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Pectolytic Enzyme Producing Ability of *Xanthomonas campestris pv. phaseoli*

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Abstract:

The bacterium *Xanthomonas campestris* pv. *phaseoli* is the incitant of the disease common bacterial blight of Bean. *In vitro* studies performed with the pathogen, indicated that it is capable of producing pectolytic enzymes. In the present study, production of macerating enzyme and polygalacturonase was observed by 11 isolates of *Xcph* cultivated in medium containing pectin. The pectolytic enzyme producing ability of *Xcph* strains was found constitutive in nature.

Key words: *Xanthomonas campestris* pv.*phaseoli*, pectolytic enzymes, polygalacturonase.

Introduction:

Bacterial diseases of vegetable plants are known to cause great damages all over the world. Bean (*Lablab niger* Medik) is the most ancient among the tropical vegetables. Among the various diseases, Common bacterial blight of Bean, is observed in Aurangabad District, which is caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye*(Xcph)*. The production of pectolytic enzymes by bacterial pathogens is an important process of pathogenesis (Vidyasekaran *et al.,* 1973).The pectolytic enzyme, degrade the middle lamella present between the plant cell walls and there by facilitate the movement of the bacterial pathogen. It appears from the literature that studies on the production of pectolytic enzymes by the bacterial pathogens of common bacterial blight of bean, has not been carried out. Therefore, the present investigation was carried out and is reported in this paper.

Materials and Methods:

From various localities of Aurangabad district ,disease samples were collected from 11 varieties of Bean, and 11 isolates of *Xcph* were isolated. The pure cultures of the isolates were maintained on Nutrient agar slants. The bacterial suspension was prepared by adding 10 ml. sterile distilled water to two days old Nutrient agar (NA) slope culture and 5 drops of it were used as inoculums in all the experiments. All treatments were in triplicate and results have been presented after repeating the

experiment at least once (Pawar and Papdiwal,2009).

In vitro production of pectolytic enzymes: For production of pectolytic enzymes, the bacterial isolates were grown in Pectin-Nitrate (PN) liquid medium containing 1% pectin, 0.25% KNO_3,0.1% KH_2PO_4, o.o5% $MgSO_4.7H_2O$ and Glucose-Nitrate (GN) medium containing Glucose 1 %, KNO_3-o.25%, KH_2PO_4-o.1% and $MgSO_4.7H_2O$-o.o5%. The pH was adjusted to 7.0. Twenty five ml of the medium was poured in 100 ml. conical flasks, sterilized and inoculated with 5 drops of cell suspension of 11 isolates of *Xcph.* The flasks were incubated for five days at 25^0C. The growth of the bacterial isolates was measured as O.D. at 600 mμ using colorimeter (Jayaraman and Verma, 2002). The culture medium was filtered through G-5 sintered glass filter and the bacterial cell free culture filtrate obtained was termed as crude enzyme solution.

a) Determination of pectolytic enzyme activity by maceration : Macerating enzyme activity was determined by the method of Brown (1915). Cylindrical plugs, 8mm in diameter, were cut from healthy potato tubers with a no. 4 cork borer. The plugs were injected with distilled water under vacuum for 15 min. Discs of 4 mm thickness were cut with sliding hand microtome from these plugs. They were washed quickly with distilled water and stored in a petridish.

Ten discs were placed in 5ml of an enzyme solution (Culture filtrate) in watch glass. At intervals of 5 min. they were subjected to slight tension by hand. As soon as the first disc had lost coherence, the mean time for the loss of coherence in all the discs was noted and taken as the reaction time (R.T.) in minutes. Macerating activity is expressed as ME, and calculated as follows:

$$ME = \frac{1000}{R.T.}$$

The tests were carried out at 25±2°C temperature and unless and otherwise stated at ph 6.5, which was the final pH of the culture filtrate.

b) Determination of pectolytic enzyme activity by viscometry: The polygalacturonase (PG) activity of the culture filtrate was studied as per the method adopted by Pawar and Papdiwal (2009). The substrate enzyme mixture had the following composition:

Substrate – 2% Pectin solution at pH 6.55ml.
Active culture filtrate at pH 6.5 … ……3ml.
Distilled water …… 2ml.

No buffer was added to the reaction mixture because it was found in the preliminary experiments that the pH of the mixture in the beginning and at the end of the experiments did not change significantly. In all experiments active crude enzyme was used to determine its viscosity reducing properties. Viscosity measurements were carried out with an Ostwald viscometer with 14 sec. as the flow rate for water, and 56 sec as the flow rates for 2% pectin solution.

Ostwald viscometer was thoroughly cleaned and dried before hand. The viscometer containing substrate solution and water was suspended in the vertical position in a water bath at 25°C and was left for a few minutes to acquire the temperature of the

bath. Three ml. of enzyme solution was then added to the substrate and the substrate-enzyme mixture was stirred quickly and simultaneously stop watch started. Viscosity readings were taken at 0, 10,20, and 30 min. after adding the enzyme solution and the observations were recorded.

From each viscosity/time observation, the percentage of loss of viscosity was calculated in the following way:

V_o = Viscosity of substrate-enzyme mixture at 0 hr.

Vt = Viscosity of substrate –enzyme mixture at `t `minutes after adding the enzyme

V_w = Viscosity of water.

Total possible loss of viscosity $= V_0 - V_w$

Viscosity loss due to the action of Enzyme = $V_0 - V_t$

$$\% \text{ Viscosity loss due to the action of enzyme} = \frac{V_0 - V_t}{V_o - V_w} \times 100$$

Results and Discussion:

The culture filtrates of 11 isolates *Xcph* obtained from GN and PN media were used as crude enzyme solutions for the assay of pectolytic enzymes. It is clear from Table 1 that eleven isolates of *Xcph* have grown on GN medium with slight change in final pH. Maximum growth was recorded with isolate *Xcph 9* and minimum with isolates *Xcph10*. Macerating enzyme activity was detected in the culture filtrates of all the 11 isolates when grown on both, GN andPN media. The isolate *Xcph2&6* have shown maximum ME activity in GN medium. However, in PN medium, isolate *Xcph 4* exhibited maximum ME activity.

The data from table 2 reveal that maximum growth was recorded with isolate *Xcph* 9, when grown on GN medium. On PN medium, isolate *Xcph2*exhibited maximum growth. The culture filtrates from GN and PN media of all the 11 isolates exhibited viscosity loss. The maximum activity was recorded with isolates *Xcph* 1and 11in GN medium and with isolate *Xcph* 7 in PN medium. Minimum enzyme activity was recorded with isolate *Xcph 6* in GN medium and with isolate *Xcph* 4 and 10 in PN medium.

It has been reported by various workers that production of pectic enzymes by plant pathogens is either constitutive (Bown,1915; Wood, 1955; Heale and Gupta, 1972 ; Harane and Papdiwal, 2011)or adaptive (Gupta,1956; Papdiwal and Deshpande, 1979; Papdiwal and Korekar, 2001, Pawar and Papdiwal,2009). In the present investigation, all the 11 isolates of *Xcph* produced macerating enzyme and polygalacturonase constitutively. It is noted that different isolates of *Xcph* have different enzyme producing ability. It is also observed that there is no correlation between growth and enzyme production by the isolates of the bacterial phytopathogen.

References:

Brown, W. (1915): Studies in the physiology of parasitism. The action of *Botrytis cinerea*. *Ann. Bot.*, ***29***:313-348.

Gupta,S.C.(1956): Studies in the physiology of parasitism-XXII. The

production of pectolytic enzyme by *Pythium debaryanum* Hasse*. Ann.Bot*.,**20:** 179-190.

Harane ,S.S. and Papdiwal,P.B. (2011): Studies on physiology of storage fungi of some medicinal seeds:II Production of pectolytic enzyme.*Bioinfolet*, **8** (2); 158-159

Heale, J.B. and Gupta, D.P.(1972): Mechanism of vascular wilting induced by *Verticillium albo-atrum*. *Trans. Brit.Mycol.Soc*., **58 (1)**: 19-28.

Jayaraman, J. and Verma,J.P.(2002): *Fundamentals of plant bacteriology.* Kalyani publishers, Ludhiana.

Papdiwal, P.B. and Deshpande,K.B. (1979): Studies on pectolytic enzyme system of *Xanthomonas malvacearum* (ed.E.F. Smith) Dowson II. Effect of physical factors. *Botanique*, **10** : 157-162.

Papdiwal, P.B.(1982): Role of Pectolytic Enzymes in Pathogenesis of *Xanthomonas campestris* pv *malvacearum*. *Indian bot. Reptr.***1**(1): 27-29.

Papdiwal, P.B. and Korekar, S.L. (2001): Pectolytic enzyme producing ability of bacterial plant pathogens. *In* : *plant disease management* (ed.J. Deshpande) Kailas Publications, Aurangabad,pp 153-155.

Pawar, B.T. and Papdiwal,P.B. (2009): Pectolytic enzyme producing ability of *Xanthomonas campestris* pv*. mangiferaeindicae. Natl. J. Life Sci.* 6(1) 21-24.

Vidyasekaran, P.; Parambaramani, C. and Govindswamy, C.V. (1973): Role of pectolytic enzymes in pathogenesis of obligate and facultative parasites causing sorghum diseases. *Indian phytopath*, **26**: 197-204.

Wood, R.K.S. (1955): Pectic enzymes secreted by pathogens and their role in plant infection. *Symp.Soc.Gen.Micro*biol., **5**: 263-293.

Table:1 Macerating enzyme activity by different strains of *Xanthomonas campestris* pv. *phaseoli* in GN and PN medium.

S.N.	Strain	Initial pH	Final pH in GN Medium	Optical density In GN Medium	M.E.activity in GN Medium (Reaction Time)	Final PH in PN Medium	Optical Density In PN medium	M.E.activity in PN Medium (Reaction time)
1	*Xcph 1*	7.0	6.5	0.06	57.1	6.5	0.06	50.0
2	*Xcph 2*	7.0	6.5	0.05	80.0	6.5	0.07	50.0
3	*Xcph 3*	7.0	6.5	0.06	66.6	6.5	0.04	50.0
4	*Xcph 4*	7.0	6.5	0.06	66.6	6.5	0.05	66.6
5	*Xcph 5*	7.0	6.5	0.05	66.6	6.5	0.06	50.0
6	*Xcph 6*	7.0	6.5	0.05	80.0	6.5	0.04	50.0
7	*Xcph 7*	7.0	6. 5	0.06	72.7	6.5	0.05	50.0
8	*Xcph 8*	7.0	6.5	0.07	61.5	6.5	0.05	50.0
9	*Xcph 9*	7.0	6.5	0.09	61.5	6.5	0.06	50.0
10	*Xcph 10*	7.0	6.5	0.04	58.8	6.5	0.05	50.0
11	*Xcph 11*	7.0	6.5	0.05	72.7	6.5	0.06	50.0

Table: 2 Viscosity reduction due to enzyme polygalacturonase by different strains of *Xanthomonas campestris* pv*. phaseoli* in GN and PN medium.

S.N.	Strain	Initial pH	Final pH in GN Medium	Optical density in GN Medium	% viscosity loss in GN Medium	Final pH in PN medium	Optical Density in PN medium	% viscosity loss in PN Medium
1	*Xcph 1*	7.0	6.5	0.06	50.0	6.5	0.06	66.6
2	*Xcph 2*	7.0	6.5	0.05	33.3	6.5	0.07	57.8
3	*Xcph 3*	7.0	6.5	0.04	37.5	6.5	0.06	31.8
4	*Xcph 4*	7.0	6.5	0.05	42.8	6.5	0.05	25.0
5	*Xcph 5*	7.0	6.5	0.07	33.3	6.5	0.04	62.5
6	*Xcph 6*	7.0	6.5	0.06	28.5	6.5	0.05	40.0
7	*Xcph 7*	7.0	6. 5	0.07	37.5	6.5	0.06	71.4
8	*Xcph 8*	7.0	6.5	0.06	40.0	6.5	0.05	50.0
9	*Xcph 9*	7.0	6.5	0.08	37.5	6.5	0.04	58.3
10	*Xcph 10*	7.0	6.5	0.05	45.0	6.5	0.06	25.0
11	*Xcph 11*	7.0	6.5	0.06	50.0	6.5	0.05	50.0