

More information on BM: Beneficial Microorganisms AB Sano Roots

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Underneath the results of the use of Beneficial Micro-organisms (= BM) on different crops. BM improves soil physical and chemical properties and produce bioavailable nutrients, increase root growth, plant growth, nutrient uptake and yield.

These effects are summarized in Table 1.

Next 7 abstracts on the use of BM in agriculture are shown as has been presented at the first World Congress on the use of Biostimulants in Agriculture, November 2012 in France.

The Beneficial Micro-organisms (BM) are one of three agricultural technologies in AB Sano Roots..

Beneficial Microorganisms for Sustainable Agriculture

After World War II was a need for food and farmers started using intensive production methods and synthetic pesticide inputs to increase the productivity. The crop yield substantially increased. However, as a side effect of the use of agrochemicals, environmental pollution and adversely affected human and animal health took place.

A growing worldwide concern for these problems was the driving force to develop alternatives to the chemical-based, conventional agriculture.

One such product is the use of beneficial microorganisms being a mix of naturally occurring, safe and efficient a microorganisms, such as lactobacilli, actinomycetes and yeasts.

Experiments conducted worldwide have shown good results using beneficial microorganisms in improving crop yield and soil fertility. Application of these microorganisms generally improves soil physical and chemical properties and favors the growth and efficiency of symbiotic microorganisms.

Although experiences of some researchers revealed that the effect of these microorganisms d wasn't evident in their (first) trials. However, this adverse effect can be overcome through repeated applications of beneficial microorganisms. Due to repeated applications the results improve. Foliar application of beneficial microorganisms avoids many of the biotic and abiotic factors and constraints of the soil environment, and thus increases the crop growth and yield significantly.

Other effects of these microorganisms is the treatment of wastewater treatment.

Research conducted so far concludes that benefits of beneficial microorganisms can be best exploited through their repeated applications for few years in combination with organic amendments (like sea weed extracts) and applying them as foliar spray. Integrated use of organic matter plus beneficial microorganisms with reduces quantities of traditional fertilizers are at least equivalent if not superior to that of full NPK fertilizers dose.

Table 1

Functions of Beneficial Microorganisms

- Production of simple organic molecules for plant uptake
- Production of bioactive compounds
- Solubilization of insoluble nutrient sources
- Production of polysaccharides to improve soil aggregation
- Fixation of atmospheric nitrogen
- Suppression of soil-borne pathogens
- Recycling and increased availability of plant nutrients
- Degradation of toxicants including pesticides
- Complexation of heavy metals to limit plant uptake

Underneath 8 abstracts are shown as presented at the 1st World Congress on the use of Biostimulants in Agriculture, November 2012, Strasbourg, France

ENDOPHYTIC COLONIZATION OF BACTERIA INDUCE DROUGHT-STRESS TOLERANCE IN MAIZE

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Drought is a potential major constraint to maize production in all areas where it is grown. Global warming, deforestation, and urbanization will all increase the severity and frequency of drought in the future, leading to a possible decrease in global food production at the same time that increasing human population demands an increase in food supplies. Therefore, there is an urgent need for drought stress tolerance to ensure optimal yield.

We here present our work on the use of bacterial inoculants to increase drought stress tolerance in maize. The plant-growth promoting endophyte *B. phytofirmans* PsJN was tested on two different maize cultivars (Kaleo and Mazurka). Surface-sterilized maize seeds were primed in bacterial suspension for 4 hours. Control seed were soaked in sterile broth. The seeds were planted in pots filled with 15 kg soil and recommended dose of NPK fertilizers were applied. Drought stress was applied by withdrawing water after 48 days of planting for 14 days. Agronomic and physiological data were recorded before/after harvesting. Plant colonization by the applied bacteria was monitored by microscopy and plate-counting assays. Microbial inoculation resulted in up to 55% increase in the biomass of maize as compared to the untreated control under drought stress. The photosynthesis activity was up to 67% higher in inoculated plants than in the untreated control.

In conclusion, our study clearly demonstrates that bacterial inoculants could be used to minimize the negative effects of drought stress on growth and photosynthesis of maize.

RhizoVital 42 – Ensures that your plants grow strong and healthy

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RhizoVital 42 contains the soil borne bacteria *Bacillus amyloliquefaciens*. These bacteria use plant sugars and amino acids for their own growth, and compete in the root area with other bacteria and fungi for nutrients and space. Thus, the application of RhizoVital 42 can effectively suppress the soil borne diseases caused by Fusarium, Rhizoctonia, Verticillium or Phytophthora. Furthermore *B. amyloliquefaciens* promotes root growth by releasing hormones and enzymes for nutrient mobilisation, which leads to a healthier growth of young plants and better yield. RhizoVital 42 can be applied in horticulture and agriculture by spraying, drenching, coating or injecting into hydroponics.

A large number of field trials on a broad range of different crops reveal the positive effect of RhizoVital 42, which will be presented in a review of results from selected crops.

Characterisation of microbial and agronomic effects of biofertilizers

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Plant rhizosphere is a preferential niche for various types of microorganisms in the soil. In the context of increasing environmental quality the use of biofertilizers for reducing chemical inputs in agriculture is a potentially important issue. Plant growth-promoting rhizobacteria (PGPR) are known to influence plant growth by various direct and indirect mechanisms. In search of efficient biofertilizers with multiple activities, microbial inoculants were evaluated either alone or together with organic and inorganic fertilizer.

For this purpose different bioassays were conducted at the laboratory scale in well controlled conditions on fertilizer formulations: (i) to assess the bioassociative effect on seed germination and root length using whinRhizo or plant growth and nutrients uptake, (ii) to screen the efficient formulation by evaluating the solubilisation of minerals such as phosphorus and by chelation of iron to make it available for the plant, (iii) to measure the effect of biofertilizers on the activity of nitrifying bacteria and the genomic structure of the bacterial communities of soil through Thermal Temporal Gel Electrophoresis (TTGE).

In addition standards based on the soil nitrification potential and the growth of microbial plant symbiosis could be performed to check the compatibility of the biofertilizer with soil microorganisms.

Examples of all these assays conducted on various biofertilizers in the framework of putting them on the market via French registration (homologation) will be presented.

Pathogenicity of 18 entomopathogenic or plant growth promoting fungal isolates to *Delia radicum* L., cabbage root fly, and their rhizosphere competence

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The entomopathogenicity of 9 entomopathogenic or potentially plant growth promoting fungal species was assessed against cabbage root fly (CRF) in soil and *in-vitro* laboratory bioassays. The fungal strains were isolated from various substrata in Slovenia. The soil experiments mimicked natural exposure pathways of the various insect life stages to the fungal strains. Spore concentrations used in soil tests were comparable to economic rates for in-furrow application (3.85×10^6 spores/g dry soil). The *in-vitro* tests were designed to screen aggressiveness of the various isolates to CRF. In *in-vitro* tests, spore suspensions with a concentration of 1×10^8 spores/ml were directly applied to CRF eggs. The following fungal species were tested: *Trichoderma atroviride* (2 strains), *T. koningiopsis* (1), *T. gamsii* (3), *Beauveria brongniartii* (1), *B. bassiana* (2), *Metharhizium robertsii* (1), *M. anisopliae* (4), *Purpureocillium lilacinum* (2) and *Clonostachys solani* (2). All isolates tested were infective to one or more of the tested life stages of CRF (eggs, larvae, imago or pupae). Abbott's corrected mortality in soil experiments ranged from $20.0 \pm 13.2\%$ to $75.0 \pm 13.2\%$ and in the *in-vitro* experiments from $15.4 \pm 6.5\%$ to $57.9 \pm 9.6\%$. The 5 most pathogenic isolates (*T. atroviride*, *B. bassiana*, *M. anisopliae* and *C. solani*) were further tested for their rhizosphere competence.

The preliminary results showed that rhizosphere competence varied considerably, possibly due to the ecological preferences of the different fungal species. The use of these fungi as an alternative to chemical insecticides in organic and integrated management programs is discussed.

Plant-growth-promoting rhizobacteria emit volatiles compounds with biostimulation activity in dicot and monocot plant species

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Despite the demonstrated capacity of certain soil bacteria to stimulate plant growth and stress response, much has still to be discovered on the mechanisms underlying these effects. In order to better understand how roots and bacteria may interact in the rhizosphere, an experimental system was designed, allowing the co-cultivation of seedlings with PGPR bacteria on compartmented Petri dishes in a shared atmosphere, with no physical contact between both organisms and with no diffusion of water-soluble compounds between compartments. Two plant species were used, *Arabidopsis thaliana* as a dicotyledonous model, and *Brachypodium distachyon* as a monocotyledonous (graminaceous) model.

Regarding the bacterial partners, 19 strains belonging to 8 genera (*Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Paenibacillus*, *Pseudomonas*, *Raoultella*, *Serratia*) were selected. Plant growth and development parameters were measured after pre-germination and ten days of co-cultivation, including biomass production and root/shoot partitioning, and architectural parameters like root branching and adventitious root production. Volatile compounds emitted by the PGPR bacteria demonstrated a strong capacity to influence all of the assessed parameters, the effects being dependent on the strains. The observed biostimulating effects include significant changes in total biomass, root / shoot ratios, and in the number of secondary and adventitious roots per plant.

Altogether, the results point to the capacity of bacterial volatile compounds to strongly biostimulate plants and roots *in vitro*, in both dicot and monocot species. Profiling of the volatile compounds is in progress, using SPME-GC-MS (Solid Phase Micro-Extraction / Gas Chromatography / Mass Spectrometry), in order to identify the bioactive components involved.

Effect of diazotrophic bacteria inoculating in *in vitro* growth of *Oncidium pumillum* Lindl

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The aim was to investigate the effect of diazotrophic bacteria inoculating in *in vitro* growth of *Oncidium pumillum* Lindl. under suitable conditions for expression of BNF and other processes. *O. pumillum* seedlings, germinated from *in vitro* seeds, were inoculated in test tubes containing Jensen medium with addition of myo-inositol, vitamins, and sucrose from WPM and activated charcoal. Two experiments were conducted for 180 days. The first experiment was conducted under N-limited conditions to test the effect of BNF. In the second experiment, all of the treatments received mineral-N to test the effect of other processes. In both experiments, 0.5 mL of each bacterial strain (per test tube) was inoculated. In both experiments, a completely randomized distribution was applied, where treatments consisted of inoculation of 24 strains; two separate uninoculated control treatments, one with and one without mineral nitrogen added. The number of leaves, shoots, and roots; shoot and root length; and plant fresh biomass were evaluated. In the first experiment, there was no effect of the strains on any of the parameters evaluated, indicating no contribution of the N₂-fixing bacteria. In the second experiment, the strains, UFLA 181S, UFLA 16, 56, 61, and 64, promoted better responses to most of the variables assessed. However, for the acclimatization period a larger root system is a key feature for success of this step, thus, the UFLA 69 strain stood out exhibiting a root length of 4.0 cm.

This study shows selected diazotrophics bacteria may contribute for *O. pumillum* growth through other processes.

MICROORGANISMS MEDIATING PHOSPHORUS AVAILABILITY IN NEW ZEALAND PASTURE SOILS

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A range of microbial taxa are believed to play important roles in soil P cycling based on the ability of various bacteria and fungi to solubilise P in in vitro assays and a few selected isolates have been developed as biofertilisers for annual crops. However, there is surprisingly little information regarding the diversity and activity of P-solubilising microorganisms in soil. In the first study of P-solubilising bacterial (PSB) populations in pasture soils, rhizosphere bacteria were isolated from pasture plants grown under high and low P levels at three long-term fertiliser trial sites in New Zealand. The frequency of the P-solubilisation phenotype was highly correlated to soil P status, with lower frequency of P-solubilisation in high P soils. Diversity was higher in the high P treatment compared with the low P soil, where Pseudomonads and Actinobacteria predominated. More detailed investigation of the P-solubilising communities at one site found that Actinobacteria and mycorrhizal fungi were clearly associated with the underlying P fertility gradient, with abundance of some mycorrhizal species being greater in high P soils than in unfertilised soils. Greater understanding of the diversity and ecology of P-solubilising microorganisms is informing strategies for selection and deployment of potential biofertiliser strains for use in pasture.

Screening of Commercially Available Micro-Organisms for the Use in Substrate

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Key words: potting soil, mycorrhiza, bacteria, algae extracts, plant growth.

In West-Europe, there is an increasing interest for the use of micro-organisms in substrates for the production of plants. Plant producers hope through the introduction of micro-organisms in their substrates to stimulate plant growth and to apply less fungicides. In order to obtain a homogenous repartition of the micro-organisms in their substrate, plant producers ask their substrate-producing company to mix the products in advance. Little research, however, has been carried out on these commercially available micro-organisms and substrate-producing companies are often not aware of what they mix in their substrate.

In this study, more than 9 different commercially available micro-organisms were screened for their beneficial effect on plant and root growth. Micro-organisms were mixed with several substrates at the recommended dose. Results of this study demonstrate a great variety in quality between the different products available on the market. Only in a few cases improved plant growth could be observed. Analysis of the roots could not always demonstrate the presence of the introduced microorganism, despite the fact that the recommended dose was applied. One product based on hyphae of mycorrhiza suppressed even plant growth. Analysis of the plant roots showed the presence of the plant pathogen *Olpidium*, which might have been a contaminant during the production of the mycorrhiza.

In conclusion, this study shows that there is a great difference in quality between the commercially available micro-organisms. Despite the fact that the recommended dose was applied, an enhanced effect on plant growth could not always be observed. Furthermore, an important effect of plant type and substrate could be observed.