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A Cocktail Enzyme – Pectinase from Fruit Industrial Dump Sites: A Review.

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ABSTRACT

Pectinases, a cocktail enzyme consists of group of enzymes that degrade the pectin substances that is present in the middle lamella of plants. The Pectin mainly constitutes D- galacturonic acid based on α- 1, 4 glycosidic linkages and forms a macromolecule. This Pectinases enzyme breakdown the complex polysaccharides into simple molecules like galacturonic acids. As the global trend was increased to minimize the environmental pollution and utilization of natural resources. The disposal of agricultural waste from industries became a threatened problem in causing pollution. So, these wastes can be used for the production of pectinolytic enzyme as it contains enough amount of pectin that can function like support and inducer by microbes. Pectinases are most upcoming enzymes in commercial sector as it has lot of applications in food and pharmaceutical industries. This article reviews about introduction of pectinase, assay methods and important applications in different sector.

Keywords: Pectin, pectinase, industries, wastes

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INTRODUCTION

In living tissues, the chemical changes are regulated by the enzymes, which are bio-active compounds. Pectinases, a cocktail enzyme consists of group of enzymes that degrade the pectin substances that is present in the middle lamella of plants (Prathyusha and Suneetha, 2011). The Pectin mainly constitutes D- galacturonic acid based on α- 1, 4 glycosidic linkages and forms a macromolecule (Naidu and Panda, 1998). This Pectinase enzyme breakdown the complex polysaccharides into simple molecules like galacturonic acids. The first application of pectinase was observed at 1930's in the production of wine and juice industry. The scientists started to use a large number of enzymes as the knowledge of chemical nature of plant tissues became apparent in 1960's. As a result, Pectinase became one of the futuristic useful enzymes in commercial sector (Kashyap *et al*., 2001). The large amount of waste from agricultural and fruit processing industries became a prominent section for biological utilization of this waste (Jose *et al*., 2008). The agro-industrial by-products can be used for the production of high value products as it prevents the environmental pollution caused by them. Recently, global trends was got increased towards the efficient utilization of natural resources (Dhillon *et al.,* 2013). The disposal of agricultural waste from industries became a threatened problem in causing pollution. So, these wastes can be used for the production of pectinolytic enzyme as it contains enough amount of pectin that can function like support and inducer by microbes (Ruiz, 2004). Pectic substances are present in the form of insoluble particles which are rich in raw press juice. These insoluble particles are known as 'Cloud particles'. This cloud particles consists of a protein nucleus with a positive surface charge and it is coated by negatively charged pectin molecules (Pilnik and Voragen, 1993). Pectinolytic enzyme degrade the pectin and expose part of the positively charged protein beneath, thus reducing the electrostatic repulsion between cloud particles which cause the particles to come close together to form larger particles. These large particles settle out, to facilitate this process flocculating agents such as tannin and gelatin can be added (Kashyap *et al.,* 2001).

The Pectinase enzyme can be broadly classified into three major types. They are as follows:

Pectinesterases: This enzyme catalyses deesterification of the methoxyl group of pectin forming pectic acid and they act mostly on a methyl ester group of galacturonate unit next to a non-esterified galacturonate unit.

Polygalacturonases: This enzyme catalyzes the hydrolysis of α- 1-4 glycosidic linkages in pectic acid. It is subdivided into two types. They are:

Endo- polygalacturonase: Catalyzes random hydrolysis of α-1,4- glycosidic linkages in pectic acid. It is also known as poly (1,4- α-D-galacturonide)glycanohydrolase.

Exo- Polygalacturonase: Catalyzes hydrolysis in a sequential fashion of α-1,4- glycosidic linkages on pectic acid. It is also known as poly (1,4-α-D-galacturonide)galacturonohydrolase.

Pectin lyase: This enzyme catalyses the cleavage of α- 1-4 glycosidic linkage in pectic acid by trans-elimination.

Endo-PGL: Catalyzes random cleavage of α-1,4-glycosidic linkages in pectic acid. It is also known as poly (1, 4-α-D- galacturonide).

Exo-PGL: Catalyzes sequential cleavage of α-1,4- glycosidic linkages in pectic acid. It is also known as poly (1,4-α-d- glacturonide).
The aim of this work is to screen the pectinase producing microorganisms from industrial waste dump sites and assaying their enzyme activity with its important application.

Assay methodology

Pectin lyase activity

The enzyme solution was then analyzed by continuous spectrophotometric rate determination method at 235 nm under 40°C and pH 5. When the crude enzyme solution added to pectin solution in the above said conditions the enzyme starts to break the glycosidic bonds of pectin by elimination. Due to this action the solution will become turbid. The increase in A was recorded at 235nm for 5 minutes. A minute using the maximum 235nm linear rate for both the test and blank was obtained. One unit of enzymatic activity (U) was defined as the amount of enzyme which released 1 µmol of unsaturated uronide/min, merely based on the molar extinction coefficient (5500) of the unsaturated products. The enzyme production was expressed in units per ml of initial dry solid substrate (U/ml) (Gopinath and Suneetha, 2012).

Pectinesterase assay

2ml of filterate is added to 10ml of 0.5%(w/v) pectin in 0.1M Nacl and the pH was adjusted to 4.5 with 0.1M NaoH and the mixture was incubated for 60 mins at 35°c. Pectin esterase activity was measured by determining the carboxyl groups released by titration with 0.02N NaoH. One unit of Pectinesterase was defined as the amount of enzyme releasing one milliequivalent of ester hydrolyzed (carboxylgroup) per minute.

Polygalacturonase assay

To 1ml of 0.9% polygalacturonic acid in 0.1M acetate buffer of pH 4.5, 1ml of sample was added. After incubation at 45°C for 30 mins, reducing sugars were determined by the dinitrosalicylic acid (DNS) method using galacturonic acid as a reference. One unit of PG was defined as the amount of enzyme that liberates one micromole of galacturonic acid of enzymatic filterate per minute (Maldonado and Strasser, 1998).

Important applications

Preparation of jams and jellies

The pectinesterase is used to demethoxylate the high methoxylated pectins to get low-methoxylated pectins. This pectin shows calcium dependent gelation. This gel formation does not require the addition of sugars. This enzyme is used in the preparation of jellies, jams, compotes, sauces and soups (Grassin and Fauquembergue, 1997).

Fruit juice extraction

Pectinase has wide range of application in fruit juice extraction and clarification process. Pectin is main substance which is normally present in cell wall of the plants and this pectin is responsible for the increase in viscosity of the juice. Thereforth, Pectinases are useful in breaking down pectin which supports in easy extraction of juice. It is also used to increase the pressing efficiency of fruit by combining the enzyme with other enzymes like amylase, xylanase, cellulase. Pectinase is commercially used in softening the citrus fruit peels (Kashyap *et al.,* 2001).

Textile processing and bio scouring of cotton fibers

On textile processing, caustic soda which is toxic used to remove the sizing particles of cotton and this technique is replaced by pectinase when it is combined with other enzymes and obtained a same result in a safe and eco-friendly manner. Bio-scouring is the method of removing non-cellulosic impurities from cotton fibers. Pectinase were used for this method without any negative side effects on cellulose degradation (Hoondal *et al*., 2000).

Degumming of plant bast fibers

Bast fibres are soft fibers were formed in groups outside the xylem, phloem like Ramie and sun hemp. These fibres hold gum, so it has to be removed for further treatment for textile processing. But, chemical treatment of degumming is toxic, polluting and non-biogradable. It can be overcome with the utilization of pectinase in combination with xylanase can be used for degumming in an eco-friendly and bio-degradable manner (Jayani *et al.,* 2005).

Waste water treatment

The pectic substances are present in the waste water of fruit industries and the treatment of these waters was carried in different steps like physical dewatering, spray irrigation, chemical agitation, activated sludge treatment and chemical hydrolysis. As, the treatment is longer as well as higher cost leading to environmental pollution from the chemicals. Henceforth, the use of pectinase from microbes which can selectively remove pectic substances from waste water became an alternative, cost-effective and eco-friendly method. Not only in fruit industries, it was also used in pre-treatment of waste water from vegetable food processing industries that facilitates the removal of pectin and made it suitable for activated sludge treatment. Alkalophilic *Bacillus sps* was mostly used for this industrial waste water treatment.

Coffee and tea fermentation

Pectinase were used in tea fermentation process by breaking down the pectin which is present in the cell walls of tea leaves and it was also used to prevent foam formation by destroying pectins in the instant tea powders. It was also used to remove mucilaginous coat from coffee beans in coffeefermentation (Vikari *et al.,* 2000)

Paper and pulp industry

During paper manufacturing Pectinase is used in depolymerising polymers of galacturonic acids and also it lowers the cationic demand of pectic solutions and the filterate from peroxide bleaching in paper manufacturing process. Alkaline pectinase from streptomyces sp. Qg-11-3 was utilized in bleach-boosting of eucalyptus kraft pulp. Pectinase was used in combination with xylanase for bio-bleaching from same organism (Vikari *et al.,* 2000),(Reid and Richard, 2004).

Purification of plant viruses

Highly pure preparations of virus were needed to carry out chemical, physical and other biological studies. Alkaline pectinase and cellulase helps in getting pure virus from the tissues in the purification of virus from phloem (Salazar and Jayasinghe, 1999).

Oil extraction

Plant cell wall degrading enzymes have been normally used in the preparation of olive oil. The enzyme was added during the process of grinding of olives by which easy removal of oil can be obtained. Pectins avoids emulsification in the presence of oils which helps in the extraction of oil (Scott, 1978).

Preparation of wines

The pectinolytic enzymes were helped in breakdown of pectins in a present grape which increased the turbidity and quality of the wines. Normally, these enzymes were added to the macerated fruits before adding wine yeast which results in improving visual characteristics like colour and turbidity of wine.

Extraction of DNA from plants

The protocols which were used earlier for extracting plant DNA or RNA do not work well for different plants, the reason is that contaminating substances co-precipitate with the nucleic acids and it also present even at the last DNA-hydration step (Rogstad,2001). Henceforth, Pectinases were used to prevent co precipitation of nucleic acids and helped in isolation of pure DNA. As it breakdown the pectin, a major component fo plant cell wall and middle lamella.

Animal feed supplement

Pectinase was utilised as animal feed supplement which helps in the reduction of feed viscosity which directly increases the absorption of nutrients that liberates from fibres by hydrolysis process and it also reduces animal faeces (Hoondal *et al.,* 2000).

Colon targeted drug delivery system

Pectin coated tablets were used in drug delivery system. The In-vitro experiments demonstrated that high methoxy pectin when applied as a compression coat it has been proven to be a protecting core from gastrointestinal environment and prevents it from enzymatic degradation. In same in-vitro conditions the colon site specificity can also be achieved as the pectin coated tablets is disintegrated at colon site (Linshu Liu *et al .,* 2007).

CONCLUSION

Pectinases are upcoming enzymes in industrial sector in future as these enzymes were used in different fields and also producing of this pectinolytic enzymes from industrial dump sites will make use of natural resources and reduces pollution caused to the environment. This enzyme will also utilized in many of industries in natural and safe manner to the environment.

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REFERENCES

[1] Prathyusha K and Suneetha V, Journal of Phytology 2011; 3(6): 16-19.
[2] Naidu GSN, Panda T, Bioprocess Engineering 1998; 19: 355-361.
[3] Kashyap DR , Vohra PK, Chopra S, Tewari R, Bioresource Technology, 77(3) 2001;215–227.
[4] Jose Rodriguez-Nogales, Natividad Ortega, Manuel Perez-Mateos, Maria Busto, Food Chem.2008; 112–119.
[5] Gurpreet Singh Dhillon, Surinder Kaur, Satinder Kaur Brar, Renewable and Sustainable EnergyReviews 2013; 27:789–805.
[6] Ruíz-Leza HA. Universidad Autónoma de Coahuila, UG thesis; 2004.
[7] Pilnik, W., Voragen, A.G.J.In: Reeds, G. (Ed.), Academic Press, New York, 1993, pp. 363–399.
[8] Gopinath R and Suneetha V, Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012; 3 (4): 484.
[9] MC Maldonado and AM Strasser de Saad, Journal of Industrial Microbiology & Biotechnology 1998; 20: 34–38.
[10] Catherine Marie Therese Grassin; United States Patent; 1997.
[11] Hoondal GS, Tiwari RP, Tiwari R, Dahiya N, Beg QK, Applied Microbiology and Biotechnology, 2000; 59(4-5):409–18.
[12] Ranveer Singh Jayani, Shivalika Saxena, Reena Gupta, Process Biochemistry 2005; 40: 2931–2944.
[13] Viikari L, Tenakanen M, Suurnakki A. In: Rehm HJ. (Eds). Biotechnology, VCH-Wiley, 2001, pp. 523–46.

[14] Reid I, Richard M. 2004. Enzyme Microbial Technology 34:499–504.
[15] Salazar L, Jayasinghe U, Training Manual,1999.
[16] Scott D. Enzymes, industrial. In: Grayson M, Ekarth D, Othmer K. (Eds). Encyclopedia of chemical technology, New York: Wiley. 1978, p. 173–224.
[17] Rogstad, Plant Genotyping II: SNP Technology , Editor Robert J. Henry,2001.
[18] LinShu Liu, Marshall L. Fishman, Kevin B. Hicks, Springer 2006.
[19] V Suneetha, M Bishwambhar, R Gopinath, S R Shrestha, G K.B. Kartik , C Pravesh, C Apoorvi, R. Kalyani , Asian Journal of Microbiology Biotechnology and Environmental Sciences 2012; 14 :405-412.
[20] Suneetha V, Ritika S, Abhishek G, Rahul G, Research Journal of Phamaceutical, Biology, Chemical Sciences, 2012;3:40-48.
[21] Suneetha V, Sindhuja KV., Sanjeev K , Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 2010; 12: 149-155.