Microbial Pectinases and their applications in industries: A review

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Abstract- *There is an ever increasing demand to replace traditional chemical processes with advanced biotechnological processes involving microorganisms or enzymes such as pectinases, xylanases, cellulases, laccases, lipases, proteases and other hydrolytic enzymes. Today, pectinases are among the most important enzymes in the commercial sector. Alkaline pectinases are important industrial enzymes of great significance in the current biotechnological arena with wide-ranging applications in textile processing, degumming of plant bast fibers, treatment of pectic wastewaters, paper making, and coffee and tea fermentations. The present review features the potential applications and uses of microbial alkaline pectinases, the nature of pectin, and the vast range of pectinolytic enzymes that function to mineralize pectic substances present in the environment. It also emphasizes the environmentally friendly applications of microbial alkaline pectinases thereby revealing their underestimated potential. The review intends to explore the potential of these enzymes and to encourage new alkaline pectinase-based industrial technology.*

Keywords: Enzymes, Industries, Pectinases, Polygalacturonase, Pectin

1. INTRODUCTION

Enzymes are highly efficient biological catalysts which perform all synthetic and degradative reactions in living organisms. The enzymes are preferred to chemicals in commercial endeavor mainly because of their high catalytic power, specific mode of action, stereo-specificity, eco-friendly nature and reduced energy requirement. Many research activities have been persued involving enzymes worldwide, for their use in the development of new important industrial processes. Literature reveals that the presence of certain potentially harmful materials, like phenolic compounds, endogenous enzyme inhibitors and proteases, limits the use of plants as source for enzyme production (Marwaha and Kaur, 2000). Animals are also not preferred for manufacturing of enzymes because the enzymes are present only in limited quantities and show a wide variation in their distribution. The most important limitations in them are the difficulties in isolation, purification and cost of enzymes, and moreover, when the enzyme is extracted from a diseased animal there is a heavy risk of contamination from diseases such as bovine spongiform encephalopathy (BSE) or mad cow disease (Ghosh, 2011). Therefore, microorganisms are favoured to plants and animals as sources for industrial enzymes, because of their low production cost, more predictability, controllability of enzyme contents and easy availability of cheap raw materials for their cultivation and high growth rate. In addition microorganisms are also an efficient and safe host where enzymes can be heterologously expressed by cloning and expressing genes from other organisms.

Enzymes were discovered in the second half of the nineteenth century, and since then have been extensively used in several industrial processes. Enzymes are extremely efficient and highly specific biocatalysts. With the advancement in biotechnology over last three decades, especially in the area of genetics and protein engineering, enzymes have found their way into many new industrial processes. Microbial enzymes are routinely used in many environmentally friendly and economic industrial sectors. Environmental pollution is no longer accepted as inevitable in technological societies. Over the past century, there has been a tremendous increase in awareness of the effects of pollution, and public pressure has influenced both industry and government. There is increasing demand to replace some traditional chemical processes with biotechnological processes involving microorganisms and enzymes such as pectinases (Bajpai1999; Bruhlmann et al. 2000), xylanases (Beg et al.2000a, b), cellulases (Bajpai et al. 1999), mannanase (Montiel et al. 2002), α-galactosidase (Clarke et al.2000), and laccases and ligninases (Bajpai 1999; Onysko 1993), which not only provide an economically viable alternative but are also more environmentally friendly (Viikari et al. 2001).
The pectinase enzymes are integral part of food industry. The estimated sale value of all industrial enzymes in 1995 was one billion dollars, of which 75 million dollars was for pectinases. Although, the commercial application of pectinases was noticed, for the first time, in 1930 for the preparation of wines and fruit juices (Oslen, 2000) but the chemical nature of the plant tissues was apparent only in 1960's (Abeles 1992). After this, the scientists began to search for the commercial utility of these enzymes. Pectinase enzymes are widely distributed in nature. They mainly occur in plants, bacteria, fungi, yeasts, insects, nematodes and protozoa. Microbial pectolysis is important in plant pathogenesis, symbiosis and decomposition of plant deposits (Lang and Domenburg, 2000). By breaking down pectin

polymer for nutritional purposes, microbial pectinases play a highly important role in nature. The biotechnological potential of pectinases from microorganisms has drawn a great deal of attention from various researchers, worldwide. These are widely used as biological catalysts in the industrial processes.

Pectin is the substrate for pectinolytic activities. Pectin was first isolated in 1820 from citrus fruits (Braconnot, 1825) and thereafter used as the key substance in making jams and jellies. The first commercial production of a liquid pectin extract was recorded in 1908 in Germany. The microorganisms, which synthesise pectic enzymes, are usually found associated with dead and decaying plant materials. Schink and Zeikus (1981) determined anaerobic breakdown of pectin by bacteria. Pectinase enzymes play a decisive role in the microbial spoilage. Many spoilage organisms are usually strongly pectinolytic and are characteristically responsible for the extensive maceration of fruits and vegetables.

Pectic substances are classified into four main types: pectic acids, pectinic acid, protopectin and pectin. Pectic acid is mostly composed of colloidal polygalacturonic acid and is essentially free from methyl ester groups, demethylated pectin is known as pectic acid or polygalacturonic acid. The salts of pectic acid are either normal or acid pectates. The pectinic acids contain upto 75% methylated galacturonate units. Under suitable conditions, pectinic acids are capable of forming gels with sugars and acid or, if suitably low in methoxyl content, with certain metallic ions. The salts of pectinic acid are either normal or acid pectinates. Protopectin is the water-insoluble parent pectic substance, located primarily in the middle lamella that serves as the glue to hold cells together in the cell walls. It yields pectin or pectinic acids upon restricted hydrolysis. Some of the reasons for insolubility of protopectin are: (1) its large molecular weight, (2) ester bond formation between carboxylic acid groups of the pectin and hydroxyl group of the other cell wall constituents, or (3) salt bonding between the carboxyl groups of pectic substances and basic groups of proteins. A model for the chemical structure of protopectin has been proposed in which neutral sugar side chains are arranged in blocks (hairy regions) separated by unsubstituted regions containing almost exclusively galacturonic acid residues (smooth regions). Pectin is the soluble polymeric material in which approximately 75% of the carboxyl groups of the galacturonate units are esterified with methanol. Like pectinic acids, pectin is capable of forming gels with sugar and acid under suitable conditions. Pectins are used in the pharmaceutical sector as detoxifying agents, and are well known for their anti-diarrhoeal effects.

1.1 Sources

Traditionally, commercial source of pectin has been the citrus peel and apple pomace. Out of which, the citrus peel has often been the preferred material for pectin manufacture due to its high pectin content and good colour properties. However, the other sources of pectin are sugar beet and sunflower. The chief raw materials for the production of pectin are the residues from the manufacture of fruit juices, apple pomace and dried citrus peel (Alkorta et al. 1994; Blanco et al. 1999). The extraction of pectin is carried out by acid hydrolysis at a pH range of 2–3 for 5 h at high temperature (70–100°C). The solid to liquid ratio is normally about 1:18. The pectin extract is separated from the pomace using a hydraulic press or by centrifugation. The extract is filtered, and finally concentrated to a standard setting strength. For powdered pectin preparation, the concentrated extract is treated with organic solvents or certain metallic salts to precipitate the polymers (Sakai et al. 1993).

1.2 Occurrence and properties

Pectic substances are prominent structural constituents of cell wall in non-woody tissues, next to cellulose, several hemicelluloses and proteins (Brillouet, 1987). In addition, these are the sole polysaccharides in the middle lamella responsible for cell cohesion (Pilnik, 1981). Pectic polysaccharides occur mainly as water insoluble protopectin. Their synthesis, beginning from UDP-galacturonic acid and taking place in golgi system (Karr, 1976), is performed mainly during early stages of growth, in young enlarging cell walls.

Texture of vegetables and fruits is strongly influenced by the type of pectin present. One of the most characteristic changes during ripening is the softening of the fruits. This change is attributed to enzymatic degradation and solubilisation of protopectic substances (Pressey, 1988; and Dick and Labavitch, 1989). However, during ripening the neutral sugar composition of the extractable pectin does not change (DeVries et al., 1981). In processing certain vegetables for example cauliflower, the excessive softening is prevented by adding calcium salts. As a consequence, insoluble pectates and pectinates are formed, giving a firm texture to the vegetables.

Pectic polysaccharides are soluble in water and other solvents, e.g. dimethyl sulphonic acid and hot glycerol and are insoluble in most organic solvents. The ease of dissolution in water decreases with increasing chain length (Deuel and Stutz 1958). Pectic polysaccharides may be precipitated by water miscible organic solvents, water insoluble basic polymers, polyvalent cations, quaternary detergents, proteins and monovalent cations (Duel and Stutz, 1958; Scott. 1978; and Doseburg, 1965).

Pectin is hydrolysed by the treatment of acids and alkali, and the hydrolysation depends on the temperature. Extensive or strong acid treatments affect complete degradation with formation of CO_2, furfural and a number of other breakdown products. Degradation also occurs by oxidizing agents and irradiation. Cross-linked pectin chains form insoluble polymers having ion exchange properties. These are very selective for calcium and heavy metal ions, e.g. Zn^{2+}, Ca^{2+} and Fe^{2+} (Doesburg, 1965; and Fogarty and Kelly, 1983).

The most unique and outstanding physical property of pectins is that these are able to form gels with sugar and acid (Fogarty and Kelly, 1983). The presence of calcium promotes the formation of gels by forming strong ion associations with carboxyl groups of neighbouring pectin chains 'salt bridges'. Pectinic acid is often added to foods and beverages, especially in diets for diabetics as non-nutritive sweeteners or sugar supplements (Forgarty and Kelly, 1983).

It has been suggested that many of the unique physical properties of pectic substances are chiefly associated with carboxyl groups of the galacturonic acid residues. Complete esterification of commercial pectin by chemical treatment means total modification of its acidic, viscosity and gel forming properties (Sakai et al., 1983).

1.3 Classification

Pectic enzymes are a group of complex enzymes, occurring in plants and microorganisms, where pectin serves as the substrate. Pectin, the main component of the middle lamella ("the cementing material" of the plant cell walls) is dissolved by the pectic enzymes. The microorganisms also produce pectic enzymes, mainly extracellularly.

Protopectinase, the enzyme that catalyses the solubilisation of protopectin was originally named by Brinton *et al*., (1927). These enzymes release pectin from protopectin. Microbial protopectinases are produced at pH 5.0 and temperature of 30°C. One unit of protopectinase activity is defined as liberation of pectic substances equivalent to 1µmol D-galacturonic acid/ml of reaction mixture at 37°C in 30 minutes (Stutzenberger, 1992).

Irrespective of the source, pectic enzymes are classified into three types based upon three criteria:

- Substrate used (whether pectin, pectic acid or oligo-D-galacturonic acid).
- Type of cleavage (whether they act by transelimination or hydrolysis).
- Mode of action (whether the cleavage is random (endo-liquifying or deploymerising enzymes) or end-wise (exo or saccharifying enzymes).

Based on this third criterion, the pectic enzymes are classified into three types: (a) Esterases; (b) Hydrolases; (c) Lyases

2. Acidic pectinases

Acidic pectinases are mainly used in the fruit industry and wine making, and most commonly these are isolated from fungal sources, especially from *Aspergillus niger*. All fruits and berries of nutritional significance contain large amounts of pectin often ranging upto 1/3rd of the total dry weight. In the unripe fruits most of the pectic substances are in the form of protopectin, as the fruit ripens the protopectins are converted to more soluble form to make the fruit soft. Because of its solubility, the pectin passes into the juice. The high pectin content in the juice makes the juice viscous and difficult to filter, as a result there is a relatively low yield of both the juice as well as the colouring and flavouring compounds. These difficulties can be overcome by the treatment of the juice with pectinase enzyme (Stutzenberger, 1992).
The juices produced commercially by these industries (Kashyap et al 2000) include:

- Sparkling clear juices.
- Juices with clouds
- Unicellular products

In the case of sparkling clear juices, the enzymes are added in order to increase the juice yield during pressing and straining and also to remove suspended particles to make the juice sparkling and clear. This principle is applied (i) in apple juice clarification and preparation of purees and nectars, (ii) in pear juice processing and preparation of purees and nectars, (iii) in grape juice and wine clarification and (iv)in strawberry, blackberry and raspberry juice clarification.

In cloudy juices, pectic enzymes containing high levels of polygalacturonase activity are added to fruit juices to stabilize the cloud, such as in cloud stabilization of orange juice, lemon juice clarification, recovery of citrus peel oils, preparation of citrus salads and dried animal feed from citrus fruits, processing of fruits like mango, apricot, guava, papaya, pineapple, banana etc.

The integrity of plant cells is preserved by selectively hydrolyzing the polysaccharides of the middle lamella. Unicellular products are substances formed by the transformation of organized tissues into a suspension of intact cells, resulting in products, which can be used as base material for pulpy juices, nectars as baby foods, as ingredients for the dairy products such as puddings and yoghurt and as protoplasts for various biotechnological applications. The enzymes used for this purpose are referred as 'macases' and this process is referred as maceration. It is likely that the best enzyme preparations for maceration contain cellulases and

hemicellulases in addition to the pectic enzymes (maceration of plant tissue, liquefaction and saccharification of biomass and isolation of protoplasts).

3. Alkaline pectinases

Alkaline pectinases are used mainly in the degumming and retting of fibre crops, pretreatment of pectic wastewater from fruit juice industry, production of paper and pulp, oil extraction and coffee tea fermentation. Pectic enzymes are also involved in wood preservation (Ward and Fogarty, 1973).

In view of the diverse applications of these acidic and alkaline pectinases, these form the backbone of biotechnology industry. The various ongoing researches are likely to find the application of these extremely important enzymes.

The enzymes hydrolyzing these pectic substances are broadly known as pectinases, and include polygalacturonases, pectin esterases, pectin lyases and pectate lyases, depending on their mode of action (Alkorta et al. 1998). Pectinases are produced from a wide variety of microbial sources such as bacteria (Dosanjh and Hoondal 1996; Kapoor et al. 2000; Kashyap et al. 2000), yeast (Blanco et al. 1999), fungi (Huang and Mahoney 1999; Stratilova et al. 1996) and actinomycetes (Beg et al. 2000a, b; Bruhlmann 1995). Microbial pectinases have tremendous potential to offer mankind. The acidophilic pectinases have extensive applications in the extraction and clarification of fruit juices and wine (Alkorta et al. 1998; Blanco et al. 1999; Gainvors et al. 1994; Pretel et al. 1997). However, work on the utilization of alkaline pectinases remains underdeveloped as only a few reports are available on applications of these enzymes. Alkaline pectinases are being used for the pretreatment of wastewater from vegetable food processing industries containing pectinacious material (Tanabe et al. 1987, 1988), and processing and degumming of plant fibers such as ramie (*Boehmeria nivea*), sunn hemp (*Crotalaria juncea*), buel (*Grewia optiva*), flax (*Lisum usitatissimum*), and jute (*Chorchorus capsularis*) (Bruhlmann et al. 2000; Cao et al. 1992; Henriksson et al. 1999; Kapoor et al. 2001; Kashyap et al. 2001a, b; Sreenath et al. 1996), as well as depolymerizing and debarking (Viikari et al. 2001). Plant fibers are commonly used for making ropes, bags, nets etc. in developing countries. This review will focus briefly on the different types of pectic substances and their utilization followed by a detailed discussion of the technological innovations and benefits of environmentally friendly industrial applications of alkaline pectic enzyme.

3.1 Biotechnological applications of alkaline pectinases

Over the years alkaline pectinases have been used in several conventional industrial processes, such as textile and plant fiber processing, coffee and tea fermentation, oil extraction, and treatment of industrial wastewater containing pectinacious material. With increased understanding and knowledge of the mechanism of pectin-degrading Microorganisms and their enzymes, alkaline pectinases have made their way into several other biotechnological processes, such as purification of plant viruses (Salazar and Jayasinghe 1999), and paper making (Reid and Ricard 2000; Viikari et al. 2001), most of which, despite sounding interesting, have yet to be commercialized. The following sections discuss some of the conventional and new applications of alkaline pectinolytic enzymes.

3.2 Clarification of fruit juice

By applying these enzymes to fruit pulp, pectin is degraded thereby the viscosity is reduced 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, fining agents such as bentonite and gelatin to remove haze and finally filtration by the diatomaceous earth to remove the fining agents. With membrane technology, juice can be clarified using depectinization followed by ultra-filtration (UF) or micro-filtration (MF).

3.3. 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 lubricate 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. The chemical treatment was not totally effective in removing the starch and also resulted in a degradation of the cotton fiber resulting in distraction of the natural soft feel or 'hand' of the cotton. The use of 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 chemical wastes to the environment,

improving both the safety of working conditions for textile workers and the quality of the fabric.

3.3 Coffee and tea fermentations

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 sometimes added to remove the pulpy bean layer consisting of pectic substances. The role of cellulase and hemicellulase enzyme preparations in enhancing digestion has also been exploited to help in digestion of the mucilage (Carr 1985; Godfrey 1985). Alkaline fungal pectinases are also reported to be used in tea manufacture.

3.4 Paper and pulp industry

With the advancement of biotechnology and increased reliance of paper and pulp industries on the use of microorganisms and their enzymes for biobleaching and paper making, the use of enzymes other than xylanases and ligninases, such as mannanase, pectinases, and α-galactosidase
is increasing in the paper and pulp industries in many countries (Bajpai 1999; Kirk and Jefferies 1996). During papermaking, 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* sp. QG-11–3 was used in combination with xylanase from the same organism for biobleaching (Beg et al. 2001). The ability of polygalacturonic acids 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).

3.5 Poultry feed

Intensive research into the use of various enzymes in animal and poultry feed started in the early 1980s. The first commercial success was the addition of β-glucanase into barley-based feed diets. Subsequently, enzymes were tested also in wheat-based diets. Xylanase enzymes were found to be the most effective in this case. The net effect of enzyme usage in feed is increased animal weight gain with the same amount of barley resulting in an increased feed conversion ratio. Usually a feed enzyme preparation is a multienzyme cocktail containing glucanases, xylanases, proteinases, pectinases and amylases. Enzyme addition reduces viscosity, which increases absorption of nutrients, liberates nutrients either by hydrolysis of non-degradable fibers, or by liberating nutrients blocked by these fibers, and reduces the amount of faeces. Recent studies in broilers conducted with and without the use of antibiotics suggest that feed additives significantly modify the immune-derived inflammatory response under stress conditions resulting in better mortality, weight gain, feed conversion and bone strength in the broilers.
Petersen (2001) studied multi-enzyme preparations containing pectinases, β-glucanases and a variety of hemicellulases to test their efficacy for improving the digestibility of a vegetable protein mixture consisting of sorghum, soy and canola in broilers for 49 days. Enzyme supplementation improved weight gain and feed conversion significantly.

3.7 Purification of plant viruses

Knowledge about 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. There are numerous purification procedures that can be adapted to many of the viruses that infect 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 the phloem, certain enzymes, such as alkaline pectinases and cellulases, can be used to liberate the virus from the tissues (Salazar and Jayasinghe 1999).

3.8 Oil extraction

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

3.9. Pretreatment of pectic waste water

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 pre-treatment 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* sp. GIR 621, was used effectively to remove pectic substances from industrial waste water (Tanabe et al., 1981).

CONCLUSION

The pectinolytic enzymes from microorganisms have generally focused on induction of enzyme production under various conditions such as fermentation process, various substrate purification and characterization and use of these enzymes for different industrial processes. The enzymes used by microbes for metabolizing and for the complete breakdown of pectin are an important tool for the elaboration of a sustainable, economical, ecofriendly and green chemical technology for using pectin polysaccharide in nature.

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