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Enzymes from Actinomycetes – Review

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Abstract : Actinomycetes are group of microorganisms produce valuable secondary metabolites like antibiotics, vitamins, organic acids and enzymes. Antibiotics from actinomycetes of different habitats have been employed extensively in pharmaceutical field. The enzymes produced by actinomycetes and applied in different industries are amylases, proteases, lipases, cellulases, xylanases, chitinases, gelatinases and keratinases. This review summarizes the application of both intracellular and extracellular enzymes of actinomycetes in different industries such as textile, biorefineries, food, pulp and paper, agriculture, detergent and pharmaceuticals.
Keywords : Actinomycetes, Enzymes, Chitinases, Amylases.

Introduction

Soil is a rich source of complex organic matter in the form of humus. Those micro-organisms which cannot transport complex molecules inside their cytoplasm depend on the action of extracellular enzymes for breakdown of these molecules into useful and essential nutrients. Enzymes can broadly be divided into intracellular enzymes and extracellular enzymes. Extracellular enzymes from microorganisms are important biocatalysts with their widespread applications in industries such as textile, biorefineries, food, pulp and paper, agriculture, detergent and pharmaceuticals. Global market for industrial enzymes was $3.3 billion in 2010 and likely to reach $4.4 billion by 2015. The active secondary metabolites produced by microorganisms are reported to be around 23,000 of which 10,000 are produced by actinomycetes. Among actinomycetes, approximately 7,600 bioactive compounds are produced by Streptomyces species[1]. Actinomycetes are of enormous importance since they possess a capacity to produce and secrete a variety of extracellular hydrolytic enzymes[2-4]. Many actinomycetes have been isolated from various natural sources, as well as in plant tissues and rhizospheric soil. Biological functions of actinomycetes mainly depend on sources from which they are isolated. Among the various genera of actinomycetes, streptomyces has been considered as the most exploited group for production of these enzymes. Physiological, biochemical and molecular characteristics and metabolic pathway of aquatic actinomycetes are different from terrestrial actinomycetes. Saline actinomycetes produced a variety of biologically active enzymes than the terrestrial actinomycetes. Identification of genes that are responsible for enzyme production, understanding the role of structural domains responsible for enzyme activity are analysed through X-ray crystallography, mass spectroscopy and NMR studies. This review summarized the studies on the extracellular and intracellular enzymes production by actinomycetes from different sources.

Amylases

Amylases are enzymes, can be divided into two categories exoamylases and endoamylases. Amylases hydrolyze the starch molecules to variety of products including dextrins and smaller polymers composed of glucose units[5]. Actinomycetes secrete amylases to the outside of the cells to carry out extracellular digestion.

Amylase starch degrading amylolytic enzymes is of great importance in biotechnological applications such as food industry, fermentation and textile to paper industries[6]. Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market [7, 8].

Amylases can be derived from plants, animals and microbes. The enzymes from microbial origin generally meet great demand in the industries. Occurrence of amylases in actinomycetes is a characteristic commonly occurred in Streptomyces[9] and the genus considered as an active source of amylases. *Streptomyces avermitilis Streptomyces* sp. SLBA-08; *Streptomyces* strain A3; *Streptomyces rochei* BTSS 1001 are used in production of amylase in starch, detergent, food and textile industries. It is effectively used in field of medicinal research[10-12]. Industrial processes of starch degradation have been improved with the help thermostable amylolytic enzymes. Extracellular amylase production by a newly isolated alkali-thermotolerant strain *Streptomyces gulbargensis* DAS 131 was studied for the highest amylase production [13]. A haloalkaliphilic marine *Saccharopolyspora sp.* strain A9 with an ability to produce surfactants, oxidant and detergent stable amylase was isolated from marine sediments[14]. The surfactant, detergent stable and calcium ion independent amylase from strains A3 was isolated which has widespread applications for detergent and pharmaceutical industry[11]. α- Amylases have potential and wide application in industrial processes such as food, fermentation, textile, paper, detergent, and pharmaceutical industries. However, with the advances in biotechnology, the amylase application has expanded widely in many fields such as clinical, medicinal and analytical chemistry, as well as their widespread application in starch saccharification and in the textile, food, brewing and distilling industries [15,16, 6].

Cellulases

Cellulases required for the hydrolysis of cellulose include endoglucanases, exoglucanases and β-glucosidases[17]. Cellulases are a collection of hydrolytic enzymes which hydrolyze the glucosidic bonds of cellulose and related cello-digosaccharide derivatives [18]. Actinomycetes are one of the known cellulase producers [19,20]. *Streptomyces drozdowiczii; S.lividans; Streptomyces longispororuber, Streptomyces rutgersensis, Streptomyces sp*. B-PNG23 are better examples for production of cellulase and used in industries such as pulp and paper, textiles, biorefineries, animal feedstocks, wine and brewing, baking[21-27]. Conversion of cellulases from agro- industrial wastages by actinomycetes also have been reported by many researchers. *Streptomyces viridobrunneus* SCPE-09 was selected as the active cellulolytic strain produces cellulose from agro- industrial residue[28]. Cellulolytic enzymes are employed in the color extractions of juices, in detergents causing color brightening and softening, in the biostoning of jeans, in the pretreatment of biomass that contains cellulose to improve nutritional quality of forage and in the pretreatment of industrial wastes[29-33]. Alkaline or alkalitolerant and cellulase producers are mainly found in the genera Streptomyces and Thermoactinomyces[34]. Cellulose is the most abundant renewable natural biological resources, and the production of bio-based products and bioenergy from less costly renewable lignocelluloses materials in the form of agro-industrial biological waste. The cost of enzyme production can be reduced by using low value biological substrates (fruit processing waste)[35].

Xylanases

Xylan is the most dominating component of hemicelluloses. It is used in the pulp and paper industry [36]. Treatment with xylanase at elevated temperatures disrupts the cell wall structure, as a result, facilitates lignin removal in the various stages of bleaching. Alkaliphilic and cellulase-free xylanases with an optimum temperature of 65°C from *Thermoactinomyces thalophilus* subgroup C was also reported recently[37]. Thermostable xylanase were isolated from a number of actinobacteria[38]. *Streptomyces sps.* have been reported to produce xylanases which are active at temperatures between 50 and 80°C. Eighty eight actinomycetes were isolated from the soil samples, India for their production and characterization of xylanase[39]. Actinomadura sp. from compost in Thailand has been reported for the production of xylanase[40]. Thermophilic Actinomadura sp. from poultry compost has been reported the production and characterization of extracellular thermostable xylanase production[41].

Lipases

Lipase is produced from a variety of actinomycetes[42]. Lipases have broad applications in the detergent industries, foodstuff, oleochemical, diagnostic settings and also in pharmaceutical fields[43]. Lipases and esterases

are a diverse group of enzymes that catalyze the hydrolysis of ester bonds in triacylglycerides to glycerol and fatty acids. Lipases have extensive range of enzymatic properties and substrate specificities produce them very useful for industrial function such as processing of fat and oils, additives, detergents, cosmetics, paper manufacturing and pharmaceuticals. Lipolytic enzymes have many industrial applications but still demand for the biocatalyst with novel and specific properties such as pH, temperature, specificity and stability is increasing some new molecular approach.

Proteases

Proteases, also known as peptidyl-peptide hydrolases, are important industrial enzymes and are extensively used in variety of industries including textiles, leather, detergents, meat tenderization, cheese making, dehairing, baking, organic synthesis, brewery and waste water treatment[44-45]. These enzymes also used in production of digestive aids and the recovery of silver from photographic film. Actinomycetes, particularly Streptomycetes are known to secrete multiple proteases in culture medium[46]. Microbial alkaline proteases for manufacturing uses are produced mostly from *Streptomyces spp*. Several studies have been made on the proteolytic enzymes of mesophilic actinomycetes[47]. *Streptomyces thermonitrificans* showed maximum protease activity[48]. Recently, alkaline protease from *Nocardiopsis sp.* NCIM 5124 [49] has been purified and characterized. Alkaliphilic acinomycete from the soil and crude components such as molasses, wheat flour, and wheat bran were found to be effective for growth and protease production[50]. The high level of enzyme production using agro-industrial by-products is commercially significant due to cheap nature of these sources. *S. gulbargensis* DAS 131 was isolated from soil samples and that was proved to produce multiple proteases [51]. There are 46 strains ofactinomyceteshave been isolated from soil samples of Northern Himalayas and studied their culture characterization, protease production and cytotoxic effects on cancer cell line[52].

Keratinases

Keratinase is specific proteases hydrolyse the keratins. Keratins, which are among the hardest-to-degrade animal proteins, are the major component proteins in poultry feathers and are characterized by a tightly packed form in α- helixes and β sheets with a high degree of disulfide bonds [53]. Keratinase is an extra cellular enzyme used for the bio degradation of keratin. Keratinase is produced only in the presence of keratin substrate. Keratinase attacks the disulfide bond of keratin to degrade it. Some microbes have been reported to produce keratinase in the presence of keratin substrate. Keratinase producing microorganisms have ability to degrade chicken feathers, hairs, nails, wool etc. [54-55]. Mostly protease positive actinomycetes are useful for studying the production of proteases. Microbial alkaline proteases for manufacturing uses are produced mostly from streptomyces and bacillus. Actinomycetes, particularly streptomycetes are known to secrete multiple proteases in culture medium [46]. The promising applications of keratinolytic proteases include enzymatic dehairing of leather, detergent industry and development of biodegradable films [56]. Amongest the industrially important enzymes, actinomycetes keratinases are stimulating tremendous interests in the enzyme market owing to the fact that there is a great demand for developing biotechnological alternatives for recycling of keratin wastes, converting unused chicken feather to useful value added products[57]. Different studies on keratinase activity of *Streptomyces sp* have been reported[58-60].

L-asparaginase

Actinomycetes have been revealed to be an excellent resource for L-asparaginase (L-asparagine amino hydrolase). A range of actinomycetes, mainly those isolated from soils such as *Streptomyces griseus, S. karnatakensis, S. albidoflavus* and *Nocardia sp.* have abilities to produce L-asparaginase enzyme [61, 62]. Microbial L-asparaginase has been generally used as a therapeutic agent in the cure of certain human cancers, mostly in acute lymphoblastic leukemia [63].

Chitinases

Chitin is the second most abundant polymer in nature. It is an insoluble linear 1, 4-linked polymer of N-acetylglucosamine. This polysaccharide is found in the cell walls of fungi and exoskeleton of insects and the shells of crustaceans. Chitinases are produced by viruses, bacteria, actinobacteria, higher plants and animals and they play important physiological and ecological roles[64]. Chitinases hydrolyze the 1, 4 linkages in chitin, yielding predominantly N-N_-diacetylchitobiose, which is further degraded by N-acetylglucosaminidases to the N-acetylglucosamine monomer [65].

Mono and oligosaccharides are converted from chitin by the enzyme called Chitinase. Amongst actinomycetes, the genus streptomyces is the best studied for chitinases [66]. Chitinolytic activity of culture filtrates of S. griseus has been reported[67, 68]. *Streptomyces thermoviolaceus* OPC-520 was isolated to extract the thermophilic chitinases[69]. Chitinase was isolated from the culture filtrate of Streptomyces sp. M-20 [70]. *Nocardiopsis prasina* showed chitinase activity[65]. Chitinase is the potential antifungal agent through its chitin degradation activity [71]. Endophytic *Streptomyces aureofaciens* CMUAc130 produced chitinase and showed antagonism against phytopathogenic fungi[72]. *Streptomyces griseoloalbus* JCM4480, *Streptomyces Clauifer* JCM5059, *Streptomyces anulatus* NBRC13369 and *S. griseus* that produced chitinase unique compounds, showing selective inhibition of the insect GlcNAcase[73]. *Streptomyces hygroscopicus*was isolated from Thailand and studied chitinase activity against phytopathogenic Fungi[74]. *S. griseus* strain (MTCC) was studied for its chitinase enzyme activity against some soil borne plant pathogens[75]. Chitinase activity against *Sclerotinia sclerotiorum* was studied with 186 endophytic actinomycetes from nine kinds of plants [76]. *Streptomyces tendae* strain TKVL 333 was isolated from laterite soils of the Guntur region, India, for chitinase production[77].

Conclusions

Recent studies on importance and application of microbial enzymes in industries proved that the enzymes from microbial origin generally meet great demand in the industries. Actinomycetes are of enormous importance since they possess a capacity to produce and secrete a variety of extracellular hydrolytic enzymes that are safer to environment. Extracellular enzymes from actinomycetes are important biocatalysts with their widespread applications in industries. Since the actinomycetes play their major role in industrial enzymes production, these could occupy their priority in different industries for giving solutions to many challengeable problems in the diverse field like textile, biorefineries, food, pulp and paper, agriculture, detergent and pharmaceuticals.

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