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Production of Cell Wall Degrading Enzymes by Isolates of *Bipolaris maydis*, the Causative Agent of Maydis Leaf Blight of Maize

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A B S T R A C T

Bipolaris maydis is a phytopathogen of maize that causes maydis leaf blight or Southern corn leaf blight disease. The *Biploaris maydis* produce extracellular enzymes that degrade cellulose, pectin, amylose and amylopectin of the plant cell wall and helps in invasion and virulence of the phytopathogen in the infected tissue. Variations among the seven isolates of *Bipolaris maydis* were found for extracellular enzymatic activity in solid medium and the amylase activity showed the highest activity index. E15 isolate of *Bipolaris maydis* showed the highest amylase, pectinase and esterase activity, whereas, E27 isolate showed highest cellulase activity index as compared to other isolates. The results indicated the possible role of cell wall degrading enzymes in the aggressiveness, virulence and increase the disease incidence of the maydis leaf blight of maize.

Introduction

Maize is the third major staple food in India and due to its high efficiency; it is regarded as queen of the cereals. Maize is susceptible to so many foliar diseases and due to the biotic stresses, the grain yield reduces to a greater extent. Maydis leaf blight is one of the most serious foliar diseases of maize and is caused by *Bipolaris maydis*. The qualitative measurement of the extracellular enzymes is indicative of the physiological process of microbes. Extracellular enzyme production by the plant pathogenic fungi is the key factor for characterization of variability among the plant pathogenic fungus. The presence of enzymatic activity exhibited by the fungus helps it to degrade the plant cell wall and facilitates its entry and mycelia growth in plants and is an important factor for virulence in the plants. The main aim of the present investigation was to study the physiological variability based on the extracellular enzymatic production by the seven isolates of *Bipolaris maydis*.

Materials and Methods

The extracellular enzymes activity by the *Bipolaris maydis*. were assessed in solid minimal medium containing 0.5 g KCl, 1.5 g KH_2PO_4, 6g $NaNO_3$, 0.5 g $MgSO_4$, 0.01 g $FeSO_4$, 0.01 g $ZnSO_4$ and one liter distilled H_2O and mycelial discs of 0.5 cm diameter of each isolate were transferred into Petri plates. These plates were incubated at 25°C for 5 days. The minimal medium were added with the enzyme substrate adjusted to pH6 as follow: pectin (1 %), carboxymethyl cellulose (CMC) (1 %), starch (1 %) and $CaCl_2$ 0.01% with Tween -80(1%) and adjusted to pH6 for pectinase, cellulase, amylase and esterase respectively. The enzymatic activity was observed after supplying the congo red solution for cellulase, 1% Ctab for pectinase, 1% Lugol(KI) for amylase and incubation at 4°C for 48h for esterase respectively.

The ratio of mean halo diameter (H) divided by the mean colony diameter(C) were used for quantification of enzyme activity.

Statistical analysis

Data obtained on various traits under laboratory were analyzed by Duncan's multiple range test (DMRT) and one-way analysis of variance (ANOVA) using Statistical Product and Service Solution (SPSS) version16.0 software Developed by SPSS Inc., now IBM SPSS and each experiment was replicated thrice. All results were expressed at $P< 0.05$ to compare difference among the treatment means.

Results and Discussion

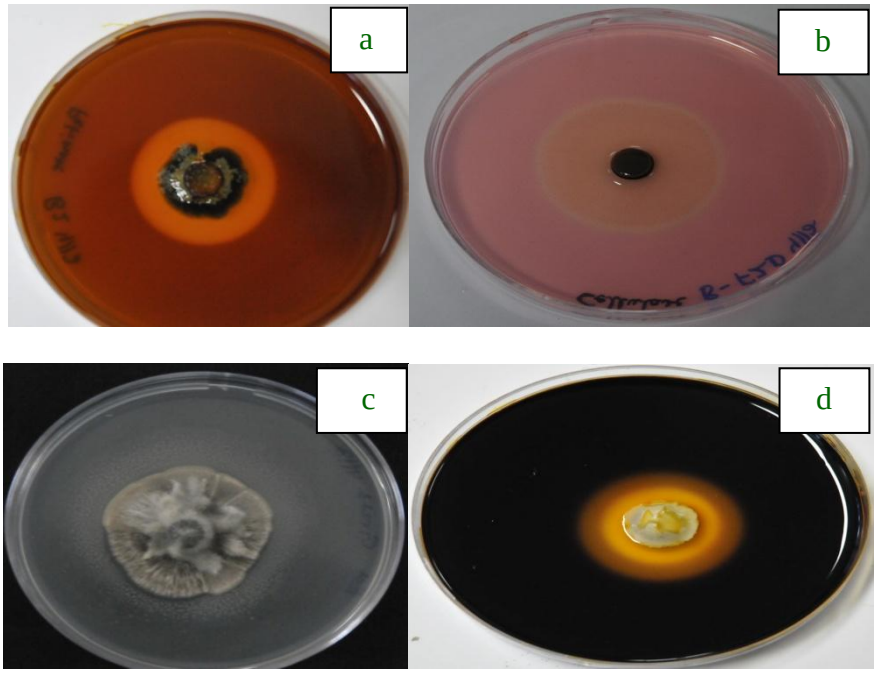
The extracellular enzymes secreted by fungal plant pathogens induce virulence (Wanyoike *et al.,* 2002 and Wubben *et al.,* 2000). In phytopathogenic fungi, the pectinase enzyme played a key role for infection (Ten Have *et al.,* 1998 and Valette-Collet *et al.,* 2003). In this process, pectic enzymes caused pectin degradation and resulted in cell lysis, plant tissue maceration and degradation of cell wall components which ultimately resulted in penetration of fungi inside the infected host tissue (Ten Have *et al.,* 2002 and Panda *et al.,* 2004). In the current investigation, all the seven isolates showed pectinase, esterase, amylase and cellulase activities (Table 1 and Fig. 1). The highest pectinase activity (H/C=1.36) was observed in E15 isolate and lowest was observed in E7 (H/C=1.09) isolate. Similar results have been reported earlier wherein *F. culmorium* and *F. graminareaum* produced pectinase enzyme that caused cell wall degradation to facilitate the pathogen entry in the infected tissue (Kang *et al.,* 2000, Kang *et al.,* 2000b and Wanyoike *et al.,* 2002). Boccas *et al.,* (1994) evaluated 248 isolates from coffee plant for assessing the production of extracellular enzymes and found that 119 isolates produced pectinase enzyme and 13 isolates were high producers of pectinase enzyme and most of them belonged to *Aspergillus* sp. and *Penicillium* sp.

The cellulase enzyme is produced by plant pathogenic fungus after appresorium formation (Deising *et al.,* 1995 and Mendgen *et al.,* 1996). Cellulase plays a major role in pathogenesis as it degrades the cellulose which is an important component of the plant cell wall and activates the process of infection, which leads to increase in virulence of the pathogen (Sipos *et al.,* 2010, Zhang *et al.,* 2014). In terms of cellulase activity, the isolate E27 showed highest index (H/C= 1.56) and the lowest was observed in E6 (H/C= 1.06).

Table.1 Extracellular enzymatic activity by *Bipolaris maydis*

Isolates	Pectinase	Esterase	Amylase	Cellulase
E6	1.21±0.02^{bc}	1.12±0.03^{d}	2.13±0.02^{c}	1.06±0.04^{c}
E7	1.09±0.02	1.34±0.01^{b}	1.53±0.04^{e}	1.05±0.05^{c}
E10	1.24±0.02^{b}	1.23±0.01^{c}	1.57±0.04^{e}	1.18±0.05^{bc}
E15	1.36±0.02^{a}	1.40±0.01^{a}	4.10±0.11^{a}	1.25±0.04^{b}
E19	1.18±0.01^{cd}	1.12±0.02^{d}	1.79±0.02^{d}	1.14±0.04^{bc}
E25	1.11±0.01^{ef}	1.23±0.01^{c}	1.75±0.01^{d}	1.14±0.05^{bc}
E27	1.16±0.02^{de}	1.14±0.01^{d}	3.06±0.07^{b}	1.56±0.04^{a}
C.D.	0.05	0.05	0.17	0.13
SE(m)	0.02	0.02	0.06	0.04
C.V.	2.42	2.16	4.26	6.27

Fig.1 Production of extracellular enzymes by *Bipolaris maydis* on plates. Clear zones representing a) pectinase b) cellulase c) esterase and d) amylase activity shown by *Bipolaris maydis*



Similar results had been observed for the production of cellulase enzymes by *Didymella bryoniae* in cultures resulted to further decaying of the host tissue and correlated with disease severity(Zhang *et al.,* 2014; Zhou *et al.,* 2016).Cellulase production observed in culture of so many plant pathogenic fungi such as *Colletotrichum acutatum* (Fernando *et al.,* 2001), *Phaeosphaeria nodorum* (Lalaoui *et al.,* 2000), *Fusarium sulphureum* (Yang *et al.,* 2012), *Gaeumannomyces graminis* (Dori *et al.,* 1995) and *Thanatephorus cucumeris* (Jayasinghe *et al.,* 2004; Zhao *et al.,* 2014), has been reported. The cell wall degrading enzymes such as pectinase, amylase, xylanase and cellulase were produced by *Fusarium* sp. (Di pietro *et al.,* 2003). In our investigation, the highest index activity of amylase was

observed in E15 (H/C= 4.10) and the lowest index activity was observed in E7 isolate (H/C=1.53). Brown *et al.,* (2001) observed that starch degrading enzymes might be indirectly responsible for pathogenecity of the fungi and α-amylase produced by *Aspergillus flavus* isolates is associated with virulence of this fungal pathogen. Verlant *et al.*, (2004) reported that the pectin methyl esterase is a pectin degrading enzymes that partially demethoxylated chains of pectin and methanol. Posada *et al.*, (2001) investigated that polygalacturonases enzyme produced by phytopathogenic fungi helps in the process of infection and colonization in the infected host tissue. In esterase activity, the isolate E15 showed the highest activity index (H/C=1.40) and least activity index was observed in E6 and E19 (H/C=1.12).

The all isolates of *Bipolaris maydis* showed a higher amylase enzymatic activity and this result revealed the potentiality of these enzymes for the invasion of plant tissues by phytopathogenic fungi.

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