N-ACETYL-L-CYSTEINE, A NEW PERSPECTIVE FOR *XYLELLA FASTIDIOSA* CONTROL

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ABSTRACT

Xylella fastidiosa (Xf) multiply and attach to the vessel walls, forming biofilm that can, when sufficiently large, occlude xylem vessels, blocking water and nutrient transport in many different plant species such as citrus, grapevine, plum, almond, peach, and coffee. Fimbrial and afimbrial proteins seem to be essential for colonization and biofilm formation in *Xf*. Disulfide bonds play an important role in the folding and stability of such proteins. N-acetyl-L-cysteine (NAC), a thiol-containing antioxidant, is an analogue of cysteine that disrupts disulfide bonds in mucus, and has been used in medical treatment of humans with chronic bronchitis for instance. NAC also decreases biofilm formation by a variety of bacteria, and reduces the production of an extracellular polysaccharide matrix, while promoting the disruption of mature biofilms. This antibacterial property, together with the ability to avoid biofilm formation and to induce its detachment makes NAC an excellent candidate to be tested against *Xf*. The effect of this molecule in a phytopathogen has never been studied, thus, the aim of this work was, in general, to evaluate the activity of NAC against *Xf* biofilm cells. More specifically, we investigated the effects of NAC on (i) biofilm cellular mass and cell viability, (ii) production of extracellular polysaccharides (EPS), (iii) CVC symptoms in sweet orange (*C. sinensis*) infected plants, (iv) uptake of NAC by those plants, (v) its environmental degradation rate, and (vi) the variation of the number of viable *Xf* cells in plants treated or not with NAC. Results of biomass quantification, number of viable cells, total exopolysaccharide content, and microscope fluorescence images of *in vitro* cultured biofilms revealed that all the tested doses of NAC (1.0, 2.0, and 6.0 mg / mL) led to a decrease in biofilm formation, and inhibited growth of *Xf*, which indicates that this substance could also be toxic for this bacterium. *In vivo* experiments showed a strong reduction in CVC symptoms in *C. sinensis* treated with different doses of NAC three months after treatment. The amount of NAC added to the plant was monitored by HPLC and it seems that the plant absorbed the analogue. The population of the bacteria was lower in plants with NAC but was still possible to detect living cells. These results indicate that NAC may have an effect on *Xf* biofilm and the symptoms remission could be a possible consequence of restoration of the xylem flow. Approximately three months after stopping the treatment with NAC, the initial symptoms returned. These results open a new perspective for the use of this molecule on *Xf* control, where the improvement of NAC absorption by the plant (low absorption would increase the availability time of NAC), the NAC association with other molecules like Cu or Zn, and the time of application, could keep the plant in the field without the disease symptoms caused by *Xf*.

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