Strategy to design microbial consortia powering compost tea beneficial properties

Eliana Dell'Olmo¹*, Giovanna Serratore¹, Massimo Zaccardelli¹, Aida Raio², Loredana Sigillo¹ 1 CREA - Council for Agricultural Research and Economics, Research centre for Vegetable and Ornamental Crops, Pontecagnano Faiano (SA), Italy

2 CNR - National Research Council Institute for Sustainable Plant Protection, Sesto Fiorentino (FI), Italy

* Corresponding author: Eliana Dell'Olmo (e-mail: eliana.dellolmo@crea.gov.it)



Background

The extensive and often improper use of chemical fertilizers and pesticides lead to severe environmental issues (1). In this scenario, there is an increasing interest in more eco-friendly methods to manage crops (2). Sustainable alternatives have been studied including beneficial microorganisms (BMs) that can contribute to plant health by improving the mobilization of nutrients and biocontrol of phytopathogens (3). Microbial diversity in soil is crucial because the ability of microorganisms to restore fertility, enhance crop yield and plant growth (2). Similarly, compost teas (CTs) can be considered organic fertilizers that can improve soil biological activity, soil quality, and promote crop growth (4).

Figure 2. Compatibility studies. Biofilm of B. *thuringiensis* (a and b); biofilm of B. halotolerans (c and d); combined biofilm including the strains of *B*. thuringiensis and B. halotolerans.

MH_PL4



MH_PL11

Objective

The objective of this study was to design novel microbial consortia with features to be used as booster improving the beneficial properties of two CTs. The analysis was focused on selecting microorganisms isolated from compost teas and other vegetable matrixes and examining their ability to counteract three legume pathogens: Fusarium solani, Rhizoctonia solani, and Macrophomina phaseolina. Moreover, the biochemical characterization of BMs and studies on their compatibility to be mixed were performed, in order to obtain the best consortium able to booster CTs.

The study

Different CTs and vegetable matrixes were used to isolate several fungi and bacteria, to be tested for their putative beneficial properties. The BMs were first analysed for the antagonistic activity against F. solani, R. solani and M. phaseolina. The most promising BMs were identified by molecular sequencing of the ribosomal 16S region and one additional specie-specific gene. The ability of BMs to produce 3indolacetic acid (3-IAA) and siderophores, solubilize phosphorus, resist abiotic stresses such as temperature, pH, and salinity, and to produce lytic enzymes was evaluated through biochemical characterization. Moreover, features regarding root colonization such as motility, biofilm adhesion and formation, analyses of chemotaxis towards root exudates were evaluated. The isolates were tested for their biocontrol properties against the pathogens included in the study by *in vivo* tests on pea and chickpea plants.

Results

The main achieved results are:

- the gathering of about 100 new isolates, with putative beneficial properties, now stored in the CREA MicroHort collection;
- the identification of 5 strains of *Bacillus thuringiensis*, 14 strains of Bacillus halotolerans, 1 strain of Pseudomonas putida and 3 of Pseudomonas chlororaphis with antagonistic activity against the pathogens included in this study;
- the finding of 3-IAA producers (strains MH_PL11, MH_897 and MH_1304) and siderophores producers (strains MH_70.2.5, MH_897) AGRARIA and MH_1304);
 - the finding of 7 phosphorous solubilizers, among the 14 strains of *B*. halotolerans
 - resistance of *B. halotolerans* to high T and acid pH while *B.* thuringiensis and P. chlororaphis prefer basic pH and lower T. B. halotolerans showed to be able to grow at 4% NaCl;
 - 23 BM isolates were able to form a stable biofilm and showed, in preliminary tests, chemotaxis towards chickpea root exudates;
 - in *in vivo* tests, *B. halotolerans* MH_70.2.13 was able to control *M.*

Figure 1. Symptoms of Fusarium solani on chickpea. (a) plants infected with F. solani; (b) protectant effect of *Pseudomonas chlororaphis* subsp. *aerofaciens* on plants infected with F. solani.



Finally, to identify the most suitable candidates for inclusion in novel microbial consortia, compatibility studies between the isolates were conducted. The analyses were performed in planktonic and biofilm growth mode.

- phaseolina on pea and chickpea and R. solani on chickpea, P. chlororaphis MH_897 was found effective against F. solani on pea and MH_1304 was able to counteract all the pathogens on chickpea;
- B. thuringiensis and B. halotolerans have the best compatibility in planktonic and biofilm growth mode; *P. putida* was compatible with all the isolates while *P. chlororaphis* strains were only compatible with other *Pseudomonas*.

Conclusions and further research

In this study, BMs suitable to be effective components of microbial consortia and to power CTs were found. Further analyses will be focused on the understanding of their mechanism of action and their persistence in the environment after treatment application to the crop. To this scope, (i) we will test microbial consortia and powered CTs in open field trials, (ii) we will study the biofilm adhesion and formation and its role in root colonization, (iii) and we will investigate on genome of *B. halotolerans*, chosen as model organism for the phenotypic variability observed.

References

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