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"Development on the margin"

Microarray Analysis of Gene Expression Induced by *Bacillus subtilis* in Tomato Leaves infected with *Phytophthora infestans*

Muna Sultan[1], Dessie Salilew Wondim[2], Dawit Tesfaye[2], Karl Schellander[2], Abd El Naser El Ashry[1], Florian M. W. Grundler[1], Heinz-Wilhelm Dehne[1], Ulrike Steiner[1]

[1] *University of Bonn, Institute of Crop Science and Resource Conservation (INRES), Germany*
[2] *University of Bonn, Institute of Animal Science, Germany*

Abstract

Bacillus subtilis, the re-isolated cells from the biocontrol agent FZB 24® (Biotechnik GmbH, Berlin, Germany) and their metabolites harvested after 72 hours of culturing, has shown promising results in biological control of late blight caused by *Phytophthora infestans*. To gain a better understanding of the mode of protection, the effect of the bacteria and their metabolites on differential expression of tomato genes in leaf tissue infected with *Phytophthora infestans* were evaluated. Tomato plants were inoculated with the *P. infestans* 24 hours after applying the microbial cells or metabolites on the lower leaf pair. Total RNA was extracted from the upper leaves 12 hours after inoculation. The plants were divided into two groups healthy and diseased plants, which were untreated as well as treated with the microbial cells or metabolites, respectively. Extracted RNA was used for the microarray analysis and the validation of the array results using quantitative real-time PCR. For the healthy plants, the data created from the cell and the metabolite-treated plants showed a minority number of re-regulated genes of 34 and 8, respectively, when compared with the healthy untreated ones. Results from microarray analysis showed that the pathogen changed the expression level of a substantial number of 682 genes from over 9200 genes on the tomato genome array when compared to the healthy plants. Application of cells and metabolites altered the expression levels of 656 and 345 genes, respectively. From those 489 and 299 genes are common between the two comparisons, which might be responsible to the infection. Interestingly, the comparison between cell or metabolite-treated and untreated diseased plants show 26 and 79 differentially expressed genes, respectively. Several genes were identified as up-regulated, including genes involved in plant defense responses, signal transduction, transcriptional regulators, and stress response. Down-regulated genes displayed identity with genes involved in cell regulation process. Differential expression of selected genes was validated by quantitative real-time PCR. Microarray analysis of gene expression showed a stimulation of systemic defense response in tomato plants induced by *B. subtilis* cells and its metabolites against *P. infestans*.

Keywords: *Bacillus subtilis*, gene expression, microarray, *Phytophthora infestans*, plant resistance, systemic protection, tomato

Contact Address: Muna Sultan, University of Bonn, Institute of Crop Science and Resource Conservation (INRES), Nussallee 9, 53115 Bonn, Germany, e-mail: muna_soltan@yahoo.com