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Histological Studies on the Efficacy of *Bacillus amyloliquefaciens* on Early Developmental Stages of *Phytophthora infestans* on Tomato Leaves

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Abstract

Bacillus amyloliquefaciens has shown promising results in biological control of late blight caused by *Phytophthora infestans*. However, the mechanisms and metabolites involved are only poorly understood. To gain a better understanding of the mode of action of *B. amyloliquefaciens* histological observations were performed.

Cells of *B. amyloliquefaciens* and their metabolites excreted in the culture filtrate after 72 hour culturing were applied on tomato plants in the greenhouse 24 h before inoculation with the pathogen (5×10^5 sporangia ml⁻¹). Samples of treated and non-treated detached leaves were taken during the early developmental stages of the pathogen. They were fixed and discoloured in saturated chloral hydrate. After staining for 24 hours in fuchsin acid solution (0.01 %) the infection structures were observed microscopically with Normaski interference contrast. The effects on pathogen development were compared to the assessments of lesion size.

Both treatments reduced significantly the development of late blight lesions. Three hours post-inoculation (hpi), the zoospores produced germ tubes which showed an increased elongation by 52 % and 12 % on cell- and metabolite-treated leaflets, respectively. Appressoria formation on treated samples decreased slightly. The ability of the pathogen to penetrate the epidermal cells and to form a primary vesicle at the infection was 6 hpi on untreated plants high with 96 %. In contrast, on treated plants this ability was reduced by more than 40 % (3hpi) and 23 % (6hpi). The results showed the effect of both the bacterial cells and the metabolites on pathogen development before penetration of the host plant cells.

However, the metabolites reduced additionally pathogens development inside of the plant tissue. The reduction of primary vesicles size was about 15 %. The number of infected cells was more than 30 % reduced, as well as the formation of haustoria and secondary hyphae. The direct effect of both cells and metabolites resulted in reduction of pathogen establishment in tomato leaf and the effect of treatments on the growth of *P. infestans* proved to be highest with metabolites. In advanced stages of the infection process, hyphal ramification and production of infection vesicles in epidermal cells were inhibited giving evidence for the induction of plant defence reactions.

Keywords: *Bacillus amyloliquefaciens*, histological studies, metabolites, *Phytophthora infestans*, pre-infection stages