Section 1 - Publishable summary

BIOFECTOR

Logo:

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

Website: www.biofector.info

Contractors involved (BIOFECTOR consortium):

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1.1 Summary description of project context and objectives

BIOFECTOR is an interdisciplinary research project with the final goal to develop novel approaches for agricultural use of so-called bio-effectors (BEs), comprising microorganisms and bio-active natural compounds, such as seaweed, compost and plant extracts with the ability to improve growth, nutrient acquisition and stress tolerance of crops without significant direct input of nutrients. The BEs are employed 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 recycling fertilisers or by fertiliser placement close to the roots as alternatives for the prevailing use of mineral fertilisers, mainly produced by direct or indirect exploitation of non-renewable natural resources. It is expected that the targeted combination of selected BEs compatible with appropriate alternative fertilisation strategies will reduce the variability of external factors affecting the efficiency of BEs in plant growth promotion, thereby improving the reproducibility of beneficial BE effects, which is still one of the major limitations for BE application in agricultural practice. Maize, wheat and tomato were selected as representative target crops.

Bio-effectors with putative plant growth-promoting potential are provided by five European companies with expertise in selection, formulation and production of BE products. For products with a proven record of plant-growth promotion, an international expert team of soil microbiologists, plant physiologists and agronomists characterises the principle modes of action and the underlying physiological and molecular mechanisms at the molecular and physiological level as well as potential impacts on native soil-microbial populations to consider putative effects on soil ecology and bio-safety. The efficiency of the selected BEs for improvement of alternative fertilisation strategies is evaluated in standardised model experiments under controlled environmental conditions, followed by small-scale field trials. Apart from single BEs, also synergistic effects of product combinations are investigated. After the initial screening phase, successful products are finally assessed within the "BIOFECTOR International Field Testing Network", providing standardised field testing facilities in nine countries under the geo-climatic conditions representative for European agriculture. The field-testing network also provides 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 in the second phase of the project. Further scenario and/or simulation analyses of representative approaches will be conducted to depict the economic efficiency under varying (world) market and price conditions to approve their economic viability and sustainability.

Perspectives for patenting, registration, and international marketing of novel BE products in different countries are investigated and developed in close cooperation of all contributing project partners. Training activities comprise organisation of training courses on application technology for BE products for extension service and farmers, as well as student workshops, master and bachelor programs on BE research. A public data base, collecting information on commercially available BE products, application modes and targets as well as evaluations in the scientific literature is installed 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.

1.2 Work performed since the beginning of the project and the main results achieved so far

1.2.1 Screening programme (WP01)

In total, 38 bio-effector-(BE)-products have been investigated within the BIOFECTOR consortium during the reporting period, using a standardized greenhouse screening protocol established in the first reporting period. Out of this pool, 13 products are already commercially available (8 microbial BEs and 5 seaweed/plant extracts) Twenty five products are representing new isolates and novel developments. Their performance in different alternative fertilization systems has been evaluated in comparison but also in combination with a selection of so-called BIOFECTOR standard BEs representative for important groups and classes of BEs with proven records for plant growth promotion. The standard BEs are based on commercial stains of *Trichoderma harzianum* (BE1), *Pseudomonas sp*. (BE2), *Bacillus amyloliquefaciens* (BE3) and a bio-active seaweed extract from *Ascophyllum nodosum* (BE4). All BEs examined within the screening programme so far are summarized in Table 1.

1.2.2 Functional mechanisms of plant-BE interactions (WP03)

Apart from the screening approach, special emphasize was placed on the identification of the principle modes of action for the representative standard BEs and other promising BEs identified within the screening program.

Interestingly, despite their origin from completely different microbial genera (*Trichoderma*, *Bacillus*, *Pseudomonas*), root growth promotion was identified as a common and most important mode of action for all microbial BEs tested in these experiments, with reproducible effects in five independent pot trials with tomato and maize on two different soils under controlled greenhouse conditions. Root growth promotion was clearly linked with root colonization density and rhizosphere survival of the respective BEs, increasing in the order BE1<BE2<BE3. Accordingly, the strongest expression of plant growth promoting effects was observed on soils with at least moderate availability of phosphate and other macronutrients, accessible via stimulation of root growth. This was associated with increased nutrient contents in the shoots of BE-inoculated plants. By contrast, all microbial BEs tested so far were largely ineffective on soils with low levels of available P, thereby excluding BE-induced solubilisation of sparingly soluble inorganic or organic P sources in the respective soils as possible modes of action. There was no indication for an interaction of tested BEs with colonization of the host plant by soil-indigenous arbuscular-mycorrhizal fungi but rhizosphere bacterial communities were changed upon inoculation of maize and tomato plants with BEs1-3.

1.2.3 Bioeffectors to mitigate abiotic stress in crops (WP04)

Plant growth promotion via stimulation of root growth by microbial BEs (1.2.1) was strongly restricted by various abiotic stress factors, such as limited nutrient (P) availability, temperature extremes, drought stress and salinity. Under these conditions non-microbial BEs seemed to be more effective and protective effects have been observed for a commercial seaweed extract from *Ascophyllum nodosum* (selected as BE4) in tomato exposed to salinity. Novel mixed seaweed extract products based on *Ascophyllum nodosum*, *Laminaria* spp and Fucus sp, rich in Zn and Mn (Algavyt Zn/Mn , Algafect) were able to mitigate cold stress-induced growth inhibition in maize. Particularly Zn was identified as active ingredient, strengthening the free radical detoxifying system of the host plant based on Zn-dependent enzymes, such as superoxide dismutase. Since these mechanisms are involved also in plant resistance to other environmental stresses it is worthwhile to test the respective BE products also under conditions of drought stress or salinity.

1.2.4 Bioeffectors to improve nutrient acquisition from organic and inorganic recycling fertilizers (WP05/06)

In total, 24 different non-microbial and microbial BEs belonging to 14 fungal and bacterial genera have been tested so far for improving plant nutrient acquisition from potential organic (manures, composts, sewage sludge, digestates) and inorganic (ashes, slugs, rock-phosphate) recycling fertilizers. The most promising results have been recorded for the standard BE2, BE3 and a BE3/*Bacillus simplex* mixture in combination with composted cow, buffalo and horse manures in two pot experiments conducted with maize on alkaline low P soils in Italy and in nursery tomato production for commercial greenhouse trials with each 1800 plants, meanwhile reproducible in the third year in Romania. However, surprisingly, no consistent effects have been observed for all other organic recycling products. There was also no indication for stimulation plant growth by nutrient mobilization from inorganic recycling fertilizers and rock phosphate, although most of the selected BEs were able to solubilize Ca-P and rock-P in plating assays and liquid culture media. In model experiments with Ca-P as exclusive P sources, it was demonstrated that the soil-buffering capacity obviously was one of the major factors limiting BE-induced Ca-P solubilization in the rhizosphere. Recent findings suggest the possibility to boost the mobilization potential for sparingly soluble Ca-P and inorganic recycling fertilisers by microbial BEs and plant roots in combination with ammonium nutrition with potential applications in fertilization systems with stabilized ammonium fertilization.

1.2.5 Bioeffectors to improve nutrient acquisition in fertiliser placement systems (WP07)

In tomato greenhouse fertigation trials, the most promising effects on biomass production and fruit yield have been observed by weekly applications of the seaweed extract (BE4) and the fungal Trichoderma-based BE1 throughout the culture period. In accordance with the lower root colonization potential as compared with BE2 or BE3 (see 2.1.2), plant growth-promoting effects of BE1 were less expressed when the fertigation period was limited to only 2-3 applications during seedling development.

A high tolerance to placement fertilizers containing ammonium sulfate (up to 250 mM) and the nitrification inhibitor DMPP was detected for BEs 1-3 and various *Trichoderma* strains. Accordingly, in fertilizer placement studies with stabilised ammonium, maize roots attracted by the depot zone were intensively colonized by BE2. On a soil with low P availability this effect contributed to improved plant acquisition of sparingly soluble soil P sources, potentially mediated by improved P solubilisation potential of BE2 supplied with ammonium (1.2.4). Moreover, in a first field experiment with stabilized ammonium sulphate placement, BE2 inoculation stimulated maize root development within the depot zone but was ineffective without fertilizer placement, indicating improved fertilizer depot exploitation achieved by BE inoculation.

1.2.6 Bioeffector-product combinations (WP02)

Indications for synergistic interactions in growth promotion of tomato were found for two novel isolates of *Trichoderma harzianum* combined with *Bacillus* (BE3) and *Pseudomonas* strains (BE2, *P. jessenii* RU47) while combinations of *Trichoderma*, *Pseudomonas* and *Bacillus strains* were ineffective in maize. By contrast maize seems to be more responsive to combined inoculations with microbial BEs (particularly BE2 and BE3) and non-microbial BEs such as humic acids, seaweed and plant extracts.

1.2.7. BE interactions with crop species/cultivars

Plant-BE interactions have been investigated in three varieties of maize and tomato and two varieties of wheat. So far positive interactions were described for maize and tomato but rarely for wheat. Effects of *Trichoderma* strains were preferentially found in tomato while *Bacillus* and *Pseudomonas* strains (including BEs2 and 3) were mainly effective in maize and particularly in combinations with *Trichoderma* also in tomato.

1.2.8 Extension to field conditions (WP08)

After the initial program for BE screening, the project is now entering the phase of field testing and promising products and applications are summarized in Table 2.

So far, nine experiments on a field scale have been conducted with tomato, maize and wheat in combination with the most promising BEs in seven countries. In accordance with the observed sensitivity of plant growth-promoting microbial BE effects to environmental stress factors (1.2.2), the most consistent results have been recorded in commercial tomato greenhouse production trials conducted under more controlled conditions in Romania with BEs for improved utilization of manure-based organic recycling fertilizers. By contrast, in a similar maize experiment conducted under field conditions in Italy, beneficial BE effects were detectable during the vegetative growth phase but were not translated into final yield.

The enormous significance of BE root colonization efficiency for plant growth promotion as demonstrated in model experiments (1.2.2), was confirmed in field tracing tests for microbial BE survival with different inoculation densities. However, while an optimum BE dosage for induction of plant growth promotion can be easily achieved in pot experiments or large-scale greenhouse trials with nursery culture, this is frequently too expensive for agricultural applications. In this context, BE placement techniques close to the target plants could offer a perspective. With suitable BE formulations for seed dressing or in granulated form, the BE application could be integrated into common agricultural practices of sowing, underfoot placement of fertilizers or depot fertilization. These options are currently under investigation. First field tests already demonstrated that BE-induced root growth promotion in maize was obtained in combination with ammonium depot fertilization but not in root zones without fertilizer depot (1.2.5.) Testing non-microbial BEs with plant growth-promoting properties or potential to increase the efficiency of microbial BEs are alternative strategies.

1.3 The expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project so far)

The broad standardized screening approach employed within the project, with a wide range of BE products and microbial strains selected from the most important BE classes, tested on different soils from different European regions under a wide range of different fertilization regimes, makes it possible to define the potential perspectives but also the limitations of BE/fertilizer combinations in the addressed fields of application. – Moreover, based on these findings, it may be possible to make more easily predictions for the most suitable application fields of novel BE product developments in the future.

Based on the information provided by the project, farmers will be able to perform a more targeted selection of suitable products for their specific culture systems including also information on yield potential, expected economic benefits and ecological impacts. In those fields of horticultural and agricultural production identified as suitable for successful implementation of BEs into the production systems, substantial improvements in fertilizer use efficiency and reductions of fertilizer inputs, greenhouse gas emissions, as ell as saving of energy and production costs can be predicted. Since many of the most promising microbial BEs identified so far within the project, apart from plant growth promotion 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.

The SME partners of the project are provided with a unique opportunity for comparative evaluation of the their product potentials 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 may unravel novel, yet unknown properties and fields of applications, already resulting in first patent applications (see WP01 report).

From the scientific point of view scientists SMEs and farmers are brought together with mutual benefits arising from the multidisciplinary and integrated research approach. Internationally recognized research experts in soil science, microbiology and plant science share their knowledge to improve the understanding of BE effects, from molecular to landscape scales. The opportunity to investigate a wide range of different BEs under different production conditions in a comparative way, will contribute to a better understanding of the frequently rather hypothetic modes of action in plant-BE interactions.The project also provides an excellent interdisciplinary education platform for students and young scientists with numerous options to contribute as scientific helper, bachelor or master student or on PhD and post-doc positions.

Table 1: Bio-effectors examined within the BIOFECTOR screening programme

Product Name	**Active ingredients / organisms**
ABI02A	*Bacillus atrophaeus*
Aegis	*Rhizophagus irregularis + Glomus mosseae*
Algafect - Seaweed extract + amino acids	*Ascophyllum nodosum, Fucus, Laminaria*
Algavyt - Seaweed extract + urea	*A. nodosum, Fucus, Laminaria, Spirulina*
Algavyt + ZnMn + urea	*A. nodosum, Fucus, Laminaria, Spirulina*
	Azotobacter chroococcum
Azoter	*Azotobacter chroococcum, Azospirillum barsilense, Bacillus megaterium Trichoderma aureoviride*
Bactoprof	*5 Bacillus strains*
Biological fertilizer DC / OC	*Penicillium sp*
	Penicillium bilaii
Biorex1	*Bacillus subtilis, B. thuringiensis* and *B. megaterium*
Biorex2	*Azotobacter chroococcum, Azospirillum lipoferum, Pseudomonas putida*
	Burkholderia sp.
	Ascophyllum nodosum
Ecolicitor - Seaweed extract	*Amino cids, peptides, proteins*
Ecoryg - Plant extract	*Azospirillum, Azotobacter, Bacillus* sp,
Geoagit	*Pseudomonas, Cellvibrio* sp, *Rhodococcus* sp
Humic acids	Artichoc compost
Humic acids	Lignite
Manek – Plant extract, secondary metabolites	*Alkaloids, Phenols, Terpenes, Glucosinolates*
Purified Seaweed extract compounds	*Laminarin, Fuccoidan*
	Paenibacillus mucilagenosus
	Piriformospora indica
	Piriformospora williamsii
Proradix (=BE2)	*Pseudomonas sp.* DMSZ 13134
Rizoset – Plant extract	*Sapindus mukorossi, Quillaja saponaria Allium sativum Ginko* biloba, *Sinapis alba*
Rhizovital FZB42 (= BE3)	*Bacillus amyloliquefaciens*
	Bacillus simplex R48
Rygex	Humic acids, seaweed extract, amino acids
Sorghum root extract	*Strigolactones, Flavonoids*
Superfifty - Seaweed extract concentrate (=BE4)	*Ascophyllum nodosum*
Triamum P (=BE1)	*Trichoderma harzianum T22*
	Trichoderma harzianum OMG08
	Trichoderma viride
Trichoderma harzianum extract	*Harzianic acid*
Vitalin T50	*Bacillus subtilis, Streptomyces sp, Trichoderma harzianum, Humic acids, Ascophyllum nodosum extract*

Table 2: Overview on promising BEs and suitable fields of application identified within the BIOFECTOR screening programme

Bio-Effector	Improved Nutrient Acquisition	Abiotic Stress Resistance	Organic Recycling Fertilisers	Inorganic Recycling Fertilisers, Rock-P	Fertiliser Placement
Single BEs					
BE1: Triamum P					Tomato Fertigation
BE2: Proradix	Maize Tomato moderate P soil	Maize moderate drought	Maize Tomato Composted manure	Maize RockP+NH_4	Maize NH_4 Depot
BE3: Rhizovital 42	Maize Tomato moderate P soil	Maize moderate drought	Maize Composted manure		
BE4: Superfifty	Maize,Tomato Lettuce, Fertilised peat substrate	Tomato Salinity			Tomato Fertigation
Pseudomonas jessenii RU47	Maize Tomato moderate P soil				
Biol. Fertiliser DC			Tomato Composted manure		
Algavyt (ZnMn)	Tomato Fertilised peat substrate	Maize cold stress			
ABI02A		Potato cold stress			
BE Combinations					
BE2 +Humic acidsA			Maize Composted manure		
BE3 +Humic acidsA			Maize Composted manure		
BE2 + Superfifty	Maize high P soil				
BE2 + Algavyt	Maize high P soil				
BE 3 + Algavyt	Maize fertilised peat substrate				
BE 3 + Manek	Maize fertilised peat substrate				
BE3 +Bacillus simplex R48			Tomato Composted manure		
TrichodermaOMG08 +Bactiprof +ZnMn Azoter	Tomato low Psoil		Tomato organic farming		

Humic acidsA = Humic acids from Artichoc compost