

EXTRACELLULAR ALKALINE PECTINASES PRODUCTION: A REVIEW

Pitambri THAKUR¹, Abhishek K. SINGH², Manjeet SINGH¹, Gunjan MUKHERJEE^{1*}

Address(es):

¹University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India.

²University Institutes of Engineering, Department of Biotechnology Engineering, Chandigarh University, Mohali, Punjab, India.

*Corresponding author: gunjanmukherjee@gmail.com

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ABSTRACT

Pectinases are most significant enzymes of commercial sector. Among all the commercially produced industrial enzymes, pectinases occupy 25% share in the overall enzyme market. Pectinases have wide applications in various processes such as fruit juice clarification, wastewater remediation and textile and paper industry etc. Pectinase is most suitable environment friendly tool of nature for sustainable development. Although, pectinases alike many other commercial enzymes are facing the imperative of low harvest and yield. Hence, pectinases have been the objective of research to accomplish the commercial scale articulation levels. To find unique physicochemical properties of pectinases often microbial pectinases are utilized among all the natural sources due to ease in production. Present study primarily centers on the different types of recent techniques for the alkaline pectinases production and optimization strategies advancement for high production yield. For achieving higher production of the extracellular alkaline pectinases by utilizing basic fermentation media and simple molecular devices along with genetic engineering approaches are included in the present study. For the improved alkaline pectinase production advanced genetic engineering techniques such as gene cloning, tools of r-DNA technology and cell immobilization technique are discussed.

Keywords: Alkaline Pectinases; Optimization strategies; Genetic engineering; Fermentation media

INTRODUCTION

In the mid nineteenth century, enzyme turned up and since then they are being spacioously used in several industrial tasks. Enzymes are tremendously effective and very particular biocatalyst. There is an expansion in the demand of using biotechnological processes in place of conventional processes. Many biotechnological processes include microorganisms and enzymes such as pectinases, xylanases, cellulases, mannase α -galactosidase, lactases and ligninases which are eco-friendly and cost effective (Hoondal *et al.*, 2002). Basically, pectins are high molecular weight acid polysaccharides, initially containing α -(1-4) linkage D-galacturonic acid residues with a short number of rhamnose residues in the main chain and arabinose, galactose, and xylose on its side chain. An enzyme family which generate breakdown of the glycosidic bonds in the pectin can also be called pectinase which is a generic name (Rangarajan *et al.*, 2010). Plant pathogens build various cell wall degrading enzymes to strike on target cells, which clear the path for entrance and evolution of pathogen in host tissue. These phyto pathogenic enzymes contain pectinases, cellulases and proteases. Pectinases take part in the breakdown of pectins. Based on mode of action and substrates used, pectinases are categorized as pectin esterase (E.C.3.1.1.11), Hydrolases (pectin methyl galacturonase (E.S 3.2.1.41) and polygalaturonases (E.S 3.2.1.15), and Lyases (pectin lyase) (E.C. 4.2.2.10) and pectate lyase (E.C. 4.2.2.2). Pectinase are divided into endo and exo pectinases relying on random or endwise cleavages (Li *et al.*, 2012). Based on the pH requirement, pectinases are divided into alkaline and acidic pectinases. There are many uses of acidic pectinases in withdrawal and fruit juice clarification. These are being broadly utilized in biopreparation of cotton fabrics, for steeping the plant fibres like flax, hemp and jute, enzymatic polishing of cotton blended fabrics, to find a solution for mechanical pulp bleaching retention problems, and for upgrading the quality of black tea (Sharma and Satyanarayana, 2006). In spite of the fact that alkaline pectinases are used in industrial works, but it is essential to purify and get all the information of biochemical characteristics of these enzymes for better understanding about the structure, and mechanism of action (Li *et al.*, 2012). Microbial production of pectinases has been described in solid state fermentation and submerged fermentation. Solid state fermentation is better than submerged fermentation in terms of practical and economical advantages but has a limited adoption (Zou *et al.*, 2014).

Pectinase and its types

Pectinases are the enzymes which hydrolyzes the pectic substances. Pectinases includes polygalacturonases, pectate lyases, pectinesterases and pectin lyases relying on the mode of action (Alkorta *et al.*, 1998). Pectinases are mostly used in food industries for clarifying fruit juices and wines to improve the level of cloudiness, haze as well as utilized to produce tea, coffee, and oil extraction. Alkaline pectinases are used to separate the mucilage from coffee beans and to speed up fermentation of tea (Willats *et al.*, 2006). They are also used for striping of plants fibers, textile and paper industry, animal feed and wastewater treatments because of their capability to demean pectic polymers (Demir and Tari, 2014). According to Taskin (2013) various types of pectinases endo and exopolygalacturonase are most important and broadly applicable pectinases and contribute as 25% of industrial sales of enzymes globally. This will continue to grow up.

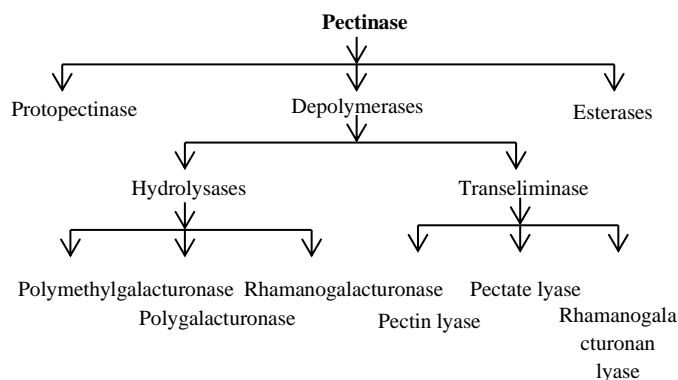


Figure 1 Classification of pectinase enzyme

Alkaline Pectinases Occurance

Pectinases are delegated in the higher plants in which they are indulged in alteration of pectinaceous materials in the time of natural maturing of various fruits. Pectinases can be acquired from different plant, animal, and microbial sources, but to satisfy the commercial needs enzymes are to be acquired from microbial sources. Due to various benefits of microorganisms such as biodiversity, faster growth represents a good resource of these enzymes (Amin et al., 2017). To produce this enzyme, which is an important enzyme for industry purposes, 30 various genus of fungi, bacteria and yeasts had been identified (Favela-Torres et al., 2006). Origin of microbial toolbox is from the filamentous fungi, yeast, non-filamentous and filamentous bacteria. Alkaline pectinases are secreted by bacterial strains, where as those pectinolytic derived from fungus are acidic in nature. Specifically, filamentous fungi are the best resource of commercial pectinolytic biocatalysts. It represents all members of polygalacturonic acid. It defines genes and shows differences in accordance with the cleavage rate, substrate specificity, temperature and pH optima for activity (Jacob, 2009). Enzyme's status from this fungus lies on the 97 pectinase genes which were glossed and 60 new genes that are generated from the whole genome of *Aspergillus niger* (Khan et al., 2013). Genus *Aspergillus* other species are also identified in the studies for the biosynthesis of the pectinases, various other acidic natured pectinase making fungi and yeast are generally from genus *Rhizomucor sp.*, *Pencillium sp.*, *Rhizopus sp.*, *Aureobasidium sp.*, *Trichoderma sp.*, *Thermotoga sp.*, *Candida sp.*, *Saccharomyces sp.*, *Pichia sp.*, *Kluyveromyces sp.*, *Schizosaccharomyces sp.*, whereas, *Klebsiella sp.*, *Bacillus sp.*, *Pseudomonas sp.* and *Cryptococcus sp.*, have been selected for alkaline pectinase production. Various types of pectinases made by bacterial, fungal and yeast cultures are present at commercial scale (Kashyap et al., 2001). Pectinase enzyme is also produced by bacteria belonged to species of Enterobacter (Enterobacter sp. MF41, Enterobacter sp. MF84, and Enterobacter sp. MF90. Enterobacter sp. MF84), Erwinia (E. carotovora and E. chrysanthemi) (Matsumoto et al., 2003), Pseudomonas (Sohail & Latif, 2016), Bacillus and Streptomyces (Ramirez-Tapias et al., 2015) Lactobacillus (Karam & Belarbi, 1995) etc. Many researchers reported that, pectinolytic properties of *Bacillus* strains are selected as the major source of pectinase enzymes (Kavuthodi et al., 2015; Sohail & Latif, 2016, Kavuthodi and Sebastian, 2018). Microbial strains are selected on various measures like number of pectinases produced, type of fermentation technique, thermostability properties and pH of enzymes and genotype of the microbial strain (Favela-Torres et al., 2006).

Substrate for alkaline pectinase

Pectin is the well layered homo polysaccharide appearing in the primary cell walls and middle lamella of terrestrial plants, inhabited in 1/3 of the dry mass of plant tissue (Gupta et al., 2008). It consists of long galacturonic acid chains along with residues of carboxyl groups and methyl esters. A pectic substance forms the important part of middle lamella and these substances are omni-present in plant kingdom. Pectic acid is the main chain of pectin which is half methyl-esterified-1, 4-D galacturonan. Pectin substances are amorphous with polymerization of 200-400. Substituents are located either on C-2 or C-3 locus of main chain. They can be sugar or non-sugar, based on sources of pectic substances, kind of branching differentiates. In the initial stages of rise in immature enlarging cell walls there appears production of pectic substances in the golgi apparatus by UDP-D-galacturonic acid (Hoondal et al., 2002; Ali et al., 2013). As compared to young actively growing tissues less than 1% of pectin substances are present in higher plants which are quite low. These mainly contribute to vegetables and fruits but comprise a huge part of algal biomass up to 30%. Pectic substances are divided as pectinic acids, pectic acids, pectin, and protopectin. Pectic acids are basically a complex designation which is given to pectic substances in colloidal polygalacturonic acids is more and there are independent of methoxy groups salts of pectic acids can be acidic pectates. Pectic acid consists of 75% methylated galacturonate units. Pectinic acid can be used to produce gels containing sugars and acids only under satisfied conditions. Protopectin is located in the middle lamella serving as a cement to bind cells altogether in the cell wall. Pectinic acid is a water-insoluble parent pectin substance. It produces pectin and pectinic acids on restriction hydrolysis. This acid is insoluble in water due to its high molecular mass, formation of ester bond in between carboxylic and hydroxyl group, and bonding of salt between the basic groups and carboxyl group of proteins. The model has been made for chemical shape of protopectin into which neutralized sugar side are organized into blocks unconnected by unsubstantial parts consisting exclusively almost galacturonic acid residues (Yoshitake et al., 1994). It is soluble in nature. It is also able to form gels with sugars and acids under certified conditions. According to Thakur and Mukherjee (2021) different agricultural waste such as lemon peel, apple waste, rice husk, wheat bran and sugar cane bagasse etc can be utilized for the production of pectinase. Residues from production of fruit juices, citrus fruits and apple pomace are used for manufacturing pectin (Alkorta et al., 1998).

Sources of Pectinases

Pectinases have variety of sources for example yeast, actinomycetes, bacteria and fungi (Hoondal et al., 2002). Basic pectinases are of two types: alkaline and acidic pectinases. Both the types have various industrial applications like fruit juice clearance and wastewater treatment, degumming of fibers such as hemp, flax, and jute etc. (Tanabe et al., 1988). And these fibers are used for various modern potential purposes such as ropes, nets etc. Various microbial sources of alkaline pectinases are *Bacillus licheniformis* (Kapoor et al., 2000), *Bacillus subtilis* (Ward and Forgarty, 2010), *Aspergillus fumigates* (Phutela et al., 2005), *Wickerhamomyces anomalus* (Martos et al., 2013), and actinomycetes etc (Bruhman et al., 1994).

Alkaline pectinases production strategies

For the production of pectinases from the microorganisms, the most common strategy is fermentation techniques. Certain strategies such as submerged fermentation (SmF), entire cell immobilization and strong state aging are used for pectinase production from various microbial sources (Couto and Sanroman, 2006).

Fermentation Techniques

Major soluble pectinase producing varieties are *Bacillus* and *Pseudomonas* sp. and at the commercial scale around 90% of enzymes are produced via submerged fermentation (Kashyap, 2001; Pedrolli and Carmona, 2010). Also, the SSF procedure is a static procedure without industrial vitality uses. In disparity, the SmF cultures fill in as homogeneous frameworks requiring expansive vitality uses to adapt to high oxygen requirements (Viniestra-Gonzalez et al., 2003). SSF is used from last two decades to produce industrially important enzymes (Couto and Sanroman, 2006). SSF in comparison to SmF produces high enzyme titers (Hoondal et al., 2002). And sadly, no extensive reports clarifying the explanation behind this are available. This absence of data makes troublesome any evaluation regarding the value of one process versus the other. For the biosynthesis of enzymes, less common technique immobilized cell culture offers many advantages. It is used in liquid-state fermentations using immobilized cells, because it provides reusability and catalytic stability of enzyme (Hoondal et al., 2002; Kapoor and Kuhad, 2000).

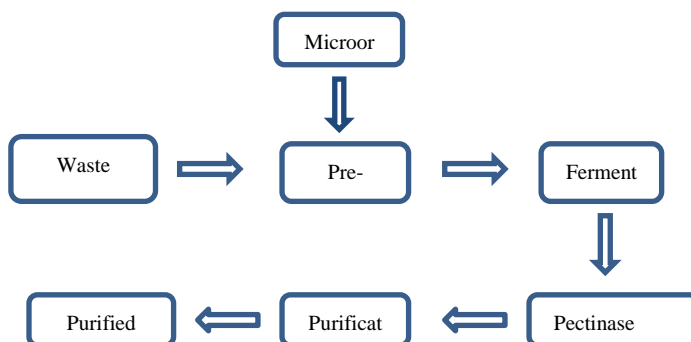


Figure 2 Overall process scheme for pectinase production from waste or pure substrate

Submerged Fermentation

To produce extracellular microbial enzymes at industrial scale, submerged fermentation is the matured process. Nonetheless, the yield of the catalyst relies upon strain type utilized and a few physical and nutritional factors, for example, pH, temperature, a substrate, incubation time, extra dietary sources, air circulation, fomentation, and extraction techniques etc. Under submerged fermentation many reports are available for pectinase production. Bacterial cultures lead to the production of alkaline pectinase and yeast pectinases are of acidic nature. Various studies unravel fungal strain alkaline pectinases production under submerged fermentation (Alimardani-Theuil et al., 2011). Various fermentation- processing factors were optimized by Glinka and Liao (2011) and recorded highest production 329 U/mL of pectin methyl esterase. 21 U/mL pectinase titer was observed by Padma et al., (2011) under liquid-state fermentation using orange peel. 1015 U/mg pectinase with PGase at neutral pH from *Bacillus* species was produced by Rehman et al., (2012). And 1122 U/L enzyme titers from *Streptomyces* species was produced by Saadoun et al., (2013). Also, in submerged fermentation utilizing food waste reported 24.18 U/mL. After utilizing cheaper growth substrate increased biosynthesis of pectinase is observed particularly for textile industry (Ahmed et al., 2016). By using agro-industrial waste PGase enzyme using various isolated bacterial strains was produced (Jahan et al., 2017).

Solid State Fermentation

In the present era of biotechnology due to certain advantages solid state fermentation is exceptionally enticing specified underneath (Jacob, 2009; Pedrolli and Carmona, 2010). It permits the use of cost-effective agro-waste substrate for the growth of microbes. As compare to submerged fermentation, solid-state fermentation has better and enhanced properties. Also, less contribution of energy is required. It is sustainable and green with high generation of microbial metabolites. SSF is very promising tool for enzymes extraction from fungi. Although, there are a few reports exhibiting the development of microscopic organisms and about great yields of the enzymes. The last yield of the pectinases relies upon a few procedure factors like sort of strain utilized, and other parameters such as extra nourishing sources, pH, temperature, incubation time and type of substrate etc. (Li et al., 2005; Ahlawat et al., 2009). Sixty fungal strains from the spoiled products of the soil observed as the high producer of pectinase with 74.0 U/mL (Sandri et al., 2013). Further, unique working factors, for example, maturation time, temperature, pH, and dampness content were applied as one-variable-at-time measurable strategy. Alkaline pectinase *B. subtilis* ZGL14 activity was optimized using RSM with Box-Behnken design (Yu et al., 2017).

Factors Affecting Pectinases Production

Nitrogen source

The effects of various nitrogen sources (organic and inorganic) on pectinase production have extensively studied. Rasheedha et al. (2010) observed that ammonium sulphate did influence production of *Penicillium chrysogenum* pectinase. Hours and coworkers (1988) reported that lower levels of inorganic nitrogen sources (K₂HPO₄) in growth medium was not influence the pectinase production. Additionally, Patil and Dayanand (2006) found that ammonium phosphate and ammonium sulphate has enhanced the pectinase production. Sapunova (1990) found that malt sprouts and ammonium salts stimulated the pectinolytic enzyme production in *Aspergillus alliaceus* BIM-83. Fawole and Odunfa (2003) reported that ammonium sulphate and ammonium nitrate have the best nitrogen source for the production of pectinase from *Aspergillus niger*. Reda et al. (2008) reported that the highest production of pectin depolymerase by *Bacillus firmus*-I-10104 in the presence of peptone. In addition, Vivek et al., (2010) reported the organic nitrogen sources showed higher endopolygalacturonase, exopolygalacturonase activities than inorganic nitrogen sources.

Carbon source

Teixeira et al. (2000) found that the best carbon sources for high pectinolytic activities from *Aspergillus japonicus* 586 were 0.5% pectin (pectinesterase), 0.2% pectin and 0.2% glycerol (endopolygalacturonase) and pectin associated to glucose (exopolygalacturonase). Pectinesterase activity was susceptible to catabolic repression with high pectin, glucose and saccharose concentrations. Aguilar and Huitron (1987) reported that the enhanced the production of pectinases from *A. niger* by the presence of glucose or saccharose association with pectin in low concentrations. Fawole and Odunfa (2003) observed that pectin and poly galacturonic acid enhanced the pectinase production. In addition, the highest pectinase production was observed using wheat bran while pure pectin give the highest pectin depolymerase production (Phutela et al., 2005).

Immobilized Cell Culture

The microbes on diverse inert polymeric solid supports gives different advantages like to use them again; distinction of product without presence of microbes, distinction of from biomass produces better enzymatic stability (Moreno-Garcia et al., 2018). A wide heterogeneity of nature such as chitin and carrageenan, agar-agar, alginate, collagen, chitosan and synthetic polymers such as polyurethane, polyacrylamide, and polyethylene glycol are being regarded as assisting resources. But ordinary biopolymers had resulted better in immobilization despite their biocompatibility attributes (Angelim et al., 2013). In both solid and liquid state, complete immobilization of cell on polyurethane foam (PUF) as immovable assistance and the attention of many researchers was derived into this (Diaz-Godinez et al., 2001). In the procedure of changing immobilization on polyurethane foam includes the immunization of sterile fermenter system containing motionless parts of PUF. When immunization is done, the germination of cell appears in a different way on the facet of PUF. This leads to immobilization of cells on the surface of PUF. Several micro-floras are employed in immobilization on PUF surface effectually (Hoondal et al., 2002). The complete immobilization of cell of *Bacillus* bacterium on PUF gave favourable outcomes also attained 1.5- folds improvement in enzyme produced under solid state fermentation than submerged fermentation (Kapoor and Kuhad, 2000; Gophanea et al., 2016). To compare exo-pectinase produced by SSF and SmF, immobilization of *Aspergillus niger* cells on polyurethane cells were used (Diaz-Godinez et al., 2001). Because of superior biomass production unaccompanied by catabolite repression, probably the higher enzyme yield was appeared in SSF. The bioprocess cost is decreased due to immobilization technology. It gives permission

to personalize target products. But execution of immobilized cells in industries is still not overcomes (Beregal et al., 2017). Attention must be given on long term preservation of immobilized cells for future purposes. New bioreactors and bioprocesses should be made that are not of complex nature, cheap and flexible and which can also be scaled up (Nedovic et al., 2015). Solid supports which are being used must also be inexpensive, eco-friendly, good quality for better industrial exploitation (Moreno-Garcia et al., 2018).

Production through genetic engineering

In the modern biotechnology era, tools of r-DNA technology like mutation, gene cloning etc. have captivated the attention of researchers to produce enzymes that can be applied in industries. These techniques had helped a lot to scientists to get all the information of a specific gene of interest and achieve required over-expression and expression of a particular selected protein. Enzymes can be widely used in industries. That is why; scientists had focused on the study of these enzymes from past many years. Enzymologists are benefitted through genetic engineering as it gives different techniques to manufacture a specific enzyme on industrial scale at a low cost. Different pectinolytic genes are cloned and then expressed in wide variety of organisms to produce homologous and heterologous expression with low cost, time, and energy (Goncalves et al., 2012; Almeida et al., 2003). High yield of pectinases are attained through genetically engineered construct of different stains such as *P. griseoseum*. *Bacillus subtilis* was utilized for the advanced level extracellular production of alkaline polygalacturonate lyase (PGL) with gene expression and PGL titers raised to 632.6 U/mL (Zhang et al., 2013). The homologous over-expression in *B. subtilis* strain of bacteria enhanced the yield of polygalacturonate lyase and with 2138 U/mL attained the highest yield of this enzyme (Zou et al., 2013). The recombinant expression of *Aspergillus* in *P. pastoris* concluded maximum yield of 2408.70 U/mL after utilizing batch fermentation of high cell density and it observed the yield was 4.8 folds higher (Abdulrachman et al., 2017).

Optimizations strategies for the production of alkaline pectinases

Pectinases production are widely influenced by the cultivation conditions, constituents of growth medium, pH, aeration, temperature, the supplementary nutrients (salts, nitrogen and carbon sources) type of strain, moisture, and inducers (Amin et al., 2017). Agriculture food industry plays a vital role as it provides all the required nutritional ingredients for the growth of microbes for biosynthesis of pectinases. US department of energy stated that approximate 500,000,000 tons of commercial waste materials takes place in USA per year. Most of this heap of agriculture and food waste is discarded in open place. But biotechnologies came with a theory to utilize this in various processes of biotechnology with focus of on fermentation as well as bio-catalysis principles in a comfortable way (Bilal et al., 2017; Djordjevic et al., 2017). Enzymes are part of this which is being produced through fermentation of agro-food wastes with microbial technology (Bilal et al., 2015). As fermentation processes are influenced by type of substrates, so several agro-industrials left over can be processed through optimization. Various agro-food waste like rice bean, wheat bran, fruit peels, leftovers etc. are utilized in industries to produce various types of enzymes (Munir et al., 2015). This agro based waste material is rich in pectin and used as important natural substrates for pectinase production (da Silva et al., 2005; Favela-Torres et al., 2006). Several authors cherished the use of this waste material as fermentation food stuffs in fermentation biotechnology (Bilal et al., 2015). Several other synthetic mediums like starch, sugar, etc and complex components played an important role to fulfill the nutrient requirements of the growth of micro-organisms and used in pectinase expression (Teixeira et al., 2000; Goncalves et al., 2012). The synthetic media can be utilized to manufacture pectinolytic enzymes (Sandri et al., 2013; Zou et al., 2013). These media mostly used to get proper production of enzymes. Various techniques like synthetic based medium and its optimizations to use the agro-waste to get sufficient yield of enzymes are being made by researchers. Some of the scientists tend to obtain the protein expressions by combining the genetic engineering with enzyme biotechnology (Zhang et al., 2013).

To obtain a crude pectinase, the fermentation medium has filtered using dry, pre-weighed Whatman filter paper, refrigerated centrifuged. Supernatant is collected for partial purification of pectinase. Crude pectinase is partial purified by using ethanol, ammonium sulphate precipitation (Barman et al. 2015). Pectinase has purified through gel filtration chromatography on Sephadex G 150 and ion exchange chromatography using DEAE-Sephacel (Kashyap et al., 2000).

Optimization of Bacterial Strains

SmF are basically used for microbial enzyme production. During fermentation, they need high water potential. Reports by author's states their consumption in solid state fermentation under ratios of high moisture and amended the procedure to acquire advantages of enhanced metabolites features given by SSF (Kashyap et al., 2003; Li et al., 2005). The manufacture of pectinase efficiently improved by *Bacillus* sp. due to various substrates generally solid, nitrogen and carbon, salts, and additional vitamin. In fermentation period, yield of pectinase is improved by 75% for 36 hours at 37 °C due to use of amalgamation of neurobin at 27 µL/g

(Kashyap et al., 2003). A bacterial strain which was recently secluded, *B. gibsonii*S-2 for optimizing yield of alkalinated pectinase utilizing pulp of sugar beet acquired 3600 U/g after 48 h at 35 °C of incubation (Ouattara et al., 2011). The fermentation media additive 1 physical variant of pH and 3 dummy variants with the help of Plackett-Burman design. Optimization of procedure concluded in 41-times of improvement in pectinase yield by *B. Pumilus* (Sharma and Satyanarayana 2006). Murugan et al, (2020) reported pectinase production from *Bacillus* species with 3.40 mg/ml of total protein and 484.70 U/mg of specific enzyme activity. In characterization studies, the pectinase demonstrated good activity at pH 6.0 and 40 °C. Also, the bacterial strain showed maximum growth when the medium pH was 7.0 and incubated 37 °C.

Optimization of Yeast Strains

The two kinds of yeasts included in the production of pectinolytic enzymes are one group which is constitutive i.e. cannot use pectic substances as carbon source. Present complex of yeast helps in secreting endo-polygalacturonases. The next group surrounds the yeasts such as filamentous fungi which can release pectinase on induction through the usage of pectinacious material, giving a more compounded enzymatic system in them. This complex is half constitutive. *Candida boidinii* and *C. albidus* have these features. Hence both the groups possess different origin of pectinases. These behaviors are disclosed by evaluating the origin of various pectin genes in yeasts. Yeast pectinases are extracellular in nature. Statistics based strategy of optimization for the better yield of pectinase using genetically modified yeast with grape skin as a source of carbon (Arevalo-Villena et al., 2011; Zhou et al., 2011). Maximum pectinolytic activity by using raw fruit as a substrate at 5°C was observed by Padma et al., (2011). Exo-polygalacturonase recorded yield 28.6 U/mL from immobilized cells and 26.9 U/mL from free cells. For 14 and 18 cycles the immobilized cell was securely used and at the end of 18 cycles recorded for polygalacturonase was a maximum yield 503.1 U/MI (Taskin, 2013).

Optimization of fungal strains

The agriculture food industrial effluents are utilized much appropriately by fungal strains to produce series of microbial metabolites. These are a great contribution of authors in fungal species culturing to produce pectinases. The two important factors for statistical yield of proper amount of 29.093 U/g pectinase were two staged optimizing strategy which utilizes D-optimal and central composite design under solid state fermentation employing *Aspergillus niger* inoculum and incubating duration (Ustok et al., 2007). After fermenting for 5 days, production of exo-polygalacturonase (1450 U/g) was successfully acquired by utilizing oil cake of pumpkin as substrate of water activity of 0.9232 (Pedroli and Carmona, 2010). The fermentation medium optimization used peel of orange as substrate by *Fusarium solani* for the improved production with peptone and glucose at 1% level was observed 102.21 U/g (Hamid et al., 2008). Two distinct substrates and strains of *A. niger* to attain the highest pectinase quantity was observed in bran of wheat resulted better than pectin (Khairnar et al., 2009). Enshasy et al., (2018) reported pectinase production from *Aspergillus niger* of 109.63 U/mL, which was about 10% higher than the uncontrolled pH culture. Furthermore, fed-batch cultivation using sucrose as a feeding substrate with a rate of 2 g/L/h increased the enzyme production up to 450 U/mL after 126 h. Qian et al, (2020), observed 1.9- to 2.3-fold higher pectinase production and 2.2- to 2.3-fold higher α -galactosidase after 72 h, at pH 6 and 7 for the utilization in environment friendly enzymatic processing.

Application of pectinase in wastewater treatment

The wastewater from the citrus-processing industry contains pectinaceous materials that are barely decomposed by microbes during the activated-sludge treatment (Tanabe et al., 1986). Tanabe et al. (1987) has tried to develop a new wastewater treatment process by using an alkalophilic microorganism. Their soil isolate an alkalophilic *Bacillus* sp. (GIR 621), produced an extracellular endopectate lyase in alkaline media at pH 10.0. Treatment with this strain has proved to be useful in removing pectic substances from the wastewater. Treatment of waste water generated through various industries such as paper processing industries, food processing industries and agro-based industries is a challenge since it releases various plant polysaccharides such as starch and pectin. Waste water treatment can be done through chemical, physical and enzymatic methods. Chemical methods utilized for the treatment waste water are harsh on the environment and cause direct or indirect environmental pollution. Alternatively, enzymes are utilized; enzymes such as pectinase is utilized for the pre-treatment of pectin rich wastewater generated through above mentioned industries before releasing the water to streams, rivers (Pitambri and Jai, 2019).

CONCLUSION AND FUTURE PERSPECTIVE

Pectinases are most potent enzyme of the commercial sector with numerous industrial applications due to its extraordinary properties to catalyze various reactions with suitable environmental parameters. Previous reports revealed that pectinolytic enzymes have two major targets: first, production of pure pectinolytic

enzyme cocktail with improved properties and stability is required for various industrial processes. Second, new and cheaper sources are required for the production of cost-effective commercial pectinases for the potential market. Alkaline pectinases are the major enzyme of textile and paper industry. Genetic engineering is promising approach to produce pure pectinolytic cocktails in very small time slot. Also, conventional optimization techniques are encouraging for the pure pectinolytic cocktail synthesis. Major stable and advanced pectinolytic enzymes can be produced using advanced protein engineering techniques to achieve improved properties and activities by in-vitro modifications of protein primary structure. It will be of utmost significance for the advancement in alkaline pectinase properties.

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