Summary of Project Activities 2013 - 2014

Within the BIOFECTOR consortium, in total 39 BE-products have been investigated in 2014. Out of this pool, 13 products are already commercially available (8 microbial BEs and 5 seaweed/plant extracts). Twenty five products (16 microbial BEs and 11 seaweed-, plant-, or compost-extracts) are representing new isolates and novel developments. These products have been evaluated in comparison, but also in combination with the representative BIOFECTOR standard BEs based on selected Bacillus, Pseudomonas and Trichoderma strains identified during the first reporting period (BE1-3 see: state of the art report 2013/14) . Tracer techniques have been established for detection of all microbial standard BEs and partially also for related microbial strains, as well as for AM fungi for application in pot and field experiments.

Main working hypotheses addressed during the reporting period 2013/2014:

1. **Microbial standard BEs, selected new microbial strains and seaweed extracts can improve nutrient (P) acquisition under different regimes of P fertilisation by:**
   1.1 stimulation of root growth to improve spatial acquisition of P and other mineral nutrients
   1.2 promotion of mycorrhizal associations
   1.3 stimulation of organic P mineralisation in the rhizosphere
   1.4 solubilisation of sparingly soluble mineral P sources (Ca-P, Rock-P, recycling fertiliser products based on ashes)

2. **Microbial standard BEs and selected new strains can improve nutrient availability and nutrient acquisition from organic recycling fertilisers:**
   2.1 manure-based recycling fertilisers
   2.2 compost-based recycling fertilisers

3. **Seaweed extracts, selected cold-resistant bacterial strains and standard BEs can improve cold resistance of maize, tomato, wheat and barley**

4. **Standard BEs, selected new strains and sea weed extracts can improve growth and nutrient acquisition under salinity stress**

5. **Microbial standard BEs and sea weed extracts can improve nutrient availability and nutrient acquisition from fertiliser depots**
   5.1 Seaweed extracts improve plant growth and nutrient acquisition of tomato in fertigation systems
   5.2 BE-induced stimulation of root growth can improve exploitation of ammonium-based fertiliser depots in maize
   5.3 Root proliferation around ammonium fertiliser depots improves root colonisation with microbial BEs by increased availability of root exudates

6. BE combinations are more effective than inoculations with single BEs

Main results

Hypothesis 1

Microbial standard BEs, selected new microbial strains and seaweed extracts can improve nutrient (P) acquisition under different regimes of P fertilisation by:

1.1 Stimulation of root growth to improve spatial acquisition of P and other mineral nutrients (YES)

Model (pot) experiments conducted within WP03 revealed plant growth promoting effects in tomato and maize for all microbial standard BEs (1-3) and a strain of *Pseudomonas jessenii* (RU47) on two different P-limited soils (loamy top-soil pH 7 grassland, Hohenheim, Germany; clay-loam orchard top soil pH 7, Karlsruhe; Germany) with different levels of soluble Pi fertilisation. Effects of shoot and root growth promotion increased in the order BE1 (TrianumP, Trichoderma) < BE2 (Proradix, Pseudomonas DSMZ13134) < BE3 (FZB42, Bacillus amyloliquefaciens) ≈ RU47 (*Pseudomonas jessenii*) associated with corresponding differences in root colonisation density of the respective BEs. Plant growth stimulation was associated with increased shoot contents (not concentrations) of P and all other macro- and micronutrients suggesting that a general stimulation of root growth was at last one of the mechanisms for improved nutrient acquisition. Accordingly, a range of repeated experiments with different levels of P fertilisation suggested that plant growth promotion occurred mainly at moderate available P levels (e.g. 80 mg P kg^{-1} soil) accessible by improved root growth, rather than under low P conditions (< 20 mg P kg-1 soil). Similarly, no BE effects were observed in maize on low P soils in Switzerland (Loam pH 6.7, 15 mg kg^{-1}), Italy (clay loam pH 8.5, 18 mg P kg^{-1} soil), Czech Republic, Denmark, and Germany (HKKalke).

Also foliar application of the seaweed extracts (Superfity (now standard BE4) and Florescence) increased shoot and root biomass production in lettuce and tomato grown on a pre-fertilised peat culture substrate in a concentration-dependent manner (BIOATLANTIS).

1.2 Promotion of mycorrhizal associations (NO)

Interestingly, mycorrhizal helper functions have been documented in former investigations at least for BEs 2 and 3. However, currently no stimulation of natural mycorrhizal infections has been observed in three experiments (Italy, Germany UHOH) with maize and tomato on three different soils but a stimulation occurred after inoculation with a commercial AM inoculum. Therefore, so far there is no indication for a stimulation of the native mycorrhizal flora by interactions with the selected bacterial standard BEs. In addition to staining techniques also molecular tracers will be imployed in the next experiments.

1.3 Stimulation of organic P mineralisation in the rhizosphere. (YES ?)

Several lines of evidence point to the possibility that apart from root growth effects at least the microbial standard BEs but also *Pseudomonas jessenii* and *Penicillium sp.* (BFDC/OC) can promote P mobilisation from organic P sources: (i) most promising effects on plant growth promotion have been observed with organic fertilisers based on manure (see Hypothesis 2). (ii) In a WP03 joint experiment on a low P grassland top soil with maize and tomato (see 1.1), inoculation with standard BEs and *P jessenii* increased soil phosphatase activities, both in the rhizosphere and in the bulk soil.

1.4 Solubilisation of sparingly soluble mineral P sources (Ca-P, Rock-P, recycling ^ fertiliser products based on ashes) (NO)

In our plating assays, many microbial BEs of different phylogenetic groups (e,g, BE2 *Pseudomonas* DMZ13134; *Paenibacillus mucilaginosus*; *Burkholedria* sp, *Rahnella aquatilis*, *Penicillium* sp, *Bacillus subtillis*, *Pseudomonas* sp, *Streptomyces* spp.) were able to solubilise insoluble tri-calciumphosphates. However, none of the tested strains contributed to P acquisition of maize in pot experiments on a calcareous Loess sub-soil with insoluble Ca-phosphates as the only P source. Accordingly, there were no or only marginal indications for P acquisition from rock phosphates or ash-based recycling fertilisers in pot experiments on P-limited soils with maize in Italy, Switzerland, Denmark, Czech Republic, Germany (HKKalke), with wheat in Northern Ireland and Denmark, with tomato in Hungary, as well as in a maize field trial in the Czech Republic (however in the latter case also no clear responses to soluble P (TSP) application). The only exception was a marked stimulation of root growth and P uptake (~40%) in a pot experiment (HKKalke) with maize grown on a low-P clayey silt soil pH 5.7 supplemented with a P-enriched BOF slag (50 mg P kg^{-1} soil), which may however, reflect a moderately increased P availability as described in 1.1).

The absence of any indication for BE-induced P acquisition in more than 10 experiments on different soils with three different crops and nine P-solubilising micro-organisms raises the question whether the potential of microbial Ca-P solubilisation in the rhizosphere has been largely over-estimated in the scientific literature. Accordingly, a closer look on publications in this context shows that a clear proof for Ca-P solubilisation as a main mechanism for plant growth stimulation is not available in most cases.

Hypothesis 2

Microbial standard BEs and selected new strains can improve nutrient availability and nutrient acquisition from organic recycling fertilisers

2.1 Manure-based recycling fertilisers (YES)

There are clear indications for strong growth promoting effects of the microbial standard BEs and Penicillium sp (BF-DC) in combination with manure-based fertilisers, observed in two

independent experiments in nursery culture for commercial tomato greenhouse production in Romania supplied with a substrate of composted cow manure (45%) a clay loam soil pH 6.7 (30%) with high P availability (550 mg kg^{-1}), sand and peat (Poster 11). In a highly fertile greenhouse production system, growth differences largely disappeared during later development probably as a consequence of compensation effects but were strongly expressed during the whole culture period under low fertility conditions. The experiment will now be repeated with moderate fertilisation levels.
Similarly, two pot experiments and one field trial with maize in Italy revealed significantly increased biomass production (35-65%) and improved early growth on low P clay loam soils supplemented with composted cow-, and horse-manure (50 mg P kg substrate) and the microbial standard BEs.

The results suggest that combination of the microbial standard BEs with manure-based recycling fertilisers could be a promising option for further field testing. However, the mechanism behind the observed effects is still not clear. Similar responses with the various fungal and bacterial BEs point to a general mechanism. Glucose soil amendments to maize plants failed to increase BE-induced growth promotions on a P-deficient soil, suggesting that the observed responses are not simply carbon source effects. The increased phosphatase activities detected after BE inoculation of maize and tomato (see 1.1) may point to an improved mineralisation of organic P forms in the manure fertilisers. However, in the experiments in Romania, growth promotion occurred even in the presence of high available mineral substrate P levels which would exclude nutritional effects via improved P supply. Moreover, the beneficial effects of manure-BE combinations were not always reproducible and only marginal growth promotion was observed in two tomato pot experiments with composted cow manure substrates similar to those used in Romania.

2.2 Other organic recycling fertilisers (NO)

In contrast, to the sometimes striking effects of standard BEs combined with manure-based fertilisers, surprisingly, no or only marginal growth and yield improvements were observed in combination with various organic fertilisers based on composted waste materials, sewage sludge or digestates in pot experiments conducted with standard BEs in Denmark, Switzerland, Germany, and Hungary using microbial standard BEs, various *Trichoderma* strains, *Penicillium* sp., *Piriformospora indica*, and a commercial *Trichoderma*/N_2-fixer combination product from Hungary in combination with maize, wheat and tomato. There are also no clear indications for a significant increase in nutrient availability during the composting process by inoculation with microbial standard BEs and various strains of *Trichoderma*, *Bacillus* and *Penicillium* in model experiments conducted in Denmark.

Hypothesis 3

Seaweed extracts, selected cold-resistant bacterial strains and standard BEs can improve cold resistance of maize, tomato, wheat and barley (YES ?)

Seaweed extracts, micronutrient (Zn+Mn) supply but not microbial standard BEs (BE2, BE3+cold-resistant *Bacillus* simplex) improved tolerance of maize to low root zone temperatures (14° C) during early growth in two pot experiments conducted in Germany. Beneficial effects were restricted to seaweed extracts with high Zn/Mn contents (Algavyt + Zn/Mn, Algafect) and Zn/Mn treatments alone. Plant nutrient analysis suggested that Zn rather than Mn supply was the critical factor.

In one out of two large open air pot experiments conducted with wheat in Northern Ireland positive effects were observed also for the combination BE3+cold-tolerant Bacillus simplex. Only marginal effects of BE2, BE3+cold-resistant Bacillus simplex were observed in a low root zone temperature pot experiment conducted with tomato in Germany.

First field experiments initiated to test Zn/Mn-rich seaweed extracts and Zn/Mn containing placement fertilisers with maize in Germany and spring barley in Northern Ireland (foliar treatments with novel seaweed extracts) revealed no beneficial effects. However, in 2014 no cold stress periods occurred during early growth in May/June.

Hypothesis 4

Standard BEs, selected new microbial strains and sea weed extracts can improve growth and nutrient acquisition under salinity stress (NO)

Salt tolerance was highly expressed in novel isolates of *Azotobacter chroococcum* (NaCl) and microbial standard BEs (Ammoniumsulfate). However, none of the selected strains showed protective effects against salinity in pot experiments conducted with tomato in Italy and Israel. A positive trend was observed for seaweed extracts, which requires further confirmation. (However, *Azotobacter* chroococcum 76a showed some effects on fruit growth of tomato under limited N supply in pot *experiments* in Italy).

Hypothesis 5

Seaweed extracts and microbial standard BEs can improve nutrient availability and nutrient acquisition in fertiliser placement strategies

5.1 Seaweed extracts and microbial standard BEs improve plant growth and nutrient acquisition of tomato in fertigation systems (YES ?)

In fertigation experiments conducted 2013 in Israel, the seaweed extract Superfifty (now standard BE 4) stimulated shoot and root growth of tomato in a concentration-dependent manner in a high fertility pot experiment with standard fertigation on a Loess soil but no effects were observed in a low fertility system or with compost fertilisation.. Also no significant effects of microbial standard BEs were detected in combination with different P fertigation levels but a trend for increased fuit biomass in BE variants was observed.

5.2 BE-induced stimulation of root growth can improve exploitation of ammonium-based fertiliser depots in maize

In a field experiment with maize conducted on a high P silty loam pH 6.9 in Germany, BE2 significantly stimulated root development induced by stabilized ammonium-sulphate depot fertilisation .

5.3 Root proliferation around ammonium fertiliser depots improves root colonisation with microbial BEs by increased availability of root exudates

In a rhizobox experiment with maize on a calcareous Loess sub-soil pH 7.5 in Germany, with ammonium-sulphate depot fertilisation vs broadcast Ca-nitrate application, BE2 was detectable in high rhizosphere concentrations even 55 days after sowing (2-4 10^{13} CFU g^{-1} root fresh weight with a trend for higher root colonisation in the depot zone.

Hypothesis 6

BE combinations are more effective than inoculations with single BEs

Based on external reports on synergistic effects of combined inoculations with plant-growth promoting *Trichoderma* and *Bacillus* strains or triple inoculations *Trichoderma*, *Bacillus* and *Pseudomonas* combinations, pot experiments and first field trials were conducted with maize and tomato in Germany and Hungary. The combination of BE1+BE3 and B1+BE2+BE3 was ineffective in a pot experiment with maize on a P-limited soil with low P fertilisation (30 mg P kg^{-1} substrate). However, positive trends were observed in pot experiments and field experiments with Tomato by combinations of BE3 and new isolates of Trichoderma (AUAS, Poster 23) and Trichoderma strains in combinations with associative N fixers in Hungary .

Another promising field are combinations of selected seaweed, plant and compost extracts with microbial BEs. Pot experiments with maize revealed positive effects of BE4 (Superfifty), novel seaweed-based (Algavyt, Algafect), plant extract-based (Manek) and artichoke-compost extract-based BEs particularly in combination with BE2 and BE3.

Interesting pre-biotic effects on B2 and BE3 were observed in plate assays with seaweed extracts (BE4 Superfifty, Ecolicitor), laminarin and fucoidan purified from seaweeed extracts. However, this effect was not confirmed in first field experiments conducted with maize in Germany .