

**Title** Microarray analysis of gene expression profiles in citrus fruit treated with chitosan

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### Abstract

Chitosan is a natural compound widely reported for its efficacy in reducing postharvest diseases of a number of fruit and vegetables. The mechanisms by which chitosan exert its activity are not fully elucidated, however it is known that it may act directly on pathogen and on host by inducing resistance. In the present study specific trials were conducted to evaluate the induction of resistance genes in citrus fruit treated with chitosan. Surface sterilized oranges were wounded, treated with chitosan or water and incubated at 20°C, high RH, for 48h. Part of the fruit were inoculated with a conidial suspension of *Penicillium digitatum* (104 conidia/ml) in a wound 0.5 cm apart from the previous one and incubated again in the same conditions for a week. Untreated fruit inoculated with the pathogen were used as a control. Three days after the pathogen inoculation the infected wounds were 62, 52, and 9% in control-, water- and chitosan-treated fruit, respectively; similarly, average lesion areas were 1.6, 2, and 0.1 cm<sup>2</sup>, respectively. Another part of the fruit treated with water or chitosan were utilized to evaluate their gene expression profile by cDNA microarray. Unwounded oranges were also included in the analysis. Total RNA was extracted from albedo and flavedo tissues. For both tissues chitosan effect was detected hybridizing RNA from chitosan and water treated tissues; similarly, wound effect was detected hybridizing RNA from water-treated and unwounded tissues against a reference sample consisting of a mixture of equal amounts of RNA from all experimental samples. A total of 70 genes were up-regulated more than 2 fold by chitosan in albedo and flavedo, while 150 were down-regulated in the same tissues. Regarding the wound effect, 517 and 512 genes were up- and down-regulated, respectively. Gene ontology (GO) annotation obtained for up- and down-regulated genes showed that many of these genes are involved in “secondary metabolism” and in “response to stimulus”. These preliminary data clearly indicate that chitosan is able to reduce the incidence of *Penicillium* rots in treated oranges by modifying their gene expression profile. Studies are in progress to identify the different metabolic pathways involved in host tissue response.