The Bioscan 9(1): 295-300, 2014 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

PATHOGENESIS OF *COLLETOTRICHUM LINDEMUTHIANUM* THE INCITANT OF ANTHRACNOSE DISEASE IN BEANS MEDIATED BY MACERATING ENZYMES

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KEYWORDS
C. lindemuthianum
Cellulase
Pectinases
Bean
Anthracnose

Received on :
25.10.2013

Accepted on :
18.02.2014

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ABSTRACT
Bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams Scrib., is a serious seedborne disease of common beans (*Phaseolus vulgaris* L.). Anthracnose has caused serious reductions in the yield of beans in many parts of the world, resulting in yield losses as high as 95%. Plant cell wall contains mainly cellulose and pectic substances which offers first line of defense. Macerating enzymes secreted by pathogens are involved in the pathogenicity of a wide range of plant pathogenic fungi. In the present study all isolates of *C. lindemuthianum* were able to grow in synthetic medium and produce cellulolytic (C1 and Cx). The maximum activity was observed in CL 06 (0.94 and 80.12%) respectively. Polymethylgalacturonase (PMG), polygalacturonase (PG) and pectin lyase (PL) activities were detected. The isolate CL06 produced higher amount of PG and PMG (0.47 and 0.91), similarly the isoaltes CL06 (153.26) showed higher activity of pectate lyase. All the enzymes were highly active in 12-day-old culture and the activities decreased with the increase of culture age. Isolate CL 06 yielded high titles in production of all the enzymes.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume crop grown throughout the world, used both as a pulse and green vegetable. In India, common bean called as "Rajmash" is one of prime grain legume crops mainly grown in hilly areas, where its cultivation is confined to mid and high hills like Nilgris, Javadu, Kinnaur, Kullu, Mandi, Shimla, Sirmour and Solan occupying an area of about 10,000 hectares with an average yield of 0.35 tones/ ha (Sharma *et al.,* 2007). Bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri and Cav. is a cosmopolitan seed borne disease in humid and cool environments of the world including Himachal Pradesh. Infection of a susceptible cultivar in favourable conditions leading to an epidemic may result in 100 per cent yield losses (Sharma *et al.,* 2008, Fernandez *et al.,* 2000).

Bean anthracnose is a common recurrence in india with wide pathogenic and molecular variability (Sharma *et al.,* 2007, 2008 Padder *et al.,* 2009) and local cultivars are susceptible to one or the other race of the pathogen (Padder *et al.,* 2010). Though different aspects of bean anthracnose in the state have been studied in detail yet diminutive information is available on yield loss estimation which is of outmost significance for crop production inventories, crop insurance policies, quarantines, variety and gene deployment.

Cellulose, hemicellulose, lignin and pectin are the main components of plant cell wall. Pectin, a heteropolysaccharide defined as galactosyluronic acid rich polymers, is composed of α-1-4 linked galacturonate chains with high percentage of methyl esterification. Cell wall degrading enzymes released by pathogens are known to be responsible for pathogenesis. The ability of a pathogen to produce cellulolytic and pectinolytic enzymes determines the degree of degradation of cell wall during pathogenesis and inhibition of these enzymes ultimately affects the disease development. A numbers of cell wall degrading enzymes have been shown to be produced by plant pathogens (Chenglin *et al.,* 1996), which are known to facilitate cell wall penetration and tissue maceration in host plants. During fungal infection a range of hydrolytic enzymes is secreted to help promoting host colonization. Depending of the ecological niche occupied by each fungus, a particular set of enzymes, mainly composed of proteases and carbohydrases, are displayed to degrade specific tissues and scavenge for nutrient resources (Yakoby *et al.,* 2000). *Colletotrichum* comprises a variety of phytopathogenic species used as models for studies concerning pathogenicity and fungal-plant interaction for their ability to produce macerating enzymes (Perfect *et al.,* 1999).

Pectinolytic enzymes, capable of degrading pectin and leading to maceration of plant tissues, are the first enzymes secreted by most fungal pathogens when attacking plant cell walls (Idnurm and Howlett, 2001). Pectin degradation can be attained by the combined action of several enzymes such as pectin methylesterases and pectin depolymerases, including hydrolases and lyases, such as polymethylgalacturonase and pectin lyase. Degradation involves the breakdown of

polygalacturonic acid through two enzimatic processes: lyases split the α-1-4 glycosidic bond between galacturonic acid residues by translimitation, while polygalacturonases catalyze a hydrolytic cleavage (Sakal *et al.,* 1995). The role of pectin degrading enzymes in causing cell wall degradation is so important that it determines the virulence of many pathogens (Rogers *et al.,* 2000). In a number of systems, correlations have been established between the presence of pectinolytic enzymes, disease symptoms and virulence.

Several *Colletotrichum* species, including *C. truncatum, C. lindemuthianum* and *C. destructivum* are intracellular hemibiotrophic pathogens and have a restricted host range, suggesting that host specifity in *Colletotrichum* might be associated with intimate contact between infective hyphae and living host cells (Ramos *et al.,* 2010). In the present work, by exploring *in vitro* pectinolytic and cellulolytic enzyme production, we wish to contribute to the physiological characterization of Indian *C. lindemuthianum* strains isolated from diseased bean pods from different geographic locations, as a preliminary step to establish the role of the macerating enzymes in the *C. lindemuthianum* and bean interaction.

MATERIALS AND METHODS

Microorganisms

The isolates were obtained from lesions of pods of symptomatic beans from several localities in Tamil Nadu, India. Pieces of symptomatic tissue bearing immature acervuli were surface sterilized in 1.5% sodium hypochlorite for 2 min, rinsed twice in sterile water and incubated in humid chambers at 22-25°C under 12 h near ultraviolet light (nuv) and 12 h of darkness, until liberation of masses of conidia, which were cultured in potato dextrose agar (PDA) plates at 19-22°C in darkness. Hyphal tips were aseptically transferred to PDA and incubated at 22-25°C under nuv light/darkness (12/12 h) to obtain pure cultures. Stock cultures were maintained on potato dextrose agar slants at 4°C.

Basal culture medium

To study the *in vitro* production of pectinolytic and cellulolytic enzymes, the pathogens were grown on Czapek-Dox (CD) liquid medium (pH 7-7.5) where the carbon source was substituted with 1% pectin (for pectic enzymes) or 1% carboxy methyl cellulose (for cellulolytic enzymes). The media were inoculated with 8 mm diameter culture disc of the pathogens. The culture filtrates were obtained after incubation at room temperature (27 ± 1°C) for 3, 6, 9, 12, 15 days and centrifuged at 3 000 rpm for 20 min. For the assay of pectinolytic enzymes, the culture filtrates were dialysed for 18 h against distilled water at 40°C. The dialysate served as enzyme source. As dialysis was found to reduce the activity of cellulolytic enzymes (Bateman 1964), the culture filtrates as such were used for the assay of cellulases.

Culture conditions

100 ml Erlenmeyer flasks with 20 ml of medium were inoculated with one agar plug (0.25 cm^2) and cut out from a colony grown on Bacto-agar 2%. Incubation was carried out at 23± 1C under stationary conditions. Cultures were harvested at different incubation periods, filtered through a filter paper using a Buchner funnel and dried overnight at 70 °C. Dry weight of mycelia was determined. The culture supernatants were used as enzyme source.

Enzyme assays

Cellulase (C_1) activity

Cellulase (C_1) activity was assayed by the method of Norkrans (1950). The assay mixture contained 1 ml of cellulose solution (the concentration which was adjusted to give approximately 0.85 absorbance at 610 nm), 4ml of 0.1M phosphate buffer (pH 7.0) and 5 ml of enzyme source. The absorbance of the assay mixture was determined at 610 nm in a Spectronic - 20 colorimeter immediately upon the addition of the enzyme source and again after the incubation period of 24 h at 27°C. The enzyme activity was expressed in units (1 unit = change in absorbance of 0.01).

Cellulase (Cx) activity

Cellulase (Cx) activity was assayed by the viscosimetric method of Hancock *et al.* (1964). Two ml of enzyme extract was added to 4ml of 1.2% carboxy methyl cellulose (CMC) solution buffered at pH 5.0 with sodium citrate buffer. The loss of viscosity of the CMC solution was determined by means of an Ostwald-Fenske viscosimeter size 150 at 5 min intervals up to 15 min. Enzyme source boiled for 10 min at 100°C served as check. The results were expressed as the percent loss in viscosity in 15 min.

$$V = \frac{T_0 - T_1}{T_0 - T_w} \times 100$$

(where, V - per cent loss of viscosity, T0 - flow time in seconds at zero time, T1 - flow time of the reaction mixture at time T1 and Tw - flow time of distilled water)

Pectinolytic enzymes

Polygalacturonase activity (endo plus exo activity) was assayed by following the release of reducing groups from 0.1% apple pectin (polymethylgalacturonase-PMG) or polygalacturonic acid (polygalacturonase-PG) in 50 mM sodium acetate buffer of pH 4.8 according to the Somogyi-Nelson method. One unit of enzymatic activity was defined as the amount of enzyme releasing 1 mmol of galacturonic acid per min at 37 °C. Enzyme activity was expressed as EU/ml of culture filtrate. Pectin lyase (PL) activity was assayed by the thiobarbituric acid method (Olutiola and Akintunde, 1979). The reaction mixture contained 3 ml of pectin (1.2% in 0.05 M Tris HCl buffer, pH 8.0) and 2ml of culture filtrate. The mixture was incubated for 1 h at 30 1C. After incubation, 1.5 ml of 1 N HCl and 3 ml of 0.04 M thiobarbituric acid were added and kept at 100 1C for 20 min. Afterwards, absorbance was measured at 550 nm. In all assays, boiled enzyme and substrate mixtures were used as controls. One unit of lyase activity was defined as the amount of enzyme causing an increase of 0.01 in absorbance during 30 min. Results are the average of three triplicate experiments with a standard error lower than 5%.

RESULTS

Growth of *C. lindemuthianum* (mg)

The result of growth on dry weight basis on various days intervals clearly indicated that the isolates differ in their growth pattern and no synchronism was found among the isolates. Maximum weight of mycelia was noticed on 15 days of post inoculation. The isolate CL 06 produced higher growth (46.53 mg) followed by CL 20 (44.12 mg) and the least was observed in iolate CL 07 (21.11mg) on 15th days (Fig. 1).

Cellulases (C1 and Cx)

Among the isolates that were tested for the production of cellulase (C1), the isolates CL06 showed higher production of cellulase on 12th day (0.94 unit), followed by CL01, CL13, CL14 and CL20 (0.87 unit), the least cellulase activity was observed in CL o4 (0.54 unit). In general the production of cellulose (C1) enzymes was increasing till 12th day and thereafter it started decreasing (Fig. 2). The activity of cellulase (Cx) enzymes was tested against 20 isolates also showed similar trend, it was increasing till 12th day and then started decreasing. The iosalte CL06 produced maximum amount of cellulase (Cx) (80.12 %), which was followed, by CL01 (76.64 %) and the least activity was noticed in islaote CL09 (52.21 %) (Fig. 3).

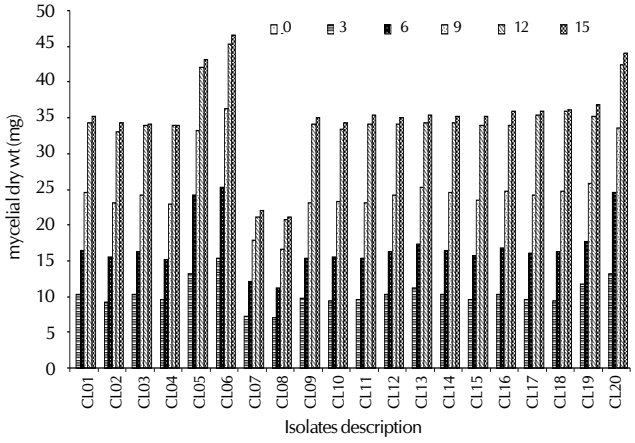

Figure 1: Kinetics of *in vitro* mean growth on defined medium, by twenty isolates of *C. lindemuthianum*

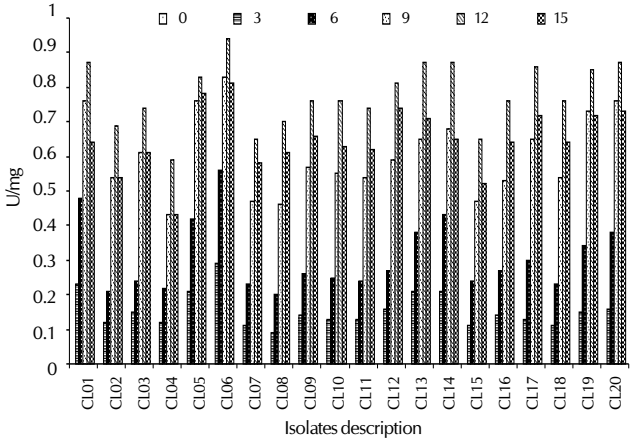

Figure 2: Kinetics of in vitro cellolytic enzyme (C1) production on defined medium based on 1% cellulose as carbon source, by twenty isolates of *C. lindemuthianum*

Table1: Places in Tamil Nadu, India where anthracnose infected bean samples were collected

S. No	Location	District	Isolate description
1	Gandhipuram	Coimbatore	CL01
2	Ukkadam	Coimbatore	CL02
3	Madampatti	Coimbatore	CL03
4	Mettupalayam	Coimbatore	CL04
5	Ooty	Niligris	CL05
6	Coonoor	Niligris	CL06
7	Cumbum	Theni	CL07
8	Gobichettipalayam	Erode	CL08
9	Kodaikanal	Dindigul	CL09
10	Perumal malai	Dindigul	CL10
11	Thadiankudasai	Dindigul	CL11
12	Tandikudi	Dindigul	CL12
13	Kotur	Thenkasi	CL13
14	Rasipuram	Namakkal	CL 14
15	Tiruchengode	Namakkal	CL15
16	Vaniyambadi	Vellore	CL 16
17	Triupatthur	Vellore	CL17
18	Yercaud	Salem	CL 18
19	Palur	Cuddalore	CL19
20	Pechuparai	Kanyakumari	CL 20

Pectinases

Polygalactronase and Pectin methyl galactronase (PG and PMG)

PG and PMG was assayed on different days, maximum amount of production was noticed on 12th day and decreased there after. The isolate CL06 produced higher amount of PG and PMG (0.47 and 0.91) on 12th day after inoculation in CD broth. This was followed by CL 07 (0.39 and 0.87) respectively. The least activity was observed in CL08 (0.19 and 0.26) respectively (Fig. 4)

Pectate lyase (PL)

The activity of pectate lyase was tested in order to find the pectin degrading ability of *C. lindemuthianum*. In general the activity of PL increases with increase in days of incubation and the maxiumum activity was noticed on 12th day of post inoculation. The sioaltes CL06 (153.26) showed higher activity

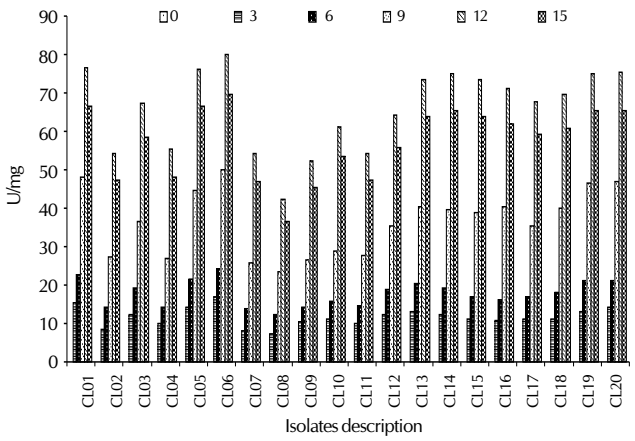

Figure 3: Kinetics of in vitro cellolytic enzyme (C_x) production on defined medium based on 1% cellulose as carbon source, by twenty isolates of *C. lindemuthianum*

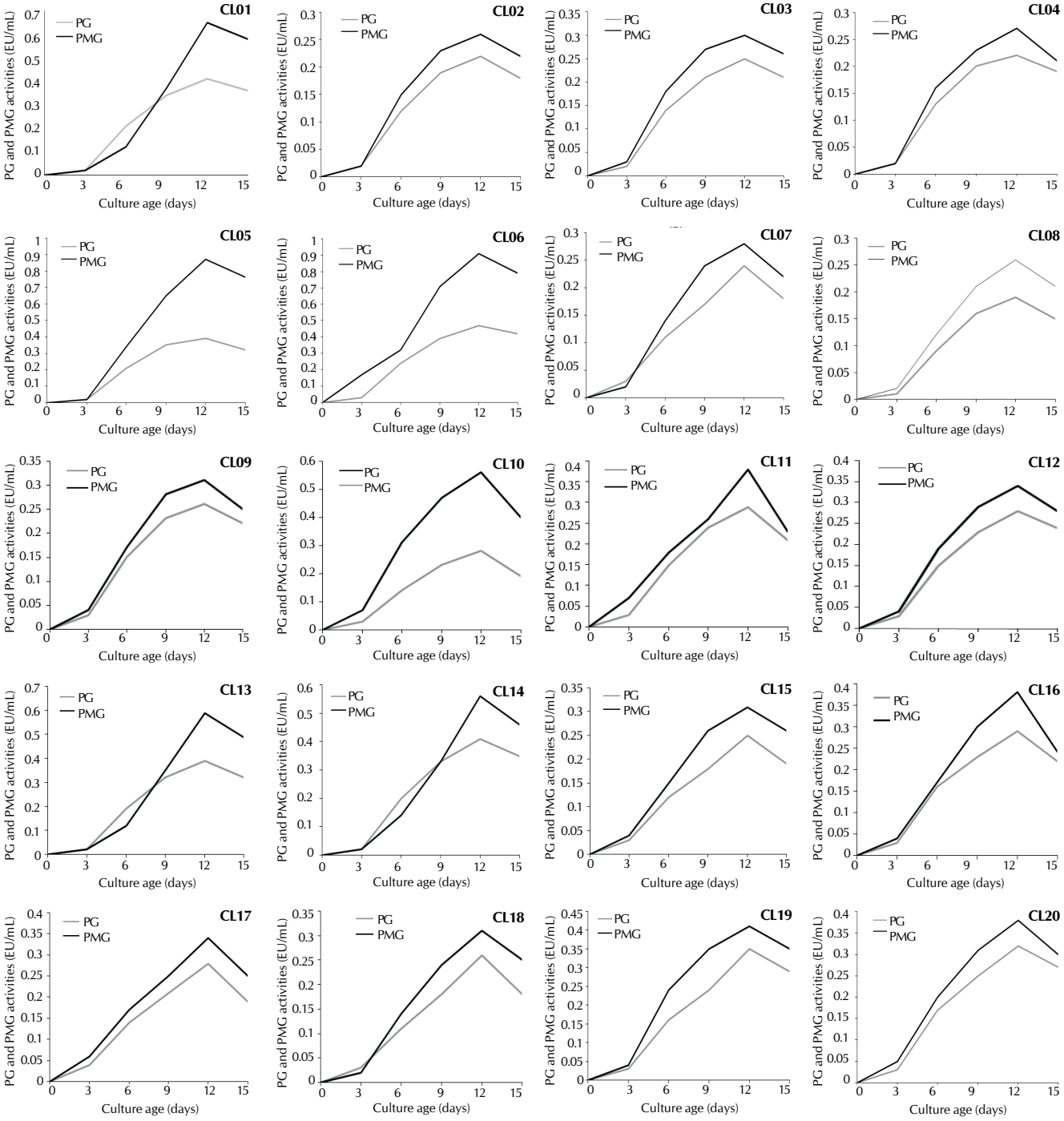

Figure 4: Kinetics of in vitro pectinolytic enzyme production on defined medium based on pectin as carbon source, by twenty isolates of *C. lindemuthianum*

of pectate lyase followed by CL05 (129.10) and the least activity is noticed in isolate CL08 (64.19) (Fig. 5)

DISCUSSION

Colletotrichum lindemuthianum is an intracellular hemibiotrophic pathogen. Following cell penetration, hyphae develop between the plasma membrane and the cell wall without penetrating the protoplast. Once a large area of the plant tissue has been colonized, necrotrophic hyphae develop (O'Connell and Bailey 1988) and this step closely correlates with the production of a set of host cell wall degrading enzymes. All isolates were able to grow in the mentioned medium and produced a complex of enzymes having the potential to degrade α-1-4 bonds in pectic substances hydrolytically, as well by a transeliminative mechanism. The disparity observed in enzyme production among strains can be attributed to fungal growth, since there is major differences in mycelial

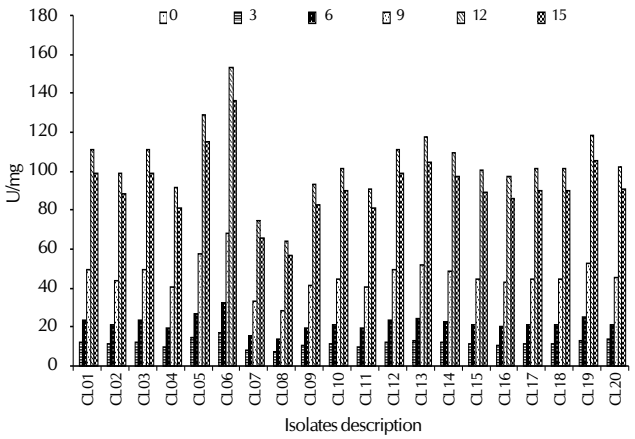

Figure 5: Kinetics of in vitro Pectate lyase production on defined medium based on pectin as carbon source, by twenty isolates of *C. lindemuthianum*

yield. Maximal growth values were around 50-70 mg/ 20 ml of medium, and were usually registered after 10-15 days of cultivation. In general, the peak of PG and PMG activities preceded the day of maximum growth. Initially, several endoPG isoforms facilitate biotrophic development without causing extensive tissue maceration, while other isoenzymatic forms predominate in the necrotrophic stage. On the contrary PL, which appears later, is more likely to play an important role during the necrotrophic phase of tissue colonization.

Cellulose is a major structural constituent of the cell wall of host plants. Many phytopathogenic fungi produce cellulases in culture adaptively which hydrolyse cellulose and its derivatives (Marimuthu *et al.,* 1974; Muthulakshmi 1990). The results obtained in the present study indicate that *C. lindemuthianum* (CL 06) produced C1 and Cx *in vitro* and the activity of these enzymes increased with increase of the culture age which coincides with the growth of the fungus. Increase in the production of cellulases was observed till twelve days and then started decreasing. Similar results were obtained by Anand *et al*. (2008) where the virulent isolate of *Colletotrichum capsici* showed maximum production of cellulases on tenth day and sudden decrease was observed indicating complete utilization of cellulose as carbon source.

As shown in Fig. 4 and 5, pectinolytic enzyme production proved to be dependent on medium composition. Synthesis of pectinases are only inducible, if the carbon source is replaced by any glucose synthesis of pectinases is reduced (Ramos *et al.,* 2010). Nevertheless, differences between *in vivo* and *in vitro* production, due to plant-pathogen interactions, cannot be ruled out. In a previous study, *B. cinerea* demonstrated constitutive production not only of endo and exoPG but also of PL, and their patterns of *in vitro* production were correlated with *in vivo* production during the infection of bean leaves. Also *Sclerotinia sclerotiorum* produced PG and PMG constitutively in a medium with glucose as carbon source. Two regulation mechanisms are thought to occur during pectinolytic enzyme secretion by pathogenic fungi: (i) the enzyme is specifically induced by the substrate (i.e. pectin) or (ii) the enzyme is constitutive, but its expression is restricted by the presence of simple sugars (catabolite repression).

Cellolytic and pectinolytic enzyems activities obtained from *C. lindemuthianum* strains fall within the range of other phytopathogenic fungi grown in a medium with pectin. *Thanatephorus cucumeris* produced up to 25 U/ml of PL (Jayasinghe *et al.,* 2004) and *Fusarium oxysporum* around 180 U/ml of PL (Blais *et al.,* 1992); PG-production by *F. oxysporum* f. sp. *niveum* reached a maximum of 0.4U/ml (Vazque *et al.,* 1993). In a previous study a comparison between pathogenic and non-pathogenic strain of *C. lindemuthianum* revealed significant differences in terms of PL production in liquid culture. On 92%-esterified pectin, the pathogenic strain had more PL activity and cell walls isolated from its host (*Phaseolus vulgaris*) induced PL only in the pathogenic strain. These authors hypothesize that differences in pathogenicity may in part reside both in the amount and time-course of PL production. In the present study, the disparity observed in enzyme production among strains can be related to fungal growth, since major differences in mycelial yield were found.

The production and activity of pectinolytic and cellulolytic enzymes detected *in vitro* suggest their active role in disease development by the pathogen in bean pods. Singh and Jain (1979) reported that the bottle gourd anthracnose pathogen (C. *lagenarium)* produced both pectinolytic and cellulolytic enzymes. Since *C. lindemuthianum* is intercellular in the host, the productions of these enzymes appears to facilitate the dissolution of host cell wall and middle lamella and help entry and establishment of the pathogen in the host and are possibly responsible for playing a vital role in pathogenesis through cell wall degradation and disintegration of tissues. In the present study, the CL 06 isolate of pathogens produced more cellulolytic and pectinolytic enzymes than the other isolates indicating the importance of the cell wall degrading enzymes in pathogenesis.

REFERENCES

Anand, T., Bhaskaran, R., Raguchander, T., Karthikeyan, G., Rajesh, M. and Senthilraja, G. 2008. Production of cell wall degrading enzymes and toxins by *Colletotrichum capsici* and *Alternaria alternta* causing fruit rot of chillies. *Journal of Plant Protection Research.* **48:** 437-451.

Bateman, D.F. 2008. Cellulase and the *Rhizoctonia* disease of bean. *Phytopathology*. **54:** 1372-1377.

Blais, P., Rogers, P. A. and Charest, P. M. 1992. Kinetics of the production of polygalacturonase and pectin lyase by two closely related Formae speciales of *Fusarium oxysporum*. *Experimental Mycology*. **16:** 1-7.

Chenglin, Y., William, S. C. and Carl, E. S. 1996. Purification and characterization of a polygalacturanase produced by *Penicillium expansum* in apple fruit. *Phytopathology*. **86:** 1160-1166.

Fernandez, M. T., Fernandez, M., Casares, A., Rodriguez, R. and Fueyo, M. Bean 2000. germplasm evaluation for anthracnose resistance and characterization of agronomic traits. A new Physiological strain of *Colletotrichum lindemuthianum* infecting *Phaseolus vulgaris* L. in Spain. *Euphytica.* **114:** 143-149.

Hancock, J. G., Miller, R. L. and Lorbeer, J. W. 1964. Pectolytic and cellulolytic enzymes produced by *Botrytis allii, Botrytis cinerea* and *Botrytis squamosa in vitro* and *in vivo*. *Phytopathology*. **54:** 928-931.

Idnurm, A. and Howlett B. J. 2001. Pathogenicity genes of phyto pathogenic fungi. *Molecular Plant Pathology*. **2:** 241-255.

Jayasinghe, C. K., Wijayaratne, S. C. P. and Fernando, T. H. P.S. 2004. Characterization of cell wall degrading enzymes of *Thanatephorus cucumeris*. *Mycopathologia*. **157:** 73-79.

Marimuthu, T., Bhaskaran, R., Shanmugam, N. and Purushothaman D. 1974. *In vitro* production of cell wall splitting enzymes by *Alternaria sesami*. Labdev. *Journal of Science and Technology*. **12:** 26-28.

Muthulakshmi, P. 1990. Studies on fruit rot of chilli (*Capsicum annuum* L.) caused by *Alternaria tenuis* Nees. M. Sc. (Ag.). thesis, Tamil Nadu Agric. Univ., Madurai, India. p. 139.

Norkrans, B. 1950. Influence of cellulolytic enzymes from hyphomycetes on cellulose preparations of different crystallinity. *Physiologica Plantarum*. **3:** 75-85.

O' Connell, R. J., Uronu, A. B., Waksman, G., Nash, C., Keon, J. P. R. and Bailey, J. A. 1993. Hemibiotrophic infection of *Pisum sativum* by *Colletotrichum truncatum*. *Plant Pathology*. **42:** 774-83.

Olutiola, P. O. and Akintunde, O. A. 1979. Pectin lyase and pectin methyl esterase production by *Penicillium citrinum*. *Transactions of British Mycological Society*. **72:** 49-55.

Padder, B. A., Sharma, P. N. and Sharma, O. P. 2009. Virulence and RAPD data-A tool to study the evolutionary trends of *Colletotrichum lindemuthianum* virulences in north western Himalayan region. *Archives of Phytopathology and Plant Protection*. **42:** 610-615.

Padder, B. A., Sharma, P. N. and Sharma, O. P. 2010. Distribution of *Colletotrichum lindemuthianum* race flora and itsimplication in deployment of resistant sources in Himachal Pradesh. *Research Journal of Agricultural Sciences*. **1:** 1-6.

Padder, B. A., Sharma, P. N., Sharma, O. P. and Kapoor, V. 2008. Ribosomal DNA analysis of *Colletotrichum lindemuthianum* virulences from Himachal Pradesh (India). *Applied Biological Research*. **10:** 6-10.

Perfect, S., Bleddyn, H. H., Connell, R. and Green, J. R. 1999. *Colletotrichum*: a model genus for studies on pathology and fungal-plant interactions. *Fungal Genetics and Biology*. **27:** 186-198.

Ramos, M.A., Gally, M., Garcý, M. C. and Levin, L. 2010. Pectinolytic enzyme production by colletotrichum truncatum, causal agent of soybean anthracnose. *Revista Iberoamericana de Micologia*. **27:** 186-190.

Rogers, L. M., Kim, Y. K., Guo, W., Gonzalez, C. L., Li. D. and Kolattukudy, P. E. 2000. Requirement for either a host or pectin-induced pectate lyase for infection of *Pisum sativum* by *Nectria hematococca*. *Proc Natl Acad Sci*. **97:** 9813-9818.

Sakal, T., Sakamoto, T., Hallaert, J. and Vandamme, E. J. 1995. Pectin, pectinase and protopectinase: production, properties and applications. *Advances in Applied Microbiology*. **39:** 2213-2293.

Sharma P. N., Sharma O. P., Padder B. A. and Kapil R. 2008. Yield loss assessment in kidney bean due to bean anthracnose (*Colletotrichum lindemuthianum*) under sub temperate conditions of north-western Himalayas. *Indian Phytopathology*. **61:** 323-330.

Sharma, P. N., Padder, B. V., Sharma, O. P., Pathania, A. and Sharma, P. 2007. Pathological and molecular diversity in *Colletotrichum lindemuthianum* across Himachal Pradesh - A north-western state of India. *Australian Jouranl of Plant Pathology*. **36:** 191- 197.

Singh R. B. and Jain J. P. 1979. *In vitro* production of pectinolytic and cellulolytic enzymes by *Colletotrichum lagenarium* the causal organism of bottle gourd anthracnose. *Journal of Mycology and Plant Pathology*. **9:** 277-278.

Somogy, M.J. 1952. Notes on sugar determination. *Biol Chem*. **159:** 19-23.

Vazquez, C., Reyes, F. and Martýnez, M. J. 1993. Comparative study of pectic activities from different Formae speciales of *Fusarium oxysporum*. *Lett Appl Microbiol*. **16:** 210-213.

Yakoby, N., Kobiler, I., Dinoor, A. and Prusky, D. 2000. pH regulation of pectate lyase secretion modulates the attack of *Colletotrichum gloeosporioides* in avocado fruits. *Applied Environmental Microbiology*. **66:**1026- 1030.