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Addition of abscisic acid increases the production of chitin deacetylase by *Colletotrichum gloeosporioides* in submerged culture

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N-Acetyl-D-glucosamine (PubChem CID: 439174)
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ABSTRACT

The activity of chitin deacetylase from *Colletotrichum gloeosporioides* was studied by the addition of phytohormones (gibberellic acid, indole acetic acid, and abscisic acid) and amino sugars (glucosamine and *N*-acetyl glucosamine) in culture media. Abscisic acid exerted a positive and significant effect on enzyme production with 9.5-fold higher activity (1.05 U mg protein^{-1}) than the control (0.11 U mg protein^{-1}). Subsequently, this phytohormone was used in batch culture with higher chitin deacetylase activity being found at acidic pH (3.5) than at neutral pH (7). Furthermore, the highest activity was determined at the acceleration growth phase. The chitin deacetylase production was ascribed to the lag phase within the spore germination process and germ tube elongation instead of during the formation of appressoria, as evidenced by the scanning electronic microscopy results. Therefore, more inoculum and medium containing abscisic acid were added to the fed-batch culture, resulting in a significant increase in chitin deacetylase activity (3.64 U mg protein^{-1}). The addition of abscisic acid led to changes in the acetylation degree of chitin extracted from the cell wall of *C. gloeosporioides*, with lower degree of acetylation (DA of 75.6%) than that determined with the culture without abscisic acid (DA of 90.6%).

1. Introduction

Phytohormones are organic molecules that act as messengers with a key role in plant development. These biological compounds interact in a synergistic or antagonistic fashion within a sensitive equilibrium in response to biotic and abiotic stresses [1]. Certain pathogens successfully induce diseases through their ability to suppress or mislead plant defense responses. The susceptibility of plant organs, particularly fruits and flowers, to an invasion by phytopathogenic microorganisms increases with aging and ripening. Phytohormones, such as ethylene, accelerate senescence and increase susceptibility, whereas those that delay senescence, such as cytokinin and gibberellin, tend to increase resistance [2]. Phytohormones might cause hormonal imbalances in the early phase of infection due to plant development, and responses to environmental cues are highly regulated. Phytopathogenic microorganisms can cause disequilibrium during the disease process by producing their own phytohormones. This effect has been well documented in the

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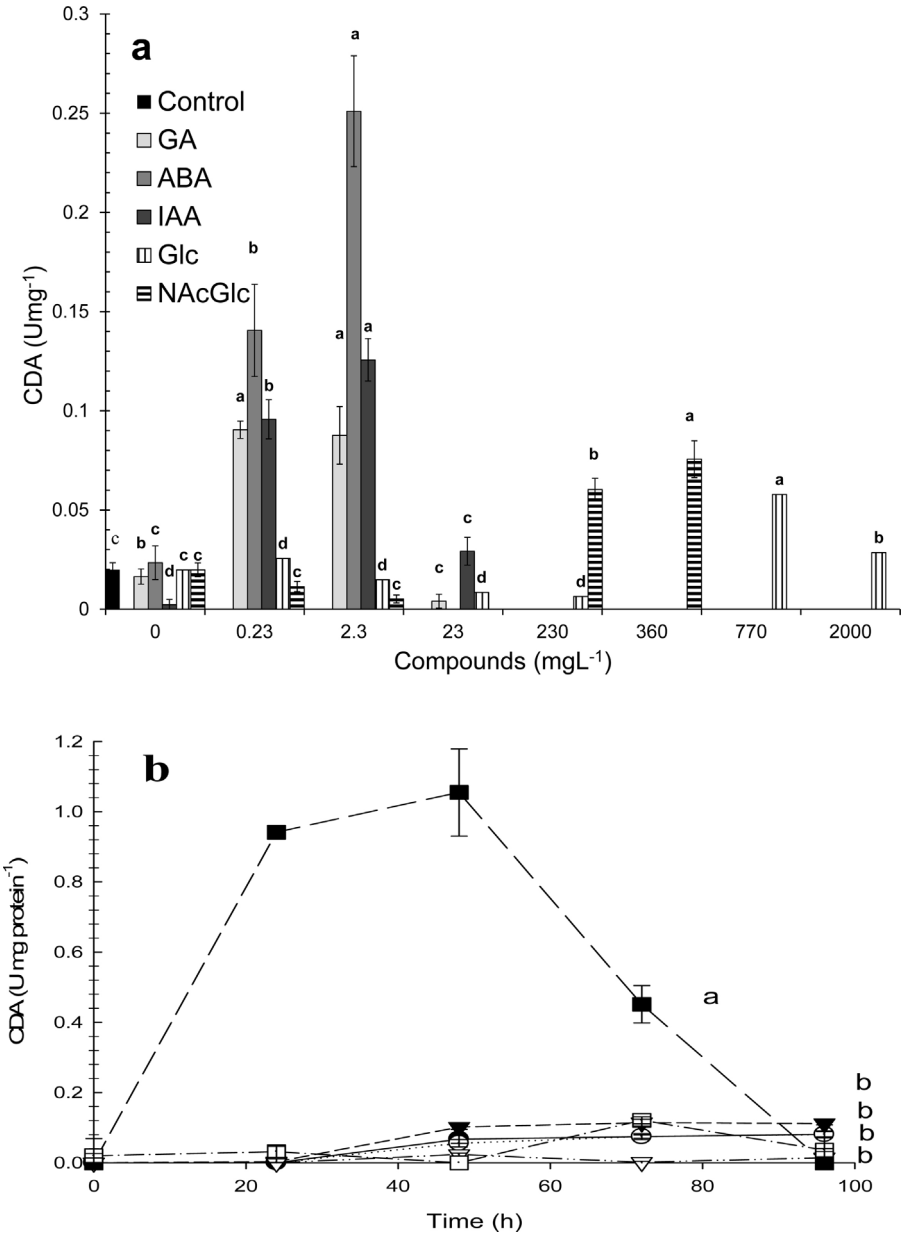

Fig. 1. SmC of *C. gloeosporioides*: a) screening of compounds at several concentrations for enhance CDA activities at 72 h and b) time course of CDA activities with added phytohormones (23 mg L^{-1}): GA (solid triangle), ABA (solid square), IAA (void triangle), and amino sugars: NAcGlc (360 mg L^{-1}) (solid circle) and Glc (770 mg L^{-1}) (void circle). Histograms with the same letter were not significantly different ($p < 0.05$) between compounds concentrations. Points with different letters were significantly different ($p < 0.05$) according to Tukeyís multiple means comparison test.

case of *Colletotrichum gloeosporioides,* which can produce indole-3-acetic acid (IAA) from tryptophan [3]. This fungus is known to produce IAA in plants, which at high concentrations inhibits the expression of plant defense molecules [4]. The application of phytohormones in plants created a suitable environment for fungal growth, as reported by Ulfers et al. [5] They applied exogenously abscisic acid (ABA) on a barley mutant with a defect in ABA biosynthesis, thus reducing its resistance against the fungus *Magnaporthe oryzae.* In another study, anthracnose produced by *Colletotrichum acutatum* was accelerated in the presence of ABA and gibberellic acid (GA) in pepper fruits. These phytohormones both accelerate or delay fruit senescence and increase the susceptibility to anthracnose [2], and also stimulate or inhibit the growth of phytopathogenic fungi [6–8]. Moreover, the production of chitin-degraded and related enzymes during the phytopathogenic process is favored as a part of the plant defense mechanisms. The phytopathogenic fungi, in turn, evade the specific plant hydrolases via the partial deacetylation of chitin in the cell wall by chitin deacetylases (CDAs), as detected in *Colletotrichum lindemuthianum* [7].

The function of phytohormones added to the culture media of fungi is unclear, and reports on the weak stimulatory or null effect of ABA on mycelial growth are limited [1]. Furthermore, increases of chitosan content in the cell walls of *Rhizopus oryzae* and *Mucor rouxii* were determined when cultivated in media supplemented with gibberellin, IAA, indole-3-butyric acid, and kinetin [9,10]. Despite these reports, to the best of our knowledge, there is no information about the effect of external phytohormones on the growth of *C. gloeosporioides* and production of CDA has not been studied yet.

In this regard, we studied the effects of phytohormones and aminosugars addition in a fed-batch culture process on