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Pectin lyases of a few indigenous fungal strains

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Four pectin lyases (PNLs), producing indigenous fungal strains (*Aspergillus flavus, A. niger, A. phoenicis and A. wentii*), have been isolated from local samples. Enzymatic characteristics of PNLs produced by above fungal strains using citrus pectin have been found to be (respectively): Km, 0.38, 0.67, 0.55 and 0.32 mg/ml; pH, 8.0, 7.0, 5.0 and 7.0; and temperature, 50 °C for all PNLs. Effect of metal ions (Ag^{+}, Ca^{++}, Co^{++}, Cu^{++}, Hg^{++}, K^{+}, Mg^{++}, Zn^{++}, Na^{+}) and protein inhibitors (EDTA, sodium arsenate, sodium azide, potassium permanganate and potassium ferrocyanide) on the activities of PNLs has been determined.

Keywords: *Aspergillus*, Fungal strains, Pectin lyase, Pectinase

Introduction

Pectin is a heteropolysaccharide composed of α, 1-4 linked galacturonate chains with a high percentage of methyl esters occupying a place in the middle lamella of the plant cell wall[1]. Food industries utilize pectinases in processing of beverages, such as wine and fruit juices[2]. In textile industries, pectinases are used for degumming of natural fibers, a process more ecofriendly than chemical processes[3]. Among all pectinases [polygalacturonase, PG (EC 3.2.1.15), pectin esterase, PE (EC 3.1.1.11), pectate lyase, PL (EC 4.2.2.2.) and pectin lyase, PNL (EC 4.2.2.10)], only pectin lyases can degrade pectin by β-elimination mechanism without complementary action of other enzymes[4]. PE and PG must act together to degrade the pectin molecule completely, and they also liberate methanol as a byproduct of PE action, which is not desirable in food industries.

This paper presents four pectin lyases (PNLs) producing indigenous fungal strains and their enzymatic characteristics like Km, pH, temperature and effect of some common metal ions and enzyme inhibitors on the enzymes produced by these fungal strains.

Materials and Methods

Citrus pectin (P-9135) was purchased from Sigma chemical company, St. Louis, USA. All other chemicals (Merck, Germany or S.D.fine, Mumbai) were used without further purification. Enzyme activity of PNLs was assayed[5] by monitoring the increase in optical density at 235 nm due to formation of unsaturated uronide product using UV/VIS Spectrophotometer Hitachi (Japan) model U-2000, which was fitted with electronic temperature control unit and had a least count of 0.001 unit. Enzyme solution (0.2 ml) was added to a reaction mixture containing 0.8 ml citrus pectin (1% w/v) and 2.0 ml of the desired buffer (100 mM conc.) maintained at 37°C. In cases of PNLs of *A. flavus, A. niger, A. phoenicis* and *A. wentii*, repective buffers used were sodium phosphate (pH 8.0), sodium phosphate (pH 7.0), citrate phosphate (pH 5.0) and sodium phosphate (pH 7.0). Optical density was measured at zero time and after 20 min. Enzyme activity was defined in terms of ¼ mole of unsaturated product released per min, based on the molar coefficient value 5500 $M^{-1}cm^{-1}$ of the unsaturated product. Protein was determined by Lowry method[6] taking Bovine serum albumin as the standard.

Fungal strains, tentatively identified as *A. flavus*, *A. niger, A. phoenicis* and *A. wentii* in the Department of Botany, DDU Gorakhpur University, Gorakhpur, were isolated from decaying wood, soil from local fruit market and diseased fruit samples by using repeated streaking method[7], maintained on Czapek Dox Agar slants and preserved at 4°C. Pure fungal strains were screened for extracellular, PNL production in submerged medium (pectin, 10; L-asparagine, 2; $MgSO_4$ $7H_2O$, 0.5; and

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KH_2PO_4, 3g/l double distilled water; pH, 4.5). One ml of spore suspension (spore density, 5 x 10^6 spores/ml) from agar slants was inoculated aseptically into sterilized liquid medium, 25 ml contained in 100 ml culture flasks. Flasks were incubated at 25°C in BOD incubator and culture was allowed to grow under stationary conditions. Aliquots (1.0 ml) of growth medium were aseptically withdrawn at 24 h intervals filtered through whatmann filter paper no. 41 and cell free filtrate (CFF) was used as an enzyme sample.

Results and Discussion

Temperature (25°C) is optimum[8,9] for PNLs production in submerged culture. Up to 4th day after incubation under static culture conditions, no appreciable production of PNLs was observed in all the four fungal strains (Fig. 1). In *A. flavus* and *A. phoenicis*, maximum production of PNL was observed on 6th day after incubation, whereas in *A. niger* and *A. wentii*, maximum productions was on 8th day of incubation. Maximum enzyme units achieved in the culture conditions were: *A. flavus*, 0.11; *A. niger*, 0.013; *A. phoenicis*, 0.47; and *A. wentii*, 0.08 IU/ml. Maximum enzyme units produced in the liquid culture media in *A.flavus* and *A.wentii* were better than the maximum enzyme units reported[10].

Km values determined for these enzymes using citrus pectin as substrate are 0.38, 0.67, 0.55 and 0.32 mg/ml respectively (Fig. 2), and are comparable to literature value[11]. pH optima of PNLs secreted by *A. flavus, A. niger, A. phoenicis,* and *A. wentii* are 8.0, 7.0, 5.0 and 7.0 respectively (Fig. 3). Most of the reported PNLs have

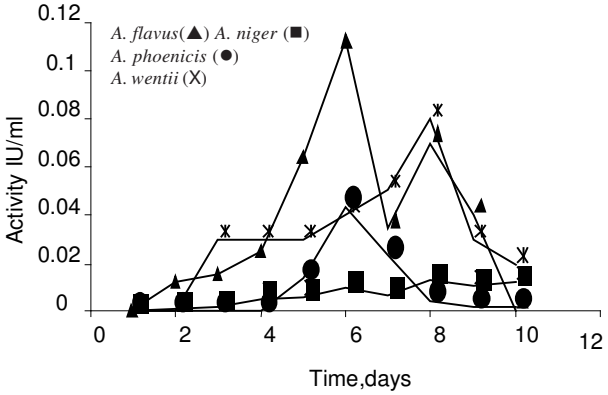
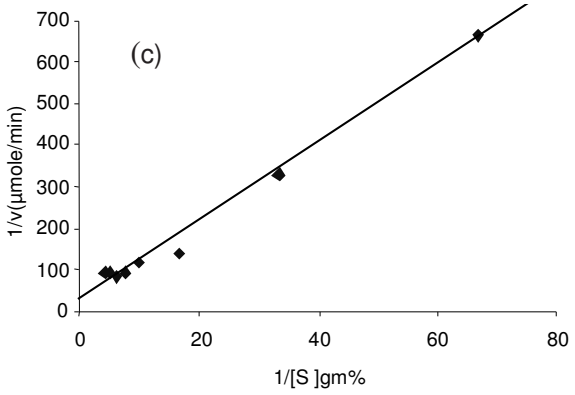


Fig. 1—Appearance of pectin lyase activity in liquid culture medium: (a) *A. flavus*(▲) (b) *A. niger* (■) (c) *A. phoenicis* (●) (d) *A. wentii* (X)

(a) (b)



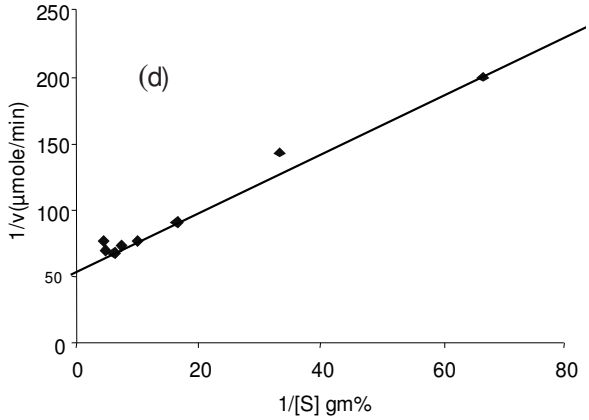


Fig. 2—Double reciprocal plots for pectin lyases: (a) *A. flavus*; (b) *A. niger* (c) *A. phoenicis* (d) *A. wentii*

Table 1—Effect of metal ions and protein inhibitors on pectin lyase activity

S.No.	Relative activity, %			
	A. flavus	*A. niger*	*A. phoenicis*	*A. wentii*
Metal ions (0.2 mM)				
1 Control	100	100	100	100
2 Ag^{+}	0.0	0.0	0.0	0.0
3 Ca^{++}	94.0	84.0	92.0	87.0
4 Co^{++}	0.0	8.6	0.0	11.3
5 Cu^{++}	0.0	0.0	0.0	13.1
6 Hg^{++}	12.5	0.0	0.0	0.0
7 K^{+}	183.0	139.0	24.6	113.0
8 Mg^{++}	177.0	130.0	73.3	58.0
9 Zn^{++}	0.0	69.5	23.3	73.8
10 Na^{+}	181.0	117.5	23.3	92.0
Protein inhibitors (0.2 mM)				
1 EDTA	57.0	55.6	21.0	79.0
2Sodium arsenate	139.0	113.0	8.6	117.0
3Sodium azide	46.8	170.0	21.3	0.0
4Potassium permanganate	0.0	0.0	0.0	0.0
5Potassium ferrocyanide	0.0	0.0	0.0	0.0

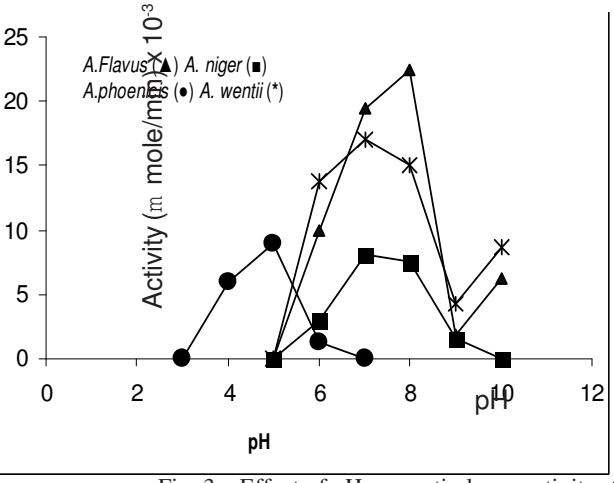


Fig. 3—Effect of pH on pectin lyase activity: (a) *A. flavus* (▲) (b) *A. niger* (■) (c) *A. phoenicis* (●) (d) *A. wentii* (X)

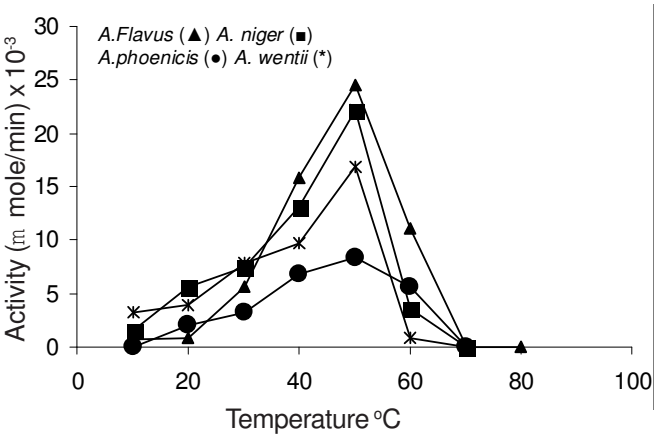


Fig. 4—Effect of temperature on pectin lyase activity: (a) *A. flavus*(▲)(b) *A. niger* (■) (c) *A. phoenicis* (●) (d) *A. wentii* (X)

pH optima in alkaline range[12-14]. In present study, PNL from *A. phoenicis* has pH optima (5.0) in acidic range suggesting that it is better suited for clarification of fruit juices[15-17]. PNL produced by *A. flavus*, which has pH optimum in alkaline range, is better suited for degumming of natural fibres in textile industries[18-21]. Though pH optima of PNLs produced by *A. niger* and *A. wentii* lie in neutral pH range but these have more activities (>60%) even at pH 8.0 and thus can be used for degumming of natural fibres.

Relative activities of PNLs from four fungal strains have temperature optima at 50°C (Fig. 4), which is comparable to reported[13] values. Effect of metal ions and some common enzyme inhibitors on the activities of 4 PNLs from four fungal strains were studied at using 0.2 mM concentrations (Table 1). K^{+}, Mg^{2+} and Na^{+} ions

promote activities of PNLs from *A. flavus*, *A. niger* and *A. wentii* but inhibit activity of PNL from *A. phoenicis*. Ag^{+}, Cu^{2+}, $Co^{2+,}$ Hg^{2+} and Zn^{2+} inhibit activities of all PNLs. Sodium arsenate promotes activities of PNLs from *A. flavus, A. niger* and *A. wentii*, whereas it inhibits PNL activity of *A. phoenicis*. Sodium azide promotes activity of PNL from *A. niger* but it inhibits activities of PNLs from *A. flavus*, *A. phoenicis* and *A. wentii*. Potassium ferrocyanide and Potassium permanganate strongly inhibit activities of all PNLs. Modification in activities of PNLs may be attributed to electrostatic bonding of metal ions with enzyme molecules, which would change tertiary structure of the enzyme[22].

Conclusions

The level of enzyme units produced in the liquid culture medium though is not enormously high but studies available can be used to isolate the gene of these enzymes and to overexpress in suitable expression system.

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