PECTIC ENZYME OF PENICILLIUM FREQUENTANS INVOLVED IN THE RETTING OF JUTE

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Penicillium frequentans secreted a pectic enzyme in the culture media with or without pectin. Of the different substrates, pectin, sodium-pectate, de-esterified pectin, starch and albumin tested, the enzyme was active only on the substrates, pectin, sodium-pectate and de-esterified pectin. The activity was optimum at pH 3.2 and 5.0 and at a temperature of 23°C. but it decreased with dilution and was not inhibited on dialysis. The enzyme macerated both potato discs and jute barks and retted jute stems. It has been identified as a mixture of pectin-polygalacturonase (PG) and pectin-methylesterase (PME). The mechanism of jute retting in terms of hydrolysis by the above enzyme has been discussed.

Introduction

Loosening of jute fibre in jute stems (*Corchorus capsularis* and *C. olitorius*) is accomplished by a process known as "retting". During this process the fibre bundles from the cortex and wood are separated, and a partial digestion of the cementing material (mostly of pectic nature) between the fibres in the bundles is effected. According to Kertesz,[1] Mitscherlich first attributed these changes to the pectic enzymes produced by micro-organisms, but comparatively little is known about these enzymes. He further added that while there are many organisms capable of attacking pectin and performing retting, only a few have been used for producing enzymes, which are capable of retting in the absence of living organisms. Katagari and Makaha compared the pectin decomposing enzymes produced by retting bacteria and found that crude preparations showed remarkable specificity in their actions towards various fibre plants. Baruah and Baruah[2] reported that an enzyme mixture named, Hiparol, secreted by *Thielaviopsis paradoxa* (De Seynes) V. Hohn were more active than bacterial enzymes in the retting of jute and coconut husk fibres. Kertesz[1] suggested that a thorough reconsideration of the role, as well as the use, of pectolytic enzyme in the retting of plant fibres would be most desirable.

This investigation, was undertaken to: (1) study the nature of the jute retting enzyme of a fungus, *Penicillium frequentans*, which retted jute stems in moist conditions in a short time and was isolated and identified in this laboratory, and (2) ascertain the feasibility of utilizing these enzymatic preparations under controlled conditions of retting.

Materials and Methods

The fungus identified as *Penicillium frequentans* was isolated from a decaying filter paper. The culture filtrate was prepared by growing the fungus for 2-4 days, on an autoclaved liquid medium, containing glucose 3%, peptone 0.3%, NH_4NO_3 0.16%, KH_2PO_4 0.34% and $MgSO_4$, $7H_2O$ 0.19% and then filtering the culture, as reported by Gupta.[3] This culture filtrate was designated as the pectic enzyme preparation.

The enzymic activity of this preparation was determined by measuring the viscosity fall of different substrates, albumin, starch and apple pectin. To 90 ml. of each of 1% solution of starch, 10% (v/v) solution of albumin and 0.5% solution of pectin, 10 ml. of the enzyme preparation were added and allowed to stand for one hour. The viscosity fall was measured with Ostwald's viscometer (16 seconds). For determining the relationship of enzyme production with dry weight of mycelia, the fungus was grown in the medium adjusted to different pH values, its dry weight was determined and the enzymic activity of the culture filtrate was measured viscometrically, using 0.5% pectin as substrate. For studying the effect of pectin in the medium on the production of enzyme, 1% pectin was incorporated in the medium and the enzyme activity was measured viscometrically.

The effect of pH on the enzymic activity was studied in two ways: (1) the mixture of culture filtrate and the substrate (0.5% pectin) in the ratio of 1:9 was adjusted to different pH values and buffered with acetate and phosphate, (2) the enzymic activity was determined by measuring the released reducing sugar from the same pectin solution, using Stiles, Peterson and Fred's method.[4] For determining the effect of temperature, the enzyme substrate mixture (10:90) was incubated at different temperatures for 20 minutes and then the released reducing sugar was measured.

The effect of enzyme preparation on the hydrolysis of pectin, sodium pectate and acid de-esterified pectin was studied by allowing the enzyme substrate mixture (10:90) for 20 minutes at 30°C. and then measuring the hydrolysis with viscometer and

reducing sugar-determination method. The sodium pectate and acid de-esterified pectin were prepared from apple pectin using Wood's method.[5]

The effect of enzyme preparation on the maceration of potato discs and jute barks was studied using Singh and Wood's method.[6] Sterilized jute bark of uniform size were placed in the enzyme preparation along with the potato discs. For studying the effect of pH on the maceration, the enzyme was adjusted to different pH values and buffered. For studying the effect of temperature, the enzyme with the potato disc and jute bark was incubated at different temperatures.

The effect of enzyme preparation on jute retting was studied by adding the enzyme preparation to 10-day leached jute stem. The leached jute stem was obtained by steeping pieces of jute stem in a flask for 10 days and then pouring off the water.

Results

Among the different substrates tested, the enzyme in the culture filtrate of the fungus, *P. frequentans* was more active on the substrate, pectin (Table 1). The enzyme production was highest in the culture filtrate of two to four days growth, was not related to the dry weight of mycelia and was independant of incorporation of pectic substance into the medium (Tables 2, 3 and 4).

Table 1.—Effect of Culture Filtrate of *P. frequentans* on the Viscosity Fall of Different Substrates.

Substrates	Viscometric readings (seconds)
Starch+Boiled culture filtrate	22.0
Starch+Culture filtrate	22.0
Pectin+Boiled culture filtrate	29.5
Pectin+Culture filtrate	17.0
Albumin+Boiled culture filtrate	15.5
Albumin+Culture filtrate	15.5

Table 2.—Effect of Pectic Enzyme of *P. frequentans* at Different Growth Periods on the Viscosity Fall of Pectin Solution.

Growth period (days)		Viscometric reading (second)	
		Reading	Difference
2	Boiled enzyme	29.5	12.5
	Enzyme	17.0	
3	Boiled enzyme	28.5	11.5
	Enzyme	17.0	
4	Boiled enzyme	28.5	11.5
	Enzyme	17.0	
6	Boiled enzyme	34.5	1.5
	Enzyme	33.0	
7	Boiled enzyme	34.0	.5
	Enzyme	33.5	
8	Boiled enzyme	33.5	0
	Enzyme	—	

Table 3.—Effect of Growth of *P. frequentans* on the Activity of its Pectic Enzyme on the Viscosity Fall of Pectin Solution at Different pH Values.

pH values	Mycelial wt. (g.)		Viscometric reading (second)	
			Reading	Difference
3.2	0		—	—
4.4	1.99	Boiled enzyme	36	
		Enzyme	17	19
5.5	1.89	Boiled enzyme	36	
		Enzyme	28	8
7.0	1.98	Boiled enzyme	36	
		Enzyme	36	0

The activity of the enzyme on different pectic substances was different under different conditions. The optimum pH, at which the enzyme hydrolysed pectin, was 3.2 (Table 5). However, when the reducing sugar released from pectin by the enzyme was measured, the pH optimum was at 5.0 (Table 6). The optimum temperature at which the enzyme released reducing sugar from pectin was between 10-20°C. (Table 6). Sodium pectate and acid de-esterified pectin were relatively more hydrolysed compared to pectin (Tables 7 and 8). The enzymic a ctivity was not inhibited

TABLE 4.—EFFECT OF PECTIN AND NON-PECTIN MEDIA ON THE PRODUCTION OF PECTIC ENZYME BY *P. frequentans*.

Media	Pectin hydrolysis, viscometric reading (second)
Non-pectin	16.8
Pectin	17.4
Check	33.0

TABLE 5.—EFFECT OF PECTIC ENZYME OF *P. frequentans* ON THE HYDROLYSIS OF PECTIN AT DIFFERENT BUFFERED pH VALUES.

pH		Viscometric reading (second)	
		Readings	Difference
3.2	Boiled enzyme	32	
	Enzyme	20	12
4.4	Boiled enzyme	28	
	Enzyme	17	11
5.5	Boiled enzyme	24	
	Enzyme	17	7
7.0	Boiled enzyme	26	
	Enzyme	26	0

TABLE 6.—EFFECT OF PECTIC ENZYME OF *P. frequentans* ON THE RELEASE OF REDUCING SUGAR FROM PECTIN AT DIFFERENT pH VALUES AND TEMPERATURES.

pH	Reducing sugar released (mg.)	Temperature (°C.)	Reducing sugar released (mg.)
3.8	0.038	10	0.239
5.0	0.158	20	0.239
6.0	0.035	30	0.217
7.0	0.030	40	0.114
		50	0.121
		60	0.000

on dialysis but it was progressively reduced on dilution (Tables 9 and 10). The enzyme macerated both potato discs and jute barks. It macerated them at a pH optimum 3.2 and temperature optimum towards 23°C. (Tables 11 and 12). The intracellular enzyme tested also had the same effect at pH 3.2 (Table 13). The retting period was remarkably reduced when the enzyme was added to leached jute stem (Table 14).

TABLE 7.—EFFECT OF PECTIC ENZYME OF *P. frequentans* ON THE VISCOSITY FALL OF PECTIC SOLUTIONS AT DIFFERENT PERIODS.

Period (Min.)	Na-Pectate	Pectin
0	24.0	25.0
15	19.0	21.0
30	18.5	18.0
45	16.0	17.0
60	16.0	17.0

TABLE 8.—EFFECT OF PECTIC ENZYME OF *P. frequentans* ON THE RELEASE OF REDUCING SUGAR OF PECTIN AND DE-ESTERIFIED PECTIN SOLUTIONS.

Pectic solutions	Reducing sugar (mg.)
Pectin ..	50.3
De-esterified pectin	64.7

TABLE 9.—EFFECT OF DIALYSIS ON THE ACTIVITY OF PECTIC ENZYME OF *P. frequentans*.

Treatments	Viscometric reading (second)
Pectin + Dialysed enzyme	29.5
Pectin + Non-dialysed enzyme	18.5
Pectin + Boiled enzyme ..	40.0

TABLE 10.—EFFECT OF DILUTION OF PECTIN ENZYME OF *P. frequentans* ON THE VISCOSITY FALL OF PECTIN SOLUTION.

Enzyme: Boiled enzyme (By volume)	Viscometer reading (second)
0 : 10	25
2 : 8	20
4 : 6	20
6 : 4	20
8 : 2	19
10 : 0	18

TABLE 11.—EFFECT OF PECTIC ENZYME OF *P. frequentans* ON THE MACERATION OF POTATO DISCS AND JUTE BARK AT DIFFERENT pH VALUES.

pH	Maceration time (minute)	
	Potato disc	Jute bark
3.2	45	240
3.8	100	No action
4.4	180	No action
5.0	720	No action
5.5	No action	No action
7.5	No action	No action

TABLE 12.—EFFECT OF PECTIC ENZYME OF *P. frequentans* ON THE MACERATION OF POTATO DISCS AND JUTE BARK AT DIFFERENT TEMPERATURES.

Temperature (°C.)	Maceration time (minute)	
	Potato discs	Jute bark
23	180	180
30	210	210
40	225	225
50	240	240
60	600	600
70	No action	No action
80	No action	No action

TABLE 13.—EFFECT OF INTRACELLULAR PECTIC ENZYME OF *P. frequentans* ON THE MACERATION OF POTATO DISCS AND JUTE BARK AT DIFFERENT pH VALUES.

pH values	Maceration time (minute)	
	Potato discs	Jute bark
3.2	240	240
4.4	No action	No action
5.0	No action	No action

TABLE 14.—EFFECT OF PECTIC ENZYME OF *P. frequentans* ON THE RETTING PERIOD OF 10-DAY LEACHED JUTE STEM.

Treatment	Retting period (days)
Jute stem+Enzyme	12
Jute stem+Tap water	18

Discussion

The results of the different tests on the properties of the enzyme in the culture filtrate of the fungus, *P. frequentans* throw some light on its indentity. Two different pH values were obtained for the optimum activity when measured in two different ways. The optimum pH value was 3.2 when it was determined viscometrically but it was 5.0 when it was determined by measuring the reducing sugar released (Tables 5 and 6). In his review of the reports of the various authors Kertesz[1] mentioned pH 3.0-5.0 to be the optimum range for the activity of pectin-polygalacturonase (PG). He said that such a discrepancy could occur because of the variations in the experimental methods. He further mentioned that fungal pectin-methyl esterase had a well defined optimum at about pH 4.5-5.0. Phaff[7] reported that the pH optimum of pectin-methylesterase (PME) from *P. chrysogenum* was 4.3. From the above reports it appears that the enzyme in the culture filtrate might be a mixture of PG and PME with the prevalence of the former. This assumption is further substantiated by the fact that the enzyme hydrolysed sodium-pectate and acid de-esterified pectin more than pectin (Tables 7 and 8). Kertsz[1] reported that the temperature optima given in the literature for pectin polygalacturonase was from

30-55°C. In this investigation the optimum was at 23°C. with a tendency towards lower value and the enzyme was inactivated above 60°C. (Table 6). Sumner and Somers[8] reported that the temperature optimum of an enzyme was a hypothetical value greatly dependant on the experimental conditions, especially the length of heating time. The temperature effect on the culture filtrate of *P. frequentans*, therefore, also indicates that the enzyme might be mostly PG.

Chaudhury[9] in his review of biochemical study on jute retting reported that during jute retting some microorganisms decomposed pectins of jute bark and the intervening tissues disintegrated. The enzyme, protopectinase hydrolysed pectin to protopectin which was broken down by pectinase to glacturonic acid. According to Kertesz,[1] Kolb reported in 1868 that the retting of flax was caused by the action of the enzyme, assumed to be protopectinase. However, he, and Winstead and Walker,[10] pointed out that the two enzyme protopectinase and PG were identical. Kertesz,[1] Chona,[11] Menon[12] and Tribe reported that the new characteristics of the so called protopectinase might be due to difference in fungal source substratum used and extra or intracellular nature of enzyme. In this investigation the enzyme of *P. frequentans* macerated both, potato disc and jute bark, with the optimum properties mostly resembling those of PG. The authors are also inclined to feel that the protopectinase should not be given a separate identity but be considered the same as PG.

The effect of enzyme of *P. frequentans* on the reduction of retting period gives a clearer picture on the mechanism of retting. It seems that during the first few days of retting known as the physical stage Eyre and Nodder,[13] soluble matters of jute stem are dissolved and it becomes soft. During this stage the actual fibres bound by pectic substances are not separated. At the end of this stage if the fibres are exposed to the appropriate enzyme, which now appears to be mostly PG, the pectic substances are hydrolysed and the fibres are released. The effect of PG on retting of jute also brings out another fact that the substance cementing the fibre bundles might not be protopectin as suspected by previous workers but mostly one of other pectic compounds with low methoxyl content. Elarosi[14] reported that the dissolution of middle lamellae by fungal attack was due to the double effect of PME and PG. Whether this situation is also true in the case of jute retting remains to be found out. The enzyme of *P. frequentans* was both adaptive and nonadaptive since it was produced in both the media with or without pectin (Table 4). Wood[15] considered adaptiveness of PG. as one of its distinguishing properties. In view of this report it seems that the enzyme in question might be a mixed one. Therefore, perhaps the retting of jute is brought about by the double effect of PG and PME.

The effect of the enzyme of *P. frequentans* on the reduction of retting period of jute indicates that perhaps this reduction in the retting period will also take place if the retting is undertaken in a wider scale. If these results can be duplicated on larger scale, then it would be greatly helpful to those contemplating to ret jute in specially prepared retting tanks. The prospect of using retting tanks would be still better if the hydrolysis products could also be utilized. To determine whether such utilization is possible, calls for further investigation into the nature of the hydrolysis products and their abundance.

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