

SCIENTIFIC NOTE REGARDING BACILLUS STRAINS ANTAGONISTIC TO *BOTRYTIS CINEREA* GREY MOULD

Sergiu Fendrihan

Research-Development Institute for Plant Protection,

correspondence address:

Research-Development Institute for Plant Protection,
8 Ion Ionescu de la Brad
013813, Bucharest, Romania
Phone: + 40 21269 32 31
Fax: + 40 21269 32 39
E-mail: ecologos23@yahoo.com

Abstract: Maximum Grey mold, *Botrytis* sp. is a very well known pathogenic fungi ubiquist and polyphage with impact on production of cultivated plants and in special in grapes production. The control requires costly treatment with chemicals, having only temporary results. The new tendency is to use as much as possible, ecological methods of control, including use of antagonists. Our preliminary tests with some *Bacillus* strain demonstrate the antagonist activity against the grey mould. The strains used also demonstrate their antagonism in the case of other pathogenic fungi.

Key words: *Botrytis*, *Bacillus*, biological control, antagonism

Grey mold, *Botrytis* is one of the most dammaging fungi- saprofit and pathogen –for grapes production and not only, attacking many other cultivated plants. The chemical treatment could contaminate in some measure the crops possible affecting health of the consumers. The grey mold can determinate, in some years, losses of about 70% in wineyards. Moreover, the fungi is polyphagous, attacking many other crops, even postharvest, as it can live as saprophyte and parasite.

Infected grapes become brownish, and conidiophores with conidia can be seen on the fruit surface. For the pathogen control, chemical treatments should be performed with Pyrus 400SC, Dithane M-45 fungicides, or with Rovral 500 SC, Cantus, Switch 62.5 WG. However, the fungicides can affect yield quality of production or wine fermentation (Caboni et al., 2010). The interest of the farmers is to obtain high quality yields, without pesticide contamination. The biological control can be a viable alternative solution, able to remove pesticide contamination risks, and also suitable in organic production.

The strains *Bacillus cereus* CRS 7 (chitinolytic) and *Pseudomonas fluorescens* CRS 31, isolated from chickpeas rhizosphere, reduced the gray mold attack, when applied as a spray inoculum on leaves of cultivated plants (Kishore & Pande, 2007). Several strains of *Bacillus*, BL1, BT5, BR8 and BF11, isolated from tomato plants, are also mentioned to have antagonistic effect against grey mould (Kefi et al., 2015). Some of the *Bacillus* strains are chitinaze producers which act against grey mold (Maachia et al., 2015). *Bacillus amyloliquefaciens* produce lipopeptides antibiotics like C17 bacillomycin D homologues (Tanaka et al., 2015) which acts against fungi too. *Bacillus amyloliquefaciens* DSM 23117, is a strain with enhanced productivity of lipopeptides in the presence of nutrients like ammonium nitrate (Pretorius et al., 2015). Similar mechanism of action was revealed in *B. cereus* B-02 by electron microscopy (Li et al., 2012). The antifungal substances released by the bacterial strain reduced the thickness of fungal cell wall, diminished the number of mitochondria, and distorted fungal cells structure.

Preliminary antagonism tests are presented in this study, on *Botrytis cinerea* isolated from grapes of Italia variety. The study was performed *in vitro*, using *Bacillus* sp. 83.2s and *Bacillus subtilis* Bce2 strains as antagonistic bacteria. Fresh mycelia plugs were inoculated on PDA in the center of Petri plates, and the bacteria were inoculated at 2.5cm from the fungal inoculum. Fungal control plates were also prepared on PDA medium. Then, all the Petri dishes were incubated at 28°C for 5 days. This experiment showed that the tested bacterial strains have antagonistic activity against *Botrytis cinerea* (figure 1), clear inhibition zones being revealed. Similar studies mention strains of *Bacillus subtilis*, *B. cereus*, *B. amylolyticus*, *Poenibacillus* spp., *Pseudomonas* spp. and many other bacteria with antifungal activity against the grey mould. Soil and endophytic environment is a permanent source of such bacteria with antagonistic effect against these pathogenic fungi.



Figure. 1 *Bacillus* sp. 83.2s (left) and *B. subtilis* Bce 2 (right) strains in co-cultivation with *Botrytis cinerea*

Although *in vitro* results are promising for grey mould biological control, several studies must be performed to improve bacteria formulation, diminish the costs of bio-preparates production and integrate the alternative method in the agricultural technology.

The analyzed strains of *Bacillus* sp. 83.2s and *Bacillus subtilis* Bce2 could be used as antagonists against the grey mould. As other strains with antifungal activity, these biocontrol strains could be used for bio-pesticides formulations.

In order to implement this alternative biocontrol technology in the organic farming, mass cultivation of beneficial bacteria must be improved at affordable costs, along with efficient formulation types and application methods.

REFERENCES

- CABONI, P., CABRAS, P. (2010) Pesticides' influence on wine fermentation. *Advances in Food and Nutrition Research*, 59:43-62.
- KEFI, A., SLIMENE, I.B., KARKOUCH, I., RIHOUEY, C., AZAEIZ, S., BEJAOU, M., BELAID, R., COSETTE, P., JOUENNE, T., LIMAN, F. (2015) Characterization of endophytic *Bacillus* strains from tomato plants (*Lycopersicon esculentum*) displaying antifungal activity against *Botrytis cinerea* Pers. *World Journal of Microbiology and Biotechnology*, 31(12): 1967-1976.
- KISHORE, G.K., PANDE, S. (2007) Chitin-supplemented foliar application of chitinolytic *Bacillus cereus* reduces severity of *Botrytis* gray mold disease in chickpea under controlled conditions. *Letters in Applied Microbiology*, 44(1): 98-105.
- LI, F.X., MA, H.Q., LIU, J., ZHANG, C. (2012) Antagonistic effects of *Bacillus cereus* strain B-02 on morphology, ultrastructure and cytophysiology of *Botrytis cinerea*. *Polish Journal of Microbiology*, 61(2):119-28.
- MAACHIA, B., RAFIK, E., CHERIF, M., NANDAL, P., MOHAPATRA, T., BERNARD, P. (2015) Biological control of the grapevine diseases 'grey mold' and 'powdery mildew' by *Bacillus* B27 and B29 strains. *Indian Journal of Experimental Biology*, 53(2):109-15.

PRETORIUS, D., VAN, ROOYEN, J., CLAKE, K.G. (2015) Enhanced production of antifungal lipopeptides by *Bacillus amyloliquefaciens* for biocontrol of postharvest disease. *New Biotechnology*, 32(2): 243-252.

TANAKA, K., AMKI, Y., ISHIHARA, A., NAKAJIMA, H. (2015) Synergistic Effects of [Ile]Surfactin Homologues with Bacillomycin D in Suppression of Gray Mold Disease by *Bacillus amyloliquefaciens* Biocontrol Strain SD-32. *Journal of Agricultural and Food Chemistry*, 63 (22): 5344–5353.