Pesticide resistance and nutrient mobilisation of legume symbiont and helper bacteria

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Introduction

Legumes, mainly beans and soybeans gain increasing importance in the agricultural production due to the national Common Agricultural Policy Agro-Environment Programs (CAP-AEP) in Hungary. These crops require symbiont bacterial species for nitrogen fixation and accordingly, proper development and yield. However, the formula of efficient nitrogen fixation is more complex than just a simple symbiosis of legumes and rhizobia. Several studies have been subjected to the research of various plant growth promoting rhizobacteria (PGPR) species that exhibit significant support in prosperity and development of legume symbionts (for example: Korir et al., Atieno *et al.*), and therefore improve crop quality and quantity. PGP bacteria are beneficial for the target organisms through mobilization of nutritive ions and molecules or by extrusion of useful organic compounds (e.g. hormones, chelators). Legumes need an elevated level of phosphorous and iron for efficient nitrogen fixation, therefore PGP bacteria mobilizing macro- and microelements are more and more often recruited for this purpose.
(Biofil technology: http://terragro.hu/img/gallery/downloads/BioFil_Sz%C3%B3ja_haszn_HUN.pdf)
Furthermore, according to Ahmad *et al.*, co-inoculation with PGPR helped in maintaining survival and function of rhizobia under abiotic stress conditions (salinity, drought). Accordingly, it is relevant to consider the addition of PGPR to newly developed rhizobial inoculants, mainly because such products may elevate the capacity of the positive symbiosis effects and improve crop yield consistency. Present work reports the first steps in the compilation of a PGPR containing symbiont inoculant family of legume crops.
Another relevant feature of legume seed inoculation is that it happens simultaneously with seed coating. During germination the bacterial strains are released into the rhizosphere, where they are contacted by the coating pesticides and also others, previously used in seedbed preparations. These, potentially harmful chemicals cannot be neglected in the production process, therefore the seed inoculating bacterial strains face a harsh selective environment. Accordingly, PGP and symbiont strain selection for use in integrated crop production requires thorough evaluation of nutrient mobilization capacity and pesticide-tolerance.
The presented work mainly concentrates on the fungicide (Vitavax, Rancona), insecticide (Force) and herbicide (Gladiator) resistance of legume symbionts (*Rhizobium leguminosarum*, *Bradyrhizobium diazoeficiens)* as well as PGP bacteria (*Bacillus spp.*, *Pseudomonas spp.*, *Agreia spp.*, *Paenibacillus spp.*, *Exiguobacterium spp.*, *Azospirillum spp.*, *Arthrobacter spp.*, *Kocuria spp.*) that may be used for macro- and microelement mobilization in soil inoculation of legume seedbed. PGP abilities of the selected bacterial species were also tested in *in vitro* experiments.

Materials and Methods

All strains were isolated and identified either by Biofil Ltd. or Saniplant Ltd. and belong to the strain collection of the respective companies.
Poison agar strain selection tests were performed on M9 minimal medium amended with the respective pesticide (Glifosate, Rancona, Vitavax, Force) starting at the field concentration advised by the manufacturer and diluted to half cc. each step. Five dilution steps were included in the tests.
Siderophore release was visualized by color reaction of the Cromazurol S (CAS), $FeCL_3x6H_2O$ and Hexadecil-trimetil-ammonium-bromid (HDTMA) painted medium.
Phosphate mobilization was detected by measuring the diameter of the clean halos around colonies on TPM agar.

Results and discussion

A total of 50 different bacterial strains were tested. All strains included in the experiments underwent a theoretical pre-selection, where symbionts and predicted helper PGP bacteria remained in focus. Pesticide amended agar tests revealed that the yet widely used herbicide glyphosate is highly toxic to the majority of the species examined. We found no resistant bacteria that could withstand the effect of glyphosate field concentrations, the most tolerant strains survived on 4-16x dilutions of the herbicide. Accordingly, microbial seed and soil inoculation technology has to adopt to herbicide application. We advise that bacterial inoculants or coated seeds should and have to be applied after the decomposition of the herbicide, in order to recover the decimated soil life. Vitavax is a commonly used seed coating fungicide of both monocot and dicot crops, currently under withdrawal, although still available on the market. This fungicide also has quite severe effects on soil bacteria, although approximately 30 % of the strains has tolerated field concentrations, and only 10 % of the species impaired growth at low (8-16x) dilutions. Rancona is a fungicide that presumably takes over the market of Vitavax for the majority of crops, and therefore is becoming relevant in microbial seed and soil inoculation. Replacing Vitavax with Rancona is a fortunate change, since according to our results only 20 % of the examined strains reacted negatively to this fungicide at field concentrations. In lesser amounts Rancona did not inhibit bacterial growth. Force is an insecticide used as soil disinfectant during seedbed preparations. This pesticide was the most “gentle” to the bacteria, basically had no harm to any strains examined, therefore it can be simultaneously applied with soil inoculants (Fig. 1.).
The iron, phosphorous and nitrogen homeostasis of plants is closely related, the availability of these nutrients has vast influence on the proper growth of legumes. Accordingly from the wide palette of bacterial PGP effects, siderophore release (iron chelation) and inorganic phosphate mobilization has been taken to the focus of our investigations. According to our results 14 out of 50 bacterial strains were good, or outstanding (11 + 3, respectively) in siderophore extrusion, whereas 25 performed well, or very well (19 + 6, respectively) in phosphate solubilization. Interestingly, good PGP performances remarkably overlapped, since 10 strains performed well in both “disciplines” (Fig. 2.).

Fig. 1. Examples of pesticide resistance of various bacterial strains. 1 – field concentration of the respective pesticide; 2 – ½ field concentration; 3 – ¼ field concentration; 4 – 1/8 field concentration; 5 – 1/16 field concentration

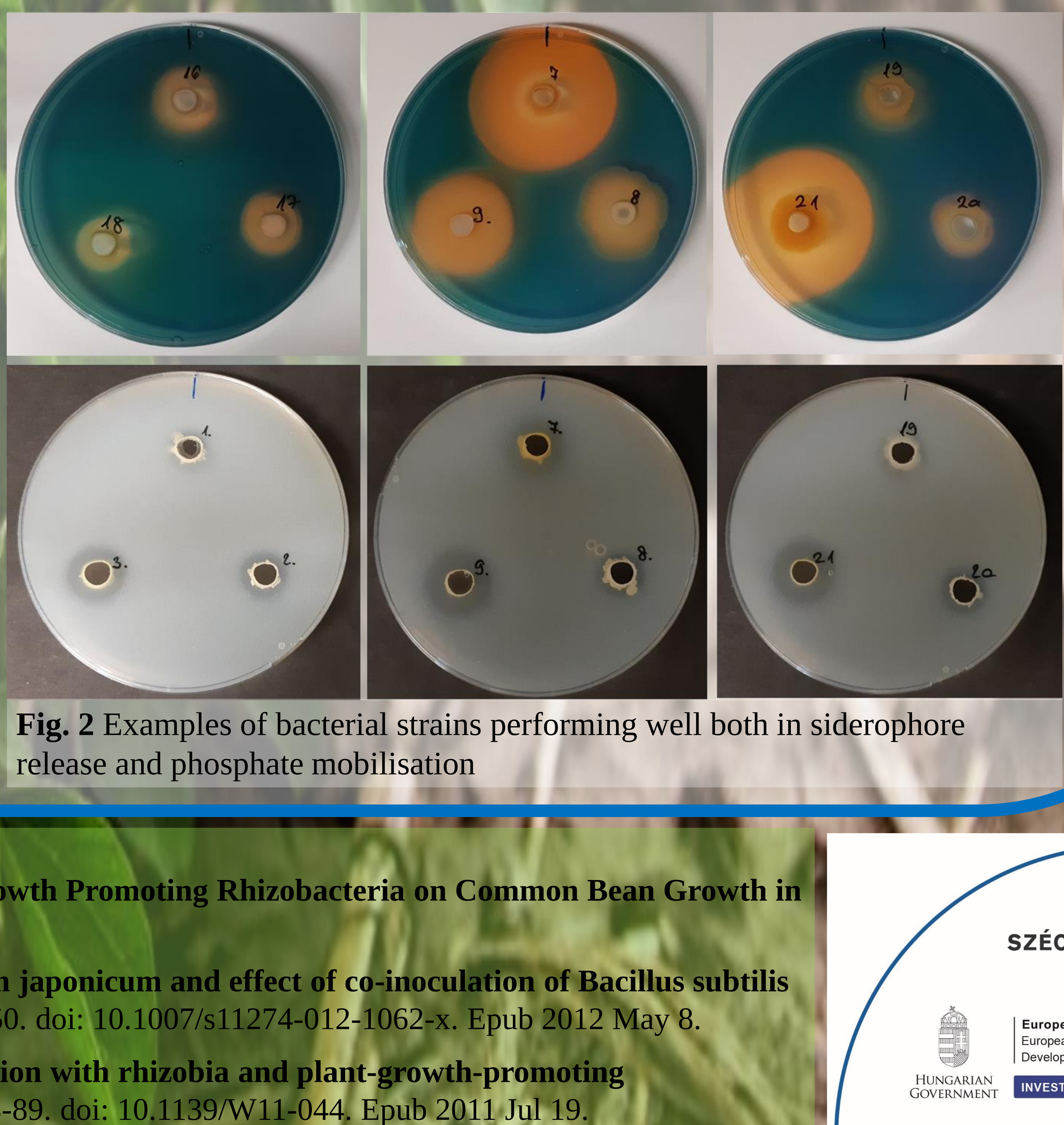

Fig. 2 Examples of bacterial strains performing well both in siderophore release and phosphate mobilisation

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