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REVIEW ARTICLE

POTENT BIOTECHNOLOGICAL APPROACH OF BACTERIAL ALKALINE PECTINLYASE ENZYME IN INDUSTRIAL SECTOR

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ABSTRACT

Microbes are potent source of different types of enzymes which are economically important in various industrial sectors. Pectinlyase which is enormously produced by many strains of bacteria, *actinomycetes*, fungi etc. Bacterial species produce this enzyme by using substrate as waste of different fruits such as peels of papaya , orange, sweet lime, guava, apple , etc. Bacterial species produced both types of pectinlyase. Acidic pectinlyase is useful in the fruit and juice processing industries but alkaline pectinlyase is useful in the textile and pulp industry mainly. Alkaline pectinlyase also useful for pretreatment of industrial waste water. Electro spray ionization and mass spectrometry are used to analyze the degradation of polygalacturonic acid.

INTRODUCTION

Enzymes which are bio-active compounds that regulate many chemical reactions in living tissues and cells (Prathyusha and Suneetha, 2011). A number of enzymes such as cellulase, protease, pectinase which are produced by plant pathogens commonly referred as phytopathogenic enzyme, these enzyme degrade plant pectic polysaccharide (Sangeeta *et al.*,2009). Enzymes are broadly used in the industrial sector such as textile industry , paper and pulp industry. Pectinlyase is used based on the composition and structure of pectinacious component in cotton pectinlyase (Etters *et al.*,1999, Hartzell *et al.*,2000, Anis *et al.*,2002 , del Valle *et al.*, 2006). Pectin is a complex polysaccharide macromolecule with high and varying molecular mass (Ridley *et al.*, 2001). This is also a versatile, structural polysaccharide of higher plants containing long Galacturonic acid chains with residues of carboxyl groups and with varying degree of methyl esters (Alphons *et al.*, 2009). Pectin is degraded by the mixture of enzymes, in which mainly two enzymes group methylesterases and depolymerases work very efficiently. Pectin lyases is very specific to pectin substances which are esterified by methyl group while unesterified polygalacturonate pectate is degraded by pectate lyases(Barras *et al.*,1994). Earlier it was seen that the majority of polymers of pectin and pectate are found in primary cell walls of all dicots and some monocots is degraded by the *actinomycetes* and fungal group (Carpita *et al.*,1993). Various type of pectinase and pectinlyase are used in food processing industries, paper and pulp industries and textile industries (Alkorta *et al.*,1998). Now a days among various enzyme pectinlyases enzymes are being incorporated into the textile industry to retting of fibers and removing of upper layer from the stem of many fiber crops such as sun, hemp , etc (Henriksson *et al.*, 1999). Acidic pectinases are generally used in the fruit juice processing sector (Rombouts *et al.*,1986) whereas alkalophilic pectinlyases are used as degumming of ramiefibers (Cao *et al.,*1992), retting of fibers(Sharma *et al.,*1987), treatment of effluent which is librated by various fruit juice industries(Tanabe *et al.*,1987). In this review article we report mainly the information related to pectin , pectinase, alkaline pectinlyase which is produced by many bacterial species such as *Bacillus sp.* RK9 , NT 33 ,*Bacillus polymyxa* ,*Bacillus sp.* P-4-N ,*Bacillus stearothermophillus* ,*Bacillus sp.* DT 7, production and application of alkaline pectinlyase in industrial sector (Fogarty *et al.*,1983 , Nagel *et al.*,1961 , Kashyap *et al.,*2000).

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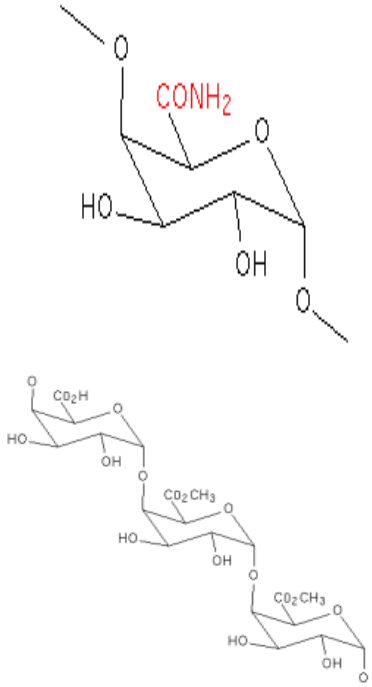
Structures and composition of Pectin

Pectins are a family of complex polysaccharide material having α (1→4)D-galactosyluronic acid residues. It has mainly two parts in which first part is known as hairy segment and another one is known as smooth segment. It is mainly three types according to structurally motifs: Homo galacturonans, Substituted galacturonans, Rhamno galacturonans (Neill *et al.*, 1990, Mohnen *et al.*, 1999). Homogalacturonans are linear polymer of α(1→4)-linked D-galacturonic acid and it is methylated at sixth position of oxygen. Substituted galacturonans are the saccharide append ant residues branching from a backbone of D-galacturonic acid residues while Rhamnogalacturonan are the repeating disaccharide of 4-α-D-galacturonic acid and α (1→2) L-

rhamnose. Rhamnogalacturonan is further divided in to two classes one is rhamnogalacturonanI and another one is known as rhamnogalacturonanII (Darvill *et al.,* 1978). Rhamnogalacturonan II contains 2-O-methyl-D-xylose, D-apiose and 2-O-methyl- L-fucose while the galacturonic acid residues of rhamnogalacturonan I are often acetylated at the C2 or C3 position (Thakur *et al.*,1997). The pectin's properties mainly determined by percentage of esterification. Generally pectin is 70% esterified. Cocktail of enzymes which is used at ''smooth region'' degradation contains mainly deesterifying enzyme.



Pectinase

Pectinases are mainly used in pectin degradation. Pectinases are the group of pectinolytic enzymes. These enzymes had a large number of application in the industrial sector such as bioscouring of cotton and degumming of plant bast fibers (Etters *et al.*,1999).

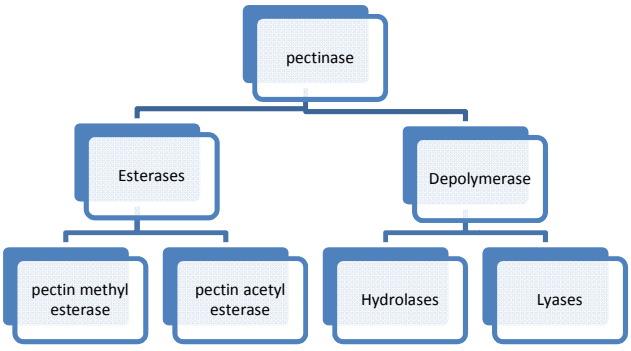


Fig.1:Classification of Pectinase based upon the action mechanism

Pectinase enzymes are divided based on the action mechanism of particular enzyme at pectin structure as shown in Fig.1. These are mainly two types: one is esterase and another one is depolymerase. Esterase enzymes are basically act on a methyl/acetyl ester of galacturonic acid unit. Esterase is further divided in to two subclass one is pectin methyl esterase which is useful to remove the methoxyl group from pectin. While another one is pectin acetyl esterase which removes acetyl residue of pectin. Depolymerizing enzymes remove complexity of polymer by depolymerization of pectin polymer. Depolymerizing enzymes is subdivided in to two groups: one is hydrolases and another one is lyases. Hydrolase enzymes are mainly hydrolyze the glycosidic bonds. Polymethyl galacturonase hydrolyze α (1→4) glycosidic linkage in pectin while polygalacturonase hydrolyze α(1→4) linkage in pectic acid. Lyase enzyme also known as enzyme of cleavage which cleave α(1→4) glycosidic linkage by trans elimination. These are sub divided in two group one is polymethyl galacturonate and another one is poly galacturonate lyase. polymethyl galacturonate catalyses breakdown of pectin while poly galacturonate lyase is catalyze breakdown of α(1→4) glycosidic bond in pectic acid. Fungul species such as *Aspergillus niger* is used to produce acidic pectinlyase which is widely used in wine and fruit juice processing industries from various fruits. Bacterial species such as bacillus species is mostly used to produce alkaline pectinlyase. Alkaline pectinlyase is produced by using peels of various fruits as a substrate and these are widely used in textile industries, paper making industries and treatment of waste water which contains a large amount of pectin substance before any type of biological treatment of waste water (Kashyap *et al.,* 2001).

Production of pectinlyase by bacterial species

Bacillus subtilis have WSHB04-02 gene which encodes pectate lyase enzyme . WSHB04-02 was amplified by PCR. Signal peptide sequence taken from periplasmic secretion and pel B from pET 22b(+) are fused with WSHB04-02. After that chimeric form of this gene is transfer *to E. coli* by using vector pHsh which can control temperature easily for cloning and expression. 5L fermentor was used to grow the recombinant form of *E. coil.* After 20 h finished in broth *E. coli* produce pectinlyase when temperature was changed from 30 degree Celsius to 45 degree Celsius. Purification was done by SDS-PAGE. Optimisation process was done at 50 degree Celsius for 30 min. and optimum pH was taken 9.4. Electro spray ionization (ESI) and mass spectrometry (MS) were used to analyze the degradation of polygalacturonic acid. which were indicate that Pectinlyase was obtained in the form of mixture . mixture is made up of unsaturated oligo galacturonides such as unsaturated bi and tri galacturonic acid(Bin *et al.*,2007). pectinlyase production is optimized under submerged fermentation (Sonia *et al.*, 2009).

Application of alkaline pectinlyase in industrial sector

Alkaline pectinlyase is used in textile industries which is produced by several bacterial species. Most of the alkaline pectinlyase is produced by the *Bacillus* species. Submerged fermentation is used for optimization process of an alkaline and thermo stable pectinase production from *Bacillus subtilis* SS and Application of alkaline pectinlyase was examined in

various industrial sector such as paper and pulp industry, textile industry etc.(Sonia *et al.*, 2009).

Degumming of bast fibrous plants

Bacillus amylobacter, *Bacillus felsineus*, *Bacillus comesii rossi*, were used in the conventional retting process. These microbes were used in the fermentation of pectinacious substance and degumming of plant bast fibers. The process of retting of fiber is complete around 70–100 h . however addition of urea accelerates process of retting and reducing the total time to only 40–50 h (Kozlowski *et al.*,1970). Pectinlyase was potentially used in various application such as retting of plant bast fiber and degumming of ramie and sun hemp. The pectinolytic and cellulolytic enzymes also have effects on the flax retting process . A modern methodology , spray enzyme retting (SER) was used to decrease the percentage of enzyme in retting . SER was worked on the principle that compress stems in to small folds due to this the penetration of enzyme (Flaxzyme) formulations into the stems tissues was increase manifold. Chelators and an optimum pH were used to improve enzyme effectiveness (Ryszard *et al.*,2006). Various Alkalophylic bacteria release polysaccharide- degrading enzymes such as pectate lyase, n-polygalacturonase, xylanase and cellulase which were used for degumming of ramie fibers (Zheng *et al.,*2001). Alkaline and thermo stable polygalacturonase from *Bacillus* sp. MG-cp-2 is used in degumming of ramie (*Boehmeria nivea*) and sun hemp (*Crotalaria juncea*) fibers (Kapoor *et al.*, 2001). Plant fibers were chemically treated before the enzymatic treatment, so due to this both enzymatic and chemical treatment were not sufficient. The improvement of retting of plant bast fibers was done by the use of enzymatic preparations (Kozlowski,1970 , Kozlowski *et al.*,2001 , Kozlowski *et al.*, 2001). The retting of bast fibers from stem of plant by enzymatic treatment was studied by Fourier transform infrared (FT-IR) micro spectroscopic mapping (Himmelsbach *et al.,* 2002).

Treatment of pectic wastewater

The waste water from citrus and other fruit juice processing industry contains high amounts of pectinaceous substances in it. These compounds are not decomposed by the microorganisms during the activated sludge treatment (Tanabe *et al.*, 1987). Alkalophilic microorganisms have been used for the waste water treatment (Tanabe *et al.*,1987).Many microbes like *Erwinia carotovora* (FERM P- 7576),are used for this purpose. *Erwinia carotovora* secrets endo-pectate lyase,which breaks the pectinaceous compounds hence it is useful in pretreatment of pectinaceous wastewater (Tanabe *et al.*, 1987). The main disadvantage of this process is that *E.carotovora* is phytopathogenecy. Pectolytic enzyme produced from the bacteria are also used for the pretreatment of wastewater having pectic substances (Kashyap *et al.*, 2001).

Application in paper and pulp industry

Fibers, fragments of fibers and inorganic filler particles are suspended in a dilute suspension after that this suspension mixture is filtered in such a way, clay or $CaCO_3$ is formed into sheets. Filter fabric contain a number of holes of large size which allow to filter the fine particles from the suspension after drainage of water. Addition of retention aids are also help full in paper making process in pulp industry. Retention aids are useful to maintain velocity of water drainage. Mostly used retention aids in paper industries were the cationic polymers (Horn, D. *et al.*,1996). Polymer of galacturonic acid is used to complex the cationic polymer but pectinlyase has the potential of depolymerization of galacturonic acid polymer (Thornton *et al.*,1994; Reid *et al.*, 2000). Pectinlyase also useful to decrease the amount of cationic polymer in the solution and filtrate (Reid *et al.*, 2000).

Oil extraction

Extraction of vegetable oils is done by the application of pectinlyase, in an aqueous phase which is formed by the liquefaction of the cell wall components of the oil-crops. Use of pectinlyase increases the percentage yield of olive oil. pH, temperature, and amount of the enzyme used play a major role in the yield of oils (Kashyap *et al.*, 2001). Commercially *A.aculeatus* is used for the production of an enzyme Olivex. it possess pectinolytic activity, along with cellulolytic and hemicellulases activity. These activities results in good oil extraction and better stability when stored. The oil formed by this way is stable against rancidity as it has also increased content of polyphenols and vitamin E.

Coffee and tea fermentation

Pectinolytic microorganisms are mainly used for fermentation of coffee. These microorganisms remove the mucilage coat of the coffee beans. Three fourth of the bean consists of pectic substances which are removed by the Pectic enzymes from the pulpy layer (Kashyap *et al.*, 2001). Presence of tea pectins destroy the foam forming property of instant tea. This problem is solved by addition of pectinlyase (Carr *et al.*, 1985).

Conclusion

In this review paper we conclude about bacterial strains which produce pectinlyase , structure of pectin and classification of pectinlyase based upon the broad range of pH. Pectinlyase is mainly found in two forms in which acidic pectinlyase is mainly used in fruit and juice processing industries while alkaline pectinlyase is mainly used in textile industries, paper and pulp industries. Alkaline pectinlyase is mainly produced by submerges state fermenter at pH 9 and optimum temperature is used 45°C to 50°C. Alkaline pectinlyase also used in pretreatment of industrial waste water.

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