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Inactivation of polygalacturonase and pectate lyase produced by pH tolerant fungus *Fusarium moniliforme* NCIM 1276 in a liquid medium and in the host tissue

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Summary

Fusarium moniliforme NCIM 1276 produced pH dependent an extracellular polygalacturonase (PG) and pectate lyase (PL) at pH 5 and pH 8, respectively. In the extracellular medium about 20.3% PG and 54% of PL protein concentrations were present in the active state at pH 5 and pH 8, respectively, whereas in intracellularly, more than 86% of both protein contents remained in the active state at all pH tested. We found two possible reasons, end-product inhibition and effect of environmental pH on conformation of the proteins after their release into the medium. Additionally, in infected tomato and cauliflower plants, the fungus secreted similar proteins which were located near to the epidermal and vascular regions of the hypocotyls. In infected tissues, between 26.9% and to 41.5% of PG and only 0.84%—13.4% of PL protein concentrations were present in active state. Thus, the medium/cell sap pH and concentrations of substrate/end products seem to play an important role in fungal invasion during plant pathogenesis are discussed with current literature.

Introduction

Plant cell wall consists of four major polysaccharides such as cellulose, hemicellulose, lignin and pectin. Pectin a heteropolysaccharide found in the middle lamella of the plant cell wall composed of α-1-4-linked galacturonate chains with a high

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percentage of methyl esterification. Hence, pectin degradation can be attained by the combined action of several enzymes such as pectin-methylesterases and pectin-depolymerases including hydrolases and lyases. Pectin-depolymerases such as polygalacturonase (PG) (EC 1.2.3.15) and pectate lyase (PL) (EC 4.2.2.1) act on homo-galacturonic regions of pectin by hydrolytic and trans-elimination cleavage mechanisms, respectively.

Pectinases are the first enzymes to be secreted by fungal pathogens when they attack plant cell walls (Collmer and Keen, 1986; Idnurm and Howlett, 2001). Pectin degrading enzymes are essential for fungal pathogens that do not have specialized penetration structures as well as for necrotrophic pathogens during the late stages of the invasion process (De Lorenzo et al., 1997). Secretion of pectin degrading enzymes during infection to the plants has been reported from various plant-pathogenic fungi such as *Fusarium oxysporum, Botrytis cinerea, Sclerotinia sclerotiorum* (Di Pietro and Roncero, 1998; Garcia-Maceira et al., 2001; ten Have et al., 2001; de las Heras et al., 2003; Li et al., 2004), from non-pathogenic fungus *Rhizoctonia* AG-G (Machinandiarena et al., 2005) and from several yeasts (da Silva et al., 2005). PG produced by bacteria *Erwinia carotovora* (Lei et al., 1985) and *Agrobacterium tumefaciens* Biovar 3 (Rodriguez-Palenzuela et al., 1991) has been known to determine virulence and cause necrosis in *Vigna unguiculata* (Cervone et al., 1987). Apart from acting as virulence factors during infection, endo-PG and PL may also function as virulence determinants by releasing their end products (oligo-galacturonides) which act as inducers of plant defense molecules such as phytoalexins (Davis et al., 1984). PL has been also implicated in pathogenicity from several fungi including *Fusarium solani* var pisi and *Colletotrichum gloesosporioides* (Crawford and Kolattukudy, 1987; Yakoby et al., 2000) and from bacterial genus *Erwinia* (Lei et al., 1985; Nasser et al., 2005) thereby suggesting that, in nature both PG and PL are agents of plant pathogenecity.

The expression of PL and PG genes in the phyto-pathogenic fungi are subjected to the ambient cell-sap pH or the available carbon source of the culture medium. For example, during ripening of *Persea americana* cv, Fuerte fruits the pericarp pH regulate PL secretion and affects the pathogenicity of *C. gloeosporioides* (Yakoby et al., 2000). PGs genes are also found to be repressed by glucose and induced by pectin or pectic substrates present in the medium (Wubben et al., 2000; Fontana et al., 2005; Radoi et al., 2005), moreover, constitutive PG expression also reported in *B. cinerea* (Van der Cruyssen et al., 1994). These evidences suggest that, environmental or host cell sap pH regulate the expression of pectinolytic enzymes and thus, play an important role against phyto-pathogenic organisms.

While searching for high pectinase producers from mangrove ecosystems, a fungal strain identified as *Fusarium moniliforme* was isolated from decaying leaves of mangrove plants (*Rhizophora apiculata* and *Avicennia officinalis*) in the saline, detritus-rich mud of a mangrove estuary on the west coast of India (18°55N, 72°54E) (Rao, 1996). Under laboratory conditions and in liquid medium containing 1% citrus pectin as carbon source, the organism produced a single PL at pH 8 and a single PG at pH 5 (Rao et al., 1996; Niture et al., 2001). In our earlier study we have shown that, when the isolate was allowed to infect tomato and cauliflower seedlings, having different cell sap pH, it caused vascular wilt in both plants. During infection in the host tissue, it secreted similar PG, PL and another PG II isoenzyme, which exhibited totally different physico-chemical properties (Niture and Pant, 2004). Hence, it can possible that PG and PL may act as virulence factors during infection in the above plants and their production as well as secretion may be pH dependent. In the present study, we have investigated the production and inactivation profiles of PG and PL as a function different pH conditions. These studies have been carried out in liquid medium as well as in host plants such as, *Lycopersicon esculentum* (tomato) and *Brassica oleracea botrytis* (cauliflower) which having a cell sap pH of 6.4 and 7.7, respectively.

Materials and methods

Pectin (citrus fruit 8% methyl-esterified), polygalacturonic acid, di, tri, and saturated D - galacturonic acid, Freund's complete adjuvant, horse radish peroxidase, were purchased from Sigma Chemical Company USA. Tetramethyl benzidine/Hydrogen peroxide (TMB/H_2O_2) was purchased from Bangalore Genei Pvt Ltd India. ELISA plates were purchased from Greiner Labortechnik Pvt. Ltd. Chandigarh, India. Wheat bran, tomato and cauliflower seeds were obtained from the local market. All chemicals and reagents were of analytical grade.

Microorganism

The present fungal strain *F. moniliforme* was isolated from decaying leaves of mangrove plants