PROJECT FINAL REPORT

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

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Section 1 – Final Publishable Summary Report

Project title: **Resource Preservation by Application of BIOefFECTORs in European Crop Production**

Project website: **www.biofector.info**

1.1 Executive Summary

BIOFECTOR (Resource preservation by application of bio-effectors in European crop production) is an interdisciplinary research project, investigating perspectives for the use of so-called bio-effectors (BEs) to improve the ability of crops to utilise nutrients from both, mineral and natural recycling fertilisers in conventional and organic farming systems. Bio-effectors comprise microorganisms (bacteria, fungi) and bio-active natural compounds (extracts from seaweed, plants and composts) with the ability to improve growth, nutrient acquisition and stress tolerance of crops, without significant direct input of nutrients. Tomato, wheat and maize were selected as important crops, representative for horticultural and agricultural production systems in Europe.

Main outcomes

- The application of BEs can significantly improve nutrient acquisition and stress tolerance of crops in a profitable way. However, this requires highly adapted and site-specific application strategies. General "easy to use approaches", applicable over a wide range of environmental conditions, are not available.
- The strongest expression of microbial BE effects was recorded in horticultural tomato production systems in combination with N-rich organic recycling fertilizers, based on animal manures, blood and bone meal. The advantage of this culture system is a stress-protected nursery phase under greenhouse conditions during the sensitive establishment phase of the plant BE-interactions, and efficient, cost-effective BE inoculation of the small soil volumes in the nursery pots.
- In agricultural production systems, the expression of microbial BE effects was frequently smaller and less reproducible. Particularly microbial BE-plant interactions are often biased by impacts of environmental stress factors during the establishment phase (drought, temperature extremes, nutrient limitations), limited responsiveness due to a frequently high inherent nutrient re-mobilization potential in many agricultural soils, higher application costs for effective inoculation due to larger soil volumes and lower market prices of the final products as compared with horticultural production. However, non-microbial BEs (seaweed/plant extracts) offer options for efficient and cost-effective applications as stress protectants by seed and foliar applications in different stages of plant development.
- Fertilizer placement and localized BE application techniques offer perspectives for a more efficient and cost-effective inoculation with microbial BEs in agricultural production systems and require further development. However, seed inoculation was the least-effective option.
- Exploitation of synergisms by BE combinations was more effective than application of single BE products. In this context, synergistic effects can be also achieved by introduction of stress-resistant BE strains, as well as combination with suitable fertilizers, such as zinc, manganese and stabilized ammonium with the potential to further increase stress protective and nutrient mobilizing effects of BEs.

- Microbial and non-microbial BEs show common modes of action, triggering adaptive stress responses of the host plants at the molecular and physiological level (stress priming effect) but the stress-sensitive establishment phase of microbial plant-BE interactions is frequently a limiting factor.
- Inoculation with microbial BEs has a significant but only transient impact on microbial communities in the rhizosphere. However, effects of the plant developmental status and the soil type are usually much stronger expressed than the impact of the inoculants.

Figure 1 summarizes the expression of BE effects in response to different fertilization strategies, culture systems, and BE types as determined in a meta-study, covering more than 150 pot and field experiments conducted with 38 BEs within the project duration.

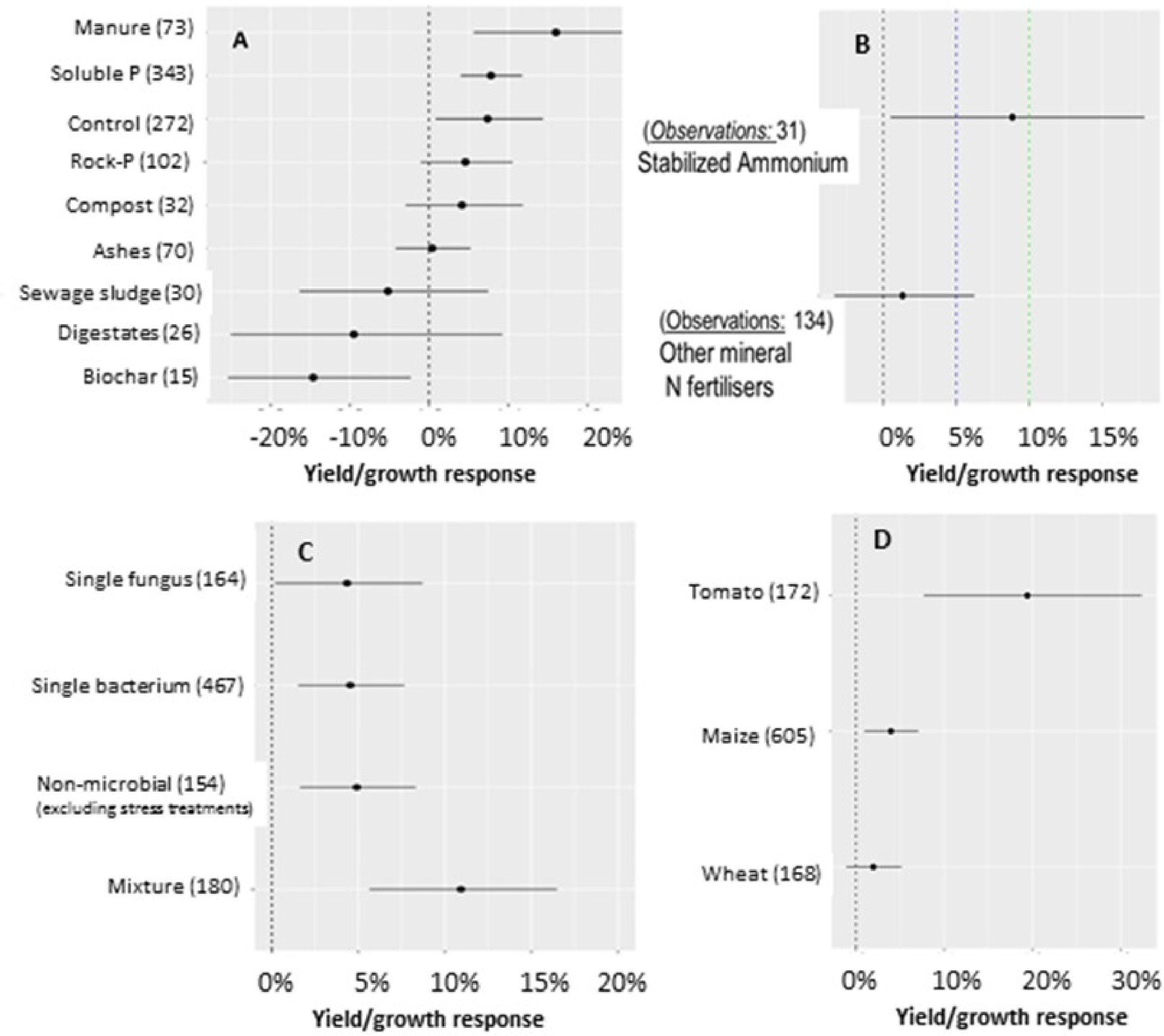


Figure 1: Yield and plant growth effects of BE applications, depending on (**A**) the type of recycling fertilizers, (**B**) the form of mineral N fertilizers, (**C**) type of BEs and (**D**) crop species. The number inside the brackets represents the number of observations included; the dashed vertical zero line indicates no difference between BE and non-inoculated control treatments, the points indicate the mean effect while the horizontal line represents the 95% confidence interval (CI). If CI lines cross the zero line, effects are not significant.

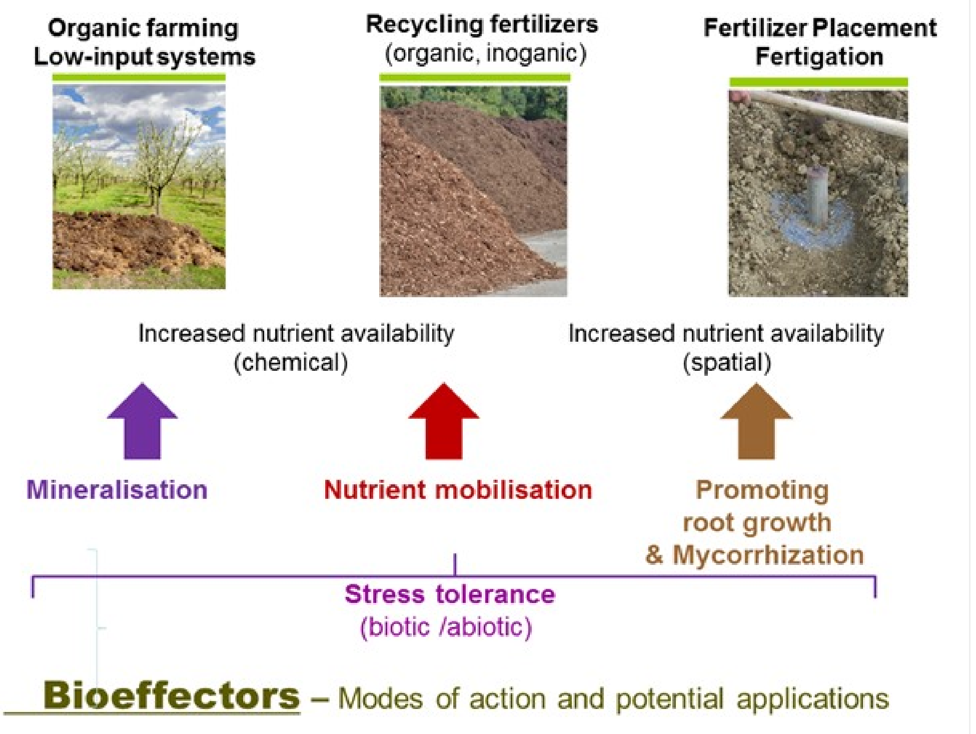
1.2 Summary description of project context and objectives

Background

Conventional agriculture relies on regular applications of mineral fertilisers containing essential plant nutrients, especially nitrogen, potassium and phosphorus. Mineral nitrogen fertilisers are made from atmospheric nitrogen, which is converted to ammonium using the energy-intensive Haber-Bosch process, consuming 1-2% of the wold energy production. Phosphorus fertilisers are produced by treating mined phosphate rock with sulphuric acid. Apart from the high energy cost of producing these fertilisers with limited natural resources, harm is also caused to the environment by their application. On average, only about half of nitrogen fertilisers and 20 per cent of phosphate fertilisers are taken up by crops. Most of the remainder is immobilised in soils, runs off into waterways, is leached into groundwater or lost in gaseous forms, contributing to global warming and after precipitation, to over-fertilisation of natural ecosystems. The liquid leachate causes pollution of groundwater sources and leads to the eutrophication of rivers, lakes and coastal zones, thereby reducing biodiversity. In turn, the limited fertiliser use efficiency in agricultural production systems further promotes the risk of over-fertilisation to maintain yield stability. Similar problems arise also from excess fertilisation in regions with high availability of manure-based fertilizers from intensive livestock production. Because of these damaging effects, many regions including Europe are introducing legislation to reduce the use of mineral fertilisers.

Objectives and aims

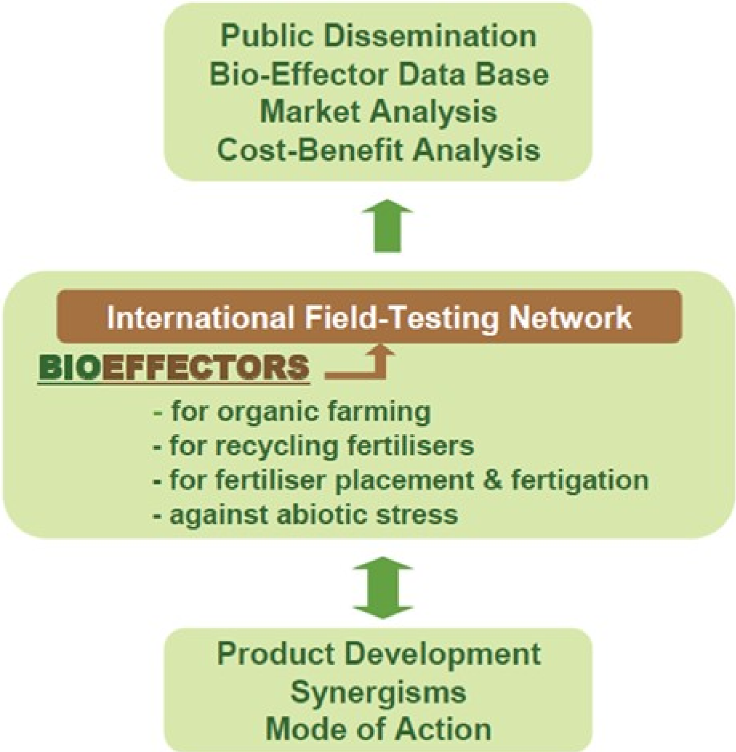
BIOFECTOR (Resource preservation by application of bio-effectors in European crop production) is an interdisciplinary research project investigating perspectives for the use of so-called bio-effectors (BEs) to improve the ability of crops to utilise nutrients from both artificial and natural fertilisers. Bio-effectors comprise microorganisms (bacteria, fungi) and bio-active natural compounds (extracts from seaweed, plants and composts) with the ability to improve growth, nutrient acquisition and stress tolerance of crops, without significant direct input of nutrients.



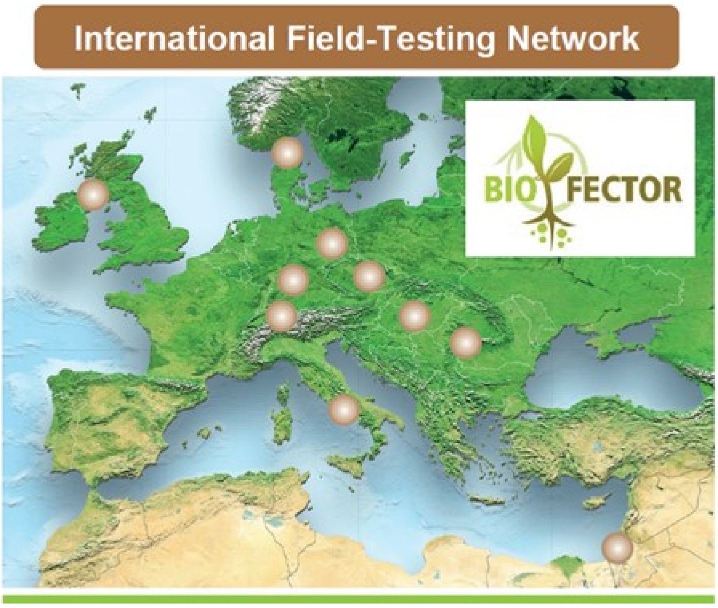
Based on these features, the project aimed to identify the most suitable combinations of BEs, crops and fertilisers, including improvement and adaptation of existing BE products, as well as the development of novel BE products with enhanced field efficiency. The final goal was the development of novel BE-based applications to optimise the productivity and particularly the nutrient use efficiency of alternative fertilisation strategies to promote a more sustainable agricultural production e.g. by organic farming, use of fertilisers based on recycling products or by fertiliser placement close to the roots. Tomato, wheat and maize were selected as important crops representative for horticultural and agricultural production systems. The project comprised a consortium of 21 industrial and academic partners from 11 countries, investigating BE applications under local production conditions in European agriculture.

Work structure

The work programme was covered by ten interrelated work packages (WP01-WP010):



Bio-effectors with putative plant growth-promoting potential were provided by five European companies with expertise in selection, formulation and production of BE products (WP01). For products with a proven record of plant-growth promotion (PGP), an international expert team of soil microbiologists, plant physiologists and agronomists characterized the principle modes of action and the underlying physiological and molecular mechanisms as well as potential impacts on native soil-microbial populations to consider putative effects on soil ecology and bio-safety (WP03). The efficiency of the selected BEs for improvement of alternative fertilization strategies was evaluated in standardized model experiments under controlled environmental conditions, followed by small-scale field trials, addressing: (i) BE applications to counteract abiotic stress factors (WP04), such as chilling, drought and salinity; (ii) applications for organic farming and low-input systems (WP05); (iii) improved utilization of fertilizers based on recycling products (WP06) and (iv) improved efficiency of fertilizer placement strategies (WP07). Apart from single BEs, also synergistic effects of product combinations were investigated (WP02). After the initial screening phase, successful products were finally assessed within the “BIOFECTOR International Field Testing Network”, providing standardized field testing facilities in nine countries under the geo-climatic conditions representative for European agriculture (WP08). Apart from assessment of individual trials, all experiments conducted within the project runtime were finally evaluated in a meta-analysis, which covered more than 150 experiments, providing more than 1100 data sets (observations) on BE effects on plant growth and yield formation for a total of 38 BE products tested in 145 treatment combinations.



The field-testing network also provided the base for public demonstration trials and the data for a cost-benefit analysis of the newly developed fertilisation strategies in comparison with conventional practice. Further scenario and simulation analyses of representative approaches were conducted to depict the economic efficiency under varying (world) market and price conditions to approve their economic viability and sustainability (WP09). Perspectives for patenting, registration, and international marketing of novel BE products in different countries were investigated and developed in close cooperation of all contributing project partners. Training activities comprised organisation of information events on application perspectives for BE products for extension service and farmers within public field days, as well as student workshops, summer schools, master and bachelor programs on BE research. A public data base was installed to collect information on commercially available BE products, application modes and targets as well as evaluations in the scientific literature as an information guide for farmers and scientists and as a platform for producers of bio-effectors to present products with a proven record of efficiency (WP10).

Scientific and administrative project management

The management of BIOFECTOR was the main task of the scientific and administrative Project Office, i.e. the coordinator and his administrative assistant at the University of Hohenheim and CMAST. The coordinator was concerned with the scientific management and the co-ordination of all research activities and ensured the smooth operation of the project and guaranteed that all efforts were focused towards the objectives. Together with the project office at CMAST, the coordinator was also responsible for administrative, financial and contractual management and the organisational co-ordination of the project activities; in this capacity, coordinator and CMAST fulfilled all administrative tasks, including submission of required progress reports, deliverables, financial statements to the European Commission, oversight of proper use and distribution of funds.

Each beneficiary in BIOFECTOR sent one representative to the General Assembly. This body was in charge of the political and strategic orientation of the project. Tasks included oversight of the rights and obligations in accordance with the contractual framework of the project and the Consortium Agreement; decision upon withdrawal, inclusion and exclusion of Participants to the project; preliminary decisions on the amendment of the Consortium Agreement (subject to ratification by the authorised legal representatives); oversight of standard operation procedure as well as of dissemination of foreground and IPR; approval of provisional budgets, discuss and approve the annual executive budget and cost claims prepared by the Steering Committee including the reimbursements to the Participants. The General Assembly met once a year unless the interest of the project required intermediate meetings.

The Steering Committee consisted of one representative per work package as well as the Coordinator; guests were invited upon necessity. It monitored the overall progress towards the global objectives of the proposal, and coordinated issues related to the project in general and issues affecting more than one WP. The Steering Committee received and approved interim progress reports and proposals from the WP leads, and prepared issues to be decided by the General Assembly. Moreover, the Steering Committee benchmarked the achievement of deliverables and milestones within each work package, defined action plans and proposed budget re-allocations. The Steering Committee met every six months during the funding period, either in person by telephone conference.

Furthermore, a scientific advisory board was implemented to ensure a high standard of research; it monitored the progress of the project by taking part in the annual project meetings.

1.3 Description of the main S&T results/foregrounds

This section summarizes the major output and the scientific findings within the different working areas of the project, covered by the work packages WP01-WP08.

WP01: Product development

The general aim of WP01 was the supply of the project partners with promising bio-effectors in readily testable product formulations and further improvement according to the requirements of the investigated applications.

1.3.1 Pre-selection, test and production of microbial BE products with nutrient-mobilizing and root growth-stimulating properties.

While intensive greenhouse and field testing of 13 fungal and bacterial BE isolates with Ca-P solubilizing potential in 8 countries with 4 crops failed to show any significant effects on P mobilizaton and nutrient acquisition of soil-grown plants (Lekfeldt et al. 2016; Thonar et al. 2017), it was demonstrated that combined application with ammonium fertilisers, stabilized with the nitrification inhibitor DMPP (3,4-dimethylpyrazolphosphate) was able to activate the plant growth-promoting potential of the inoculants. This was related with synergistic effects on ammonium-induced rhizosphere acidification, increased root surface area by ammonium-induced promotion of root hair development and microbial root growth stimulation, associated with an increased auxin production potential of the host plant and the bacterial inoculants. Superior performance of BE combinations with stabilized ammonium fertilisers was also confirmed in the BIOFECTOR Meta-analysis (Fig. 1.1). Obviously, N fertilisation management can be employed as a tool to improve the efficiency of plant-BE interactions. These findings resulted in a joint patent application with EUROCHEM Agro GmbH (Mannheim, Germany) as license holder for the nitrification inhibitor DMPP, submitted in April 2017 to the European Patent Office (EPO) with the title "*Method and composition for improving nutrient acquisition of plants*".(No 17167762B-1375).

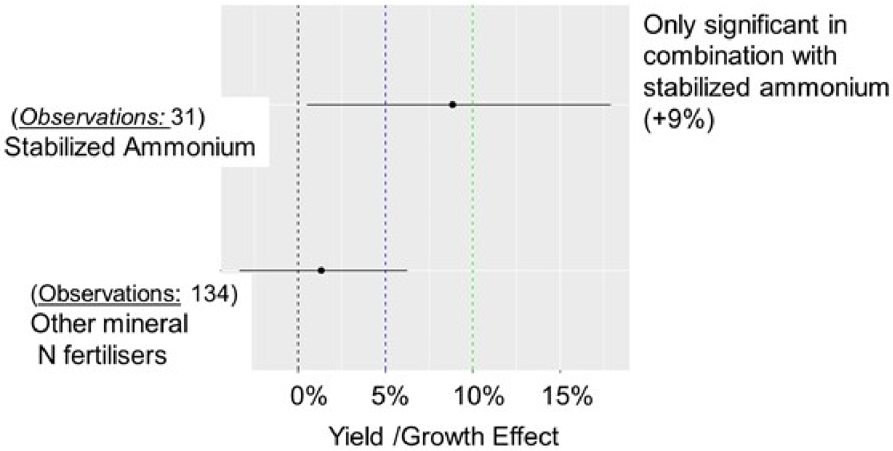


Figure 1.1: Yield/plant growth effects of BE applications depending on the type of mineral N fertilizers added in the experiment. The number inside the brackets represents the number of observations included; the dashed vertical zero line indicates no difference between BE and non-inoculated control treatment, the points indicate the mean effect while the horizontal line represents the 95% confidence interval (CI). If CI lines cross the zero line, effects are not significant.

Novel microbial strains with significant plant and root growth-promoting potential, characterized within joint WP01, WP02 and WP03 activities have been formulated and are ready for large scale production and market introduction: *Bacillus atrophaeus* Abi05 (ABI), *Pseudomonas jessenii* Ru47 (SP, JKI) and a combination product based on *Trichoderma harzianum* OMG16 and Bacillus amyloliquefaciens FZB42 in a formulation containing Zn and Mn as stress protectants (CombiFect-B, AUAS). Two further patent applications ("*Applications of a mycorrhizal fungus of the genus Trichoderma for increasing the efficiency of nutrient acquisition and improving stress tolerance of crop plants*") are scheduled for submission in October 2017 (AUAS).

1.3.2 Pre-selection and production of plant growth-promoting microorganisms with tolerance to low temperature, drought and high salt concentrations

A patent describing a "*bio-formulation of PGPRs growing under extreme conditions (low temperature, drought, salinity*)" was registered by the EPO under PCT DE 2016/000159. Synergistic effects of stabilized ammonium fertilization or micronutrient (Zn/Mn) starter treatments on the stress (chilling/drought)-protecting and plant growth-promoting potential of BEs have been identified within WP02 and these findings were included in the EPO patent application No 17167762B-1375 and in the development of a novel combination product CombiFectB. Partners in Italy isolated the *Azotobacter chroococcum* strain A76, which increased tomato growth and fruit yield under salt stress (50 and 100 mM NaCl) and improved wheat growth under drought conditions with reduced N-input. The strain is ready for large scale production. Similar effects were reported for the commercial *Trichoderma harzianum* strain T22.

1.3.3 Manufacturing and characterization of natural extraction products

A drought and salt stress-protective potential has been identified for the seaweed extract products “Superfifty”, Rygex and Algavyt-ZnMn in wheat, tomato and maize. Cold-stress-protective effects were recorded for “Superfifty, Algavyt-ZnMn, and the plant extract Manek in wheat and maize. The underlying modes of action have been characterized at the transcriptional and metabolic level. Humic acids preparations extracted from artichoke compost, repeatedly showed synergistic plant growth-promoting effects with various Bacillus, Pseudomonas and *Trichoderma* strains investigated within WP03.

Table 1.1: Novel bio-effector products developed during the BIOFECTOR project duration

Bio-effector	Reported effects	Application mode	Stage of Marketing
Bacillus atrophaeus, B. simplex strains (Combi product ABI)	Plant growth promotion, stress resistance	Seed dressing, soil incorporation, placement	Patented
B. amyloliquefaciens FZB42 + Trichoderma harzianum OMG16 +Zn/Mn (AUAS/ABI)	Plant growth promotion Cold/drought stress resistance	Soil incorporation, placement	Ready for patenting
Trichoderma harzianum OMG16 (AUAS)	Plant growth promotion Stress resistance	Soil incorporation, placement	Ready for patenting
*Pseudomonas jesseni*i RU47 (JKI, SP)	Plant growth promotion, P mobilization, Biocontrol	Seed dressing, Soil incorporation	Ready for large scale production
Azotobacter chroococcum A76 (UNINAa)	Salinity/drought resistance	Soil incorporation	Ready for large scale production
Algavyt ZnMn. Seaweed extract combi product (AGRIGES)	Cold/drought resistance	Soil incorporation, foliar spray	Commercial product. Novel application perspectives in agriculture
Manek: Plant extract combi - product (AGRIGES)	Cold/drought resistance	Foliar spray	Commercial product: Novel application perspectives in agriculture
Superfifty (BIOAT) Seaweed extract	Cold/drought resistance	Foliar spray	Commercial product: Improved formulation
DMPP-Ammonium + microbial BEs (UHOHa EurochemAgro)	P mobilization Plant growth promotion# Cold/drought stress resistance	Soil incorporation, placement	Patented
DMPP-Ammonium + non-microbial BEs (UHOHa)	Plant growth promotion Cold/drought stress resistance	Soil incorporation, placement foliar spray	Ready for large scale production

WP02: Synergisms and Product Combinations

Activities in WP02 focussed on the development of novel BE products, based on combined or synergistic interactions of single BEs identified within WP01.

2.1 Identification of functional combinations of promising BEs based on the strain and product collections already available from WP01 partners tested in WP03-WP07.

Microbial and non-microbial BE combinations, as well as BEs combined with selected fertilizers (stabilized ammonium, micronutrients: Zn/Mn) have been tested in greenhouse and field experiments in comparison with a set of pre-selected commercial standard BE products (BE1-4). The standard BEs were based on strains of Trichoderma (*Trianum-P*), *Pseudomonas* (*Proradix*), *Bacillus* (*Rhizovital42*), and the *Ascophyllum nodosum* seaweed extract "*Superfifty*", representative for important groups of bio-effectors. Competitive survival was tested in combination with

(i) newly isolated BEs, such as *Trichoderma* strains and complex bacterial formulations (BioRex) containing different Bacilli and N-fixing bacteria,
(ii) arbuscular mycorrhizal fungi, with *T.harzianum* and *T.virens* combined with Gram-positive (Bacilli) and Gram-negative (Pseudomonades) bacteria.
(iii) different Bacilli such as *B.simplex*, *B. atrophaeus*.
(iv) combined inoculations of *Trichoderma* (BE1) and *Bacillus* (BE3) or triple inoculations with *Trichoderma* (BE1, *Bacillus* (BE3) and *Pseudomonas* (BE2) in pot experiments with maize, tomato and wheat on P-limited soils.
(v) Non-microbial BEs in combination with living BEs were tested with seaweed extracts, humic acids and fungal cell wall preparations as lipo-chitooligosaccharides from *Sebacinales* fungi.

2.2 Characterization of the conditions (e.g. formulation, environmental factors, etc.) required for the best performance of most promising bio-effector combinations

Compatible combinations of bio-effectors have been identified, selected and favourable conditions regarding product formulations, application methods and abiotic factors influencing performance have been characterized. With regard to production methods spray-drying of fungal conidiospores and freeze-drying of bacterial endospores have been selected as most effective and economic procedures, also considering product stability and shelf life. As favourable application method, spraying and soil incorporation of the re-suspended dry formulation or a more economic direct application into the seeding furrow (targeted application) have been identified.

A bio-formulation containing a consortium of selected Bacillus strains with tolerance against low temperature, drought and salinity and growth-promoting properties demonstrated in pot and field experiments in maize was patented in 2017.

2.3 Synergistic interactions of BEs with fertiliser components.

Two novel combination products CombiFect (CFA and CFB) developed within WP01/WP02, based on the Trichoderma stain OMG16 combined with different strains of Bacillus, have been intensively tested within WP04-08, with superior performance of CFA in tomato and similar efficiency of CFA and CFB in maize. The preferential form of nitrogen fertilization (nitrate vs ammonium) and micronutrients such as Zn and Mn have been discovered as major nutritional factors, determining synergistic interactions with microbial BEs. Accordingly, CFA in combination with stabilized ammonium fertilization was particularly effective in supporting rock phosphate acquisition in maize. Also cold and drought stress-protective properties especially in maize, have been proven and efficacy was further enhanced by combination with micronutrients (Zn,Mn) and stabilized ammonium fertilisation. Similar synergistic effects with ammonium and Zn/Mn fertilisation were recorded also for non-microbial BEs such as seaweed extracts (Superfifty, Algavyt +Zn/Mn, compost extracts and fermentation products, as cold and drought stress protectants in maize.

Production and formulation procedures are straightforward and handling recommendations easy to follow. Products are compatible with conventional farming machinery and are currently tested in on-farm experiments. The products improved growth of maize and tomato in model experiments and first field trials with clear synergistic effects due to the combinations and their single components. Patenting and marketing of the newly developed combination products was recently initiated. Superior performance of combination products has been identified also in the BIOFECTOR metastudy (Fig. 2.1).

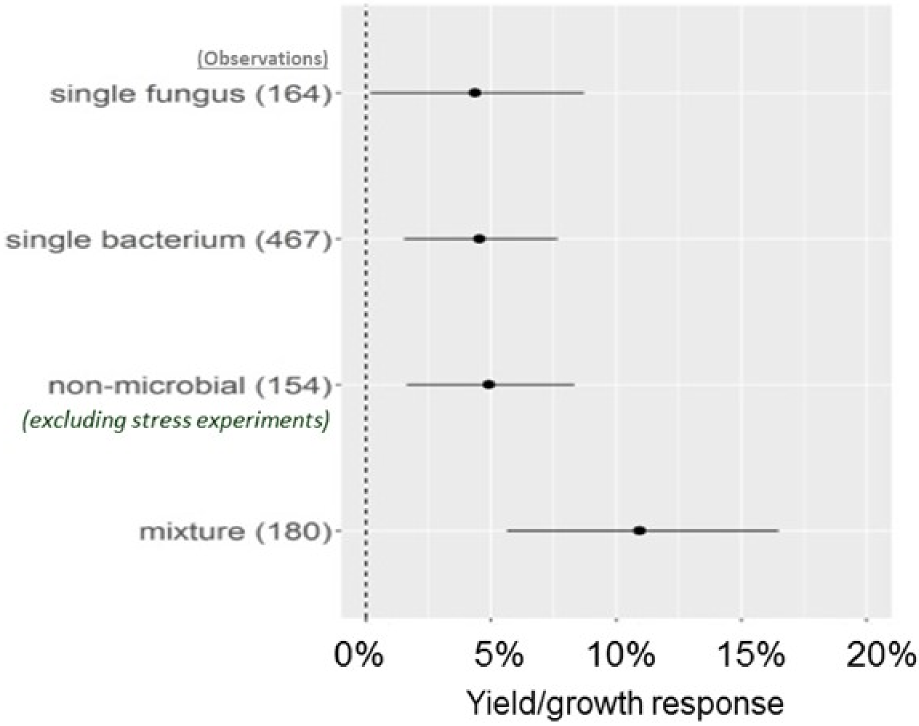


Figure 2.1: The effect of single or combination product (mixture) application on the mean effect of BEs on plant growth/yield. A total of 965 observations from BIOFECTOR pot and field experiments were included into the analysis. The number inside the brackets represent the number of observations included, the point indicates the mean effect, while the horizontal line represents the 95%confidence interval. If CI lines cross the zero line, effects are not significant.

WP03: Functional Mechanisms

Activities in WP03 were targeted to (i) tracing the fate of microbial BEs in the rhizosphere, (ii) identification of physiological and molecular targets for plant-BE interactions and (iii) characterizing their interactions with other rhizosphere organisms.

3.1 Tool development: Standardisation of new or already existing methodologies for the purposes of: (i) for measuring effects of bio-effectors on plants, (ii) for determining the most important sites for colonization of BE strains, and (iii) for assessing their potential impacts on plant-associated microbial communities.

Research efforts within WP3 were mainly focussed on a set of model experiments conducted on field soils under greenhouse conditions. Seven participating partners contributed interdisciplinary approaches and concepts to unravel working mechanisms of plant-BE interactions with respect to plant adaptations to abiotic stress (chilling, drought) and nutrient (P) limitation. Tomato and Maize and Arabidopsis thaliana were chosen as model plants. Microbial strains with proven plant growth-promoting potential, representing important groups of PGPMs (*Trichoderma harzianum* T22 and OMG16, *Pseudomonas* sp. DSMZ 13134, *Bacillus*. *amyloliquefaciens* FZB42, *Pseudomonas*. *jessenii* RU47 were selected as microbial BEs, while non-microbial BEs were represented by selected seaweed extracts (Superfifty, Algavyt+Zn/Mn), plant and compost extracts (Manek). BE effects were determined at the plant transcriptional and metabolic level. Root colonization of microbial BE strains was studied in the rhizosphere and inside the plant tissues (endosphere). Impact of BEs on bacterial community composition and rhizosphere processes were investigated by metagenomics, characterization of soil marker enzyme activities and expression of functional genes in the rhizosphere.

3.2 Describing effects of BEs on plant performance; physiological and molecular responses to BE application, P acquisition, root exudation and resistances against stresses and diseases

Transcriptome and metabolome analysis of soil-grown maize seedlings inoculated with selected microbial BEs (*Pseudomonas* sp.”Proradix”; *Bacillus amyloliquefaciens* “FZB42”), just prior to the appearance of visible effects on plant growth (14 days after sowing), revealed significant upregulation of genes related with stress adaptation: stress hormones (ethylene, salicylic acid jasmonic acid); secondary metabolism (phenylpropanoids, lignin biosynthesis) and oxidative stress-related genes. This was confirmed also by metabolic investigations. The results showed striking similarities with the gene expression patterns reported for application of non-microbial BEs based on plant and seaweed extracts (Manek, Superfifty) in Arabidopsis, leading to the hypothesis that priming of stress adaptations is a common primary plant response, both to application of microbial, as well as non-microbial BEs (Fig. 1). More specifically, certain BEs selectively increased root growth and rhizosphere phosphatases, the production of phenolic antioxidants or hormones, such as Indole acetic acid, gibberellic acid, abscisic acid and jasmonic acid. Combination with selected fertilizers, such as stabilized ammonium or Zn/Mn was a measure for further synergistic activation of the adaptive stress responses.

3.3 Demonstration of preferred sites for colonization of BE strains in plants and interactions with soil-microbial communities

Plant root colonization by microbial BEs was identified as a key factor for plant growth promotion, with a clear preference for rhizosphere instead of endosphere colonization. Investigations on four different soils revealed clearly significant but transient effects of the inoculants on rhizosphere bacterial communities but not on endosphere bacteria. Similarly, there was no indication for BE effects on root colonization by native populations of endo-mycorrhizal fungi. However, *B. amyloliquefaciens* FZB42 clearly stimulated the endophytic root colonization by *Trichoderma harzianum* OMG16 in tomato and maize.

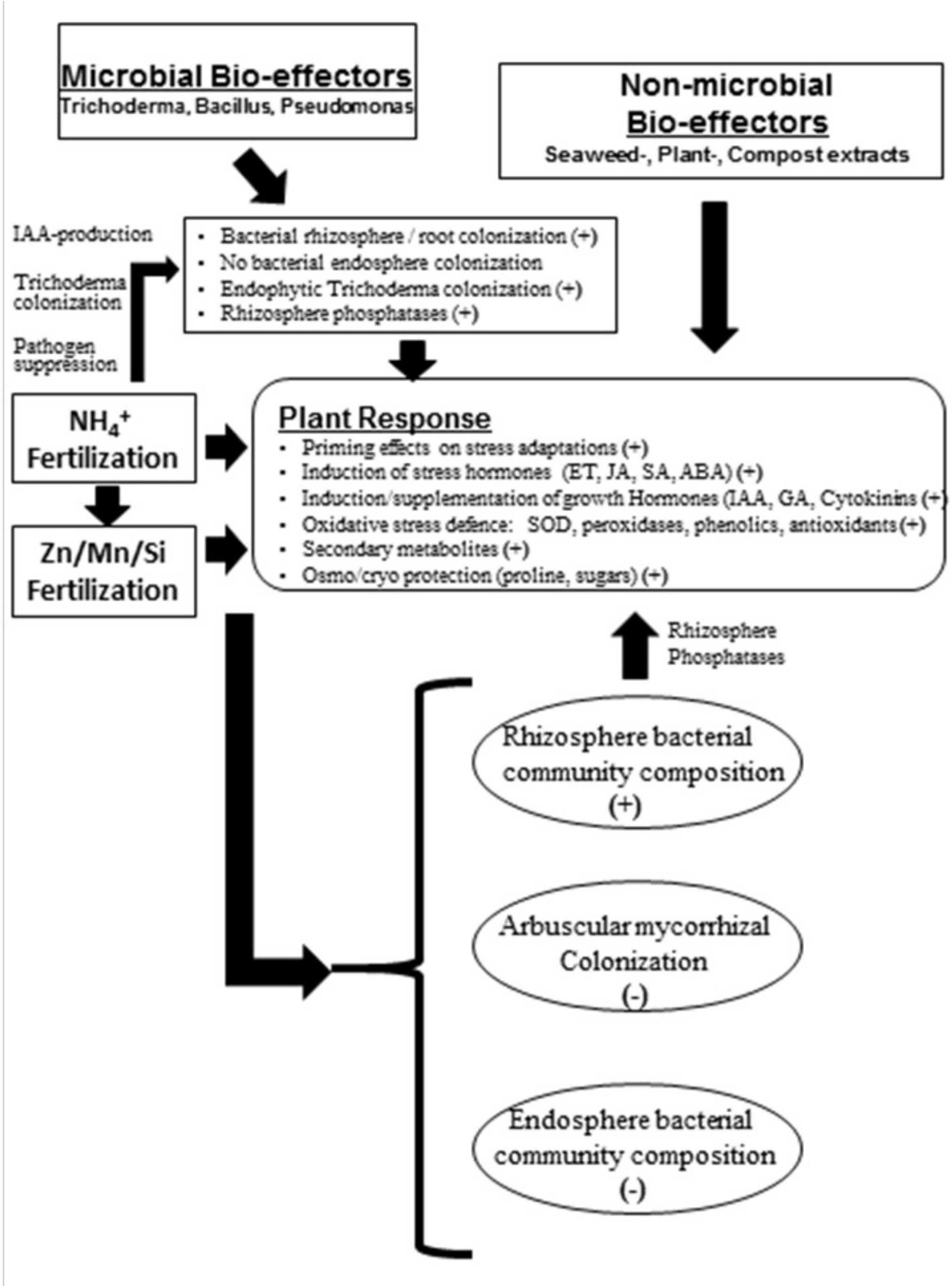
In general, effects of different soil types or the plant developmental stage on bacterial community structures were more intensively expressed than the transient effects of BE inoculations, indicating a comparatively low risk for long-term interferences with soil microbial communities.

Effects on rhizosphere processes involved in nutrient cycling were mainly detected for phosphatases involved in P mineralization. Inoculation with living or heat-inactivated populations of *Pseudomonas jessenii* RU47 similarly increased plant growth in P-limited tomato, as well as the activities of rhizosphere alkaline phosphatases of bacterial origin. These findings suggest indirect effects on P mineralization, mediated by shifts in the microbial community structure in response to RU47 inoculation in response to the input of easily soluble organic P applied with the inoculation of dead bacterial cells. As a consequence, P availability for the host plant may be increased, which is further improved by strong root-growth promoting effects of RU47.

The effects on P turnover seem to be mainly restricted to the rhizosphere. Accordingly, there was no indication for a generally improved availability of native soil P sources by BE inoculation on low P soils, even in cases of high soil-organic matter contents, since in these cases organic P fractions are frequently dominated by insoluble forms such as phytate, not easily available for enzymatic hydrolysis. However, a promotion of P acquisition from organic P fertilizers, providing high levels of soluble organic P seems to be likely (seeWP05 Report).

The various interactions between fertilizers, BEs and the target plants identified in WP03 are summarized schematically in Figure 3.1.

Figure 3. 1: Schematic overview on interactions detected between fertilizers, microbial and non-microbial BEs and target plants detected in the framework of WP03 activities.



WP04: Abiotic Stress

WP04 aimed to assess and specifically adapt selected BEs for improved tolerance of crop plants against abiotic stress conditions, such as drought, low root zone temperatures and high salt concentrations, thus facilitating agricultural production under difficult soil and climate conditions.

4.1 Target drought stress: Identification of BEs that can reduce mineral fertilization under drought –*Trichoderma harzianum* T22 has been identified as a stress-tolerant strain that increased durum wheat biomass in a pot experiment under optimal water availability (+22% DM) and under 50% reduction of water supply (+40%DM), demonstrating an effect as growth enhancer and stress protectant at moderate drought. Moreover, *Trichoderma* T22 also increased wheat biomass relative to control in the absence of N fertilization (+25% DM).

In model experiments with maize, foliar application of selected seaweed- (AlgavytZn/Mn; Super-Fifty), plant-(Manek) and compost extracts (compost tea) was able to reduce drought stress-induced leaf damage (chlorosis, necrosis, wilting after two weeks 50% water supply) particularly in combination with stabilized ammonium fertilization.

4.2 Target salt stress: Identification of BEs that can reduce mineral fertilization under salt stress – A stress-tolerant bacterial strain **Azotobacter chroococcum** 76A (UNINAa) was isolated in Southern Italy. In a model system, tomato roots were inoculated with **A. chroococcum** 76A under reduced nutrient input and 50mM NaCl stress. **A. chroococcum** 76A treatment was able to increase fruit yield by 40% in terms of fresh weight and by 35% in terms of fruit number. Therefore, inoculation with **A. chroococcum** 76A may offer a solution for low-input systems where environmental constraints and limited chemical nutrients for fertilization may affect the potential yield and counteract detrimental effects of increasing irrigation water salinization, as an increasing problem in Mediterranean countries.

Also, algal derivatives could serve as stress protectants in saline soils. Seaweed extracts (Rygex and SuperFifty) provided via irrigation in a greenhouse experiment did not preserve tomato growth and yield under salinization, but they enhanced the accumulation of minerals, antioxidants and essential amino acids in tomato fruits, with an overall improvement of their nutritional value. Field experiments with a tomato-wheat rotation under production conditions in Southern Italy (saline irrigation for tomato; residual salinity for wheat) revealed that application of SuperFifty increased fruit yield in non-stressed tomato plants (+47% total fruit fresh weight vs. control). Fruit number was enhanced by 29% compared to the untreated plants. Wheat plant height under residual salinity was increased by 11% and 8% with Rygex and SuperFifty treatments, respectively as compared to the untreated control.

4.3 Target cold stress: Identification of BEs that can improve root growth and reduce mineral fertilization under cold stress - Selected seaweed extracts and fermentation products rich in Zn, Mn and Si exerted cold-protective effects in maize, provided that the application was performed during seed imbibition. Stimulation of root growth, detoxification of reactive oxygen species, accumulation of protective solutes, improved micronutrient acquisition and normalization of disturbed hormonal balances were identified as common modes of action. Field testing with early sowing during mid of April, to provoke chilling stress revealed a significant average yield increase by 17.6% in three out of four field experiments.

Perspectives to exploit potential synergisms of fertilization strategies and stress-protective BEs have been investigated. A patent application for a superior combination product with Zn/Mn and selected strains of Bacillus and Trichoderma (CombiFect-B) is currently under preparation and the combination with stabilized ammonium fertilization is part of a submitted patent application. Extension to other crops has been performed in an external cooperation with oilseed rape breeders in Germany (Rapool Ring, Germany) with a first commercial application introduced for rape seed dressing introduced in 2016 and extended as a standard treatment for the whole portfolio of hybrid seed production in 2017.

Approaches to improve winter hardness of cereals have been investigated in model and field experiments in Germany and Northern Ireland, using foliar applications of selected seaweed and plant extracts (SuperFifty, Manek). Between 2015 and 2016 three field experiments in Northern Ireland and Germany revealed an average yield increase of 16%.

WP05: Organic Farming and WP06: Recycling Fertilizers

Activities within WP05 addressed the selection and specific adaptation of BEs for the amelioration of insufficient availability of mineral nutrients under organic and low-input farming conditions. WP05 was closely interlinked with WP06, focused on selection and adaptation of BEs for the efficient exploitation of mineral nutrients contained in organic and industrial recycling products, frequently used as alternative fertilizers in organic farming systems.

5.1 Selection of promising BE products

The application of bio-effectors (BEs) for organic and low input farming systems has been assessed by nine WP05/WP06 partners in seven countries, conducting about 40 experiments by testing a range of different BEs and organic and inorganic recycling fertilizers on soils with different characteristics typical for European agro-ecosystems. During the first two years, model experiments have been conducted with maize and wheat, following previously elaborated standard guidelines for pot experiments to enable a common data analysis. Three commercial microbial strains, pre-selected as representative standard BEs for important groups of PGPMs, derived from the genera *Trichoderma*, *Bacillus* and *Pseudomonas* (BE1-3), were used as inoculants.

5.2 Assessment of the potential efficacy of BEs in combination with organic amendments based on recycling products, rock phosphate and mineral recycling fertilizers to promote plant growth and nutrient uptake in various soils of different European regions.

Experiments have demonstrated the efficiency of the two bacterial products Proradix (BE2, Pseudomonas) and RhizoVital (BE3, Bacillus) in combination with organic recycling fertilizers, such as composted animal manures, fresh digestates of organic wastes and sewage sludge on the growth of maize plants. Across all soils and fertilizers Proradix (BE2) and RhizoVital (BE3) increased maize shoot biomass by 14% and 13%, respectively, but only by 6.9 % with Trianum-P (BE1, *Trichoderma*) with a clear preference for BE combinations with manure composts (Thonar et al. 2017). Similarly, joint pot experiments were conducted with spring wheat, including also inorganic recycling fertilizers based on slugs and ashes. However, inoculation with BEs did not result in increased growth or P uptake in combination, neither with any of the tested recycled fertilizers nor with rock-P or native soil P sources (Lekfeldt et al. 2016). Interestingly, a perspective for improved utilization of sparingly soluble inorganic P fertilisers, such as ashes or rock phosphates, was indicated by combining microbial BEs with a stabilized ammonium fertilisation. Although this is not an option for organic farming systems, it may promote the use of recycling fertilisers also in conventional farming.

5.3 Development of innovative plant nutrition strategies to improve plant growth and nutrient acquisition in organic and low-input system and enhance the nutrient use efficiency of organic and sparingly available mineral fertilizers

In order to test promising BEs, as identified within the model experiments, a total of 14 field experiments growing maize or tomatoes on various European soils have been conducted, following the guidelines for field trials as elaborated by the project partners. Most promising results have been obtained in experiments conducted with tomato in Romania and Hungary with a positive effect on shoot growth and tomato yield and quality after BE inoculation when fertilizers based on manures and animal waste recycling products (blood/horn meal) were applied. Similar, positive effects of BEs on maize growth performance under field conditions were observed in Italy on alkaline soils, in Switzerland on a neutral soil and in Romania (maize and wheat: WP08/09 report) on moderately acidic soils when manure-based fertilizers were applied, although the effects were less pronounced than in tomato.

In WP04, different options of BE combinations with micronutrients (Zn/Mn) and stabilized ammonium-fertilization to improve tolerance against chilling and drought stress have been investigated. In this context, also alternative options suitable for applications in organic farming systems were investigated. For improving cold tolerance during early growth of maize, starter fertigation with micronutrient-rich seaweed extracts (AlgavytZn/Mn; Algafect) but also seed treatments with silicic acid have been identified as promising approaches (Bradacova et al. 2016), while foliar application of plant and seaweed extracts (Manek, Superfifty) increased cold-hardness of winter wheat, both, in pot and field experiments. First field experiments in Southern Italy suggest also an option for seaweed extracts (Superfifty, Rygex) on salt-, and drought-affected soils.

Similar to the evaluation of single experiments, also the BIOFECTOR meta-study confirmed best efficacy of microbial BEs in combination with manure-based fertilizers (Fig.5.1A). By contrast, no significant solubilizing potential of the tested microbial BEs was detected for Rock-P or other sparingly soluble mineral fertilizers, and root-growth promotion was identified as a major mode of action for BE-induced nutrient acquisition under these conditions; (Lekfeldt et al., 2016; Thonar et al. 2017). Accordingly, the BIOFECTOR meta-study revealed the most pronounced BE effects at moderate availability of soluble P sources, easily acquired by improved root growth, and not on soils with low levels of soluble P (Fig. 5.1B). These findings were confirmed by an additional meta-analysis, covering 169 published field studies in the scientific literature. The respective study further indicated that BEs were more effective (i) at low levels of soil organic matter compared to soils with a high soil organic matter content and ii) in dry climates over other climatic regions.

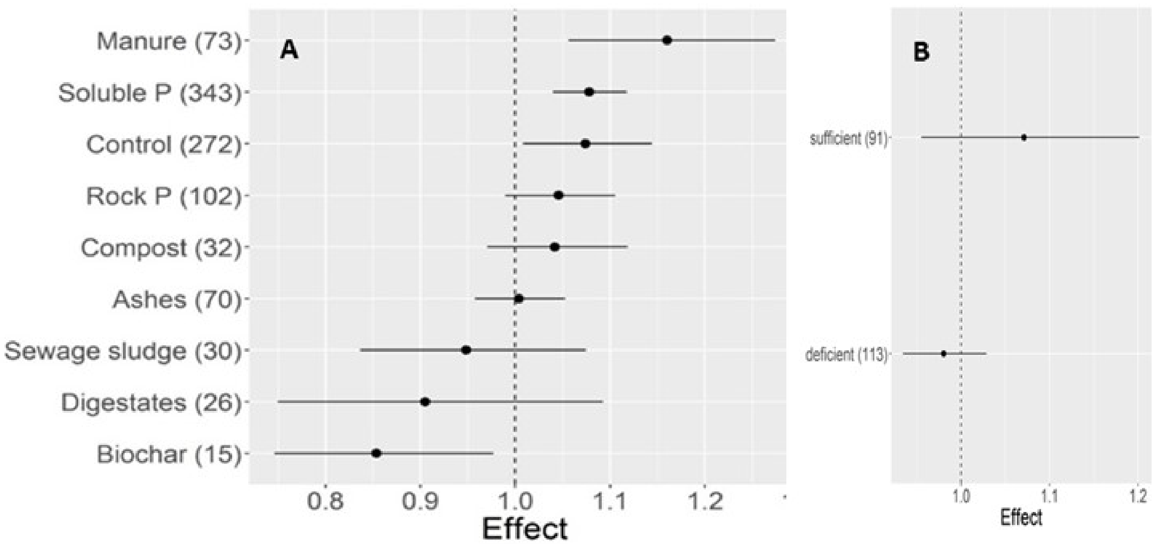


Figure 5.1: Effect of BE applications as a function of the type of P fertilizer added in the experiment (A) or the level of soil P availability (B) on either, grain, fruit or shoot dry matter. The number inside the brackets represents the number of observations included; the dashed vertical line indicates no difference between BE and non-inoculated control treatment; the points indicate the mean effect while the horizontal line represents the 95% confidence interval (CI). If CI lines cross the vertical line, effects are not significant.

WP07: Fertilizer Placement and Fertigation

WP07 investigated perspectives to adapt selected BEs for combination with fertilizer placement and fertigation technologies towards a more efficient use and reduction of fertiliser inputs. An extended literature survey, including a general review on the use of bio-stimulants to enhance nutrient uptake in crops (Halpern et al. 2015).and a meta-study on the efficiency of fertilizer placement strategies in agricultural practice (Nkebiwe et al., 2016) provided the scientific background for the WP07 activities.

7.1 Compatibility of BEs with placement fertilizers

As a next step, compatibility tests of ammonium-based fertilizers used for fertilizer placement were performed with 11 microbial BE strains, belonging to the genera Trichoderma, Bacillus and *Pseudomonas* (as representative groups of plant growth-promoting microorganisms widely used in practical applications) and revealed tolerance even to high ammonium concentrations up to 50-250 mM as a prerequisite for a combined BE application close to fertilizer hotspots, frequently based on ammonium sulphate or ammonium phosphates.

7.2 Spatial control of the rhizosphere (development of “rhizosphere hotspots”) by placed application of fertilisers, fertiliser depots and drip fertigation techniques. Establishment of bio-effectors in the rhizosphere hotspots.

Pot and rhizobox experiments with maize and wheat revealed that placement of ammonium sulphate fertiliser depots stimulated the formation of “rhizosphere hotspots” (zones of intensive root growth), which was further promoted by inoculation with microbial BEs (*Pseudomonas* sp. *Proradix*; *Bacillus amyloliquefaciens* FZB42, *Trichoderma harzianum* T22 and *Paenibacillus mucilagenosus*). This effect contributed to improved acquisition of native soil P and sparingly soluble P sources, such as rock phosphates on low P soils (Nkebiwe et al. 2017). Although the responses were most pronounced when the BEs were present in root zones close to the fertilizer depot, significant BE-induced root growth stimulation in the depot zone was detectable even in cases of preferential BE root colonization in more basal parts of the root system in a larger distance from the depot. These findings suggest a promotion of rhizosphere hot spots also by systemic effects of BE inoculation.

The effects of BE applications in fertigation systems in response to different levels of P application were tested in greenhouse experiments with tomato. Microbial BEs increased P uptake and vegetative plant growth (Proradix), fruit biomass (FZB42) and similar effects were recorded also for the sea weed extract “Superfifty”.

7.3 Evaluation of BE applications combined with placed fertilisation and fertigation under a wide range of field conditions.

Improved establishment of rhizosphere hotspots around fertiliser depots by BE (Proradix) inoculation was demonstrated in maize also under field conditions (Fig. 7.1A), using localized application of stabilized ammonium fertilizers but also with an ammonium-rich organic pellet fertilizer, based on poultry manure.

In 2016/17, a final set of field experiments was conducted with maize and tomato. Stabilized ammonium fertilisation and underfoot-placement of di-ammonium phosphate combined with, micronutrients (Zn/Mn), silicon and various microbial and non-microbial BEs in silo maize revealed significantly improved emergence particularly for Si and Zn/Mn seed treatments after exposure to a cold period in April. Final biomass was significantly increased by Si, Zn/Mn, the cold-resistant *Bacillus atrophaeus* strain ABI02 and the Zn/Mn-rich seaweed extract “Algavyt+ZnMn”.

Similarly, biomass yield of silo maize was increased by 8.5-19.7% after combined application of *Trichoderma harzianum* OMG16, supplied with an ammonium-rich biogas-digestate depot fertilization in comparison with untreated digestate or digestate stabilized with the nitrification inhibitor Piadin.

A drip-irrigated field experiment was conducted in the Negev desert in Israel on a sandy low-P soil pH 7.9, with tomato, supplied with underfoot band placement of DCD-stabilized ammonium sulphate, three levels of underfoot P fertilization and inoculation with two microbial single-strain BEs (Proradix, FZB42) or two combination products (CombiFectB and HYT-A). Superior performance in terms of plant biomass production and final yield was recorded for the combination product HYT-A (25 microbial strains + seaweed extract + micronutrients), but only in combination with low P supply (Fig.7.1B).

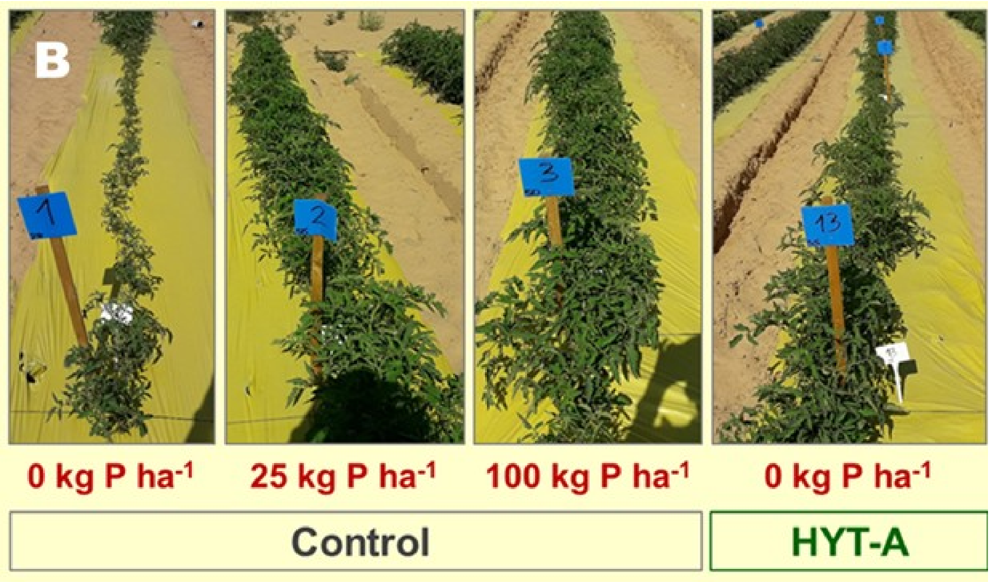


Figure 7.1 (A): BE-assisted formation of rhizosphere hot spots in field-grown maize plants, induced by ammonium sulphate placement. (B) Stimulation of plant growth by inoculation with the microbial consortium product HYT-A of drip-irrigated tomato on a low P soil with underfoot band placement of stabilized ammonium sulphate (Negev, Israel).

Taken together, the WP07 findings suggest promising perspectives for BE-assisted-fertilizer placement strategies towards improved stress tolerance and nutrient acquisition of crops with positive yield effects in four out of six field experiments, although the most suitable application conditions still need to be defined more clearly.

WP08: BIOFECTOR-International-Field-Testing Network

The major focus of the activities within the International Field-Testing Network of WP08 was the final evaluation of the most promising approaches for the development of alternative plant nutrition strategies that have been identified in WP01-WP07 under real production conditions and in different geo-climatic situations.

i. In particular, these approaches comprised
   - (i) the **combination** of microbial BEs with stabilized ammonium fertilizers and sparingly soluble phosphate fertilizers (WP01, WP02) and **BE combinations** with organic recycling fertilizers (WP05, WP06).
   - (ii) **Combination** of different BEs with complementary properties in mixed products (WP01, WP02, WP03).
   - (iii) development of cost-efficient and effective application strategies for the agricultural crops wheat and maize. In this regard, **product saving placement strategies. such as banding or seed treatments** for the application of microbial bio-effectors and fertilizers were investigated in maize and tomato (WP01, WP07), whereas in wheat crops **foliar spray applications** of non-microbial bio-effectors were tested (WP03, WP04).
   - (iv) Especially under **stress conditions**, such as low temperatures or drought, seed treatments with micronutrients (manganese, zinc) or silicates in maize and foliar

spray applications of plant or seaweed extracts in wheat were considered as viable strategies based on the results from previous pot and field experiments.

Field and greenhouse production experiments to test the agronomic effectiveness and economic value of bio-effector application in alternative plant nutrition strategies under practice conditions have been conducted with tomato, wheat and maize.

8.1 Combination strategies

The most consistent improvements in crop yield and quality were achieved in tomato production under greenhouse conditions in Romania, where microbial bio-effectors were combined with manure-based organic fertilization strategies. Investigations within WP03 suggest that different microbial agents may promote plant growth by increasing resistance against stress factors associated with manure based fertilisation regimes. The microbial inoculants may also contribute to improved utilization of soluble organic P sources supplied with the organic fertilisers by increasing the potential for P mineralisation, mediated increased activities of rhizosphere phosphatases and/or microbial root growth promotion. In this context, the relative contribution of direct BE effects or indirect effects via interactions with rhizosphere microbiomes, remains to be elucidated.

Critical for the success of microbial BE treatments in tomato appeared to be that first application was performed at BBCH 12 when the 2nd leaf on main shoot was unfolded, which is associated with increasing photosynthetic activity, release of root exudates and root development, which is frequently not optimal during the early stages of seedling development. When this application scheme was adapted, the yield potential of organic greenhouse tomato production (approx. 150 kg ha-1) could be reached even at 70% of the standard fertilizer input. Also in a field production system in Hungary significant improvements in tomato yield and quality could be achieved in combination with a commercial organic recycling fertiliser based on animal waste products (blood meal/bone meal) plant residues and seaweed.

In a field experiment conducted in Israel with a drip-fertigated tomato production system on a low P soil in the Negev desert, combined with underfoot band placement of stabilized ammonium sulphate and superphosphate, particularly the inoculation with a microbial combination product (HYTA) significantly increased shoot biomass production with a clear trend for increased yield in the treatment with low P supply. This demonstrates that the combination with organic fertilizers is not necessarily a critical factor for the effectiveness of the microbial agents. Accordingly, strong growth-promoting effects were also found in combination with a slow release compound fertilizer based on stabilized ammonium (DuraTec® Starter). Generally, a common feature of the most successful BE-fertiliser combinations identified within the project, was a comparatively high ammonium availability, - either directly or indirect via mineralization of organic N sources, which has been identified as strong promoting factor for plant-BE interactions within WP01, WP02 and WP03 activities. In WP06, the combination with stabilized ammonium fertilizers was recognized as a promising strategy to support the effectiveness of microbial agents in stimulating root growth and activity or mobilizing sparingly available mineral nutrients from native soil sources or mineral recycling fertilizers based on slags and ashes.

8.2 Bio-effectors as stress protectants

Impressive increases in the yield of winter wheat (up to 25 %) could be achieved with foliar spray applications of selected seaweed or plant extracts in field experiments in Northern Ireland and

Germany. Associated investigations in WP03 and WP04 showed that these products can enhance the stress resistance of plants and exert growth regulatory effects via effects on hormonal balances. Application schemes and dosages need to be further optimized with respect to variable soil and weather conditions and optimal timing to achieve more reliable effects with the use of these products.

In maize, starter treatments with manganese, zinc, silicates, Zn/Mn-rich seaweed extracts and selected cold-resistant microbial BEs were effective to improve the cold stress resistance at early growth stages, which resulted in reproducible average yield increases of 17.6 % under conditions of cold stress during emergence and early growth but not under more optimal culture conditions.

The development of microbial combination products with complementary properties in combination with stress protective micronutrients (WP02) was identified as a promising approach to improve the effectiveness under field conditions by synergistic exploitation of beneficial properties,

8.3. Improved application strategies

Remarkable progress was made in the development of adapted application strategies to benefit from the beneficial potential of microbial BEs in wheat and maize production. Together with WP07, it was investigated if the combination of BE and fertilizer placement strategies, such as the underfoot placement of ammonium phosphate fertilizers or the insertion of ammonium depots according to the CULTAN (Controlled Uptake Long Term Ammonia Nutrition) method, can provide favourable conditions for intensive root colonization, localized stimulation of root growth, resulting in a more efficient acquisition of mineral nutrients in maize. However, under practice conditions it is evident that an intensive cooperation with WP01, 02 and 09 is necessary to develop adequate formulation and application technologies that support the successful establishment of introduced microbial strains in the rhizosphere for an effective expression of their beneficial traits. In this context the currently widespread use of seed treatments was found to be least effective in terms of microbial root colonization as a prerequisite for the establishment of successful plant-BE interactions. Placement techniques by nursery inoculation or banding were found to be more effective but require a careful assessment of the application conditions in terms of economic benefits (see WP09 report).

Table 8.1 summarizes the outcome of the various approaches, tested within the WP08 activities.

Table 8.1: General overview on bio-effector- based innovative plant nutrition strategies tested under practice conditions in WP08 and the achieved results in terms of plant responses (**black** = no clear effects, red = variable, temporary or moderate effects, green = persistent and reproducible yield effects)

Country	Crops	Bio-Effectors	Fertilizers	Soil P level	Plant growth results
Germany	Wheat	NH_4^+ + Microbial	Min. / org.	Low	Tendential increases
Germany	Maize	NH_4^+ + Microbial	Min. / org.	Moderate but not limiting	Improved early growth
Italy	Maize	NH_4^+ + Microbial+ Algae +Humic acids	Mineral	very low	Improved early growth and yield
Italy	Maize	Microbial	Organic	Very low	Improved early growth
Czech Republic	Maize	NH_4^+ + Microbial	Mineral	Low but not limiting	No improvement
N. Ireland	Wheat	Algae/plant extracts Si ZnMn	Mineral	moderate	Improved yield, but variable
N. Ireland	Wheat	Algae/plant extracts Si ZnMn	Mineral	Low	No improvement
Germany	Wheat	Algae/plant extracts Si, Zn/Mn	Mineral	Low	Improved yield, but variable
Germany	Maize	Si, Mn/Zn, Algae, cold-resistant microbial BEs	Mineral	High	Improved early growth and yield under stress
Romania	Wheat	Microbial	Organic	Low	Annual variations
	Maize	Microbial	Organic	Low	Annual variations
	Tomato	Microbial	Organic	High	Improved yield
Hungary	Tomato	Microbial	Organic	High	Improved yield + quality
Israel	Tomato	NH_4^+ + Microbial	Mineral	Low	Increased biomass and yield

WP09: Economic Evaluation

The overall objective of WP09 was the economic evaluation of bio-effector applications in tomato, wheat and maize production trials, established within the International Field Testing Network (WP08) at different sites in the EU. The final evaluation mainly considered those experiments, providing data sets for three and more years field-testing of promising BE-assisted fertilization strategies identified within WP04-WP07.

The investigated approaches comprised: (i) Improved utilization of organic recycling fertilizers based on manures and animal waste products with microbial inoculants in tomato greenhouse production in Romania and organic tomato field production in Hungary; (ii) improved utilization of manure-based fertilizers in combination with different application techniques of microbial BEs in winter wheat and maize production in Romania and Germany and (iii) improved cold hardness and stress tolerance by application of selected seaweed / plant extracts and micronutrients in Germany and Northern Ireland.

Major findings can be summarized as follows:

The application of bio-effectors can induce remarkable improvements in yield performance, nutrient use efficiency and stress tolerance. However, it has to be clearly stressed, that the economic impact is highly selective and can be influenced by various factors, such as environmental conditions, fertilization strategies, application technologies and the whole range of costs for production means, and particularly for BE products themselves.

9.1 BE applications in organic tomato production

A major benefit of BE applications was found in tomato production systems, where microbial BEs can increase profitability by inducing yield and quality growth, by improving seasonal yield distribution and also by allowing nutrient savings. Furthermore, gross margins of high-value products like vegetables can more easily cover additional costs for production means like BEs. Particularly production systems requiring nursery culture, such as tomato, are highly compatible with microbial BE application strategies since the required concentration of BEs for a successful root colonization can be achieved by applying rather small dosages into the small substrate volumes of the nursery pots at the optimal plant-developmental stage. Moreover, the host plant is subsequently cultivated under greenhouse conditions, protected from environmental stress factors detrimental for the establishment of the plant-BE interactions.

9.2 BE applications in agricultural production systems

But, also in agricultural production systems, specific BE applications can be profitable. However, in these cases the appropriateness of the applied BE product as well as the optimal application technology are notably essential for their profitability. Especially for wheat and maize, the field experiments showed impressing benefits of seaweed and plant extracts applied as stress protectants with rather low BE prices and convenient foliar application possibilities or cost-saving seed dressings.

In contrast, the application of microorganisms was in many cases not profitable, as high dosages and repeated applications were necessary for efficient inoculation of the larger soil volumes as compared with the nursery culture systems in tomato. Product-saving seed treatments were usually associated with limited root colonization efficiency, repeatedly demonstrated in pot experiments and under field conditions. For maize, profitable applications could be found particularly for microbial BEs inoculated as comparatively stress-resistant spore formulations (Bacillus, Trichoderma) but only for low-price products at small input rates, which could be achieved by placement strategies such as seed furrow application. Although, WP03 activities demonstrated a stress-protective potential for microbial and non-microbial BEs as well, plant-microbial interactions were particularly sensitive to environmental stress factors affecting root development and activity (extreme nutrient limitation, drought, temperature extremes etc) during the sensitive establishment phase in the early stages of host plant development - a well-documented problem also for the establishment of symbiotic associations with mycorrhizal fungi or nitrogen-fixing bacteria. In wheat production trials, the lowest profitability of microbial BE applications was recorded with the current market price for winter wheat of approximately 150 € t^{-1} but higher economic benefits may be achieved with an increasing market value of the crop.

9.2 Perspectives and limitations

Over all, the results demonstrate a clear potential of BEs to improve nutrient acquisition and stress tolerance of crops in a profitable way. However, this requires highly adapted and site-specific application strategies and general “easy to use approaches” for a wide range of environmental conditions are not available. Currently, the probability for profitable applications of microbial BEs is highest in horticultural production systems in combination with organic recycling fertilisers, while the exploitation of stress protective functions of BE products seems to be more promising for agricultural applications, particularly in combination with selected non-microbial BEs. The increased abundance of extreme climatic conditions related with global change and declining availability of chemical plant protection agents may further promote the exploitation of general stress protective BE functions to reduce production risks by abating yield fluctuations. To quantify this contribution to yield assurance, long-term experiments on yield development have to be carried out.

Some other promising strategies, more recently developed within WP01-WP07 activities were not considered in the final WP09 evaluation, since the data availability from model experiments allowed only one year field testing before termination of the project. These data suggest that localized and product-saving BE application strategies can be further optimized and synergistic effects of micronutrients, Si and stabilized ammonium fertilizers in combination with selected non-microbial and microbial BEs can further improve stress resistance and nutrient acquisition of crops. Of course, these scenarios offer additional perspectives to increase the profitability of BE application strategies.

Beyond the economic profitability, which has been mainly addressed so far, positive environmental impacts of BE applications due to their contribution to improved nutrient efficiency and stress resistance would require more detailed investigations on effects of nutrient balances, leaching losses, greenhouse gas emissions etc. These external effects could only be identified and mentioned here but need to be included into a global evaluation of BE applications in agriculture.

1.4 The potential impact

Scientific impact

The broad screening approach employed within the project, covering a wide range of 38 BE products selected from the most important BE classes, tested on different soils under a wide range of different fertilization regimes and geo-climatic conditions relevant for European agriculture, makes it possible to define potential perspectives but also the limitations of BE-assisted production strategies. The final meta-study, covering more than 150 pot and field experiments conducted within the project, provides a comprehensive data set for final interpretation and evaluation of the results and to identify critical factors determining successful plant-BE interactions on a broader scale, not limited to single observations of individual experiments. In contrast to many meta-studies based on published data, this approach offers the unique opportunity to include also all observations lacking BE effects or even negative results, frequently not published in the scientific literature, which makes the data interpretation more reliable.

The most promising perspectives identified so far comprise:

(i) Improved utilization of organic recycling fertilizers, preferentially based on manures and animal waste products, with microbial inoculants mainly in horticultural production systems (tomato) but with perspectives also in maize and wheat. Manures are still the most widely used organic recycling fertilizers but the application is also associated with various risks, including volatilization of ammonia, greenhouse gas emissions and nitrate leaching. In this context, a more efficient use of nutrients, as demonstrated in the BIOFECTOR tomato trials by combinations with BE inoculations, could be an interesting option which requires further investigations. There are also uncertainties concerning contaminations with organic pollutants such as veterinary drugs in manure-based fertilizers. More recently, manures have also been identified as potential pools for spreading resistances against antibiotics and resistance genes have been also identified in a commercial poultry manure fertilizer product, investigated within the project. Nothing is known about potential impacts of microbial inoculants on this type of contaminants.

(ii) The strong impact of certain mineral fertilizers, such as stabilized ammonium or micronutrients on the efficiency of plant-BE interactions is a novel largely unexploited field of BE research, which offers a wide range of potential applications in terms of synergistic interactions with both, microbial and non-microbial BEs, BIOFECTOR could demonstrate beneficial effects on nutrient (P) mobilization, cold and drought stress resistance. Since adaptive plant stress responses show numerous similarities at the molecular and physiological level, it is feasible to assume that beneficial effects can be expected also against other abiotic and maybe even biotic stress factors, particularly in face of the fact that many of the investigated microbial BEs also exhibit pathogen antagonistic properties. Further investigations towards more sustainable options to increase stress tolerance of crops are of particular interest, due to increased abundance of weather extremes and declining availability of chemical plant protection agents. Moreover, the strategy is compatible increased integration of fertilizer placement strategies as starter or depot fertilization, which is frequently based on stabilized ammonium fertilizers.

(iii) Exploitation of synergistic effects by combining BEs and also fertilizers with compatible properties is an emerging field in BE development and the benefits could be demonstrated in numerous experiments within the project and also in the meta-analysis.

(iv) An unexpected observation was the strong dominance of the culture conditions as determinant for the expression of BE effects. In many cases, BEs of different origin showed very similar effects in different plant species and cultivars when the culture conditions were suitable. This offers a higher flexibility in terms of BE product selection. A better understanding of the critical factors determining these common BE responses may offer key information for more reproducible BE application strategies.

(v) The tested microbial inoculants had a significant impact on the composition rhizosphere bacterial communities. Although these effects were only transient, following the declining root colonization of the inoculants over time, potential feedback loops of the microbiome shifts on the expression of BE effects are still largely unexplored. The transient effects of BE inoculations on soil microbial communities suggest only a limited risk of long-term disturbances of soil microbiomes. However, a final assessment of potential risks requires long-term experiments with BE applications over several years. The finding that BEs of different origin frequently show similar plant growth-promoting effects under suitable application conditions (iv) could offer an option to minimize these risks by application of different products over time.

Socio-economic impact and wider societal implications

As a major “take home message” arising from the project, the successful implementation of BEs into horticultural and agricultural production systems requires highly-adapted and site-specific application strategies, much more specific than originally expected. Unfortunately, general “easy to use approaches”, applicable over a wide range of environmental conditions, are not available.

Based on the currently available information, farmers will be able to perform a more targeted selection of suitable BE/fertilizer combinations for their specific culture, including also information on yield potential, expected economic benefits and effects on product quality. In those fields of horticultural and agricultural production, identified as suitable for successful implementation of BEs into the production systems, BEs can significantly improve stress resistance, fertilizer use efficiency and reduce fertilizer inputs. Since many of the most promising microbial BEs with plant growth-promoting potential also provide proven records of bio-control activities against soil pathogens, a beneficial impact on disease resistance may be expected as a side effect, associated with reduced consumption of pesticides. Taken together, this can be translated into consumer benefits in terms of price stability, product quality and product safety and ecological benefits by reduced input or more efficient use of agrochemicals.

The industrial SME partners were provided with a unique opportunity for comparative evaluation of their product portfolio and of pipeline products, even in combination with BE products from other producers under a wide range of agricultural production conditions in Europe, using the infrastructure of the project for standardized lab and field testing. Apart from the originally intended use of the BE products, this unravelled novel, yet unknown application fields. Respective patent applications are in preparation or have been launched for novel cold- and salt-resistant Bacillus strains, for novel isolates of Trichoderma with plant growth-promoting and stress protecting properties for novel combination products based on selected Trichoderma/Bacillus strains supplemented with micronutrients and for exploitation of synergistic interactions between microbial plant growth promotion in combination with stabilized ammonium fertilization.

External industrial co-operations have been initiated for testing promising BE-assisted-applications in combination with fertilizer products available on the market. Eurochem Agro GmbH, Mannheim, Germany; Compo Expert GmbH Münster, Germany; Yara Germany, Dülmen, Germany; Landor, Birsfelden, Switzerland; Lebosol GmbH, Elmstein, Germany; Vitalin Pflanzengesundheit GmbH, Oberramstadt, Germany; Koppert BV, The Netherlands, are among the companies actively supporting the investigation of BE-based fertilization strategies within the project.

In an ongoing external research cooperation with oilseed rape breeders in Germany (NPZ Innovation GmbH / Rapool Ring, Isernhagen, Germany), practice implementation of WP04 knowledge on starter treatments with cold/drought stress protectants has been further developed into a commercial application for rape seed dressings, released in 2016 and meanwhile used as a standard treatment for hybrid seeds for the European market.

Scientists SMEs, extension services and farmers were brought together with mutual benefits arising from the multidisciplinary and integrated research approach, resulting in research cooperations and common activities. Internationally recognized research experts in soil science, microbiology and plant science shared their knowledge to improve the understanding on BE effects, from molecular to field scales. The opportunity to investigate a wide range of different BEs under different production conditions in a comparative way, contributed to a better understanding of the frequently rather hypothetic modes of action in plant-BE interactions. The project also provided an excellent interdisciplinary education platform for students and young scientists with numerous options to contribute as scientific helpers, exchange students bachelor or master student or on PhD and post-doc positions with a final output of 56 Bachelor and Master theses, 17 PhD degrees and 3 PostDoc projects.

Main dissemination activities and exploitation of results

Web-based dissemination channels

The BIOFECTOR website (www.biofector.info) introduces background information on BEs, application fields in agricultural practice, and the goals of the project, currently presented in 9 languages. An introductory video presentation was produced in 2015 and is available on the BIOFECTOR website, on the websites of the contributing partner institutions and on a BIOFECTOR channel at the Youtube platform. Summaries of the project outcomes as well as a continuously updated publication list and key publications are available as downloads. The website installed in 2012 (57 active pages) had been visited 26,000 times by the end of the reporting period and is regularly updated with novel project publications, conference contributions, announcements of public field days, summer schools and other dissemination activities.

The BIOFECTOR website also provides a link to the bio-effector data base installed by FIBL-Projekte, which offers the opportunity for BE producers to provide information on commercially available BE products and their application fields. The continuously growing data base currently contains information on 147 BE products (http://www.biofector-database.eu/en/biofectors-homepage.html). Both, BIOFECTOR website and database will be further available and updated also after the end of the project runtime.

Publications

A total 87 manuscripts (69 peer reviewed) on BE effects investigated within the project activities have been published or submitted by members of the consortium to scientific journals and public magazines during the project runtime and made available to the public on the BIOFECTOR web page and by preferential publication in open access journals.

Compilations on the current scientific BE knowledge were addressed in 11 review papers and one meta-study, published in peer-reviewed international journals. A monthly series of articles on “Plant-growth promoters, biostimulants and bioeffective solutions was published in the Hungarian farmer´s journal “Agrarsector” and the project outcome was a topic in contributions to scientific articles in public newspapers, public television reports (Bayrischer Rundfunk, “Unser Land” 14.10.2017) and farmer´s journals in The Netherlands. Project results were reported 58 peer-reviewed articles in international scientific journals.

Conference contributions

BIOFECTOR activities have been presented as oral presentations and posters by all members of the consortium in more than 30 national and international conferences and symposia in nine countries. Apart from directly BE-related topics, special emphasis was placed on a broader integration also into conferences covering different thematic areas, including plant nutrition, agricultural management, and rhizosphere biology. Satellite sessions were organized at the “Rhizosphere 4 Conference”, 2015 in Maastricht, The Netherlands and at the “German Plant Nutrition 2016 International Conference” in Hohenheim, Germany.

Educational projects

The BE topic was integrated into teaching modules within the International Master Programme “Crop Science” at the University of Hohenheim, with two courses on function and applications of BEs, student seminar presentations, covering recent scientific BE publications and excursions to field demonstrations and BE-producing SMEs. As a direct output of these educational activities, 28 bachelor and master- have been completed or initiated within project runtime and a total number of 56 master and bachelor theses, 17 PhD and 3 PostDoc projects were completed or initiated within the whole consortium.

Four international summer school projects with bio-effector related topics have been organized in the Czech Republic; Romania, Hungary and Italy.

Project presentations, demonstration trials and public field days

Field demonstration trials and presentations have been regularly organized since 2014 for extension service staff, farmers, scientists, industry partners, students, politicians and the interested public during field days at the Ihinger Hof Research Station, University of Hohenheim, Germany, University of Timisoara; the Experimental Research Station of Szent Istvan University, Soroksár, Hungary, DLG field days 2014, Bernburg, Germany and the Organic Field Days 2017, Frankenhausen, Germany.

BIOFECTOR was presented within various contact workshops: “BioPro Baden-Württemberg 2016, Freiburg, Germany”; “BioValley 2016, Suttgart Germany”, “ProBio 2015, Brussels, Belgium”, and Biocontrol Andermatt, Switzerland.

Registration and marketing

The importance of registration and marketing channels for BEs is a current subject of political discussion. In particular, a harmonization of registration regulations and procedures is being addressed by the “Association of Biostimulants in Agriculture (ABISTA)” , which was initiated under guidance of Madora (WP10) as an outcome of the symposium “Plant Protection and Plant health in Europe 2014” Braunschweig, Germany, by representatives of SMEs and scientists working in the field of BE research, to establish an interest group for promotion of market placement and BE application in agricultural and horticultural practice. Moreover, Madora acted as co-organizer and presenter on symposia on registration issues of bio-stimulants: Plant Protection and Plant health in Europe 2015, German Phytopathological Society DPG, Berlin, Germany and KABS e.V. Workshop 2015, Speyer, Germany. In 2017, the group is co-organiser of the “8th International Symposium on Plant protection and Plant Health in Europe. Efficacy and risks of „biorationals“ in organic and integrated pest management – acceptable ?” at the Julius Kühn Institute Braunschweig Germany. The conference aims to discuss the scientific base for a regulatory infrastructure towards a targeted risk assessment and approval procedure of so-called biorationals (including bioeffectors, biostimulants and biocontrol agents) with a panel of scientists, producers, international experts and representatives of registration authorities.

Future research and outlook

Future perspectives for practice implementation and exploitation of BIOFECTOR results have been addressed in a workshop in cooperation with “i-con innovation GmbH, Ostfildern, Germany”, within the framework of the H2020 project “ProBio” (Professional support to the uptake of bioeconomy RD results towards market, further research and policy for a more competitive European bio-economy) held on May 11th 2017 in Hohenheim, Stuttgart, Germany.

Apart from the research perspectives addressed in 1.4.1., potential applications of BE-based fertilization strategies as drought stress protectants in combination with limited availability of phosphate and nitrogen are currently addressed in the framework of a H2020 follow-up project SolACE: (www.SolACE-EU.net). Further development of BE-based strategies in organic farming is part of the H2020 proposal “BreedOrganic”, currently under evaluation. Perspectives of BEs in concepts to replace chemical plant protection agents are addressed within a joint BMBF Proposal (NOcsPS) of the Faculty of Agriculture, University of Hohenheim, Germany. Additionally, there are ongoing research co-operations on practice implementation of BEs with different companies: EurochemAgro GmbH, Mannheim Germany (microbial consortium products); NPZ Innovation GmbH, Holtsee, Germany; Compo Expert GmbH, Krefeld, Germany, BioAtlantis Ltd, Tralee, Ireland (stress protectants).

Section 2 – Use and dissemination of foreground

Please see the corresponding sections of the Final Report in the Participant Portal

- Publications (Table A1)
- Dissemination Activities (Table A2)
- Patents (Table B1)
- Exploitable Foreground (Table B2)

Section 3 – Report on societal implications

Please see the corresponding section of the final report in the Participant Portal