

Modes of action of biocontrol agents of postharvest diseases: challenges and difficulties

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Numerous biological control agents (BCAs) against post-harvest diseases have already tentatively been used in the past. Unfortunately, practical application is still limited because BCAs don't meet biological, technological and economical requirements. In this context, better knowledge on the modes of action of BCAs are crucial for improving successful post-harvest biocontrol strategies in order to (1) develop rational selection procedures yielding a second generation for more effective antagonistic strains, (2) optimize the method and timing of antagonist application, (3) achieve appropriate production and formulation enhancing antagonistic efficacy, and (4) provide a quality control procedure.

The protective action of BCAs can rest on one or several of the following processes: nutrient or site competition, antibiosis, direct interactions between the BCA and the pathogen, and induced host resistance. Moreover, multiple interactions between antagonist, host, pathogen, and other components of natural epiphytic microflora can interfere with the elucidation of BCAs mode of action. And last but not least, this elucidation is also hampered by the difficulty of interpretation of *in vitro* studies. While several features of the pathogen-antagonist interactions are relatively easy to assess *in vitro*, it is much more problematic to prove the involvement of a mechanism at the site of action. Hence, most conclusions are often based on indirect evidence.

Different experimental strategies based on several techniques (microscopy, biochemical and molecular tools) have been developed to overcome these difficulties. For example, the following progressive steps are currently used to determine if a particular compound such as hydrolytic enzymes or toxins is directly involved in biological control of fungal pathogens: (1) the purified compound shows fungicidal or antimicrobial properties, (2) the compound may be detected *in situ*, when producing strains are present, (3) the biocontrol ability of mutants defective in compound of interest is reduced in the laboratory and in practical conditions, (4) the complementation of the mutant with DNA sequence restoring the synthesis of the compound of wild strain also restores biocontrol ability.

Since it is easier genetically manipulate prokaryotes, all of these approaches have been already adopted to determine the implication of metabolites produced by antagonistic bacteria. However, the steps involving molecular biology were rarely reported to analyze the implication of a compound produced by antagonistic yeast's due to their greater genetic complexity and the scarcity of molecular tools in comparison with bacterial agents. In this context, the modes of action potentially involved in the antagonistic activity of bacterial and yeast strains against post-harvest pathogens on fruits will be reviewed with respect to the methodology and the techniques used to their study. Special attention will focus on the mode of action of *Pichia anomala* strain K, an antagonistic yeast against *Botrytis cinerea* on apples. In this particular case, biochemical and molecular tools were used to determine the possible implication of 1,3- β -glucanases in the antagonistic relationship (Jijakli *et al.*, 1998; Grevesse *et al.*, 1998).

Table 2. Incidence of fungi on acorns in blotter tests, 20 acorns per treatment. Test of acorns treated with biocontrol agents and/or thermo-therapy following 4 months of storage at -1°C.

Frequency (%) of taxon on seeds

- Jijakli, M.H. & Lepoivre, P. 1998: Characterization of an Exo- β -1,3-glucanase Produced by *Pichia anomala* Strain K, Antagonist of *Botrytis cinerea* on Apples. *Phytopathology* 88: 335-343.
- Grevesse, C., Jijakli, M.H. & Lepoivre, P. 1998: Study of Exo- β -1,3-glucanase activity production by yeast *Pichia anomala* in relation to its antagonistic properties against *Botrytis cinerea* on postharvest apples. Twelfth Forum for Applied Biotechnology, Med. Fac. Landbouw. Univ. Gent 63: 1685-1682.