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Pectinases from Actinomycetes: A Thorough Study

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Abstract: Enzymes are most significant products for human needs in the broad area of environmental, industrial and food technology through microbial sources. Pectinase are cluster of enzymes that help in breakdown of pectic substances which are mostly present in plant tissues. They have enormous applications in paper, fruit and textile industries. Microbial pectinase makes a quarter sales of global food enzymes. Actinomycetes are active degraders of plant debris thereby it can produce extracellular enzymes like xylanase, cellulase and pectinase. About 80% of the metabolites produced by Actinomycetes species rise from *streptomyces sp.* Only limited information is available on the pectinase enzyme system of actinomycetes. This review article mainly emphasis on the production of pectinase enzyme from actinomycetes mainly concerned about *streptomyces sp*. and their applications in industries. Due to their enzyme activity over a vast range of pH and good thermal stability it may be desired option to be utilized in industries in mere future.
Keywords: Enzyme, Pectinase, Actinomycetes, *Streptomyces.*

Introduction

In modern food industry, enzyme is one of the significant tools because while processing of various intermediate processes are simplified due to utilization of enzymes. Most of the industrial enzymes fall into different groups among these pectinase is most salient group used in vegetable and fruit processing industries. The history of pectinase starts with an understanding of the structure of pectin and the mechanism by which pectinolytic enzymes degrades pectic substances. The commercial application of pectinase for the preparation of fruit juices and wines was first observed in 1930 which made its remark for the first enzymes to be used in homes[1]. Pectinases, a repertoire of pectinolytic enzymes that helps to the break down the pectin by different mechanisms and can be categorized as eliminative depolymerases (Pectin lyase), hydrolytic depolymerases (polygalacturonases) and esterases (pectin esterase). The Pectin, an indispensable component present in between the middle lamella and cell wall of plant cells composed of as many as 17 various monosaccharide and at least seven various polysaccharides forming a most complex bio-macromolecule in nature[2]. Pectinases are naturally produced by different organisms which include fungi, bacteria, yeasts, nematodes, protozoa, plants and insects. A quarter sales of the global food enzymes depend on microbial pectinases. Even though fungal pectinases are being industrially exploited, pectinase production from actinomycetes has also been reported earlier[3, 4].

Actinomycete plays a major role in the plant residue degradation. Actinomycetes produce extracellular enzymes like cellulase, xylanase and pectinase as they are efficient degraders of plant debris. It belongs to a distinct class of gram-positive bacteria (Actinomycetales) of prokaryote. In 16SrDNA tree, actinomycetes form a definite phylogenetic line and have been a major technological importance in the past years, with the

discovery of numerous number of metabolites produced by its various genera[5]. The diversity in actinobacterial population in sediment and water samples from the marine environment of Tamil Nadu and diverse genera viz. *Streptomyces, Actinopolyspora, Actinomadura, Nocardiopsis, Micromonospora* and *Actinomycete* have been seen in earlier reports[6]. They have ability to synthesize chemically diverse and commercially important bioactive compounds like pigments, antibiotics and enzymes, etc[7]. *Streptomyces sp*. is salient one in soil ecology and present worldwide in soil. They are responsible for the characteristic earthy smell of soils[8]. They are considered as one of most significant bacteria, because of their capacity to develop the soil properties as well as producing several extracellular substances (enzymes) as secondary products[9, 10]. This review provides understanding of structures of pectic substance, pectinase enzymes and previous works related to production, optimization and application of pectinase from Actinomycetes.

Pectic Substances

Pectin is heterogeneous structural polysaccharides present in nature of about 35% of primary walls in dicots and non-graminaceous monocots, 5% of walls in woody tissue and 2-10% of grass and other commelinoid primary walls[11, 12]. The fresh weight of plant material constitutes 0.5-4.0% of pectic substances[13]. A D-galacturonic acid residue forms the backbone of pectinase[14]. These residues are linked by α (1-4) linkage with a main chain consists a small number of rhamnose residues and arabinose, galactose and xylose on its side chains. Pectin maintains structural integrity by contributing involuntary strength and physical properties of primary cell wall. It also provides inter cellular fixation due to the presence of non- esterified carboxyl groups which may be linked through divalent cations such as Mg^{2+} and ca^{2+} causing pectin to form gel[15].

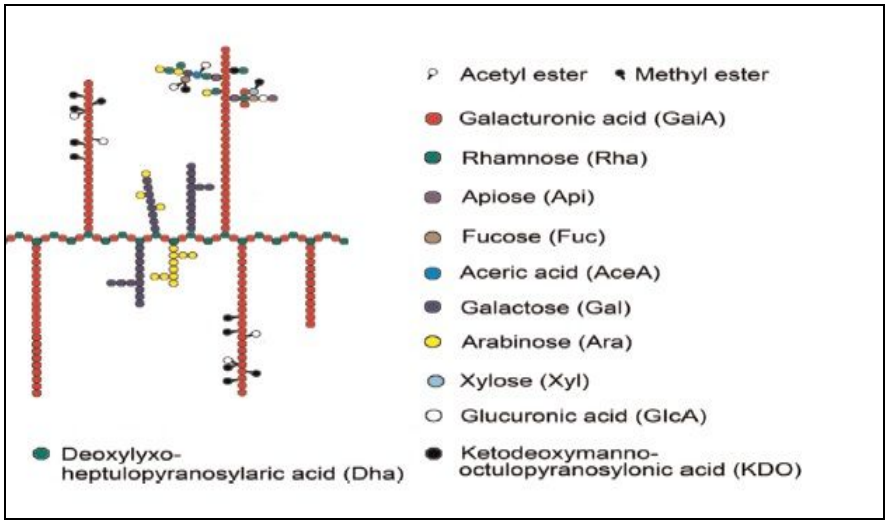

Figure1. Schematic representation of the recently proposed alternative structure of pectin, showing major domains present in most pectins other than definitive structures[16].

Classification of pectic substance

1. Homogalacturonan (HG)

It can also called as smooth regions of pectin in which a D- galacturonic acid forms a linear polymer that may be methyl esterified or acetylated. This molecule is classified based on its level of esterification.

(a) Pectin:

At least 75% of carboxyl groups are methylated

(b) Pectinic acid:

Less than 75% of carboxyl groups are methylated

(c) Pectic acid:

Absence of methyl-esterified carboxyl groups

2. Rhamnogalacturonan I (RGI)

It is comprised of disaccharide rhamnose-galacturonic acid in repeating units. The neutral sugars such as galactose, arabinose and xylose are present as side chains in galacturonic acid residues[17].

3. Rhamnogalacturonan II (RGII)

RG II is a homogalacturonan chain in which galacturonic acid is attached by group of side chains. The RGI and RGII are also known as hairy regions of pectin molecule. Vincken and co-workers[18] have suggested a structure model of pectin molecule in which RG II and HG forms a long side chains of RG I backbone.

Pectinolytic enzymes

Pectin Lyases (PL)

Pectin lyase catalyzes the high esterified pectin which produces unsaturated methyloligogalacturonates through trans-elimination of glycosidic linkages. They do not require ca^{2+}.

Pectin Methyl Esterases (PE)

Pectin esterase or pectin methyl esterase catalyzes the de-esterification of pectin's methoxyl group forming pectic and methanol. One of the advantage of PE, it acts on a methyl ester group of galacturonate unit next to a non-esterified galacturonate unit. On the other hand, PG and PL requires non-esterified substrates.

Polygalacturonases (PG)

It catalyzes the production of D-galacturonate by the hydrolysis of alpha 1, 4- glycosidic linkages in polygalacturonic acid.

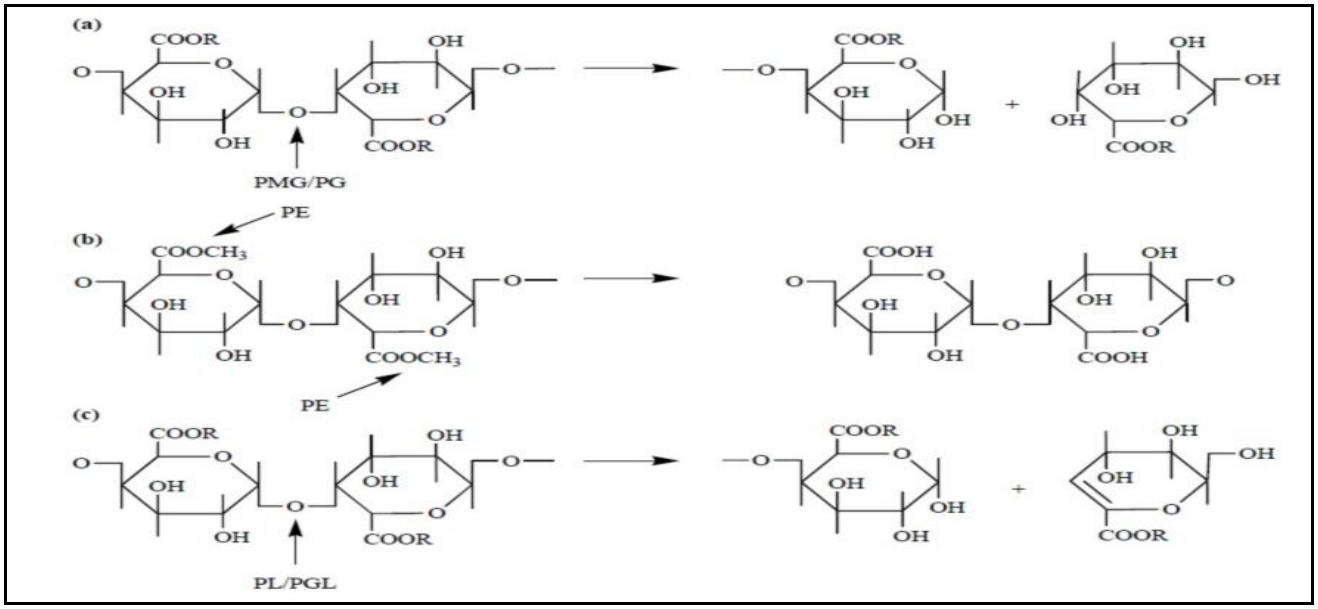

Figure 2. Mode of action of pectinases: (a) R=H for PG (b) PE (c) R=H for PGL and CH3 for PL. The arrow indicates the place where the pectinase enzyme reacts with the pectic substances[19].

Actinomycetes for Pectinases

Actinomycetes are present as omnipresent which is one of the superior group of gram positive bacteria. They have been commercially utilized for the nutraceuticals, pharmaceuticals, enzymes, anti-tumor agents, enzyme inhibitors production and so forth[20].A broad range of enzymes and their products used in biotechnological and biomedical industries has been reported from diverse genera of actinomycetes. Since,

there is an advent of protein and genome sequencing data of actinomycetes provides essential information which makes them repeatedly exploited for the production of pectinase and other enzymes.

***Streptomyces*: Source of pectinase enzyme**

Streptomyces species have cosmopolitan distribution among the antibacterial population because of the dispersion of spores[21]. Approximately 80% of the metabolites produced by actinomycetes rise from *streptomyces* species[22]. Various industrial applications requires use of elevated temperature and extreme pH and ionic concentrations since pectinases produced from *streptomyces* have activity over a wide range of pH and good thermal stability it can be desired option to be utilized in industries. For better enzymatic production, it is necessary to study indigenous flora and explore unique habitats of *streptomyces*.

Isolation of pectinolytic *streptomyces*

For the isolation of pectinolytic *streptomyces sp*. different media have been employed in the earlier reports[3, 23] have reported using actinomycete isolation agar for the isolation of *Streptomyces sp.* QG-11-3. *Streptomyces lydicus* MTCC 7505 was maintained on starch casein agar[2].Starch casein nitrate (SCN) agar medium was used for isolation of *streptomyces sp*. GHBA 10 [24].

Optimization of nutritional factors

Complex carbon sources such as agro-industrial wastes and pectin in fermentation medium have been reported as substrates that induce the pectinase activity in *streptomyces* sp. have been reported[25, 26]. Pectin is the important source that induces pectinase activity and it can be enhanced by supplying organic nitrogen sources such as peptone, yeast extract, casein, tryptone, beef extract and malt extract[2, 4].

Effect of amino acids

Pectinase production by *streptomyces sp*. QG-11-3 was stimulated by DL-isoleucine, DL-norleucine, L-leucine, L-lysine monohydrochloride and DL-b-phenylalanine upto 2.78-fold. The combination of L- leucine, DL- norleucine and Dl- isoleucine synergistically stimulated the pectinase production upto 5.62-fold whereas, DL- norvaline, Glycine, DL- methionine and DL- aspartic acid showed no significant stimulation effect on pectinase production[25].

Immobilization of pectinase

Immobilization of pectinase from *streptomyces sp*. was also observed from the previous reports. The Polyurethane foam (PUF) as the inert support matrix for immobilization of streptomyces sp. RCK-SC was carried out. The pectinase production was enhanced by 32%. The optimum conditions for partially purified pectinase were 60°C and retained 80% of its activity at 50°C after 2h of incubation time. The half life of enzyme was 3h at 70°C. And, moreover, pectinase was stable at alkaline pH ranging from 6.0 to 9.0 at room temperature for more than 8hr retaining more than 50% of its activity[23].

Purification of pectinase from *streptomyces sp*.

The crude pectinase from *streptomyces sp.* can be easily purified by salt precipitation, dialysis followed by gel filteration chromatography. The pectinase enzyme showed a molecular weight of about 32kDa[24]. The purified exo-polygalacturonase from *streptomyces lydicus* revealed a molecular weight of about 43 kDa. The purified thermostable Polygalacturonase from Streptomyces halsteedii ATCC 10897 showed a molecular weight of 48kDa[27] which is more compared to enzyme produced by *Bacillus sp.* in previous reports.

Application of pectinase

Paper making waste waters

The alkaline and thermostable polygalacturonase from *Streptomyces halstedii* ATCC 10897 helps in depectinization in pulping mill and papermaking waste waters[27].

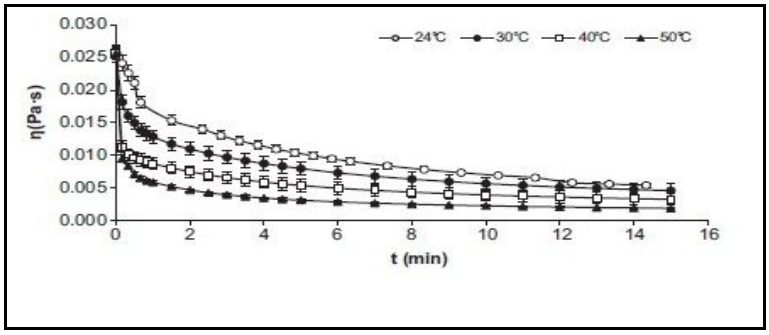

Figure.3 Effect of pectinase from *streptomyces halstedii* ATCC 10897 in treatment of waste water from paper making process at different temperatures[27].

Degumming of ramie bast fibers

The pectinolytic actinomycete strains act along with xylanolytic and cellolytic strains were helpful in the degumming of ramie bast fibers[3].

Conclusion

The Actinomycetes are active degraders of pectic substance present in nature. The pectinase enzymes which they have produced are of stable and it can withstand broader range of pH and moreover thermo-stable makes an advantage over pectinase produced by other species. The Actinomycetes utilize agricultural wastes for the production of pectinase enzyme therefore, it is potential to make enzyme in cost-effective and eco-friendly manner. The enzyme production and application in industries using *streptomyces sp*. are very scanty. Henceforth, Actinomycetes can be employed to produce pectinase as it has numerous benefits and optimistic alternative than other species in future.

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