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# The Efficacy of *Bacillus amyloliquefaciens* on Late Blight Development and Biomass of *Phytophthora infestans* in Tomato Leaf Tissue

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**Abstract**

*Bacillus amyloliquefaciens*, re-isolated from the biocontrol agent FZB 24® (Biotechnik GmbH, Germany) has shown promising results in biological control of late blight caused by *Phytophthora infestans*. However, the mechanisms and metabolites involved are only poorly understood. In order to gain a better understanding of the mechanisms of action of the bacteria or their metabolites in reducing the disease severity of late blight, directly or indirectly by induced resistance, real time quantitative PCR were performed to determine the effect of foliar application on the pathogen biomass in tomato leaf tissues.

*B. amyloliquefaciens* cells and the excreted metabolites (culture filtrate) harvested after 72 hours of incubation time were applied on foliar parts of tomato plants in the greenhouse 24 h before inoculation with the pathogen ($10^5$ sporangia $ml^{-1}$). The effects were investigated on attached leaves as well as on detached leaves which were cut immediately after inoculation and incubated in plastic boxes under the same environmental conditions as the plants. Samples of attached and detached leaves were taken 3 h, 6 h, 12 h, 24 h, 48 h, 96 h (4 days), and 144 h (6 days) after inoculation corresponding to different developmental stages after infection.

From frozen dried leaf tissues DNA was isolated using the Plant Mini Kit Method. Real-time PCR reactions were performed with PinfRAS-Forward primer (CATTACATTGCT-CACATGGCTTTC) and PinfRAS-Reverse primer (ATCACGCGGGG ACAAATG) in an ABI Prism®7000 SDS instrument. The results were reported as the absolute amount of DNA of *P. infestans*. The correlation coefficient (R2-value) of the standard curve was at least 0.99 while the slope ranged from -3.1 to -3.8.

Both, bacterial cells as well as the metabolites were effective in preventing infection; they inhibited the pathogen biomass development in the tissue of the tomato leaves and significantly reduced the expansion of existing late blight lesions. The suppression of disease symptoms and pathogen growth was evident from the first stages of infection.

The efficacy of the bacteria or their metabolites in reducing the development of *P. infestans* was higher in attached than detached tomato leaves. 6 Days after inoculation, compared to untreated leaves, both treatments reduced the pathogen biomass by 83 % on attached leaves compared to 40 % (cells) and 60 % (metabolites) on detached ones. The amount of pathogen DNA detected in detached leaves was 4.7 (untreated samples), 17 (cells) and 10 (metabolites) times higher than in attached leaves. There was an increase in tratment efficacy to suppress the pathogen development. The results provide evidence for an additionally activation of plant defense responses.

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