Pectinolytic Enzyme - A Review of New Studies

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Abstract

Pectinase is a general term of enzymes such as pectolyase, pectozyme and polygalacturonase. These are enzymes breakdown pectin, a polysaccharide substrate that is found in cell walls of plants. Pectinase enzymes are produced from a wide variety of microbial sources such as Bacteria, Fungi, Yeast and Actinomycetes of them the major producer is Fungi. In the present review of focused on the initiation of pectinolytic enzymes production under different substrate, fermentation conditions and application of these enzymes in different industries such as Food industry, Textile industry, Paper industry, Poultry industry etc.

Keywords: Pectinolytic enzyme, Microbes in pectinase, Fermentation condition and Industrial Application.

Introduction

Pectin is a complex polysaccharide consisting mainly of esterifies D-galacturonic acid resided in (1-4)-chain. The acid groups along the chain are largely esterifies with methoxy groups in natural product. There can also be acetyl groups present on the free hydroxyl groups. The galacturonic acids main chain also has the occasional rhamnose group which disrupts chain helix formation. Pectin is also known to contain other neutral sugars which are present in side chains. The most common side chain sugars are xylose, galactose and arabinose (Shembekar *et al.,* 2009).

Pectinase are a group of at least seven different enzymatic activities that contribute to the breakdown of pectin which is a structural polysaccharide found in primary cell wall and middle lamina of fruits and vegetables. Pectolysis is one of the most important processes for plant, as it plays a role in cell elongation and growth as well as fruit ripening. Microbial pectolysis is important in plant pathogenesis, symbiosis and decomposition of plant deposits (Lang and Dornenberg, 2000). The main source of the microorganisms that produce pectinolytic enzymes are yeast, bacteria and large varieties of fungi and particularly *Asperigillus species* endopolygalacturonase production was first reported in 1951 using *Saccharomyces fragilis* (Luh *et al.,* 1951).

Pectic enzymes have two classes namely pectinesterases and pectin depolymerases. Pectin esterase has the ability to de-esterify pectin by the removal of methoxy residues. Pectin depolymerases readily split the main chain and it was further classified as polygalacturonase (PG) and pectinlyases (PL). Thus on the whole pectinases are hydrolytic enzymes, which hydrolyze the pectin molecules and are readily soluble in water. (Ramanujam *et al.,* 2008).

Pectic Substances

Pectin substance consists of pectin and pectic acid. The main chain of pectin is partially methyl-esterified-1, 4, D-galacturonan. Demetylated pectin is known as pectic acid or polygalacturonic acid. Pectic substances are commonly amorphous; with a degree of polymerization of about 200-400 substituents can be found at the C-2 OR C-3 position of the main chain. Substituent's can be either non-sugar (acetyl) or sugar (D-galactose, D-xylose, L-arabinose and L-mannose). The degree and type of branching varies depending upon the source of the pectic substance. The synthesis of pectic substances occurs in the Golgi apparatus from UDP-D-galcturonic acid during early stages of growth in young enlarging cell walls. (Sakai *et al.,* 1993). Compared with young, actively growing tissues, lignified tissues have a low content of pectic substances. The content of the pectic substances is very low in higher plants usually less than 1%. They are mainly found in fruits and vegetables, constitute a large part of some algal biomass (up to 30%) and occur in low concentration in forestry or agricultural residues. Polysaccharides from cell walls of ripe pears were reported to contain 11.5% pectic substances, 16.1% lignin, 21.4% glucosan, 3.5% galactan, 1.1% mannan, 21% xylan and 10% arabinan (Horikoshi, 1990).

Role Of Microbes In Pectinase Production

Pectolysis is one of the most important processes for plant, as it plays a role in cell elongation and growth as well as in fruit ripening. Pectolytic enzymes are wide spread in nature and are produced by Bacteria, Fungi, Yeast, Insects, Nematodes and Protozoa. For example Bacteria like *Bacillus species,* Clostridium species, Fungi like *Aspergillus species, Penicillum species,* Yeast like *Saccharomyces, Candida* etc., microbial pectolysis is important in plant pathogenesis, symbiosis and decomposition of plant deposits (Lang and Dornenburg 2000). Thus by breaking down pectin polymer for nutritional purposes, microbial pectolytic enzymes play a

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important role in nature. The enzymes are inducible i.e. produced only when needed and they contribute to the natural carbon cycle.

Microbial pectinolytic enzymes are not only enzymes available to attack plant polysaccharides. However, pathogenic attack on plant tissue in normally initiated by pectic enzymes because pectic substances are most readily accessible. Other carbohydrates appear sequent and attack the available polysaccharides. Final result in a sequence of appearance of microbial carbohydrates during microbial attack on plant cell walls (Sakai *et al.,* 1993).

Substrate For The Production Of Pectinase

Substrates that are employed in the production of enzyme should be solid as solid substrate can give good encourage to the growing cells. Substrates should provide all needed nutrients to the microorganisms for its growth. Other factors like particle size, moisture levels are also to be taken for consideration. Generally agro-industrial wastes are employed for the pectinase production. Various substrates that are being used are sugarcane bagasse, wheat bran, rice bran, wheat straw, rice straw, saw dust, corn cobs, coconut coir pith, banana waste, tea waste, sugar beet pulp, apple pomade, orange peel etc (Pilar *et al.,* 1999).

Fermentation Condition

Pectinase are constitutive or inducible enzymes that can be produced either by submerged (Aquilar and Huitron 1990) or solid state fermentation (Acuna-arguelles *et al.,* 1995). Various factors related to environment affect the production of pectinase. Some of them are concentration of nutrients, pH, temperature, moisture content, influence of extraction parameters on recovery of pectinases and the effects played by the inducers. Both carbon and nitrogen sources show overall effect on the productivity of pectinases (Catarina and Almeida *et al.,* 2003). Pectin, glucose and sucrose when added to the media in higher concentration have a repression effect on the studied enzyme activity (Maria.F *et al.,* 2000) of the various nitrogenous matters that can be used. Optimum sources are $(NH_4)_2SO_4$, yeast extract, soya bean pulp powder, soya peptone.

Temperature and pH are also important parameters to be taken care. Since the system used is solid-state fermentation, the conjugation of temperature and pH are highly important. The pH is regulated using a mixture of source of nitrogen as when *Aspergillus niger* is being used pH turns to be acidic. Besides the nature of the substance also plays a vital role in the pH maintenance. Generally the pH is maintained at 7 and temperature in solid state fermentation is maintained at 30-32°C, as it cannot be precisely controlled due to reason that solid-state fermentation has solid substances have limited heat transfer capacity. Moisture content in the substrate also plays a significant role (Martin *et al.,* 2004). The previous studies show that it was generally maintained around 50-55% for the production of pectinases by microbial means (Leda *et al.,* 2000).

Two types of fermentations can be carried out for the pectinase production. They are solid state fermentation and submerged fermentation. In comparison between these two fermentations, solid state fermentation presents a serious of advantages over submerged state fermentation. Culture conditions are similar for the filamentous microorganisms as in the case of solid state fermentation. The growth of organisms is very high with large quantities of enzyme being produced (Ramanujam *et al.,* 2008).

Pectinolytic Enzymes Production

Production of pectinase from pectin rich agro waste, viz. lemon peel, sorghum stem and sunflower head used as substrate for *Aspergillus niger* DMF 27 and *Aspergillus niger* DMF 45 in submerged fermentation and solid state fermentation system, respectively. The maximum amount of endo and exo pectinase was obtained from sunflower head followed by lemon peel in solid state fermentation. The increased level in the production of pectinases was noticed when the agro wastes were supplemented with additional carbon and nitrogen sources and supplementation of sucrose was more effective than glucose in solid state fermentation (Patil *et al.,* 2006).

Identification of growth phenotypes in *Aspergillus niger* pectinase producing mutants using image analysis procedures. Relative growth rates of four strains of *Aspergillus niger* were estimated by image analysis of colonies grown with mineral salts and 10g pectin/liter. Water activity levels were 0.96 and 0.99. 2-deoxy-D-glucose (2DG) was added up to 0.1g/liter. These techniques proved useful to select mould strains for pectinase production in culture media with different water activities (Loera and Gonzalez, 1998).

Pectinase were produced by *Aspergillus species* using various pretreated lemon peel as the carbon source instead of pectin. It was found that the production of polygalacturonase was about the same and that of pectin esterase sustain higher when unwashed fresh lemon peel was used instead of pectin (Maldonado *et al.,* 1986).

Using various carbon source and nitrogen sources as well as natural products was investigated as inducer for the production of amylases and pectinase using *Aspergillus niger.* Wheat bran extract was best for the production of both amylase and pectinases. High pectinase activities were also observed when polygalacturonic acid, fructose, mannose, saccharose and cellobiase were used as stimulators. Optimum pH for the production of pectinase was 6.0 and temperature was 35°C (Fiedurek *et al.,* 1989).

Bacillus sps DT7 isolated from soil, it has been found to produce significant amounts of an extra cellular pectinase subsequently characterized as pectinlyase. By optimizing growth conditions, *Bacillus sps* DT7 produced higher amount of pectinlyase using gel filtration and ion exchange chromatography (Kashyap *et al.,* 2000).

The production of pectinase and expression of genes encoding pectinase by *Candida* and *Germlings* of *Blumeria graminis* were investigated by pectate plate assay, the activity of polygalacturonase was detected in homogenates from ungerminated *Conidia* and *Germlings* grown on an artificial substratum. We could amplify the fragments of two endo-polygal; acturonase genes, two pectinlyase gene and a pectatelyase gene from genomic DNA of the fungus by the polymerase chain reaction (PCR) (Suzuki *et al.,* 1999).

Friedrich *et al.,* 1992 studied the effect of different sugars as carbon source on *Aspergillus niger* to synthesis pectinolytic enzymes. It was found that pectin esterase and pectinlyase activities were found similar to those obtained in the medium containg pectin. By increasing sugar concentration from 1.5-15% the activities

are increased as follows:

Polygalacturonase - 1.8 to 20U/ cm^3

Pectinlyase - 0.14 to 0.65U/ cm^3

Pectin esterase - no effect

Endopolygalacturonase lyase was produced from *Streptomyces thermovulgaris* CR42. It is an endopolygalacturonase lyase with in 48 hours of fermentation. Complete degradation of pectin was observed and optimum temperature for growth and enzymes production was 55°C, the optimum pH of the medium was 7.6 and there is no product of enzyme if the initial pH of the medium is less than 6.7 (Niranjan and Dhala, 1981).

The microbial flora of coffee beans collected in the regions of Sao Paulo contained *Clodosporium, Fusarium and Aspergillus sps.* The pectolytic enzymes liberated from the fungi are capable to breakdown pectic acid and galactoarabinan (Woriaki *et al.,* 1973).

Aureobasidium pullulans LV10 produced extracellular pectinolytic enzymes when grown on medium containing apple pectin as a carbon source. Maximum enzyme production was 22U/cm3 for polygalacturonase and 9U/cm^3 for pectin lyase was obtained after 4 days of fermentation (Manachini *et al.,* 1988).

Dried sweet whey was used as a complete medium for production of polygalacturonase by *Kluveromyces fragilis.* The optimum concentration of whey for enzyme production was 0.5% (w/v), two days of fermentation at 25°C. supplementation of whey with sodium polypeptone does not increase enzyme production (Donaghy *et al.*, 1994).

Application Of Pectinolytic Enzymes

Clarification Of Fruit Juice

By applying these enzymes on fruit pulp, it degrades pectin thereby reducing the viscosity and the fruit juice can be handled easily. These enzymes play an important role in maceration and Solubilization of fruit pulps and in clarification. The traditional method of clarification of pectin containing juice involves a number of steps, including centrifugation to remove suspended solid, enzymatic treatment for depectinization, finding agents such as bentonite and gelatin to remove haze and finally filtration by the diatomaceous earth to remove the finding agents. With membrane technology, juice can be clarified using depectinization followed by ultra filtration (UF) or micro filtration (MF).

Pectinase In Textile Industries

Textile processing has benefited greatly in both environmental and product quality aspects through the use of enzymes. Prior to weaving of yarn in to fabric, the warps yarns are coated with a sizing agent to lubricated and protect the yarn from abrasion during weaving. Historically, the main sizing agent used for cotton fabrics has been starch because of its excellent film-forming capacity, availability, and reality low cast. Before the fabric can be dyed, the applied sizing agent and the natural non-cellulosic materials present in the cotton must be removed. Before the discovery of amylase enzymes, the only way to remove the starch-based sizing was extended treatment with casting soda at high temperature.

Clarified orange juice

Clarified orange juice

The chemical treatment was not totally effective in removing the starch and also result in a degradation of the cotton fiber resulting in distraction of the natural soft feel or 'hand' of the cotton the use enzyme such as pectinase in conjugation with amylases, lipases, cellulases, and other hemicellulolytic enzymes to remove sizing agents has decreased the use of harsh chemicals in textile industry, resulting in a lower discharge of waste chemicals to the environment, improving both the safety of working conditions for textile workers and the quality of the fabric.

Degumming Of Plant Fibers

The most upcoming application of pectinolytic enzymes use in the degumming of plant fibers such as ramine, sunn herm, jute, flax and hemp (Bruhlmann *et al.,* 1994; Cao *et al.,* Henriksson *et al.,* 1997, Kapoor *et al.,* 2001). The enzymatic processing result in no damage to the fibers and most importantly in addition to being energy conservative is environmentally friendly (Gurucharanam and Deshpande 1986). A high pH optimum of pectinase from microorganisms is reported to be desirable for degumming of plant fibers since a high pH not only prevents contamination but also allows an open fermentation system to be adopted (Zheng *et al.,* 2001).

Retting Of Plant Fibers

In recent years, a few fundamental studies have been initiated on the enzymatic retting process. These employ purified enzymes on defined substrates and characterization of the resulting products. A pectinase from *Rhizomucor pumilis* was used for flax retting (Henriksson *et al.*, 1999). To ensure maximum strength of the thread manufactured from retted flax, only a small fraction of the pectinases belonging to the fiber bundles needs to be hydrolyzed. In developing nation and particularly in countries where forest lands are endangered from over exploitation, better use might be made of herbaceous fibers for paper production. Such feedbacks should be amenable to enzymatic pulping and the resulting processes should give together yields with fewer environmental problems.

Pretreatment Of Pectic Waste Waters

Environmentally, the treatment of waste water from citrus processing industries containing pectic substances is carried out in multiple

steps, including physical dewatering, chemical coagulation, direct activated sludge treatment and chemical hydrolysis, which lead to formation of methane. These have several disadvantages, such as the high cost of treatment and longer treatment times in addition to environmental pollution from the use of chemicals. Thus, an alternative, cost effective, and environmentally friendly method is the use of pectinases from bacteria, which selectively remove pectic substances from the waste water. The pretreatment of pectic wastewater from vegetable food processing industries with alkaline pectinase and alkalophilic pectinolytic microbes facilitates removal of pertinacious material and renders it suitable for decomposition by activated sludge treatment (Horikoshi 1999; Tanabe *et al.,* 1987, Tanabe *et al.,* 1988). An extracellular endopectate lyase from an alkalophilic soil isolate, *Bacillus sps* GIR 621, was used effectively to remove pectic substances from industrial waste water (Tanabe *et al.,* 1987).

Coffee And Tea Fermentation

Pectinase treatment accelerates tea fermentation and also destroys the foam forming property of instant tea powders by destroying the pectins (Carr 1985). Pectinolytic microorganisms are used in the fermentation of coffee to remove the mucilaginous coat from the coffee beans. Pectinases are some time added to remove the pulpy bean layer consisting of pectic substances.

Paper And Pulp Industry

With the advancement of biotechnology and increased reliance of paper and pulp industries on the use of microorganisms and their enzyme for biobleaching and paper making, the use of enzyme other than xylanases and ligninases, such as mannanase, pectinases is increasing in the paper and pulp industries in many countries (Bajpai 1999; Kirk and Jefferies 1996). During paper making pectinase can depolymerize polymers of galacturonic acids, and subsequently lower the cationic demand of pectin solutions and the filtrate from peroxide bleaching (Reid and Ricard 2000; Viikari et al., 2001). An overall bleach-boosting of eucalyptus Kraft pulp was obtained when alkaline pectinase from *Streptomyces sps*. QG-11-3 was used in combination with xylanase from the same organism for biobleaching (Beg *et al.,* 2001). The ability of polygalacturonic acid to complex cationic polymers depends strongly on the degree of polymerization. Pectinases depolymerise polygalacturonic acids and consequently decrease the cationic demand in the filtrate from peroxide bleaching of thermo mechanical pulp (Viikari *et al.,* 2001).

Poultry Feed

Intensive research in to the use of varies enzymes in animal and poultry feeds started in the early 1980s. The first commercial success was addition of -glucanase in to barley-based feed diets. Usually a feed enzyme preparation is a multi enzyme cocktail containing glutanases, xylanases, proteinases, pectinases and amylases. Enzyme addition reduces viscosity which increases absorption of nutrients, librates nutrients either by hydrolysis of non degradable fibers, or by librating nutrients blocking by these fibers, and reduces the amount of faces (Petersen 2001).

Purification Of Plant Viruses

A virus prior to purification is very limited. Very pure preparations of viruses are required in order to carry out chemical, physical, and other biological studies. The need numerous purification that can be adapted to many of the virus that infects plants. However, there are several different purification systems that can be selected for use according to the type of virus. In those cases in which the virus is restricted to phloem, certain enzymes, such as alkaline pectinases and cellulases can be used to liberate the virus from the tissues (Salazar and Jayasinghe 1999).

Oil Extraction

Citrus oil such as lemon oil can be extracted with pectinases as this enzyme destroys the emulsifying properties of pectin. Which interfere with the collection of oils from citrus peel extracts (Scott 1978). Plant cell wall-degrading enzyme preparation as begin to be used in olive oil preparation. The enzyme is added during the process of grainding of olives by which easy removal of oil is accomplished is subsequent separation procedures.

Conclusion

The pectinolytic enzymes from microorganisms have generally focused on induction enzyme production under various conditions, fermentation process, various substrate purification and characterization and use of this enzyme for different industrial process. The enzyme system used by microbes for metabolizing and for complete breakdown of pectin are most important tools for elaborating the economical, ecofrienly and green chemical technology for using pectin polysaccharide in nature.

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