structural determination of the carbohydrate moiety of several glycoproteins and for studying their biochemical functions and biosynthesis [31]. Generally, actinobacteria producing enzymes are synthesized at extracellular region including endo- β -N-acetyl-D-glucosaminidase H [32] and endo- β -N-acetyl-glucosaminidase L [33] isolated from *Streptomyces griseus* (formerly *S. plicatus*). The enzyme were designated as endo- β -N-acetyl-D-glucosaminidase L [34; 35] and appear to be extremely useful for structural determination of ovoalbumin and several other glycoproteins [30; 36].

3.4. 1, 3- α and 1, 3- β Glucanase

Endo-1, 3- α -D-glucanases (1, 3- α -D-glucan glucanohydrolase) hydrolyzes fragments of polysaccharides that contain consecutive 1, 3-linked α -D-glucosyl residues. Consequently, 1,3- α -D-glucanases are useful in detection of 1,3- α -D-linkages sequences in dextran as well as provide a route for 1,3- α -D-glucans study in fungal cell walls [37; 38]. These enzymes are produced by fungi and bacteria and are quite common among actinobacteria. Therefore, these enzymes may be useful as protective agents for odontological purposes. The presence of mutan-hydrolyzing enzymes was detected in *S. chartreusis* and *S. werraensis* [39]. Endo-1,3- β -D-glucanases (1,3- β -D-glucan glucanohydrolase) occur in bacteria, fungi, higher plants, and actinobacteria. Investigations of 1,3- β glucanases isolated from actinobacteria have been mainly carried out for their ability to degrade the cell walls of yeasts and fungi [40]. Several species of actinobacteria excrete 1, 3- β -glucanases together with chitinases. A laminarinase system consisting of three different types of 1,3- β glucanases and chitinases was isolated from *S. rimosus* [41]. When laminarin (1,3- β glucan) was used as substrate, laminaritriose was obtained as the major product of one type of endo-1,3- β glucanase in addition to oligomeric breakdown products. The second laminarin-degrading (exo-splitting) enzyme yields predominantly laminaribiose. Another exo-1,3- β glucanase liberates glucose but not oligosaccharides from the nonreducing end of laminarin. The mycolase system produced an extracellular complex when the strain was grown on media with crab-shell chitin and fungal mycelia. δ 1,3- β glucanases have also been isolated from *Streptomyces (Actinomyces) cellulosa* [42] and *Streptomyces* sp. [43]. Some of them, such as 1,3- β glucanase isolated from *S. murinus*, are used in wine preparation [44]. The preparations of 1,3- β glucanases have also been used to obtain some saccharides. The enzymatic preparation of laminaribiose (3-O- β gluco-pyranosyl-D-glucose) was achieved by the hydrolysis of curdlan (1,3- β -D-glucose) with the 1,3- β -glucanase system from *Streptomyces* sp. K.27-4 [45]. The hydrolysate obtained consisted mainly of glucose and laminaribiose in an approximate ratio of 1:1 by weight. The application of the yeast *Schizosaccharomyces pombe*, which selectively metabolized all the glucose present in the hydrolysate, resulted in crystalline laminaribiose at 30% of yield.

3.5. Cellulase

Cellulose, which forms almost half of the dry weight of the earth's biomass, is an unbranched polymer consisting of D-glucose units linked by 1,4- β glycosidic bonds. This macromolecule has a complex crystalline structure, is insoluble in water, and is quite resistant to depolymerizing enzymes and chemical reagents. Under natural conditions, cellulose is almost always combined with hemicellulose and lignin [46], which makes its degradation by microorganisms even more difficult.

Investigations on the mechanism of cellulose degradation and its possible applications have been carried out for many years [47]. Recently, the enzymatic hydrolysis of cellulose for D-glucose production has aroused an ever-increasing interest [48; 49]. Cellulose-degrading enzymes are excreted by microorganisms into the surrounding environment and as with most enzymes-degrading biopolymers they constitute a multicomponent lytic complex that acts synergistically on the cellulose. The cellulolytic system consists of three major components: 1,4- β glucan glucanohydrolase acting as endoglucanase, 1,4- β -D-glucan cellobiohydrolase displaying exoglucanase activity, and β -glucosidase, which splits cellobiose. The enzymatic system of cellulases operates synergistically, i.e., endoglucanases make random scissions of the cellulose chain yielding glucose and oligosaccharides; exoglucanases attack the nonreducing end of cellulose forming cellobiose; and finally cellobiases hydrolyze cellobiose to glucose [50-52]. Members of several mesophilic and thermophilic actinobacteria have been studied for their ability to degrade cellulose. *Thermomonospora* species have been found to be highly cellulolytic [51-55]. Cellulases produced by representatives of *Thermomonospora* species are multiple, extracellular exoglucanases and endoglucanases at pH of 6.0 and temperature ranging from 60° to 70°C; they also display considerable heat stability. The mesophilic actinobacteria known to produce cellulolytic complexes include *Streptomyces antibioticus* [56], *S. flavogriseus* [57-62], and *S. viridosporus* [63]. Mesophilic streptomycetes also produce complex cellulases at pH between 5 and 7; they show their highest activity at 40°-55°C (Table 2). Both mesophilic and thermophilic actinobacteria produced cellulolytic complexes when cultivated on media supplemented with powdered cellulose.

3.6. Protease

Proteases, generally classified into exopeptidases (cleave off peptide bonds from the ends of the protein chain) and endopeptidases (cleave peptide bonds within the protein) (Figure 2.), are the major industrial enzymes and fulfill more than 65% of the global market need [64]. These enzymes are extensively used in the food, pharmaceutical, leather, and textile industries [64; 65]. Among the extremophilic sources, thermostable proteases have been reported from certain haloalkaliphilic bacteria and actinobacteria [66; 67]. With the increasing demand of the enzymes, there will be an ever-increasing need for stable biocatalysts capable of withstanding extreme conditions of operation. Proteases generally activate a nucleophile, which will in turn attack the carbon of the peptide bond. The electrons in the carbon-oxygen double bond migrate onto the oxygen as the nucleophile attaches itself. This tetrahedral intermediate is a highly energetic intermediate, and the protease will stabilize this intermediate. The intermediate will then decompose, usually releasing the two peptide fragments.

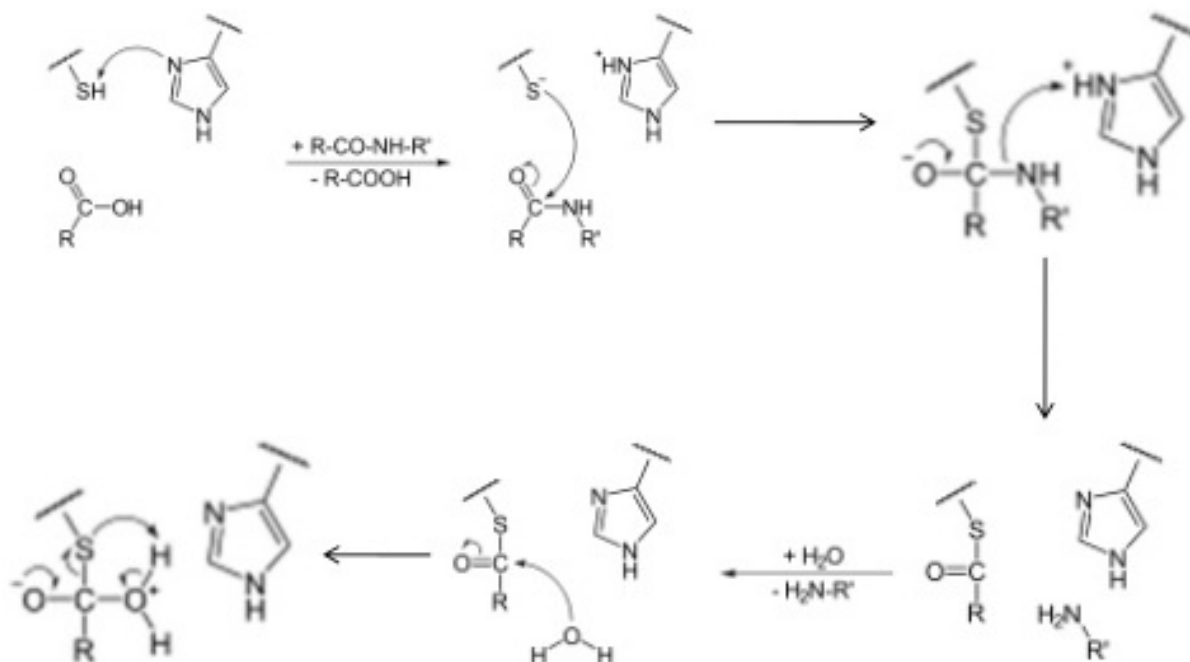


Figure 2. Catalytic mechanism of protease

The ability to produce a variety of proteolytic enzyme is a well-known phenomenon in mesophilic actinobacteria; *Streptomyces* protease including “pronase 7M” (*S. griseus*) and “fradiase 7M” (*S. fradiae*) are commercially useful. While alkaline proteases from bacteria are extensively characterized, similar attention has not been paid to alkaliphilic actinobacteria namely *S. nigellus*, *S. albidoflavus*, and other genus *Nocardiopsis*, *Thermomonospora*, and *Thermoactinomyces* [68].

3.7. Chitinase

Chitin, a polymer occurring in crustaceans, fungi, and insects, is a fibrillar 1,4 linked 2-acetamido-2-deoxy- β -D-glucan with acetyl groups attached to nitrogen to various extents. It is found in three polymeric forms with various degrees of crystallinity. Fully deacetylated chitin is known as chitosane [69]. Enzymatic hydrolysis of chitin, liberating free N-acetyl-D-glucosamine, is caused by the chitinolytic complex which consists of chitinase (poly β -1,4- (2-acetamido-2-deoxy)-D-glucose glycanohydrolase) and chitobiase (β -N-acetyl-D-glucosaminidase) (Figure 3.). As a result of the action of chitinase complex, chitobiose and chitotriose are released. Chitinases are specific to linear polymers of N-acetylglucosamine, but they do not split chitobiose. They hydrolyze chitin to chitobiose and to a lesser extent to chitotriose [70].

Chitinolytic complexes commonly occur in bacteria, fungi, and especially in actinobacteria. The chitinase have been isolated from culture filtrates of *S. griseus* [71], *S. antibioticus* [70], *Amycolatopsis* (*Streptomyces*) *orientalis* [72], and several strains of *Streptomyces* spp. [73-75].

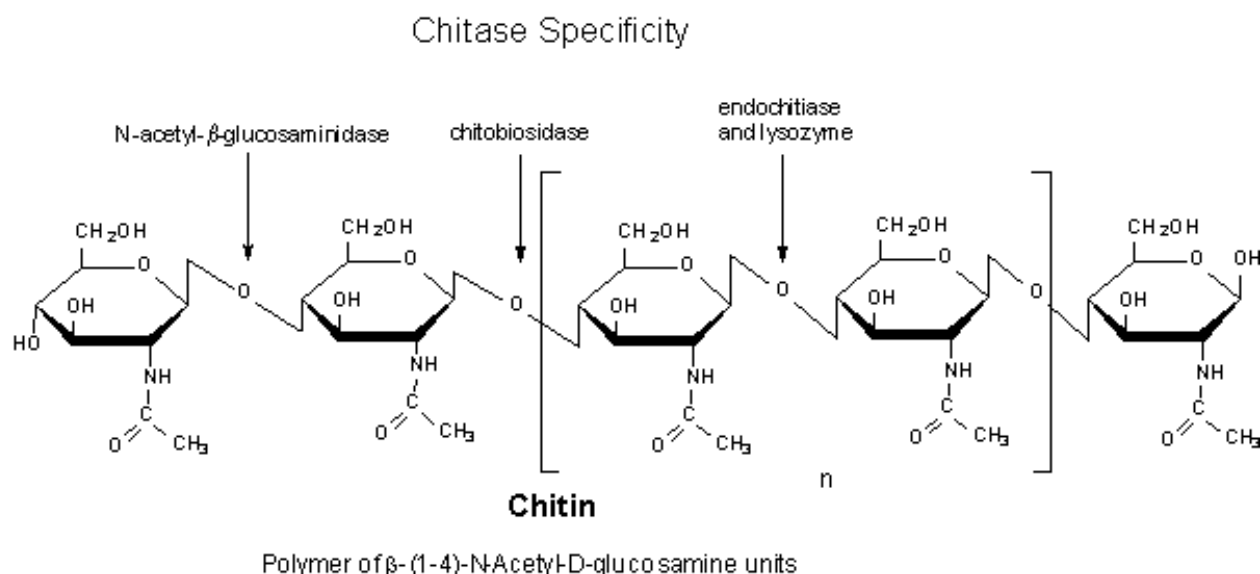


Figure 3. Polymer of β -(1-4)-N-acetyl-D-glucosamine units

Chitinases are produced in abundance where strains are cultivated on chitin-supplemented media. Purified chitinases are more active at pH 5.0 but they are not heat-stable. Their properties have been considered from the view of fungal cell wall degradation [76] and utilization of chitin wastes [77].

3.8. Lipase

Cholesterol esterase, which converts cholesterol esters into free cholesterol, is used in clinical tests for determining the cholesterol level in blood serum [78]. Until now, little is known about the properties of cholesterol esterases. These enzymes differ not only in their optimum pH for production but also in their substrate specificity. For example, cholesterol esterase isolated from *S. lavendulae* had lipolytic activity [79]. A lipase of the arylesterase group able to hydrolyze specifically phthalate esters to a free phthalic acid and simple n-alcohols was isolated from a *Rhodococcus (Nocardia) erythropolis* [80]. The enzyme, which was most active at pH 8.6 and at 42°C, hydrolyzed several phthalate esters and to a lesser extent olive oil and tributyrin. The lipase production by several *Streptomyces* strains was reported by Chakrabarti *et al.* [81] but details were not given on substrate specificity. There is an increasing interest in lipases, especially those which display high stereo-specificity and may be useful for resolution of racemic acids and alcohols applied as chiral substrates in organic synthesis [82]. More attention has been paid to lipases from actinobacterial origin since these microorganisms are known for their ability to produce various secondary metabolites and hence provide a potential source of enzymes with substrate specificity.

3.9. Phospholipases

Phospholipases, the enzymes capable of selective cleavage of ester bonds in glycerophosphatides, occur widely in both animal and plant kingdoms. Because of their high specificity, they

are used for the analysis of phospholipid components of biological membranes as well as for clinical diagnostic tests. Phospholipases are classified into four groups, A, B, C, and D (Table 3). Serum choline phospholipids are hydrolyzed by phospholipase-D and the amount of liberated choline can be estimated quantitatively. Phospholipase-D from streptomycetes has been found useful for the determination of serum choline-phospholipids and in clinical diagnostic tests [83; 84].

Enzyme	Name of the producer	Optimum pH	Leading references
Phospholipase - A ₂	<i>Streptomyces cinnamomeus</i>	7.0	Okawa and Yamaguchi (1976a)
Phospholipase - B	<i>S. hiroshimensis</i>	9.0	Walker and Walker (1975)
Phospholipase - C	<i>S. griseus</i>	7.5	Verma and Khuller (1983)
Phospholipase - D	<i>S. chromofuscus</i>	8.0	Imamura and Horiuti (1979)

Table 3. Practical significance of phospholipases isolated from actinobacteria

3.10. Xylanase

Xylan, a hemicellulose, is composed of 1,4- β -linked D-xylose units that form a linear backbone to which 4-O-methyl-D-glucuronic acid and L-arabinose are attached as side chains (Figure 4). This polymer, which occurs together with cellulose, is degraded by xylanases. Xylanases can be found in large amounts in both microorganisms as well as several invertebrates [85]. Together with other carbohydrases, xylanases play an important role in the degradation of terrestrial biomass [86]. Like cellulases they occur in microorganisms in the form of extracellular complexes, which consist of endo and exoxylanases that differ in substrate specificity. Xylanases produced by mesophilic actinobacteria belong to the endotype (1,4- β -D-xylan xylanohydrolase). They have been isolated and purified from several species of streptomycetes, such as *S. flavogriseus* [67], *S. lividans* [87], and *Streptomyces* sp. [88]. These enzymes were produced by microorganisms grown on media with xylan or its hydrolysates as a carbon source and/or in the presence of nonmetabolizable inducer [89]. The isolated xylanases exhibited their higher activity at pH 5.0-7.0 and at 40-60°C. Little attention has been given to xylanases produced by thermophilic actinobacteria [90; 68]. Thermostable xylanases isolated from *Thermomonospora* strains are heat-stable and most active at 60-70°C and at pH from 5.0 to 8.0. Apart from attempts to apply them to biodegradation of hemicelluloses and xylanases, they have also been used in the food industry for the production of D-xylose. Since this is not assimilated by mammalian organisms, it is used as an artificial sweetener in dietetic preparations [86]. An original method of obtaining xylobiose was developed by Kusakabe *et al.* [91] who prepared a pure xylobiose using a xylan hydrolysate from corncobs and rice straw and xylanase produced by *Streptomyces* sp. E-86. D-xylose, formed during the hydrolysis, was eliminated by the yeast *Candida parapsilosis*, which utilized xylose as a carbon source.

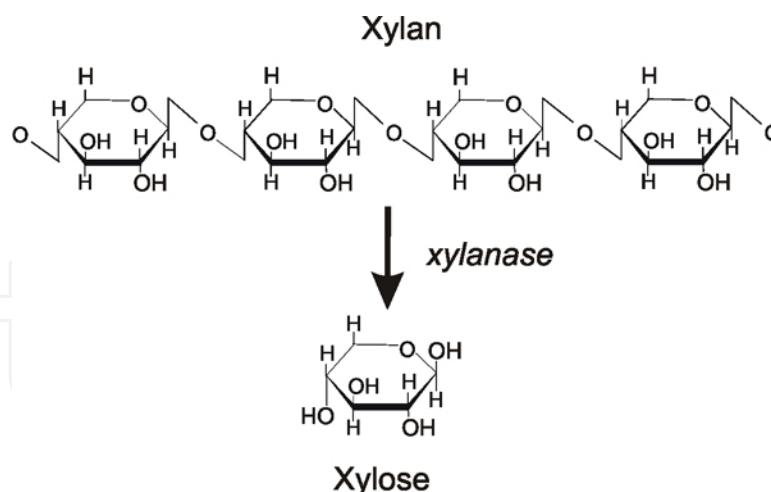


Figure 4. Structure of xylan and digestion of xylose by using xylanase

3.11. N-Acetylmuramidase

N-acetylmuramidase, an enzyme resembling lysozyme in action, cleaves the N-acetylmuramyl- β ,4-N-acetylglucosamine bonds of the polysaccharide chain of peptidoglycan, liberating free-reducing groups of N-acetylmuramic acid. N-acetylmuramidase (mucopolysaccharide N-acetylmuramoyl-hydrolases) belongs to the group of bacteriostatic enzymes comprising glycosidases that hydrolyze peptidoglycan (murein), which is basic component of the bacterial cell wall. Murein, composed of glycan strands consisting of alternating acetylated amino sugars, N-acetylglucosamine and N-acetylmuramic acid linked by β (1-4) glycosidic bonds mutually cross-linked by peptide chains, forms a mono- or multilayer net covered with lipopolysaccharides, phospholipids, and lipoproteins. The peptide moiety of murein is composed of short chains of unbranched aliphatic amino acids and/or amino acids that form stem peptides linked to the carboxyl group of N-acetylmuramic acid and cross-linked by interpeptide bridges [92]. Peptidoglycans, especially available high in Gram-positive bacteria, are highly diversified. The determination of the primary structure of peptidoglycans has revealed differences between the bacteria and it provides significant taxonomic tools [93]. The enzyme was isolated from *Streptomyces globisporus* [94; 95], and is also found in other streptomycetes, including *Streptomyces* sp. [96] *S. griseus* [97], *S. erythraeus* [98], *S. (Actinomyces) levoris* [99], and *S. rutgersensi* [100].

3.12. Neuraminidase

Neuraminidase (acylneuraminydase) splits 2,3-, 2,6-, and 2,8- and 2,9-glucosidic linkages which join terminal nonreducing N- or O-acetylated neuraminyd residues present in oligosaccharides and glycoprotein. Neuraminidases or sialidases occur widely in bacteria, viruses, animal tissues, and biological fluids [101]. These enzymes, isolated from various sources and differing in their substrate specificity, are applied in a wide area of biological and immunological research, particularly in cell surface and clinical studies [23; 102]. In actinobacteria, neuraminidases have been found in representatives of the genera *Corynebacterium*,

Mycobacterium and *Nocardia* [101], *Streptomyces griseus* [103; 104], *Actinomyces naeslundii*, and *A. viscosus* [105]. Neuraminidases isolated from *Streptomyces* strains showed optimum activity at pH 3.5-5.0 and at 50°-60°C. They differed notably in their properties, including substrate specificity. Neuraminidase from *Clostridium perfringens* and *Vibrio cholerae* are able to split all types of sialic acid linkages.

3.13. Peptide hydrolase

Proteolytic enzymes of microbial origin were classified by Morihara [106], on the basis of their catalytic mechanism, into serine, thiol, metallo, and acid proteases according to the general systematic scheme introduced by Hartley [107]. The ability to produce a variety of proteolytic enzymes is a well-known phenomenon in mesophilic actinobacteria [106]. There is also an increasing interest in proteases derived from thermophilic actinobacteria including members of the genera *Thermoactinomyces* (*Micromonospora*), *Thermomonospora*, and *Streptomyces*. These actinobacteria are still not fully exploited as a potential source of thermostable enzymes acting not only over a wide range of pH but also great number of proteases with wide spectrum of substrate specificity. An increasing interest is observed in the application of actinobacterial proteases in bioorganic chemistry. For commercial purposes, they are routinely obtained as by-products formed during biosynthesis of antibiotics in the logarithmic phase of growth [107], from the fermentation broths of *Streptomyces fradiae* [109], *S. griseus* [110], and *S. rimosus* [108]. The preparations obtained are enzymatic complexes that contain a mixture of endo- and exopeptidases; as commercial preparations they are known as pronase (*S. griseus*) or fradiase (*S. fradiae*). Actinobacterial proteolytic complexes provide an excellent source of protease in various substrates specificity. Of the actinobacterial protease complexes available, the most attention has been given to pronase obtained from *S. griseus*.

Pronase like enzymes are produced not only by *S. griseus* but also by members of several other species of streptomycetes. A trypsin-like serine protease was isolated from *S. erythraeus* [111] and *S. fradiae* [112]; carboxypeptidase T, which has a mixed specificity compared with pancreatic carboxypeptidase A and B, is produced extracellularly by a *Thermoactinomyces* strain [113]. Aminopeptidases have been isolated from culture filtrates of *S. rimosus* grown under conditions conducive to the industrial biosynthesis of oxytetracycline [114] and from other *Streptomyces* sp., including *S. mauvecolor* [34], *S. peptidofaciens* [115], and *S. sapporonensis* [116].

3.14. L-Asparaginase

The important application of the L-asparaginase enzyme is in the treatment of acute lymphoblastic leukemia, Hodgkin's disease, acute myelocytic leukemia, acute myelomonocytic leukemia, acute and chronic lymphocytic leukemia, lymphosarcoma treatment, reticulosarcoma, and melanosarcoma [117]. L-asparaginase broadly distributes among the plants, animals, and microorganisms. The microbes are a better source of L-asparaginase, because they can be cultured easily and the extraction and purification of enzyme from them is also convenient, facilitating large-scale production [118]. L-asparaginase has been arousing considerable interest as it displays an antineoplastic activity against a variety of murine neoplasms. As an antineoplastic agent, L-asparaginase from *Escherichia coli* has been the most widely available

[119], but the enzyme also occurs in actinobacteria. It has been isolated from several actinobacteria including *Mycobacterium bovis* [120] and *M. tuberculosis* [121]. These enzymes were active at pH 8-9. L-asparaginase obtained from *Streptomyces karnatakensis* [122], like others, was also intracellular and showed stereospecificity not only toward the L-isomer but was also able to hydrolyze D-asparagine to a smaller extent. The L-asparaginase production was reported from various actinobacteria namely *Pseudonocardia endophytica* VUK-10 [123]; *Streptomyces* sp. WS3/1 [124]; *Streptomyces* sp. (SS7) [125]; *S. halstedii* [126]; *Streptomyces acrimycini* NGP [127].

3.15. Penicillin amidase

Penicillin amidase (penicillin amidohydrolase) is an enzyme that hydrolyzes penicillins to 6-aminopenicillanic acid (6-APA) and carboxylic acid. The cleavage of penicillin into 6-APA and side chain is a reaction in which the penicillin nucleus, the basis for the production of semi-synthetic penicillins, is obtained. Penicillin amidases in the form of immobilized preparations are applied in the production of 6-APA on an industrial scale [128]. Penicillin acylases are classified into three groups on the basis of their substrate specificity: the first group includes the enzymes that hydrolyze phenoxymethyl-penicillin (penicillin V); the second, those that act on benzylpenicillin (penicillin G); and the enzymes of the third group display specificity with respect to D-a-amino-benzylpenicillin, ampicillin [129]. The penicillin hydrolysis reaction proceeds in an alkaline medium and at lower pH values and is reversible. This property was exploited to synthesize semisynthetic penicillins and cephalosporins by the application of penicillin amidase preparations [128] occur in bacteria and actinobacteria and to a minor extent in yeasts and moulds [129-131]. The majority of those found in actinobacteria such as *Mycobacterium*, *Nocardia*, and *Streptomyces* [131; 132] are able to hydrolyze phenoxymethyl-penicillin [133]. These enzymes are mainly intracellular and they display optimum penicillin hydrolysis at pH 7.0 to 8.0. Similarly to acylases isolated from the other sources, they catalyze penicillin synthesis in an acidic medium at pH from 4.0 to 5.5 [131]. So far they have not been reported to be of commercial significance.

4. Conclusion

Enzymes are considered as a potential biocatalyst for many biological reactions. Particularly, the microbial enzymes have extensive uses in industries and medicines. The microbial enzymes are also more active and stable than plant and animal enzymes. In addition, the microorganisms, particularly actinobacteria, represent an alternative efficient source of enzymes because they can be cultured in large quantities by fermentation and owing to their biochemical diversity and susceptibility to gene manipulation. Industries are looking for new microbial strains in order to produce different enzymes to fulfill the current enzyme requirements. Hence, the actinobacteria as biofactory of potential enzyme as well as secondary metabolites production, fulfill the requirements of several industrial enzymes. In a world with a rapid increasing of population and approaching exhaustion of many natural resources, enzyme technology offers a great potential for many industries to help meet the challenges they will face in the years to come.

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