International Research Journal of Microbiology Vol. 2(5) pp. 153-158, June 2011
Available online http://www.interesjournals.org/IRJM

Full Length Research Paper

Production of pectic acid lyases (pal) by three isolates of *syncephalastrum racemosum*

B. Vijayapal Reddy, K. Narasimha Rao, M. Srinivas and S.M. Reddy

Department of Microbiology, Kakatiya University, Warangal – 506 009, A.P., India.

Accepted 11 March, 2011

Production of pectic acid lyase (PAL) by three isolates of *Syncephalastrum racemosum* was studied under difference cultural conditions. The PAL secreted by all the three isolates were adaptive as their secretion was maximum in pectin containing media. The present three isolates of *S. racemosum* differed significantly in their choice for pH, temperature, carbon and nitrogen sources for production of PAL enzymes. Similarly different growth substances and fungicides had varying effect on the three strains of *S. racemosum* under investigation for productions of PAL. In general alkaline pH favoured the activity of these enzymes. A temperature of 30^{0}C was optimum for PAL (Exo-and Endo) production. The three isolates under study were stimulated by malt extract and 2,4-DNP and corn steap liquor stimulated Exo-PAL production by orange and mosambi isolates respectively.

Key words: Pectic acid lyases, *Syncephalastrum racemosum, p*H, temperature, carbon and nitrogen source, fungicides and growth substances.

INTRODUCTION

Pectic enzymes have been receiving increased attention both in plant pathology as a agent helpful in the establishment of pathogen in host plant. Since the report of production of transeliminases by Albersheim *et al.* (1960), a large number of plant pathologists including Alana, *et al.* (1989); Bugbee, (1990); and Jacobo Ortega, (1996) have implicated these enzymes in the cause of plant diseases. These enzymes were also implicated in the decomposition of plant parts (Neeraj Saxena, 1983). Narania and Reddy (1982) reported the synergistic production of pectin lyases and polymethyl galacturonase by *Spegazzinea tersortbra* and accelerated the decomposition of substratum. Samiappan and Vidyashekaran (1981) could find a positive correlation between pathogenicity and production pectin lyase by two isolates of *macrophoma phaseolina,* in accordance with their pathogencity. As well as commercially in canning and production of fruit juices and textile industrial (Alkorta et.al, 1998; and Kashyap at.al, 2001). Dube and Gour 1975 and Charya 1980 have reported the adaptive nature of pectin lyase secreted by *macrophoma Phaseolina.* On the other hand, *Phoma exigua* reported to secrete pectin lyases constitutively. The fungi are reported to vary in their potential to secrete these enzymes not only among different species of same genus but also among strains of same species. Ramraj, Vidyasekaran, 1982; Dube and Bordia, 1982; Hasija and Batra, 1981 have tried to correlate the pathogenicity and pectic enzyme producing potential. Therefore, in the present investigations production of pectic acid lyase by three strains of *Syncephalastrum racemosum* under different culture conditions was studied.

METERIALS AND METHODS

Monosporic cultures of three strains of *Syncephalstrum racemosum* (cohn) schroet, isolated from lemon (citrus medica v.medica linn.), mosambi (*c.sinonsis* osbeck) and orange (*c. reticulate* blanco) fruits and maintined on Ashana and Hawker's mediuma A (l mg) glucose 5.0, Kno3 3.5, KH_2PO_4 1.75g $MgSO_4$ $7H_2O$ 0.75g, distilled water 1 liter was employed in the present studies. These fungi were grown in 100 ml, Erlenmayer conical flark containing 25ml of different synthetic media (table-1) for 12 days at 27-29^{o}C. At the end of 4, 8 and 12 days

*Corresponding author E-mail: kollu2006@yahoo.com

incubation period, cultures were harvested on previously dried and weighed Whatman filter paper No-42, The filter papers along with fungal mycelium were dried at 60-70^{o}C for 48 hrs and weighed to a constant weight after cooling to room temperature in a desiccators.

The experiment was repeated and run in replicates. Since the difference among replicates was in significant, the average of three replicates was taken as a criterion for expressing the growth of fungus.

The culture filtrate thus obtained was centrifuged and dialyzed against glass distilled water at room temperature over night and taken as an enzyme sample.

Endo-peptic acid lyase was assessed as suggested by wood (1995). The reaction mixture consisting of 10.5ml of sodium polypectate (0.5%), 1ml of tris-HC1 buffer (pH-8) and 3.5ml of enzyme and incubated at 30 ± 0^{o}C. The loss in viscosity was measured for every 10 min over a period of 30 min. The reaction enzyme and water served as controls. The percentage of loss of viscosity was calculated by the formula.

$$\text{An min} = \frac{t1 - ta}{t1 - to} \text{ X } 100$$

Where,

An min = Percentage of loss of viscosity

t1 = Flow time of reaction mixture + inactive enzyme

ta = Flow time of reaction mixture + active enzyme

to = Flow time of reaction water + active enzyme

Enzyme activity is expressed in relative viscosity units (RVU) = 1000/$\mathbf{t_v50}$ where $\mathbf{t_v50}$ represent the time required in min to reduce the viscosity to 50% of the initial viscosity.

Exco-pectic acid lyase (Exo-PAL) was assayed as described by Sherwood (1967). The reaction mixture consisting of 4ml of sodium poly pectate (0.5%), 1ml of tris-Hcl buffer (pH-8) and 2.0ml of enzyme was incubated at 30 ± 0^{o}C for 4hrs. At the end of incubation period 1ml of reaction mixture was with drawn into of test tube and 5ml thiobarbutric acid (TBA) was added followed by 1.25ml 1N Hcl. After boiling for 1hr in a water both, the test tubes were cooled and the absorbance was read, at range of 475-575nm. A peak at 547 nm was taken for the percentage of lyase activity due to the formation of unsaturated products.

Influence of pH (3.5, 4.5, 5.5 and 6.5) on production of PAL was studied by adjusting pH of the medium with the help of 6N HCl/6N NaOH Just before the inoculation of fungus. Similarly fungal inoculated flasks were incubated at 5, 15, 25, 30, 35 and 40^{o}C for 12d. Influence of carbon and nitrogen source was studied by substituting glucose and potassium nitrate of the basal medium so as to supply 480mg and nitrogen respectively. Different growth substances and fungicides (200 and 400 mg/ml) as listed in table were added to the medium aseptically just before the inoculation. The rest of the details were similar to those described earlier Narania and Reddy (1997).

RESULTS AND DISCUSSION

Table 1 reveals that medium E supported maximum production of exo-PAL, while medium C was the poor substratum. Orange isolate was versatile in the production of exo-PAL as it secreted on all the media tried except medium C. Maximum production of exo-PAL on medium E which contained pectin as one of its constituents suggests that adaptive nature of these enzymes. Similarly production was more on medium E. Charya and Reddy (1983) have also reported the adaptive nature of PAL secreted by *Phoma exigue.*

Lemon and Orange isolates failed to produce exo-PAL, when pH of the medium was below 6.5, but produced increasing amount with the increase in pH with a maximum at pH 7.5 and 8.5 respectively (Table 2). In contrast to these strains, mosambi isolate secreted exo-PAL over a wide pH range (3.5 to 9.5) with a maximum at pH 7.5. Endo-PAL production by lemon and orange isolates was maximum at pH 6.5, while for mosambi isolate it was at pH 5.5. It is interesting to note that endo-PAL activity was more at acidic pH rather exo-PAL was more at alkaline pH.

The activity of exo-PAL secreted by all the three isolates of S.*racemosum* was witnessed between temperature of 40 to 45^{0}C with a maximum at 30^{0}C. However, the activity of exo-PAL secreted by lemon isolate was inactivated at 40^{0}C. On the other hand, endo-PAL of all the three isolates was active with in the temperature range of 25-35^{0}C with a optimum at 30^{0}C. Lemon isolate opted mannitol followed by sorbitol for production of exo-PAL, while lactose was the primary choice of orange and mosambi isolates (Table 3). L-sorbose supported minimum activity of exo-PAL of all the three isolates under study. Rest of the carbon sources supported intermediate quantity of exo-PAL. Most of the carbon compounds failed to induce endo-PAL in all the three isolates of *S. racemosum* understudy. Only glucose, sucrose and galactose could induce little amount of endo-PAL in lemon isolate, while orange isolate could produce this enzyme during its growth on lactose, sucrose and glucose. Similarly mosambi isolate secrete this enzyme only on fructose and glucose.

Exo-PAL production by lemon isolate was maximum during its growth on L-arginine, while for orange isolate preferred peptone and L-asparagine for production of this enzyme. Mosambi isolate did the same on casein (Table 4). Rest of the compounds induced varying amount of exo-PAL. Ammonium nitrate was poor substratum for induction of endo-PAL in all the isolates under investigation. When casein and urea were favorable substances for production of endo-PAL by lemon isolate, glycine, DL-aspartic acid and casein were responsible for

Table 1. Production of pectice acid lyase (Exo and Endo- PAL) by three isolates of *S. racemosum* in different synthetic media.

Medium	Incubation (in days)	Lemon isolate		Orange isolate		Mosambi isolate	
		Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL
Asthana and Hawker's Medium A (A)	4	3.5	---	17.0	33.33	---	13.33
	8	---	---	18.0	---	---	---
	12	---	---	6.0	---	8.5	---
Richards Medium (B)	4	---	---	---	---	---	---
	8	---	---	13.0	8.33	---	---
	12	---	---	---	---	---	---
Modified Czapek's Medium (C)	4	---	---	---	---	---	---
	8	---	---	---	---	3.0	16.68
	12	---	---	---	---	---	---
Modified Czapek's Medium+0.5% CMC (D)	4	---	11.10	8.0	---	6.5	---
	8	---	---	6.0	---	3.0	---
	12	12.0	---	20.0	---	---	---
Singh & Wood Medium (E)	4	4.0	22.22	9.0	22.22	3.0	15.14
	8	34.0	44.44	30.0	33.33	28.0	22.22
	12	47.0	33.33	31.0	50.00	36.0	33.33

'+' = Expressed in units (0.01 OD change taken as 1 unit of enzyme activity)
'++' = Expressed in relative viscometric units (RVU)

Table 2. Influence of different pH and temperature on pectic acid lyase (Exo- and Endo-PAL) production by three isolates of *S. racemosum*

pH	Lemon isolate		Orange isolate		Mosambi isolate	
	Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL
3.5	---	36.85	---	24.99	6.0	29.62
4.5	---	39.51	---	26.66	6.0	34.21
5.5	---	37.03	---	24.28	25.0	36.65
6.5	34.0	44.44	30.0	33.33	28.0	22.22
7.5	36.0	27.66	38.0	31.99	29.0	17.10
8.5	33.0	18.44	39.0	26.66	17.0	17.10
9.5	---	9.20	30.0	13.33	14.0	8.54
Temperature (in °C) 5	---	---	---	---	---	---
15	---	---	---	---	---	---
25	13.0	---	16.0	26.08	21.0	7.40
30	34.0	44.44	30.0	33.33	28.0	22.22
35	13.0	16.66	30.0	18.18	21.0	22.22
40	---	---	21.0	---	17.0	---

'+' = Expressed in units (0.01 OD change taken as 1 unit of enzyme activity)
'++' = Expressed in relative viscometric units (RVU)

Table 3. Influence of different carbon sources on pectic acid lyase (Exo-and Endo-PAL) production by three isolates of S. racemosum

Carbon Source	Lemon isolate			Orange isolate			Mosambi isolate	
	Exo-PAL	Endo-PAL		Exo-PAL	Endo-PAL		Exo-PAL	Endo-PAL
Glucose	34.0	44.44		30.0	33.33		28.0	22.22
Frutose	36.0	---		32.0	---		33.0	16.66
Galactose	33.0	13.33		30.0	---		25.0	---
Sorbose	22.0	---		27.0	---		21.0	---
Sucrose	32.0	22.22		20.0	44.44		42.0	---
Lactose	38.0	---		41.0	22.22		48.0	---
Starch	38.0	---		31.0	---		32.0	---
Sorbitol	44.0	---		30.0	---		41.0	---
Mannitol	48.0	---		35.0	---		28.0	---

\+ = Expressed in units (0.01 OD change taken as 1 unit of enzyme activity)
\+ + = Expressed in relative viscometric units (RVU)

Table 4. Influence of different nitrogen sources on pectic acid lyase (Exo-and Endo-PAL) production by three isolates of S. racemosum

Source	Lemon isolate			Orange isolate			Mosambi isolate	
	Exo-PAL	Endo-PAL		Exo-PAL	Endo-PAL		Exo-PAL	Endo-PAL
Potassium nitrate	25.0	25.00		14.0	20.00		39.0	33.33
Ammonium nitrate	11.0	---		6.0	---		9.0	---
Ammonium sulphate	---	---		7.0	8.33		6.0	20.00
L-Glycine	21.0	3.33		25.0	66.66		16.0	44.44
DL-Alanine	31.0	44.44		23.0	33.33		19.0	25.00
DL-Aspartic acid	31.0	25.00		---	66.66		14.0	3.33
L-Arginine	40.0	16.66		---	44.44		18.0	20.00
L-Methionine	21.0	---		5.0	6.66		4.5	25.00
Tyrosine	28.0	25.00		9.0	33.33		17.0	25.00
Urea	25.0	50.00		18.0	33.33		15.0	16.66
Peptone	9.0	20.00		34.0	44.44		7.0	66.66
Casein	36.0	66.66		22.0	66.66		43.0	3.70
L-Asparagine	34.0	44.44		30.0	33.33		28.0	22.22

\+ = Expressed in units (0.01 OD change taken as 1 unit of enzyme activity)
++ = Expressed in relative viscometric units (RVU

Table 5. Influence of different growth in promoting substanceds on pectic acid lyase (Exo-and Endo-PAL) production by three isolates of S. racemosum

Name of the substance	Lemon isolate		Orange isolate		Mosambi isolate	
	Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL
Gibberellic acid	---	---	30.00	---	31.00	9.52
Indole acetic acid	23.00	---	61.00	9.52	15.00	---
Colchicine	---	9.52	---	9.52	---	---
2,4-DNP	30.00	---	---	---	68.00	8.33
Malt extract	---	8.33	25.00	9.52	72.00	---
Yeast extract	---	19.04	---	19.04	---	---
Corn steap liquor	---	---	64.00	8.33	---	---
Control	34.00	44.44	30.00	33.33	28.00	22.22

\+ = Expressed in units (0.01 OD change taken as 1 unit of enzyme activity)
++ = Expressed in relative viscometric units (RVU)

Table 6. Influence of different fungicides substances on pectic acid lyase (Exo- and Endo-PAL) by three isolates of S. racemosum in different synthetic media.

Name of the Fungicide	Concentration (ug/ml)	Lemon isolate		Orange isolate		Mosambi isolate	
		Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL	Exo-PAL	Endo-PAL
Dithance M-45	200	---	11.1	---	13.33	---	22.22
	400	---	---	---	5.55	---	16.66
Baviston	200	---	22.22	---	24.49	---	13.33
	400	---	16.66	---	11.10	---	11.10
Miltox	200	34.00	44.44	22.00	20.50	---	22.22
	400	32.00	27.77	14.00	---	---	---
Brassical	200	---	---	---	---	---	7.68
	400	---	---	---	---	---	5.55
Antrocol	200	---	8.33	---	14.48	---	11.10
	400	---	5.55	---	5.55	---	---
Control		34.00	44.44	30.00	33.33	28.00	22.22

\+ = Expressed in units (0.01 OD change is taken as one unit of enzyme activity)
++ = Expressed in relative viscometric units (RVU)

maximum production of this enzyme by orange isolate. Mosambi isolate opted peptone as a nitrogen source for maximum production of endo-PAL. Thus three isolates differed significantly in their choice for nitrogen sources. When glycine was the best nitrogen source for orange isolate, it was poor for lemon isolate. Similarly DL-aspartic acid was of primary choice for orange isolate but it failed to induce endo-PAL is mosambi isolate in significant quantity.

When IAA had partial inhibitory effect on production of exo-PAL by lemon and mosambi isolates, it simulated the exo-PAL production by orange isolate (Table 5). Similarly 2,4-DNP stimulated the exo-PAL production by mosambi isolate, while it has partial inhibitory effect on lemon isolate. Orange isolate failed to notice the presence of 2,4-DNP in the medium. All the above substances were inhibitory to endo-PAL production by three isolates of *S.racemosum* under study. However, the degree of inhibition varied both with the isolate and the substance.

All the fungicides employed in the present investigations were toxic and inhibited the production of exo-PAL and endo-PAL (Table 6). The degree of toxicity however, varied both with the fungus and the fungicide.

ACKNOWLEDGEMENTS

Thanks are due to Head, Department of Botany and Microbiology, Kakatiya University, Warangal for providing necessary facilities and encouragement.

REFERENCES

Alana A, Gabilondo, F Remade MD, Moragues, JB, Minguez, DO, Liana MJ, Serva JL (1989). Pectin lyase production by *Pencilium italicum* strain Environ. Microbial, 55 : 1612-1616.

Albersheim P, Muhlethaler K and Frey-Wyssling A (1960). Stained pectin as seen in the electron microsope. J. Biophys. Biochem. Cytol. 8: 501-506.

Alkorta I, Garibisu CL, Llama MJ, Serra JL (1998). Industrial applications of pectic enzymes. A review process Biochem. 33: 21-28.

Bugbee WWM (1990). Purification and chalactroiztion pectin usage from *Rhizoctinia* solani physiol. Mol. Plant pthol. 36 : 15-26.

Chary MAS, Reddy SM (1980). Production of cell wall degrading enzymes by two seed-borne fungi curr. Sci, 49 : 557-558.

Charya MA, Reddy SM (1982). Production of Lyases by *Phoma exigua* associated with seed-rot of *Vigna radiate*. Folia Microbiol. 28: 100-105.

Dube HC, Gour NW (1975). Extra cellular pectic enzyme of *macrophomina phaseolina*, the incitant of root-rot of *Sesamum indicum*. Proc. Indian Natl. Sci. Acad, 41 : 576-579.

Dube HC, Bordia S (1982). Extra cellular pectolytic enzymes of *heliminothosporium sacchari* the incitant of eye-spot, diseases of sugarcane, Indian Phytopathol, 35(1) : 115-119.

Hasija SK, Batra S (1981). Invivo production of pectic enzymes by *Phomadestructiva*. Indian Phytopathol, 34 : 230-231.

Jacobo Ortega (1996). Pectolytic enzymes produced by the phytopathogenic fungus *Collectotrichum glocosporioides.* The Texal J Sci.

Kashyap DR, Vohar PK, Chopra S, Tewari R (2001). Applications of pectinases in the commercial sector, review Bioresource Techn. 77: 215-227.

Narania K, Reddy SM (1982). Synergistic production of pectin lyase and polymethyl glacturonase by *Spegazzinia tessarthra*, Curr. Sci. 31: 615.

Neeraj Saxena KU (1983). Production of pecticnolytic and cellutolytic enzymes by *Rhizopus nodus* and *phytophthora nicotiance* in different culture media. Geobios, 10(3): 108-112.

Olutiola PO, Akintunde OA (1979). Pectin lyase and pectin methylesterase production. Mycol. Soc. 72: 49-55.

Ramraj B, Vidyasekaran, (1982). Possible involvement of pectic enzyzmes in *Betelvinewilt* development, Indian Phytopathol, 35(1): 71-72.

Sherwood RT (1967). Pectin lyase and polygalacturonase production by *Rhizocotonia solani* and other fungi. Phytopathol. 56; 279-286.

Simiappan R, Vidyasekaran P (1981). Differences between *Macrophomina phaseolina* isolates causing root-rot and leaf blight of urbid bean. Indian Phytopath. 34: 407-409.

Wood RKS (1955). Pectin enzymes secreted by pathogens and their role in plant infection p. 263 in Mechanism of Microbial pathogenicity (J.W.Howle, A.J.O.Hea, Eds.), University Press, Cambridge.