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Production of Pectinases and Pectinolytic Enzymes: Microorganisms, Cultural Conditions and Substrates

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Abstract

Pectinase comprises a heterogeneous group of enzymes that catalyze the breakdown of pectin containing substrates. The most important enzymes of the pectinase complex are polygalacturonase, pectin lyase, pectate lyase and pectin esterase. Solid state fermentation is considered as an efficient method for the synthesis of pectinolytic enzymes over submerged fermentation. Microorganisms that are particularly suitable for pectinase production through fermentation are the filamentous fungi followed by bacteria. This article aims to present an overview of potential pectinolytic microorganisms, their fermentation conditions, factors involved in maximum pectinase activity and interesting summary of substrates used in the fermentation processes. In conclusion, recent developments in industrial biotechnology offer several opportunities for the utilization of low-cost substrates for the pectinolytic enzyme production using fungi and bacteria through fermentation.

Keywords: Pectinase; Pectinolytic enzymes; Polygalacturonase; *Aspergillus*; Bacillus; Solid state fermentation

Introduction

Enzymes have found their way into many new industrial processes. Demand is increasing to replace some traditional processes such as chemical bleaching with biotechnological processes involving microorganisms. Use of microbial enzymes has been an increasing trend in different industries. Fermentation technique is considered as a powerful tool for the synthesis of valuable products by the help of microbes. Pectin comprises of D-galacturonic acid occurred in α-1,4 chain, naturally esterifies with methoxy groups and natural sugars occupy the side chains. Pectinase comprises a heterogeneous group of enzymes that catalyze the breakdown of pectin-containing substrates. The most important enzymes of the pectinase complex are polygalacturonase, pectin lyase, pectate lyase and pectin esterase. Pectin esterase catalysis the de-esterification of the methoxy group of pectin, forming pectic acid. Hydrolases (Polygalacturonases and Polymethyl galacturonases) catalysis the hydrolytic cleavage of α-1,4-glycosidic linkage in pectic acid and pectin, respectively, while Lyases (Polygalacturonate Lyase and Poly methyl galacturonate Lyase)-Catalysis the cleavage of α-1,4-glycosidic linkage in pectic acid and pectin, respectively by trans-elimination reaction and forming unsaturated galacturonates and methyl galacturonates, respectively. Pectinases are widely used in the food industry to improve the cloud stability of fruit and vegetable nectars, for production and clarification of fruit juices and for haze removal from wines [1]. Pectinases are also involved in clarification of wine, oil extraction, removal of citric fruit peels, and degumming fibres [2-4]. Pectinase usage accelerates tea fermentation and also destroys the foam forming property of instant tea powders by destroying pectins. They are also used in coffee fermentation to remove mucilaginous coat from coffee beans [5-7]. Considering the advantages of pectinase enzymes in food industries, this article aims to present an overview of potential pectinolytic microorganisms, their fermentation conditions, factors involved in maximum pectinase activity and interesting summary of substrates used in the fermentation processes.

Microorganisms

The most important factors to be considered during the pectinase production are the choice of microorganisms. Microorganisms that are particularly suitable for pectinase production through fermentation are the filamentous fungi, since the technique simulates their natural habitat. During fermentation, they are able to synthesize considerable amounts of enzymes and other metabolites. Although filamentous fungi (*Aspergillus* and *Penicillium*) are considered the most appropriate microorganisms for pectinase production, there are also some species of bacteria (Bacillus, *Erwinia*, Enterobacter) which have been reported to successfully produce pectinases.

Pectinase was produced by solid state fermentation with *Aspergillus niger* using sugar beet pulp as carbon source and the

wastewater from monosodium glutamate production as nitrogen and water source [8]. *Aspergillus oryzae* was cultivated in a pilot-scale packed-bed bioreactor containing citrus pulp and sugarcane bagasse [9]. Pectinase yields of 33-41 U g-1 were obtained and indicated the potential for using solid-state fermentation to produce pectinases in a citrus waste biorefinery. Banana peel was used as substrate to cultivate *A. niger* for pectinase production by Barman, et al. [10]. The optimum substrate concentration, incubation time and temperature of incubation were 8.07 %, 65.82 h and 32.37 °C respectively, and the polygalacturonase activity achieved was 6.6 U/ml for crude pectinase. The use of three *Aspergillus* species for pectinolytic enzyme production in solid state fermentation was studied by Heerd et al. [11]. All strains produced pectinases with the highest yield reached between the fourth and fifth day of cultivation. The highest exo-pectinolytic activity with 33.4 U/g Polymethylgalacturonases and 28.3 U/g polygalacturonase, as well as the highest endoenzyme activity of 32.9 U/g PMG and 30.1 U/g PG was observed by *A. sojae* ATCC 20235. *A. oryzae* showed considerable prospective for the production of industrially important pectin lyase in SSF of lemon peel waste (residual waste from citrus field crop) with maximal 875 6.2 U/mL activity [12]. Pectinase production from *A. niger* on orange peel waste by submerged fermentation was studied by Ahmed et al. [13]. The maximum enzyme activity of 99.4 ± 1.1 µM/mL/min was observed under optimum (4%) substrate concentration. pH of 5 and 50°C was found optimum for maximum activity of pectinase enzyme produced from *A. niger*.

Fermentation conditions

There are two fermentation methods that can be used for pectinases production, which are Solid State Fermentation (SSF) and Submerged Fermentation (SmF). Solid State Fermentation (SSF) is a microbial involved process which takes place under minute moist conditions or in the complete absence of free-flowing water contents in the growth/fermentation media. In contrast, in submerged fermentation (SmF) the nutrients and microorganisms are both submerged in water. Solid state fermentation due to its low water activity has advantage over submerged fermentation for pectinase production by fungi. Other advantages include reduced wastewater output, simpler fermentation technology and higher product concentration [14-16].

SSF processes are interesting for countries with abundant agricultural and industrial solid wastes. Agro industrial waste materials can be used both as source of energy for growth and as carbon for synthesis of cell biomass and other products. A wide range of agricultural/ agro-industrial wastes and by-product residues such as sugar cane bagasse, oranges, rice, banana and coffee are potentially suitable feedstocks for their possible bioconversion into a range of value-added product of interests like enzymes. With this regard, solid state fermentation permits the use of agricultural and agro industrial residues as substrates which are converted into bulk chemicals and fine products with high commercial value. In Asian countries, application of SSF technology in industrial scale for enzyme production is considered as a reliable fermentation process. However, the selection of a substrate for enzyme production depends on several factors including cost and availability of the substrate.

Factors affecting microbial pectinases production

pH

Acidic pH was found optimum for the production of fungal pectic enzymes [17,18]. For example, polygalacturonase produced from Lentinus edodes has a relatively lower pH of 5.0 [19]. Similarly, [20] & Silva, et al. [4] observed that *Pencillium griseoroseum* and *P. viridicatum* had produced higher levels polygalacturonase and pectin esterase at pH of 4.5 and 5. Rasheedha, et al. [21] found that *P. chrysogenum* exhibited maximum polygalacturonase production at initial pH of 6.5. *Aspergillus* niger had the optimum pectolytic activity at pH 5 [22] whereas Debing, et al. [23] reported pH 6.5 as optimal for pectinase production. Maximum pectinase and polygalacturonase (1116 and 1270 Ug-1) activity was at pH 4.0 5.0 respectively by *Aspergillus* fumigatus [24]. Both polygalacturonase and pectin lyase from *A foetidus* using mango peel as substrate had optimum activities at pH 5 and 5.5 [25]. Reda, et al. [26] found that the polygalacturonase productivity by *Bacillus firmus* reached its maximum at initial pH 6.0 and 6.2. Similarly, for *Bacillus sphaericus,* the optimal pH for polygalacturonase production was found to be 6.8 [27].

Cultivation time

The fermentation period for the production of pectinase varied among earlier reports and had a profound effect on product formation. Maximum production of pectic enzyme from different moulds varies from 1 to 6 days [28]. Castilho, et al. [29] reported that the highest polygalacturonase activities were obtained by *A. niger* after 70 h of fermentation period. Fawole & Odunfa [22] reported that optimum production of pectin methylesterase by *A. niger* was obtained after 4 days of fermentation under submerged fermentation condition. Patil & Dayanand [30] observed a gradual increase in the production of pectinase by *A. niger* after 72 h of fermentation period in submerged and up to 96 h in solid-state conditions. In a study by Dhillon et al. [31], maximum pectinase activity by *A. niger* was obtained after 120 h of fermentation. The optimum production of pectinase enzyme by a fungus *Aspergillus* niger was observed at 48 h of fermentation as reported by Khatri et al. [32]. *Aspergillus* foetidus has produced polygalacturonase and pectin lyase after 120 h and 96 h in solid state and submerged fermentations, respectively [25]. In agitated cultures supplemented with 0.5% citrus pectin and initial pH of 2.5, extracellular pectinase was produced by *Penicillium* frequentans after 48 h at 35 °C [33]. Reda et al. [26] found that the level of polygalacturonase increased gradually with increasing the incubation period up to a maximum of 96 h by *Bacillus firmus*. Maximal quantities of polygalacturonase were produced by Bacillus sphaericus when a 16-hours-old inoculum was incubated in shaking condition for 72 hours [27]. *Chryseobacterium*

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indologenes strain was found to produce maximum pectinase at 37°C with pH 7.5 upon incubation for 72 hours, while cultured in production medium containing citrus pectin and yeast extract as carbon and nitrogen sources [34].

Nitrogen source

The effects of organic and inorganic nitrogen sources on the production of pectinase were extensively studied. Both ammonium phosphate and ammonium sulphate did influence production of pectinase positively [24,30,35,36]. Rasheedha et al. [21] found that ammonium sulphate has enhanced the production of *P. chrysogenum* pectinase. In contrast, Sapunova [37] found that ammonium salts stimulated the pectinolytic enzyme production in *Aspergillus* alliaceus. Fawole & Odunfa [22] found that ammonium sulphate and ammonium nitrate were good nitrogen sources for pectic enzyme production from *A. niger* while glycine and tryptophan did not support enzyme production. On the other hand, report of Aguilar et al. [38] showed yeast extract as the best inducer of exopectinases by *Aspergillus* sp. Yeast extract, peptone and ammonium chloride were found to enhance pectinase production up to 24% and addition of glycine, urea and ammonium nitrate inhibited pectinase production [39]. For *Bacillus firmus*, peptone has resulted in the maximum value of polygalacturonase productivity (350 U m L^{-1}) [26]. Vivek et al. [40] found that organic nitrogen sources showed higher endo, exo pectinases activities than inorganic nitrogen sources. Soybean meal (4%) showed the maximum Exopectinase activity of 5128 IU g^{-1} and endo-pectinase activity of 793 IU g^{-1}.

Carbon source

Aguilar & Huitron [41] reported that the production of pectic enzymes from many moulds is known to be enhanced by the presence of pectic substrates in the medium. Fawole & Odunfa [22] found that pectin and poly galacturonic acid promoted the production of pectic enzyme. Phutela et al. [24] stated that wheat bran supported maximum pectinase production (589 U g-1) while pure pectin give the maximum production of polygalacturonase (642 Ug-1). Patil & Dayanand [30] reported that glucose (4-6%) increase the production of pectinase in submerged condition whereas 6-8% sucrose gives better yield of pectinase in solid-state condition. Reda et al. [26] reported that Solanum tuberosum peels was the best carbon source for polygalacturonase production by *Bacillus firmus*. In a study, 88 ± 9 IU/mL pectinase was produced by *Bacillus pumilus* after inoculating into media containing 2% each of wheat bran and Citrus limetta peel, 0.5% peptone, 10 mM $MgSO_4$, pH 7.0 [42-65].

Substrates

The most promising residues for pectinase activity include agricultural residues and fruit peels. The choice of the most appropriate microorganisms (Fungi and bacteria) to be cultivated in the agro residue depends much on its composition. Usually, these agro residues are not only a solid support for nutrients absorption and biomass growth, but they are also a source of carbon and nutrients. Sometimes, supplementation is needed in order to provide all necessary nutrients for optimum growth [66-75] (Table1&2).

Table 1: Substrates used for Pectinolytic enzymes production by bacterial species.

Micro organism	Substrate	Product	Source
Aeromonas salmonicida		Pectinase	Pavan, et al. [43]
Bacillus sp.	Wheat bran	Pectinase	Kashyap et al. [39]
Bacillus pumilus		Thermostable pectinase	Sharma and Satyanarayana [44]
Bacillus subtilis	Wheat bran	Thermostable pectinase	Ahlawat, et al. [45]
Bacillus subtilis	Wheat bran	Alkaline Pectinase	Ahlawat, et al. [46]
Bacillus subtilis	Pectin	Pectinase	Swain and Ray [47]
Bacillus sphaericus	Citrus pectin	Polygalacturonase	Jayani, et al. [27]
Bacillus subtilis	Date syrup	Pectianse	Qureshi, et al. [48]
Bacillus sp	Cassava waste	Pectinase	Mukesh Kumar et al. [49]
Bacillus firmus	Pectin	Pectinsae	Roosdiana et al. [50]
Erwinia cartovora	Pectin	Pectinase	Kothari and Baig [51]
Bacillus pumilus	Agricultural wastes	Exo-pectinase	Tepe and Dursun [52]
Bacillus licheniformis	Apple pectin	Pectinase	Rehman et al. [53]
Bacillus subtilis	Hazlenut Shell hydrolysate	Pectinase	Uzuner & Cekmecelioglu [54]
Bacillus subtilis	Pectin	Pectinase	Takci & Turkmen [55]
Bacillus pumilus	Wheat bran, citrus limetta peel	Pectinase	Kaur, et al. [42]
Bacillus licheniformis	Pectin	Polygalactouronase	Jahan, et al. [56]
Bacillus sonorensis	Pectin	Pectinase	Mohandas, et al. [57]
Chryseobacterium indologenes	Citrus pectin	Pectinase	Roy, et al. [34]

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Enterobacter tabaci		Pectinase	Obafemi, et al. [58]
Bacillus subtilis	Pectin	Pectinase	Mahto, et al. [59]

Table 2: Substrates used for Pectinolytic enzymes production by fungal species.

Micro organism	Substrate	Product	Source
Aspergillus niger	Sucrose	Pectinase	Friedrich, et al. [60]
Penicillium occitanis	Apple and citrus	Pectinase, pectin methyl esterase	Jain, et al. [61]
Penicillium frequentans	Citrus pectin	Pectinase, pectin esterase	Said S, et al. [33]
Aspergillus foetidus	Wheat bran	Pectinase	Sebastian, et al. [62]
Aspergillus niger	Soy and wheat bran	Pectinase	Castilho, et al. [29]
Aspergillus niger		Ectopectinase	Diaz-Godinez, et al. [15]
Aspergillus awamori	Wheat	Pectinase	Blandino, et al. [63]
Penicillium viridicatum	Orange bagasse and wheat bran	Pectinase	Silva, et al. [4]
Thermoascus aurantiacus	Orange waste, sugar bagasse, wheat bran	Pectinases	Martin, et al. [64]
Aspergillus niger		Invertase, pectinase and tannases	Gonzalez et al., [65]
Aspergillus niger		Pectinase	Patil & Dayanand [30]
Aspergillus niger	Citrus peel	Pectinase	Dhillon, et al. [31]
Aspergillus niger	Sugar beet pulp	Pectinase	Bai, et al. [8]
Aspergillus awamori	Grape pomace	Pectinase, xylanase	Botella, et al. [66]
Penicillium decumbens	Wheat bran	Pectinase	Sun X, et al. [67]
Aspergillus fumigates	What flour	Pectinase	Palaniyappan, et al. [68]
Aspergillus heteromorphus	Orange peel	Pectin methyl esterase	Mandhania, et al. [69]
Fomes sclerodermeus	Soy and wheat bran	Polygalacturonase	Salariato, et al. [70]
Mucor circinelloides	Pectin methyl ester	Polygalacturonase	Thakur et al. [71]
Penicillium chrysogenum	Sucrose	Pectinase	Banu et al. [72]
Aspergillus foetidus	Mango peel	Pectinase	Kumar et al. [25]
Rhizomucor	Pectin	Pectinase	Siddiqui et al. [73]
Rhodotorula glutinis	Citrus pectin	Pectinase	Taskin [74]
Aspergillus foetidus	Mango peel	Pectinase	Yannam et al., [75]
Aspergillus oryzae	Lemon peel	Pectin lyase	Koser, et al. [12]
Aspergillus sojae	Wheat bran	Polygalacturonase	Demir & Tari [76]
Trichoderma viridae	Orange peel	Pectinase	Irshad et al., [77]
Aspergillus niger	Banana peel	Pectinase	Barman, et al. [10]
Aspergillus niger	Orange waste peel	Pectinase	Ahmad, et al. [13]
Aspergillus oryzae	Citrus waste	Pectinase	Biz, et al. [9]
Aspergillus terreus	Banana peel	Pectinase	Sethi, et al. [78]
Aspergillus niger	Orange pomace	Pectinase	Mahmoodi, et al. [79]
Aspergillus niger	Orange peel	Pectinase	Rangarajan, et al. [80]

Conclusion

Microbial fermentation allows the re-use of agro-industrial and/or sub-products as substrate support for biotechnological production of pectinase group of enzymes. However, it is clear that some limitations for the scale-up are still observed. In this case, the choice of the microorganism, substrate and other factors which influence the pectinase production are predominant. The economic viability industrial fermentation of pectinolytic enzymes depends on a careful selection of the microorganism and the substrate used. Recent developments in industrial biotechnology offer several opportunities for the utilization of low-cost substrates for the pectinolytic enzyme production using fungi and bacteria through fermentation [76-80].

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