Selection of Pectinolytic Fungi Suitable for Retting

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Abstract

Among fifteen local isolates of different fungi investigated, it was determined that ***Aspergillus niger*** (106) and *A. niger* (107) are the best producers of total pectinase (TP) and polygalacturonase (PG). The production of constitutive cellulase along with the pectinases was found to be insignificant rendering them to be suitable for degumming of raw fibers in retting.

Introduction

Microbial pectinases are of substantial importance to industry since they play a significant role in food manufacture and are involved in different biotechnological processes [1-3]. They have been used intensively in textile industries for the treatment of raw fibers of flax, hemp or jute causing degradation of middle lamella and the primary cell wall, a process commonly known as retting [4]. Although, it is an age old process, it has progressed little scientifically. To our knowledge, information on the use of pectinolytic enzyme mixture has not been reported in industry. Therefore, when employing microorganisms for such a treatment it is important that cellulosic fibers should not sustain damage normally caused by constitutive cellulases consequently, the system should be comparatively free of these enzymes.Both aerobic and anaerobic retting has been performed by pectinolytic bacteria belonging to the genera *Bacillus*, *Paenbacillus* and *Clostridium* [5], respectively. Dew retting however, is a aerobic form in which fungi and yeasts are the pectinase producing microorganisms more than bacteria.These included species of *Penicillium*, *Aspergillus*, and *Rhodotorula* [4] Our main objective in this work is to search for pectinolytic fungi capable of producing economically appreciable amount of pectinases devoid of cellulases for utilization of degumming of fibers.

Materials and Methods

Microbial Isolates and Growth Conditions:

The fungal isolates tested (Table 1) are local strains (isolated from different sources of rotten food and classified in our laboratory), grown on solid media at 28°C until sporulation (6-9 days. The culture medium used contained : (g/l : (NH_4)$_2$ SO_4 , 1.0 , KH_2PO_4 , 2.0, NaH_2PO_4 3.0, $MgSO_4$; 1.0, $FeSO_4.7H_2O$. 0.1, $CaCl_2$ 0.0005, Yeast extract 0.6, Pectin 5.0, pH 6.9). solidified with 1.5% agar when required. Inoculation was performed under sterile conditions: one loop of spores transferred to one liter Penicillium bottles containing 300 ml of media and incubated at 28 °C for 6 days.

Determination of Enzyme Activity

Extracellular polygalacturonase, total pectinase, and cellulase activities were measured in the supernatant of each culture. Readings represent the mean value of three replica. Enzyme activity was determined using dinitrosalicylic acid reagent (DNS), assay is based on the release of reducing groups [7].Total pectinase and polygalacturonase activities were carried out with galacturonic acid as a standard. The reaction mixture consisted of 3.0 ml of the supernatant to which 2.0 ml of either 1% pectin solution (1.0g apple pectin dissolved in 100 ml buffer M: (g/l: KH_2PO_4 0.6, $MgSO_4.7H_2O$: 0.1, $(NH_4)_2SO_4$, 1.0, pH6), or 2.0 ml of 0.1% polygalacturonic acid (dissolved in buffer M, pH 5.5) solution respectively. The mixtures were incubated at 40 °C for 1 hr. The unit enzyme is defined as the micromoles of the galacturonic acid released per microgram protein per hour. Similar procedure was used for the determination of cellulase activity implying glucose as a standard. The reaction mixture contained 3.0 ml of the supernatant into which a strip of filter paper immersed. Incubation performed at 4 °C for 1hr. Cellulase unit was defined as the micromoles of glucose released per microgram protein per hour.

Results and Discussion

The data reveals a considerable variation in the enzyme production between isolates of the same or different species screened (Table 1). A tremendous difference in pectinase production from 0.018 to 0.466 TPU, polygalacturonase production from 0.008 to0.115 PGU; and cellulase production from 0.001 to 0.097 Cellulase Units (Table 1). ***Aspergillus niger*** (106) is the best producer of pectinase and polygalacturonase (0.466 TPU, and 0.115 PGU, Table 1). The second in order is ***A. niger*** (107)(0.400 TPU and 0.082 PGU, Table) which is considered better suited since its cellulase production is approximately one half (0.024) that of the former (0.045) Cellulase Units(Table 1). Polygalacturonases and hemicellulases have been regarded as the major enzymes for the separation of cellulosic fibers from the cell wall [9]. The

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quantities of PG formed by *A. niger* (106) and *A.niger* (107) are relatively low as compared with the work of others [8,9]. Nevertheless, if other pectinases provide similar functions, the quantities of pectinase produced by the above microorganisms are considered satisfactory. Baracat et al. [9] showed that *Penicillium versicolor*, among other fungi tested, is the best for pectinase production (0.636 TPU) which is relatively higher than that produced by *A. niger* in the present work (0.466 and 0.400 respectively, Table 1). On the other hand, cellulase production by *P. versicolor* was abundantly higher (0.092 Cellulase Units) than that formed by *Aspergillus niger* 106 and 107 (0.045 and 0.024 Cellulase Units, Table 1); a property rendering them more suitable for retting. Table1: Polygalacturonase, total pectinase and cellulose production by species of *Aspergilus niger*, *Trichoderma* and *Mucor* expressedin enzyme units.

Table (1):

Fungal Isolates *	Total Pectinase Units	Polygalacturonase Units (PGU)	Cellulose Units
Aspergillus niger	0.400	0.082	0.024
1. 107			
2. 2	0.196	0.021	0.034
3. B22	0.296	0.039	0.091
4. 106	0.466	0.115	0.045
5. 103	0.300	0.026	0.043
6. 104	0.283	0.024	0.043
7. 118	0.271	0.069	0.032
8. D	0.109	0.051	0.017
9. G	0.021	0.012	0.019
10. x	0.018	0.008	0.007
11. *Aspergillus flava*	0.1[illegible]5	0.041	0.030
12. *Aspergillus terreus*	0.064	0.012	0.004
13. *Trichoderma viride* (1)	0.130	0.030	0.005
14. *Trichoderma viride* (2)	0.080	0.022	0.003
15 *Mucor* sp.	0.042	0.013	0.002

* Local designation

References

1. Fogarty, W. M. and Kelly, C. T. (1983). " Microbial enzymes and Biotechnology" . In W. M. Fogarty (ed.). Chapter 3. Applied Science Publisher. London. New York.
2. Kashyap, D.R., Vohra, P. K., Chopra, S., Tewari, R. (2001). Application of pectinases in the commercial sector, a review. Biosresour. Technol. 77: 215
3. Hoondal, G., Tiwari, R., Tewari, R.,Dahiya, N. and Beg, Q. (2002). Microbial alkaline pectinases and their industrial applications: a review. Appl. Microbiol. & Biotechnol. 59: 409.
4. Tanabe, H. and Kobayashi, Y. (1987) Plant tissue maceration caused by pectinolytic enzymes from *Eriwinia spp.* under alkaline condition. Agric. Biol. Chem. 51: 2845.
5. Tamburini,E., Gardillo Leon. A., Perito, B. and Mastromei, G.. (2003). Characterization of bacterial pectinolytic strains involved in the water retting process. Env. Microbiol. 5:730.
6. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Analyt. Chem. 31:.
7. Ceci, L. and Lozano, J. (1998). Determination of enzymatic activities of commercial pectinases for clarification of apple juice. Food Chem. 61: 237-241.
8. Kobayashi, Y. and Matsuo, R. (1984). Rapid retting of kazoo "paper mulberry" best with *Erwinia caratovora*. Agric. Biol. Chem. 48: 1353.
9. Baracat, M. C. Valentim, C. Muchovej, J. J. and Silva, D. O. (1989). Selection of pectinolytic fungi for degumming of natural fibers. Biotech. Letters 11: 899

الخلاصة

تم فحص 15 عزلة محلية من الفطريات و تبين أن العزلتين أسبرجلس نايجر 107 و 106 هما الأفضل لإفراز الأنزيمات المنتجة للبكتيز و البولي كالاكتورونيز. كما تبين أن إنتاج الانزيم السليوليز الفطري و المركز لأنتاج البكتينيز قليل مما يجعلهما مفيدين في إستخلاص الألياف في عملية التعطين.