Title Real time RT-PCR expression analysis of *syr*B and *syp*A genes in the interaction between *P. syringae* biocontrol strains and *P. digitatum*

Author Scuderi G, Panebianco S and Cirvilleri G

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Abstract

Some strains of *Pseudomonas syringae* van Hall are effective in controlling postharvest diseases of citrus fruits, and their antagonistic activity has been correlated with the production *in vitro* of lipodepsipeptides. Syringomycin (syr) and syringopeptin (syp) lipodepsipeptides are produced through a nonribosomal peptide synthetase system, and *syr-syp* genes are subjected to coordinated control by SalA and SyrF in response to environmental signals such as nutrient availability and presence of plant signal molecules. In the present study, the expression of syringomycin (*syr*B1) and syringopeptin (*syp*A) synthetase genes from seven antatgonistic *P. syringae* strains was evaluated *in vitro* on different culture media and *in vivo* on citrus fruits during the interaction with *P. digitatum*. The relative transcript level was evaluated by real-time RT-PCR one-step using the 16S rRNA as housekeeping gene. Expression analyses revealed that *syr*B1 and *syp*A genes can be differentially expressed under induced and non-induced conditions. A basal level of *syr*B1 gene expression was detected when *P. syringae* strains were grown on NB or PDB (no-induced) media. Similar results were reported for the syringopeptin synthetase gene *syp*A. Both genes were more actively expressed when bacteria were grown on orange peel extract media (induced) as compared to NB and PDB culture conditions. The presence of *P. digitatum* during *P. syringae* growth on PDB, on orange peel extract and in vivo on citrus fruits resulted to be strongly stimulatory only to *syr*B1 expression, suggesting that at least *syr*B1 gene is involved in biocontrol activity. Such up-regulation was correlated with inhibition of conidial germination *in vitro* and with antagonistic activity *in vitro* and *in vivo*. This is the first example of monitoring expression of *syr*B-*syp*A genes in interactions between *P. syringae* biocontrol agents and pathogen *P. digitatum in vitro* and *in vivo*.