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PECTINOLYTIC ENZYMES: CLASSIFICATION, PRODUCTION, PURIFICATION AND APPLICATIONS

Anil Ramdas Shet*, Shivalingasarga Vijaykumar Desai, Sharanappa Achappa

Department of Biotechnology, K.L.E. Technological University,

Hubballi, Karnataka, India.

ABSTRACT: Microbial Pectinase has drawn great attention from various researchers worldwide as a biological catalyst in a variety of industrial processes. Pectinase is an enzyme that breaks down pectin, a polysaccharide found in plant cell walls. Pectinolytic enzymes are classified as Protopectinases, Esterases and Depolymerases according to their mode of attack on the galacturonan part of the pectin molecules. Pectinolytic enzymes are among the most important industrial enzymes with wide-ranging applications in fruit juice industry, degumming of plant bast fibers, wastewater treatment, wine industry, textile processing, paper making and coffee and tea fermentations. The present review discusses the structure, properties and applications of pectin, classification of Pectinase, production of microbial Pectinase, purification and biotechnological applications of microbial Pectinase.

KEYWORDS: Pectin, Microbial Pectinase, Pectinolytic enzymes.

***Corresponding Author: Anil Ramdas Shet**

Department of Biotechnology, K.L.E. Technological University, Hubballi, Karnataka, India.

Email Address: anil_shet@kletech.ac.in

1. INTRODUCTION

Enzymes are protein molecules in cells which work as catalysts. Enzymes speed up chemical reactions in the body, but do not get used up in the process. Pectinases are a heterogeneous group of related enzymes that hydrolyze the pectin substances. Pectin substances form the major components of middle lamella, a thin layer of adhesive extracellular material found between the

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primary cell walls of adjacent young plant cells. Pectinase are widely distributed in higher plants and microbes [1]. They are importance for plants as they help in cell wall extension [2] and softening of some plant tissues during maturation and storage [3, 4]. Pectinases accounts for about 10% of total enzyme production in the world market [5]. Pectinases are classified according to their mode of secretion as extracellular and intracellular pectinases. An extracellular enzyme is secreted outside the cell into the medium in which that cell is living. Extracellular enzymes usually convert large substrate molecules into smaller molecules that can then be more easily transported into the cell, whereas an intracellular enzyme operates within the cell membrane. Pectinolytic enzymes are divided into three groups according to the cleavage site as hydrolases consisting of polygalacturonase, lyase/trans-eliminases comprising pectinlyase and pectate lyase, pectin esterase [6]. Pectinases are produced from different microorganisms such as bacteria [7, 8], yeast [9], fungi [10] and actinomycetes [11, 12]. Amongst these, the filamentous fungi are most commonly employed. *Aspergillus niger* is the most commonly used fungal species for industrial production of Pectinolytic enzymes [13, 14, 15]. Intracellular as well as extracellular enzymes are produced by fungi. All fungi are heterotrophic, which depend on carbon compounds produced by other living organisms. Small molecules like mono disaccharides fatty acids and amino acids can easily pass through but for breaking down of larger complex compounds like pectin, fungi secrete extra cellular enzymes. The extra cellular enzymes are easier to be extracted when compared with intracellular enzymes. The extraction of Intracellular enzymes requires more time and costly chemicals. Pectinolytic enzymes can be produced by both submerged and solid state fermentation. The materials of plant origin like grains such as rice, corn, root, tubers and legumes are used as substrates for solid state fermentation. The pectinases have extensive applications in the extraction and clarification of fruit juices and wine [16, 9, 17, 18].

Pectin- its structure, properties and applications

Pectin is a high molecular weight (194.139g/mol) heterogeneous and acidic structural polysaccharide [19] which is one of the major constituents of cereals, vegetables, fruits and fibers. It is the component of middle lamella and primary cell wall in plant cell wall and within the wall forms a matrix in which a network of cellulose and hemicelluloses are embedded [20]. Pectin has been characterized as having a backbone of D-galacturonic acid residues [21], linked by α(1–4) linkage with a small number of rhamnose residues in the main chain and a rabinose, galactose and xylose on its side chains. Pectin contributes to the mechanical strength and physical properties of primary cell walls there by maintaining structural integrity. Pectic substances represent between 0.5-4% of fresh weight plant material [2, 22]. In addition to their role as cementing and lubricating agents in the cell walls of higher plants, they are responsible for the texture of fruits and vegetables

during growth, maturation and their storage [16, 20]. Furthermore, pectic substances are involved in the interaction between plant hosts and their pathogens [23]. The generic name of pectic substances is used for referring to four types of molecules: protopectin: pectic substance in intact tissue, pectinic acids: polygalacturonan containing >0-75% methylated galacturonate units, pectic acids: polygalacturonan that contains negligible amount of methoxyl groups, and pectins: pectinic acid with at least 75% methylated galacturonate units. Protopectines are insoluble in water, while the rest are wholly or partially soluble in water [16, 24]. Pectins are used in the food and pharmaceutical industries. In the food industry, it is primarily used as a gelling agent, replacing sugars and/or fats in low-calorie food and as nutritional fiber [22, 25]. In pharmaceutical industry, it is used to reduce cholesterol or to act as a lubricant in the intestines thus promoting normal peristaltic movement without causing irritation. In addition, pectin's are used as drug delivery systems, which can also reduce the toxicity of these and make their activity longer lasting without altering their therapeutic effects [26, 27, 28, 25].

Pectinase enzyme and its Classification

Pectinolytic enzymes comprise a group of enzymes that catalyze the breakdown of substrates containing pectin. These are the one which hydrolyze pectic substances [9]. Pectic enzymes are widely distributed in nature and are produced by bacteria, yeast, fungi and plants [21, 1]. In plants, pectic enzymes are very important since they play a role in elongation and cellular growth as well as in fruit ripening [3, 1]. Pectolytic activity of microorganisms plays a significant role, firstly, in the pathogenesis of plants since these enzymes are the first to attack the tissue [23, 1]. In addition, they are also involved in the process of symbiosis and the decay of vegetable residues [30, 29]. Pectinases are classified into three classes: pectin esterases, depolymerizing enzymes (hydrolases, lyases), and protopectinases according to the following criteria: (1) whether, pectin, pectic acid or oligo-D-galacturonate is the preferred substrate; (2) whether they act by trans-elimination or hydrolysis; or (3) whether the cleavage is random (endo-, liquefying or depolymerizing enzymes) or 'endwise' (exo- or saccharifying enzymes) [16]. Detailed classification and properties of pectolytic enzymes are discussed in other literature on pectinases [16, 31, 22]. Protopectinases solubilizes protopectin forming soluble pectin. Esterase (pectin methyl esterases and pectin acetyl esterases) eliminates methoxyl and acetyl residues from pectin giving rise to polygalacturonic acid. Depolymerases breaks down the glycosidic α-(1- 4) bonds between galacturonic residues via: 1. Hydrolysis (polygalacturonases) 2. Transelimination (pectin lyases and pectate lyases) Also, the latter enzymes are subdivided into endo- if its pattern of action is random or exo-if its pattern of action is at the terminal end [32, 33, 3, 1].

Production of Microbial Pectinases

Pectinolytic enzymes are produced by many organisms like bacteria, fungi, yeasts, insects, nematodes, protozoan and plants. The microbial world has shown to be very heterogeneous in its ability to synthesize different types of Pectolytic enzymes with different mechanisms of action and biochemical properties [21]. Microbial production of pectinases has been studied during recent years [31]. Pectic enzymes are produced by both prokaryotic microorganisms, which primarily synthesize alkaline pectinases, and by eukaryotic microorganisms, mostly fungi that synthesize acid pectinases [30, 2, 31].Production of Pectinase has been reported from bacteria [11], yeast [34] and fungi. All the commercial preparations of pectinases are produced from fungal sources [35]. *Aspergillus niger* is the fungal species for industrial production of Pectinolytic enzymes [21, 2]. Most extracellular induced enzymes are produced in higher quantities when inducers are present in the cultivation medium [16]. Microbial enzymes are produced either through submerged fermentation (SmF) or solid state fermentation (SSF) techniques. Submerged fermentation is a well-developed system used in industrial scale to produce a large variety of microbial metabolites. The SmF techniques for enzyme production are generally conducted in stirred tank reactors under aerobic conditions using batch or fed batch systems. Submerged fermentation requires high volumes of water, continuous agitation and generates lot of effluents. On the other hand solid state fermentation involves microbial growth and product formation on or within particles of a solid substrate under aerobic conditions, in the absence or near absence of free water and does not generally require aseptic conditions for production of enzymes [36] Some of the advantages of SSF processes over liquid-batch fermentation are that a lower volume of liquid is required for product recovery, cheap media can be used for fermentation, and there is a lower risk of contamination due to the inability of most contaminants to grow in the absence of free-flowing substrate. Higher fungi and their enzymes, as well as spores or metabolites, are well adjusted to growth on solid wet substrates. For instance, fungal spores produced by SSF show higher stability, are more resistant to drying and exhibit higher germination rates for extended periods of time after freeze-drying than do spores produced by SmF [37]. Solid state fermentation has only found restricted applications in processes using unicellular organisms. The main obstacles are the low amenability of the process to regulation, the strongly heterogeneous fermentation conditions and the ensuing frequently unsatisfactory reproducibility of the results, difficult scale-up, the often unfeasible biomass determination and complicated product purification by downstream processes resulting from the use of heterogeneous organic growth substrates [38]. Agricultural and food processing wastes such as wheat bran, cassava, sugar beet pulp, citrus waste, corn cob, banana waste, saw dust and apple pomace are the most commonly used substrates for SSF for Pectinase

production [30].

Purification of Microbial Pectinases

Microbial pectinases must be purified for the complete understanding of the properties and their characterizisation studies. Pectinases from a diversity of sources of microorganisms have been purified. An *Aspergillus niger* strain producing exo-Polygalacturonases separated by eluting from DEAE cellulose with 0.2 M sodium acetate buffer at pH 4.6 with 209-fold increase in specific activity with a recovery of 8.6%. A second pectinase was isolated with 205- fold increase in specific activity with a recovery of 1%. Partial purification of polygalacturonase and α-Larabinofuranosidase from *Sclerotinia fructigena*, using ion exchange and CM-sephadex gel filtration chromatography was conducted [39]. The purification and characterization of two endo-polygalacturonases from *Sclerotinia sclerotiorum* was done using gel filtration, iso-electric focusing and anion exchange chromatograph. An extracellular Pectinolytic enzyme from *Sclerotinia sclerotiorum* was purified using gel filtration, cation or anion exchange chromatography and again by gel filtration along with the characterization, amino acid sequencing and immunological studies of the enzyme [40]. Many enzymes were purified by salting out with ammonium sulphate and precipitated with ethanol after gel filtration through Sephadex G-25. Homogeneous preparation of enzyme can be obtained by performing repeated chromatography on DEAE-cellulose column. Endopectate lyase produced from *Bacillus macerans* was purified using ammonium sulphate precipitation followed by DEAE-Sephadex A-50 chromatography and CM-cellulofine chromatography [41].The purification of Endopectate lyase I-IV from *Erwinia carotovara* was done by CM Sepharose CL 6B chromatography, Sephadex S-200 gel filtration and isoelectric focusing. An acid stable polygalacturonase from *Corticium rolfsii* was reported and which showed the optimum pH for enzyme activity at 2.5 and stability at storage pH of 1.5. A 60-fold purification of *Verticillium albo-atrum* polygalacturonase was achieved by chromatography on columns of CM-cellulose and hydroxyapatite, gel filtration and preparative electrophoresis. Determination of molecular masses of exopolygalacturonase and polygalacturonase from *Aspergillus foetidus* EGEK145 was carried out [42] and revealed as 54 and 31 kDa, respectively. In recent years, considerable progress has been made in purifying pectinases to homogeneity using different purification methods. Apart from the advent of new affinity matrices for isolation of Pectinase, definite and improved purification methods such as immunochemical techniques are essential. Endo-PG from *Trichosporon pencilillanum* was crystallized by the hanging-drop method of vapor diffusion using ammonium sulphate as precipitant and carried out X-ray analysis. A novel Pectinase (PECI) from *Acrophialophora nainiana* was purified and characterized using MALDI-TOF, FS and CD measurements. Likewise Circular dichroism studies were used to investigate the

effect of pH on medium on inactivation of enzyme and its structural conformational changes [43]. The application of MALDI TOF fragment ion analysis was used for the identification and prediction of accurate mass of polygalacturonase from *Fusarium graminearum*[44].

Biotechnological applications of Microbial Pectinase

Pectinase production occupies about 10% of the overall manufacturing of enzyme preparations. Over the years, pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater, containing pertinacious material, etc

Acidic Pectinases

Acidic pectinases are widely used in extraction, clarification, and removal of pectin in fruit juices, in maceration of vegetables to produce pastes and in winemaking, are often produced by fungi, especially *Aspergillus niger sp*.

Fruit Juice Extraction

The largest industrial application of pectinases is in fruit juice extraction and clarification. Fruit juice viscosity and turbidity is contributed by the pectins. Fruit juices are clarified by a mixture of pectinases and amylases. It decreases 50 % filtration time [9].Treatment of fruit pulps with Pectinase showed an increase in banana, grapes and apple fruit juice volume [45].Pectinases with other enzymes, viz., cellulases, arabinases and xylanases, have been used to increase the pressing efficiency of the fruits for juice extraction [46]. Vacuum infusion of pectinases is used to soften the peel of citrus fruits for removal.

Alkaline Pectinases

Alkaline pectinases are generally produced by bacteria, particularly species of *Bacillus,* but are also made by some filamentous fungi and yeasts [47, 48, 4]. They are used in the pretreatment of waste water containing pectin residues from vegetable food processing and treatment of paper pulp. They are also useful in the processing of textile fibers such as flax, jute and hemp, coffee and tea fermentation and extraction of vegetable-oil [32, 49].

Textile processing and bio-scouring of cotton fibers

Pectinases have been used in conjunction with amylases, lipases, cellulases and hemicellulases to remove sizing agents from cotton in a safe and ecofriendly manner, replacing toxic caustic soda used for the purpose earlier. Bio-scouring is a novel process for removal of non-cellulosic impurities from the fiber with specific enzymes [2].

Degumming/retting of plant blast fibers

Bast fibers are the soft fibers formed in groups outside the xylem, phloem or pericycle, e.g. Ramie and sun hemp. The fibers contain gum, which must be removed before its use for textile making.

The chemical degumming treatment is toxic, polluting and non-biodegradable. Eco-friendly and economic alternative to the above problem is the biotechnological degumming using pectinases in combination with xylanases [50]. Pectinases are involved in the retting and degumming of jute, flax, hemp, ramie, kenaff (*Hibiscus sativa*), and coir from coconut husks [51, 52]. Retting is a fermentation process, in which certain bacteria (e.g., *Clostridium sp., Bacillus sp.*) and certain fungi (e.g., *Aspergillus sp., Penicillium sp.*) decompose the pectin of the bark and release fiber.

Waste water treatment

For treatment of wastewater from citrus processing industries various processes have been investigated, which include: physical dewatering, spray irrigation, chemical coagulation, direct activated sludge treatment and chemical hydrolysis followed by methane fermentation [53]. These processes have low efficiency due to chemical resistance of the pectic substances, high treatment cost, long treatment periods and complexity of the process. Vegetable processing industries release pectin, containing wastewaters as by product. Pretreatment of these wastewaters with Pectinolytic enzymes facilitates removal of pectinaceous material and renders it suitable for decomposition by activated sludge treatment [30]. The wastewater from the citrus-processing industry contains pectinaceous materials that are barely decomposed by microbes during the activated-sludge treatment [53]. Pectin containing waste waters is released by vegetable food processing industries as by-product. These wastewaters are pretreated with Pectinolytic enzymes which facilitate the removal of pectinaceous material and render it suitable for decomposition by activated sludge treatment [2].

Coffee and tea fermentation

Pectinase treatment accelerates tea fermentation and also destroys the foam forming property of instant tea powders by destroying pectin [54]. In coffee fermentation, it is used to remove mucilaginous coat from coffee beans. Enzymatic treatment accelerates tea fermentation, wherein the enzyme dose is carefully adjusted to avoid damage to the tea leaf.

Paper and pulp industry

Pulp and paper mills are beginning to use enzymes to solve problems in their manufacturing processes. Pectinase produced by *Bacillus sp.* and *Erwinia carotovora sp.*[55] due to its strong macerating activity, has been used for retting of Mitsumata bast [53]. During papermaking; Pectinase depolymerize polymers of galacturonic acids, and lowers the cationic demand of pectin solutions and the filtrate from peroxide bleaching [34].

Animal feed

Pectinases are used in the enzyme cocktail, used for the production of animal feeds. This reduces the viscosity of feed, which in turn increases nutrient absorption; nutrient liberation, either by

hydrolysis of non-biodegradable fibers or by liberating nutrients blocked by these fibers, and reduces the amount of faeces [2].

Oil extraction

Oils from rape seed, coconut germ, sunflower seed, palm, kernel and olives are traditionally produced by extraction with organic solvents. Hexane, a potential carcinogen is the most commonly used solvent. Recently, the plant cell-wall-degrading enzyme preparation has begun to be used in olive oil preparation. During grinding of the olives, the enzymes are added, thereby releasing the oil easily [56].

Chromaticity and Stability of Red Wines

Pectinolytic enzymes added to macerated fruits before the addition of wine yeast in the process of producing red wine resulted in improved visual characteristics (color and turbidity) as compared to the untreated wines. Red wines which are enzymatically treated showed chromatic characteristics better than the control wines. These wines also showed greater stability as compared to the control [57].

2. CONCLUSION

The exploration of microbial biodiversity has allowed identifying and characterizing new pectic-enzyme-producing microorganisms with different biochemical characteristics. Pectinases have been classified based on their catalytic activity to pectin or its derivatives and in terms of industrial use. Pectinases have acquired great attention in industries like textile industry where they have replaced the use of huge amount of water, energy demand and harsh chemicals. Most of the studies performed so far have been concentrated with the screening, isolation, production, purification, characterization and applications of Pectinolytic enzymes in increasing the fruit juice yield and its clarification. Research should be focused on protein engineering in order to obtain pectic enzymes more robust and versatile as well as the optimization of production processes with new strains are necessary for the successful completion of this new approach for the production and use of microbial Pectinase.

REFERENCES

1. Whitaker JR. Microbial Pectinolytic enzymes. Microbial enzymes and biotechnology (2nd Edition), Elsevier Science Ltd, England, 1990, pp. 133-176.
2. Jayani RS, Saxena S, Gupta R. Microbial Pectinolytic enzymes: a review. Process Biochem. 2005; 40: 2931-2944.
3. Sakai T. Degradation of Pectins. In: Microbial degradation of natural products, G Winkelmann (Ed),Weinheim & New York, Germany & USA, 1992, 57-81.
4. Aguilar G, Huitron C. Stimulation of the extracellular production of pectinolytic activities of

Aspergillus sp. by galacturonic acid and glucose addition. Enzyme Microbiol Technol. 1990; 9: 690-696.

5. Kohli P, Gupta R. Alkaline pectinases: A review. Biocatalysis and Agricultural Biotechnology. 2015; 4: 279-285.
6. Yadav S, Yadav PK, Yadav D, Yadav KDS. Purification and characterization of alkaline pectin lyase from *Aspergillus flavus*. Process Biochem. 2008; 43: 547-552.
7. Kapoor M, Beg QK, Bhushan B, Dadhich KS, Hoondal GS. Production and partial purification and characterization of a thermo-alkali stable polygalacturonase from *Bacillus* sp. MGcp-2. Process Biochem. 2000; 36: 467-473.
8. Kashyap DR, Chandra S, Kaul A, Tewari R. Production, purification and characterization of Pec tinase from a*Bacillus* sp. DT7. World J Microbiol Biotechnol. 2000; 16: 277-282.
9. Blanco P, Sieiro C, VillaTG. Production of pectic enzymes in yeasts. FEMS MicrobiolLett. 199 9; 175: 1-9.
10. Stratilova E, Breierova E, Vadkertiova R. Effect of cultivation and storage pH on the production of multiple forms of polygalacturonase by *Aspergillus niger*. Biotechnol Lett. 1996;18: 41-44.
11. . Beg QK, Bhushan B, Kapoor M, Hoondal GS. Production and characterization of thermostable xylanase and pectinase from a *Streptomyces* sp. QG-11–3. J Ind Microbiol Biotechnol. 2000; 24:396-402.
12. Bruhlmann F. Purification and characterization of an extracellular pectate lyase from an*Amylo cota* sp.Appl Environ Microbiol. 1995; 61: 3580-3585.
13. Senthilkumar PK, Uma C, Saranraj P. Amylase production by *Bacillus* sp. using cassava as sub strate. International Journal of Pharmaceutical and Biological Archives. 2012; 3(2): 274-280.
14. Naidu MA, Saranraj P. Bacterial Amylase: A Review. International Journal of Pharmaceutical and Biological Archives.2013; 4(2): 274 - 287.
15. Saranraj P, Stella D. Fungal Amylase: A Review. International Journal of Microbiology Research. 2013; 4(2): 203-211.
16. Alkorta I, Garbisu C, Llama M J, Serra J L.Industrial applications of pectic enzymes: a review. Process Biochem. 1998; 33:21-28.
17. Gainvors A, Karam N, Lequart C, Belarbi A. Use of *Saccharomyces cerevisiae* for the clarification of fruit juices. Biotechnol Lett. 1994; 18: 1329-1334.
18. Pretel MT, Lozano P, Riquelme F, Romojaro F. Pectic enzymes in fresh fruit processing:optimization of enzymatic peeling of oranges. Process Biochem. 1997; 32: 43-49.
19. O'Neil MA, Warrenfeltz D, Kates K, Pellerin P, Doco T, Darvill AG, Albersheim P. Rhamnoga

lacturonan-II, a pectic polysaccharide in the cell walls of growing plant cells form a dimer that is covalently cross linked by a borate ester. Invitro conditions for the formation of dimer. J Biol chem. 1996; 271 (37): 22923-30.

20. Caffall KH, Mohnen D. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr. Res. 2009; 344: 1879-1900.
21. Gummadi SN, Panda T. Purification and biochemical properties of microbial pectinases: a review. Process Biochem. 2002; 38: 987-996.
22. Sakai T, Sakamoto T, Hallaert E, VandammeEJ. Pectin, pectinase and protopectinase: production, properties and applications. Adv Appl Microbiol. 1993; 39: 213-294.
23. Collmer A, Keen NT. The role of pectic enzymes in plant pathogenesis. Annual Review of Phytopathology. 1986; 24: 383-409.
24. Kertesz ZI.The pectic substances. Interscience Publishers, NewYork, USA, 1951.
25. Thakur BR, Singh RK, Handa AK. Chemistry and uses of pectin: a review.Critical Reviews in Food Science and Nutrition. 1997; 37: 47-73.
26. Morris G, Kök S, Harding S, Adams G. Polysaccharide drug delivery systems based on pectin and chitosan. Biotechnology & Genetic Engineering Reviews. 2010; 27: 257-284.
27. Pilnik W, Voragen AGJ. The biochemistry of fruits and their products. In: Hulme AC (ed) vol2 Academic Press, New York, 1970, 57-87.
28. Schols HA, Visser RGF, Voragen AGJ. Pectin and Pectinases. Wagenigen Academic Publisher Wagenigen, Netherlands, 2009.
29. Lang C, Dornenburg H. Perspectives in the biological function and the technological application of polygalacturonases. Appl Microbiol Biotechnol. 2000; 53: 366-375.
30. Hoondal GS, Tiwari RP, Tewari R, Dahiya N, Beg QK. Microbial alkaline pectinases and their industrial applications: a review. Applied Microbiology andBiotechnology.2002; 59: 409-418.
31. Kashyap DR, Vohra PK, Tewari R.Application of pectinases in the commercial sector: a review. Bioresour Technol. 2001:77: 215-227.
32. Fogarty WM,Kelly CT. Pectic Enzymes. In: Fogarty, W.M. (ed.) Microbial Enzymes and Biotechnology Applied Science Publishers, London, 1983, 131-182.
33. Rexová-Benková L, Marcovic O. Pectic enzymes. Advances in Carbohydrate Chemistry. 1976 33: 323-385.
34. Reid I, Ricard M. Pectinase in papermaking: solving retention problems in mechanical pulps bleached with hydrogen peroxide. Enzyme Microb Technol. 2000; 26:115-23.
35. Singh SA, Plattner H, Diekmann H. Exopolygalacturonate lyase from a thermophilic *Bacillus* sp. Enzyme Microb Technol. 1999; 420-425.

36. Pandey A, Selvakumar P, Carlos RS, Poonam N. Solid State Fermentation for the Production of Industrial Enzymes. Current Science.1999; 14:1069-1087.
37. Kawano CY, Chellegatti MA, Said S, Fonseca MJ. Comparative Study of Intracellular and Extr Acellular Pectinases produced by Penicillium Frequentans. Biotechnology and Applied Biochemistry. 1999; 29: 133-140.
38. McMillan GP, Johnston DJ, Perombelon MCM. Purification of homogeneity extracellular poly Galacturonase and isoenzymes of pectate lyase of *Erwinia carotovorasub* sp. Atroseptica by Column chromatography. J Appl Bacteriol. 1992; 73: 83-86.
39. Fielding AH, Byrde RJ. The partial purification and properties of Endopolygalacturonane and α-L-Arabino furanosidase secreted by *sclerotinia fructigena.* J Gen Microbiol.1969;58:84
40. Riou C, Georges F, Michel F. Purification and characterization of a β-galactosidase from Scler Otinia Sclerotiorum. Microbiology letters. 1992; 95: 37-41.
41. Miyazaki Y. Purification and characterization of an endopectate lyase from *Bacillus macerans*. Agri Biol Chem. 1991; 55:25-30.
42. Taskin E, Stratilova E, Turk J. Polygalacturnases produced under solid state and submerged fermentation conditions by the strains of *Aspergillus foetidus*, Turk JBiochem.2008; 33(4): 190-196.
43. Niture SK. Comparative biochemical and structural characterization of fungal polygalacturonases.Biologia. 2008; 63 (1): 1-19.
44. Ortega LM, Kikot GE, Rojas NL, Copez LM, Astoreca AL. Production,characterization and identification using proteomic tools of a polygalacturonase from *Fusarium graminearum*. J. Basic. Microbiol.2014; 54: 5170-5177.
45. Kaur G, Kumar S, Satyanarayana T. Production, characterization and application of a thermos Table polygalacturonase of a thermophilic mould *sporotrichum* thermophile Apinis. Bioresour Technol. 2004; 94: 239-243.
46. Gailing MF, Guibert A, Combes D.Fractional factorial designs applied to enzymatic sugar beet pulps pressing improvement. Bioprocess Eng. 2000; 22: 69-74.
47. Foda MS, Hussein MF, Gibriel AY, RizkLRS, Basha SI. Physiology of polygalacturonase form formation by *Aspergillus aculeatus and Mucor Pusillus.* Egypt. J. Microbiol. 1984; 19: 181.
48. Young MM, Moriera AR, Tengerdy RP. Principles of Solid state Fermentation in Filamentous fungi Fungal Technology, Arnold E. London,1983, 117-144.
49. Sara SP, Torres EF, Gonzalez GV, RojasMG. Effects of different carbon sources on the synthesis of pectinase by Aspergillus niger in submerged and solid state fermentations. Appl Microbio Biotechnol. 1993; 39:36-41.

50. Kapoor M, Beg QK, Bhushan B, Singh K, Dadhich KS, Hoondal GS. Application of an alkaline and thermostable polygalacturonase from *Bacillus* sp. MG-cp-2 in degumming of ramie (Boehmeria nivea) and sunn hemp (Crotalaria juncea) bast fibers. Proc Bioche.2001; 36:803-807
51. Chesson A, Codner RC. Maceration of vegetable by a strain of *Bacillus subtilis*. J Appl Bacteriol. 1978; 44: 347-364.
52. Bruhlmann F, Kim KS, Zimmerman W, Fiechter A. Pectinolytic enzymes from actinomycetes for the degumming of ramie bast fibers. Appl Environ Microbiol.1994; 60:2107-2112.
53. Tanabe H, Yoshihara Y, Tamura K, Kobayashi Y, Akamatsu T. Pretreatment of pectic wastewater from orange canning process by an alkalophilic Bacillus sp. J Ferment Technol. 1987; 65:243-246.
54. Carr JG.Tea, coffee and cocoa. In: Wood BJB (ed) Microbiology of fermented food, vol 2. Elsevier, London, 1985, 133-154.
55. Paloma¨ki T, Saarilahti HT.Isolation and characterization of new C-terminal substitution mutation affecting secretion of polygalacturonases in *Erwinia carotovora* sp. FEBS Lett. 1997; 400: 122-126.
56. West S. Olive and other oils. In: Industrial enzymology(2nd edition), T. Godfrey & S.West, (Eds.), Stockholm Press, New York, USA,1996, 293-300.
57. Revilla I, Ganzalez-san jose ML. Addition of pectolytic enzymes: an enological practice which improves the chromaticity and stability of red wines. Int J Food Sci Technol. 2003; 38:29-36.