ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Actinomycetes Excellent Source of Industrially Important Enzymes: Review.

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ABSTRACT

The taxonomic and ecological positions of antibiotic producing actinomycetes are well recognized for their metabolic flexibility, commonly accompanied by the production of primary and secondary metabolites of economic the metabolites generated in the process. Enzymes produced by Actinomycetes and applied in different industries are cellulases, proteases, amylases, lipases xylanases, chitinases, cutinases and pectinases. Actinomycetes identified from the extreme environments are known to be producers of novel enzymes with great industrial potential. This review attempts to summarize the applications of enzymes from Actinomycetes in different industries such as food, medicine, pulp and paper, detergent, textile, agriculture and biorefineries.
Keywords: Antibiotic, Actinomycetes, Metabolites, Enzymes, Biorefineries.

https://doi.org/10.33887/rjpbcs/2019.10.3.39

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ISSN: 0975-8585

INTRODUCTION

To meet the increasing demand of robust, high turnover, easily and economically available biocatalyst, research is always channelized to get novelty in enzyme or improvement of existing enzymes by engineering at gene and protein level [1]. Enzymes produced by microorganisms are considered as potential biocatalysts for a large number of reactions. Enzymes derived from microbial source are generally regarded as safe and they are functional at wide range of temperature, pH, salinity or other extreme conditions. Actinomycetes are one of the most diverse groups of microorganisms that are well characterized and recognized for their metabolic versatility. They play a vital role in decomposition of organic matter, e.g. cellulose, chitin and pectin, therefore, they play an important part in carbon cycle and help to maintain the soil structure [2,3]. Actinomycetes produce a wide variety of chemical compounds, e.g. enzymes, antibiotics, neutraceuticals, antitumor agents, plant growth regulators and vitamins [4,5]. Various genera of Actinomycetes have been reported to produce a wide array of potential industrial enzymes that can be used in biotechnological applications and biomedical fields in particular [6]. Continuing advances in sequencing technology and bioinformatics tools make it possible to study the microbial enzyme production by using proteomics and metaproteomics [7]. Actinomycetes have been continuously studied and employed for production of amylases, cellulases, proteases, chitinases, xylanases and pectinase. This review summarized the production of industrially important enzymes by Actinomycetes and representative examples of these enzymes, their characteristics and industrial uses (applications in biomedicine, food, detergent, pulp and paper, agriculture, textile and waste management) are enlisted in (Table 1).

Structure of Actinomycetes:

The actinomycetes (sing. actinomycete) are a large group of aerobic, high G-C percentage gram-positive bacteria that form branching filaments or hyphae and asexual spores. These bacteria closely resemble fungi in overall morphology. Presumably this resemblance results partly from adaptation to the same habitat. Studies of the fine structure of actinomycetes spores during germination have been confined to the genera Streptomyces (8). The latter genus forms endospores which behave in a similar way to those of Bacillus, a new wall layer being synthesized inside the cortex of the spore and extending to form the germ-tube wall.In the Streptomyces species studied, the spores had a two-layered wall and the inner one extended to form the germ-tube wall. It is not clear if this layer is newly synthesized during germination or if it is formed by reorganization of wall material existing in the dormant spore. Ultra structural changes during the germination of fungal spores have been studied more extensively. Most fungi fall into one of two groups: (i) those in which the germ-tube wall is formed from a layer which is synthesized de now within the existing spore wall; (ii) those in which the germ-tube wall is formed by the extension of a wall layer already present in the dormant spore (9). Some conflicting results have been obtained and closely related species have been reported to fall into different groups (10). This may be partly due to the use of different fixatives, potassium permanganate giving inferior results to those obtained with osmium tetroxide or aldehydes (11).Marked changes in spore wall layers can also be induced by hydration during specimen preparation .When grown on an agar-surface, the actinomycete branch forming a network of hyphae growing both on the surface and under-surface of the agar. The on-the-surface hyphae are called aerial hyphae and the under-surface hyphae are called substrate hyphae.Septa normally divide the hyphae into long cells (20 mm and longer) possessing many bacterialchromosomes (nucleoids). These are the aerial hyphae that extend above the substratum and reproduce asexually. Most actinomycetes are non-motile. When motality is present, it is confined to flagellated spores.

ISSN: 0975-8585

CLASSIFICATION OF ENZYMES		
Group of Enzyme	**Reaction Catalysed**	**Examples**
1. Oxldoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another.	Dehydrogenases Oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl etc.) from one substrate to another.	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate.	Estrases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate.	Phospho hexo isomerase, Fumarase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate.	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds.	Citric acid synthetase

Table 1 : Classification of Enzymes(12)

Enzyme Classification According To Their Sources

Biologically active enzymes may be extracted from any living organism. A very wide range of sources are used for commercial enzyme production from *Actinoplanes* to *Zymomonas*, from spinach to snake venom. Of the hundred or so enzymes being used industrially, over a half are from fungi and yeast and over a third are from bacteria with the remainder divided between animal (8%) and plant (4%) sources. A very much larger number of enzymes find use in chemical analysis and clinical diagnosis. Non-microbial sources provide a larger proportion of these, at the present time. Microbes are preferred to plants and animals as sources of enzymes because:

1. They are generally cheaper to produce.
2. Their enzyme contents are more predictable and controllable,
3. Reliable supplies of raw material of constant composition are more easily arranged, and plant and animal tissues contain more potentially harmful materials than microbes, including phenolic compounds (from plants), endogenous enzyme inhibitors and proteases.
4. Attempts are being made to overcome some of these difficulties by the use of animal and plant cell culture.

ISSN: 0975-8585

Table 2: Some important industrial enzymes,sources and their applications(13).

Types	Growth characteristics	Environment/source/ geographical location	Enzymes	Applications
Acidophile	Organism with a pH optimum for growth at or below 3-4	Acid mine drainage, volcanic springs, USA	Amylase, glucoamylase	Starch processing. Single-cell protein from shellfish waste
			Proteases	Animal feed for the improvement of digestibility
			Cellulases	Removal of hemicellulosic material from feed Feed component
			Oxidases	Desulfurization of coal
Alkaliphile	Organism with optimal growth at pH values above 10	Soda Lakes, Utah USA.	Proteases, cellulases	Detergents, food, and feed Fermentation of beer and wine, breadmaking, and fruit juice processing
Halophile	Organism requiring at least 1 M salt for growth	Salt Lakes, Utah USA	Proteases	Peptides synthesis
			Dehydrogenases	Biocatalysis in organic media Asymmetric chemical synthesis
Neolith	Organism that lives inside rocks	Upper subsurface to deep subterranean, Mediterranean and Japan Seas	[a]NI	[a]NI
Hyperthermophile	Organism having a growth temperature optimum of 80°C or higher	Submarine Hydrothermal vents, East Pacific, Porto di Levante, Vulcano, Italy	[a]NI	[a]NI
Hypolith	Organism that lives inside rocks in cold deserts	Desert, rock, Cornwallis Island and Devon Island in the Canadian high Arctic	[a]NI	[a]NI
Metallophile	Organism capable of tolerating high levels of heavy metals, such as copper, cadmium, arsenic, and zinc	Heavy metals, Latin America, and Europe	[a]NI	[a]NI
Oligotroph	Organism capable of growth in nutritionally deplete habitats	Carbon source, or carbon concentration, Antarctic	[a]NI	[a]NI
Piezophile	Organism that lives optimally at hydrostatic pressures of 40 MPa or higher	Deep ocean, Mariana Trench, Antarctic ice	To be defined	Food processing and antibiotic production
Psychrophile	Organism having a growth temperature optimum of 10°C or lower, and a maximum temperature of 20°C	Ice, snow, Antarctic ice and Arctic Ocean	Proteases	Detergents, food applications
			Amylase	Detergents and bakery
			Cellulases	Detergents feed and textiles
			Dehydrogenases	Biosensors

Amylases:

Amylases are categorized into exoamylases and endoamylases, these 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 play their major role in biotechnological applications such as food industry, fermentation and textile to paper industries[6]and having approximately 25% of the demand in 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 gulbargensisDAS* 131 was studied for the highest amylase production [14]. A haloalkaliphilic marine

Saccharopolyspora sp.strain A9 with an ability to produce surfactants, oxidant and detergent stable amylase was isolated from marine sediments[15]. The surfactant, detergent stable and calcium ion independent amylase from strains A3 was isolated which has widespread applications for detergent and pharmaceutical industry11. α-Amylases have potential and wide application not only in industrial processes but has been applied widely in many fields such as clinical, medicinal and analytical chemistry. Amylaes have been utilized effectively in starch saccharification and in the textile, food, brewing and distilling industries.

Cellulases

Cellulases are important industrial enzymes for sustainable production of biofuel as they convert the cellulose into fermentable sugars. Cellulases from *Streptomyces spp.* like *S. ruber, S. lividans and S. rutgersensis* are highly thermostable [16]. These enzymes are mostly used as a supplement in detergents, textile, animal additives and paper and pulp industry [17,18]. Some members of genera *Thermobifida* and *Micromonospora* also produce cellulases that exhibit industrial potential to be used commercially [19]. Cellulases from e*xtremophiles* like *Thermobifida* are stable at high temperature and pH and are used for degradation of cotton and avicel. They have ability to use rice, wheat and other crops as substrates [19,20].

Proteases:

Several studies reported production of proteases from Actinomycetes like members of genera, *Streptomyces, Nocardia and Nocardiopsis* [21]. Mostly proteases show tolerance to various abiotic stresses like high pH, temperature and salinity [22]. Proteases from *Streptomyces* spp. can be used in processing of different agro-industrial wastes like feathers, nails, hair and plant wastes [23]. Proteases produced by Nocardiopsis spp. are known as important industrial enzymes and have potential to be extensively used in leather, baking, textile, detergent, brewery, cheese and dehairing industry [24]. More than 48 strains of soil Actinomycetes have been reported for production of proteases along with their cytotoxic effects on cancer cells [25].

Keratinases:

Keratinases are industrially important enzymes produced by a number of Actinomycetes strains like *Streptomycetes spp*. and *Actinomadura* [26]. These enzymes are mostly used for the hydrolysis of keratin. There is a great demand for developing biotechnological alternatives for recycling of keratic wastes, converting unused chicken feather, hairs, nails and wool to useful products with the help of Actinomycetes keratinases [27].

CONCLUSION

Industries are looking for new microbial strains, including Actinomycetes in order to produce novel enzymes to fulfil the current requirements because of till now, only 20 enzymes produced by microorganisms are utilized by various industries. However, many of the rare genera of Actinomycetes have been neither manipulated nor explored for their biotechnological potential. Studies on the microbial potential of extreme environments can be utilized to produce novel enzymes that can become future harbingers of green biotechnology.

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