BIOFECTOR Research Document

The Use of Bio-Effectors for Crop Nutrition

BIOFECTOR 2012-2017 funded by the European Commission within the 7th Framework Programme | Grant Agmt. No. 312117

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Vorwort / Foreword

Zahlreiche Institutionen in Europa forschen zur Thematik wie Düngungs- und Pflanzenschutzmaßnahmen im Pflanzenbau reduziert werden können ohne dabei deren Wirksamkeit zu reduzieren. In diesem Zusammenhang besteht auch zwischen der Universität Hohenheim und der madora GmbH Lörrach seit 1999 eine enge Forschungskooperation. Aufgrund der Vorarbeiten und des Forschungsnetzwerkes der Universität Hohenheim konnte die Projektausschreibung der europäischen Kommission gewonnen werden.

Die nachfolgende Darstellung zeigt die Struktur, Ergebnisse sowie Akteure des Biofector Projektes zum Projektende.

Im Zusammenhang mit dem Einsatz von Biostimulanzien besonders von lebenden Organismen und deren Zulassung für die Landwirtschaft in der Europäischen Union sind erhebliche Fragen offengeblieben, die in weiteren Projekten geklärt bzw. einer Lösung zugeführt werden sollten bzw. müssen

Die wissenschaftliche Verantwortung des Biofector Projektes lag in den Händen von Günter-Neumann-Universität Hohenheim, die administrative Abwicklung lag in den Händen von Kathrin Prebeck Cmast. Für Training und Desimination ist die madora gmbh Lörrach zuständig.

Als Pflanzenstärkungsmittel oder Bioeffektoren gelten verschiedenste Produkte auf der Basis von Mikroorganismen, Huminstoffen, Aminosäuren, Pflanzen-, und Algenextrakten etc., Sie sollen ohne wesentlichen Nährstoffeintrag Böden und Pflanzen biotisch, chemisch oder physikalisch beeinflussen, die Düngemittelausnutzung verbessern, schwerlösliche Nährstoffe besser verfügbar machen, das Pflanzenwachstum stimulieren und deren Stressanfälligkeit verringern. Die Bewertungen von Bioeffektoren sind so vielfältig wie die ihnen zugeschriebenen Wirkungen und reichen von kompletter Wirkungslosigkeit bis hin zu Ertragssteigerungen im zweistelligen Prozentbereich. Im Hinblick auf den zunehmend drängenderen Bedarf nach der Entwicklung effizienterer Produktionssysteme mit nachhaltigerer Ressourcennutzung, vermindertem Flächenverbrauch und verbesserter Stressresistenz ist eine klarere Bewertung der Einsatz-möglichkeiten solcher Produkte erforderlich. In den vergangenen fünf Jahren hat sich das EU-Verbundprojekt BIOFECTOR (www.biofector.info) mit dieser Frage beschäftigt. Unter der Beteiligung von 7 Universitäten, 5 Forschungsinstituten und 9 Firmen und Verbänden wurde die Frage untersucht, inwieweit Bioeffektoren in der Lage sind, die Ausnutzung von mineralischen und organischen Düngern zu unterstützen und die Stresstoleranz von wichtigen Kulturpflanzen (Mais, Weizen, Tomate) zu verbessern. Insgesamt wurden mehr als 150 Gewächshaus- und Feldversuche mit 38 kommerziellen Produkten, Neuentwicklungen und Produktkombinationen in elf Ländern durchgeführt. Bei mehr als 1100 Behandlungsvergleichen wurde in 30 Prozent der Fälle eine Wirksamkeit nachgewiesen, die allerdings stark von den jeweiligen Anwendungsbedingungen abhing. Eine wichtige Rolle spielte in diesem Zusammenhang die Auswahl geeigneter Kombinationen von Bioeffektoren und Düngemitteln: So wurden zahlreiche organische und anorganische Recyclingdünger untersucht, für die aufgrund der propagierten Eigenschaften der Bioeffektoren eine verbesserte Ausnutzung erwartet werden konnte. Überraschenderweise war aber beispielsweise in den Fällen von Aschen, Schlacken und auch von Rohphosphat, die durch säurelösliche Phosphatformen charakterisiert sind, die Anwendung sogenannter phosphatlösender Mikroorganismen völlig wirkungslos. Eine verbesserte Düngerausnützung wurde in diesen Fällen nur dann erreicht, wenn die mikrobiellen Bioeffektoren in Kombination mit einer ammoniumbetonten Stickstoffdüngung mit Nitrifikationsinhibitoren angewendet wurden. Durch Ammonium-vermittelte Ansäuerung des

Wurzelraumes konnte das phosphatlösende Potenzial der Mikroorganismen unterstützt und die Wurzelbesiedelung sowie die Produktion wachstumsstimulierender Hormone gefördert werden, was letztendlich die Nährstoffaufnahme durch verbessertes Wurzelwachstum begünstigte. Auch bei der Nutzung organischer Dünger ergab sich eine unerwartete Selektivität der Wirksamkeit von Bioeffektoren: Die besten Ergebnisse zeigten sich im Tomatenanbau, bei Verwendung von Mikrobenpräparaten in Kombination mit organischen Düngemitteln hoher Stickstoffverfügbarkeit (z.B. Stallmistkompost, Guano Blut-, Haar- und Federmehl). Sie wiesen in 4 Versuchsjahren in Rumänien und Ungarn profitable Ertragssteigerungen von 15 bis 40 Prozent auf. Dabei wurden neben dem Ertrag auch Qualitätsfaktoren wie Fruchtgrößenverteilung oder die Zuckergehalte beeinflusst. Im Tomatenanbau kann die Vorkultur unter geschützten Gewächshausbedingungen in kleinen Kulturgefäßen erfolgen, was sich positiv auf die sensible Etablierungsphase mikrobieller Bioeffektoren im Wurzelraum auswirkt. Bei Ackerkulturen waren die Effekte der Pflanzenstärkungsmittel dagegen weniger deutlich und variabler Das ist darauf zurückzuführen, dass im Ackerbau viele, oft schwer kontrollierbare Faktoren die Wurzelbesiedelung, das Pflanzenwachstum und den Ertrag beeinflussen. Die Wirksamkeit mikrobieller Bioeffektoren wird wesentlich durch eine erfolgreiche Wurzelbesiedelung bestimmt. Diese wiederum ist abhängig von der Fähigkeit der Pflanze, eine solche Besiedelung durch Abgabe von Signalsubstanzen und Wurzelabscheidungen als Nährstoffquelle zu fördern. Alle Stressfaktoren mit negativen Wirkungen auf die Aktivität der Wurzeln und der beteiligten Mikroorganismen wirken sich daher auch negativ auf das Zustandekommen einer erfolgreichen Interaktion zwischen Pflanze und Stärkungsmittel aus. Das gilt für Temperaturextreme, Trockenheit, Staunässe, Toxizitäten, konkurrierende Bodenmikroorganismen oder starken Nährstoffmangel, aber auch für ausreichende

Nährstoffverfügbarkeit, wenn keine weitere Stimulierung des Pflanzenwachstums erreicht werden kann. Erfolgreich etablierte Bioeffektoren tragen allerdings zu einer Verbesserung der Stresstoleranz bei, wobei besonders hormonelle Stressanpassungen sowie die Produktion von Antioxidanzien und Radikalfängerenzymen und Interaktionen mit der Bodenmikroflora auch schon vor Eintritt einer Stresssituation eine Rolle spielen. Sie wirken damit ähnlich wie ein leichter Stress-Stimulus aktivierend auf die pflanzeneigenen Abwehrsysteme, die dann im Falle einer Stresssituation besonders schnell und intensiv wirksam werden können. Diese Mechanismen wurden für mikrobielle und nichtmikrobielle Bioeffektoren gleichermaßen nachgewiesen, und die Testpflanzen zeigten bei Anwendung unterschiedlicher Produkte (Mikroorganismen, Algen-, Pflanzen- und Kompostextrakte) ähnliche physiologische Reaktionen in ähnlich starker Ausprägung. Offensichtlich können solche Anpassungsreaktionen durch unterschiedliche Stimuli induziert werden. Auch in diesen Fällen konnte eine Verbesserung der Wirksamkeit durch geeignete Düngerkombinationen erreicht werden, wobei hier Mikronährstoffe wie Zink, Mangan, aber auch Silizium und Ammonium besonders effektiv waren. Bei den Ackerbaukulturen wurden besonders vielversprechende Effekte mit nichtmikrobiellen Bioeffektoren wie Algen- und Pflanzenextrakten erzielt. Im Gegensatz zu den mikrobiellen Präparaten können sie flexibler und einfacher auch in geschlossenen Beständen durch Blattspritzungen eingesetzt werden, während für Bodenmikroorganismen eine Einarbeitung erforderlich ist. So konnte die Kältetoleranz von Mais sowie die Winterhärte und die Frühjahrsentwicklung von Weizen in mehrjährigen Feldversuchen in Nord Nordirland und Deutschland mit profitablen Ertragssteigerungen von 13 bis 16 Prozent verbessert werden. Aufgrund ähnlicher physiologischer Anpassungen besteht hier auch Potenzial zur Verbesserung der Trockentoleranz. Für zukünftige Anwendungen wird eine besondere Herausforderung darin bestehen, die Anwendungsbedingungen für einen erfolgreichen Einsatz von Bioeffektoren noch genauer zu charakterisieren. Gerade im Ackerbau besteht für den Einsatz mikrobieller Präparate Optimierungsbedarf bei den Applikationstechniken, die eine effiziente Wurzelbesiedelung mit ökonomischen Aufwandmengen verbinden und sich in bestehende Arbeitstechniken integrieren lassen. Kostengünstige Saatgutbehandlungen sind nach den bisherigen Erfahrungen dafür nur bedingt geeignet. Kombinationen mit Düngemittelplatzierungen könnten hier bessere Ergebnisse erzielen. Im Hinblick auf die Stressresistenz bieten die Selektion toleranter Stämme, aber auch die Kombination verschiedener Stämme mit unterschiedlichen Ansprüchen und mit Schutzsubstanzen wie Mikronährstoffen, Silizium oder nichtmikrobiellen Bioeffektoren weitere Ansatzpunkte. In jedem Fall ist für eine erfolgreiche praktische Nutzung des unbestreitbaren Wirkpotenzials von Bioeffektoren die Entwicklung angepasster und standortspezifischer Anwendungsstrategien erforderlich. Welche Produkte letztlich zur Verfügung stehen, wird aber auch stark von der Umsetzung der geplanten europaweiten Harmonisierung der Zulassungsrichtlinien abhängen.

Manfred G Raupp
madora gmbh

BIOFECTOR Project Overview

Reducing mineral fertilizers to improve soil and produce

Conventional agriculture relies on regular and liberal applications of artificial mineral fertilisers containing essential plant nutrients, especially nitrogen and phosphorus. Nitrogen fertilisers are made from atmospheric nitrogen, which is converted to ammonium using the energyintensive Haber-Bosch process, while phosphorus fertiliser is made by treatingmined 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. Only about half of nitrogen fertilisers and 20 per cent of phosphate fertilisers are taken up by crops. Most of the remainder is immobilised, runs off into waterways, isleached into groundwater or lost in gaseous form. The liquid leachate causes pollution of groundwater sources and leads to the eutrophication of rivers, lakes and coastal zones, thereby reducing biodiversity and producing toxic algal blooms. Because of these damaging effects, many regions - including Europe - are introducing legislation to reduce the use of mineral fertilisers.

In response to the need to maintain crop yields whilst reducing artificial inputs, a number of projects are being funded by the European Commission to investigate more natural ways of sustaining agricultural production. BIOFECTOR (Resource preservation by application of bio-effectors in European crop production) is a major project investigating the use of bio-effectors (BEs) to improve the ability of crops to utilise nutrients from both artificial and natural fertilisers. Coordinated by the University of Hohenheim in Germany and benefiting from the dedicated project management skills of consultancy company CMAST, the project comprises a consortium of 21 industrial and academic partners BIOFECTOR is now approaching the end of its five-year duration, in which it has tested the effects of 36 BEs in over 150 laboratory and field experiments.

Introduction

BIOFECTOR is an integrated project with the aim to reduce input of mineral fertilisers in European agriculture by development of specifically adapted bio-effectors (BEs) to improve the efficiency of alternative fertilisation strategies, such as organic and low-input farming, use of fertilisers based on waste recycling products and fertiliser placement technologies.

Bio-effectors addressed comprise fungal strains of Trichoderma, Penicillium and Sebacinales, as well as bacterial strains of Bacillus and Pseudomonades with well-characterized root growth promoting and nutrient-solubilising potential. Natural extraction products of seaweed, compost and plant extracts, as well as their purified active compounds with protective potential against biotic and abiotic stresses are also tested in various combinations. These features offer perspectives for a more efficient use of nutrients by strategic combination with the alternative fertilisation strategies. Maize, wheat and tomato are chosen as representative crops. Laboratory and European-wide field experiments assure product adaptation to the various geo-climatic conditions characteristic for European agriculture.

The final goal is the development of viable alternatives to the conventional practice of mineral fertilisation as contribution to a more efficient management of the non-renewable resources of mineral nutrients, energy and water, to preserve soil fertility and to counteract the adverse environmental impact of agricultural production.

The project has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 312117 (BIOFECTOR). Project Duration 60 Month 01.09.2012-31.08.2017
EU-Contribution €5,999,821

Video https://www.youtube.com/watch?v=Nd4uwx0ShPc

BIOFECTOR Participants:

P 01: University of Hohenheim (UHOH)

P 02: Julius Kuehn-Institute Federal Research Centre for Cultivated Plants (JKI)
P 03: Czech University of Life Sciences (CULS)

P 04: Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara (BUAS)
P 05: Corvinus University Budapest, Hungary (CUB)

P 06: WUR Plant Research International (DLO)

P 07: University of Naples, Department of Agricultural Engineering and Agronomy (DIAAT-UNUNA7a) &
The Università of Napoli Federico II (DIAAT-UNUNA7b)

P 08: University of Copenhagen (UCPH)

P 09: Agri-Food Biosciences Institute (AFBI)

P 10: Bioatlantis Ltd. (BIAT)

P 11: Anhalt University of Applied Sciences (AUAS)

P 12: Research Institute of Organic Farming (FiBL)

P 13: DR. RAUPP E. K. & madora gmbh (madora)

P 14: ABitep GmbH (ABI)

P 15: Arbeitsgemeinschaft Huettenkalk e.V. (HKKalke)

P 17: Bayer Crop Science Biologics /Prophyta GmbH (PROPH)

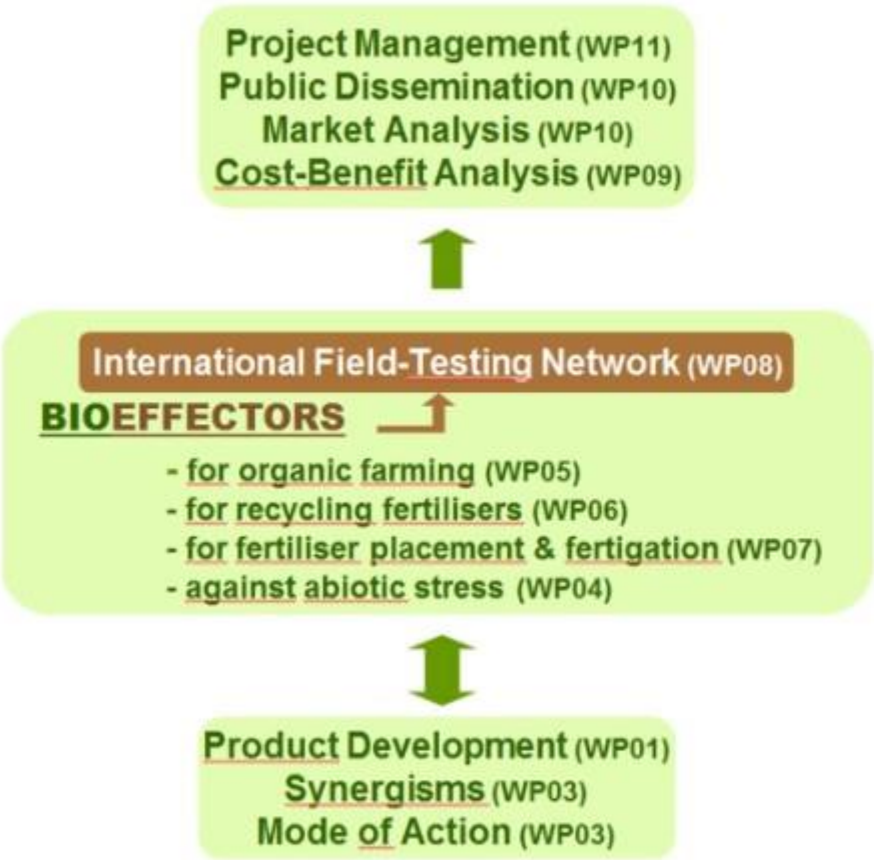
P 18: Sourcon Padena GmbH & Co. KG (SP)

P 19: FIBL Projekte GmbH (FIBL-Projekte)

P 20: The Agricultural Research Organisation of Israel – the Volcani Centre (ARO)
P 21: Agriges s.r.l (AGRIGES)

P 26: CMAST bvba (CMAST)

BIOFECTOR
Project structure



WP01: Product Development

This work package comprises a network of experts from science and industry with long-lasting experience in production of commercially used bio-formulations for plant growth promotion and plant protection. As a main task, partners in WP01 will provide bio-effector products based on plant growth promoting microorganisms or active natural compounds, to be tested for beneficial effects on plant growth and fertiliser savings in the different fertilisation strategies addressed in WP04-WP08.

The starting point is a selection of the currently best-characterized plant growth promoters under commercial use; they are able to promote root growth by interfering with plant-hormonal signalling, mobilise sparingly soluble nutrients fixed in soil particles and complex organic compounds and strengthen plant defence mechanisms against pathogens and abiotic stress factors.

Based on their performance under the test conditions investigated in WP04-WP06, the products will be further adapted and optimised by modifications e.g. of the formulation and application technology or of the biologically active ingredients. Important information required for product optimisation in WP01 is also provided by WP03 and WP02, investigating the functional mechanisms behind plant growth-promoting effects and interactions of product combinations. The final goal are bio-effector products, specifically adapted to the requirements of the different fertilisation systems investigated in WP04-WP07 with final field testing under the variable conditions in European agriculture performed in WP08.

Participants: ABI, UHOHa, JKI, CULS, CUB, SP, PROPH, AUAS; UNINA, AFBI, BIOAT, AGRIGES

WP02: Synergisms and Product Combinations

In many cases bio-effectors are not only applied as single products but also as product combinations to induce multiple responses of the target plants (e.g. stimulation of root growth in combination with mobilisation of sparingly available sources of phosphate and other nutrients). Therefore, the overall objective of WP02 is the development of bio-effector products based on combined or synergistic interactions of single bio-effectors identified in WP01, to combine multiple beneficial properties according to the requirements of the different alternative fertilisation systems addressed within WP04-WP07.

Starting point is the identification of functional combinations of promising bio-effectors based on the products, microbial strain and product collections already available from partners with expertise in bio-effector production. In a second phase, also combinations of promising bio-effectors identified in the WPs addressing the different alternative fertilisation systems (WP04-WP07) will be included. A special task is the investigation of positive synergistic interactions of bio-effectors with the native microflora (e.g. promotion of already existing symbiotic interactions).

Pilot experiments are conducted in controlled environments to characterise the conditions required for the best performance of the most promising bio-effector combinations and to dissect the detected synergistic effects as a prerequisite to elucidate the functional mechanisms behind within WP03.

Participants: ABI, CUB, UHOH,DLO, UNINA, AUAS, PROPH, AGRIGES

WP03: Functional Mechanisms

A major problem related with high variability and often limited reproducibility of plant bio-effector responses under field conditions is the limited knowledge concerning environmental factors determining the performance of bio-effectors and the functional mechanisms behind the observed effects. Therefore, WP03 will address all aspects related with the function of the selected bio-effector products and product combinations. The major tasks comprise:

(i) Standardisation of new or already existing methodologies for measuring effects of bio-effectors on plants.

(ii) Tracing colonization of microbial bio-effectors along or inside the roots or even in aboveground plant parts.

(iii) characterisation of responses to bio-effector applications at the physiological and molecular level, both, in target plants and also in the applied microbial bio-effectors.

(iv) demonstrate the impact of bio-effector applications on the composition of plant-associated microbial communities especially with respect to nutrient cycling, plant pathogens, and bio-safety issues.

Participants:DLO, UHOH, JKI, CUB, UNINA, AFBI, AUAS, FIBL, ABI

WP04: Abiotic Stress

The limitation of readily plant available nutrients is a common feature of many alternative fertilisation systems avoiding the use of mineral fertilisers and nutrients are frequently sequestered in complex organic or sparingly soluble inorganic compounds. Other common stress factors with increasing significance for European agriculture comprise drought and high temperatures linked with global climate change particularly in Mediterranean areas but also in Central Europe. Also low soil temperatures during early spring in Central and Northern Europe cause limitations particularly for crops of tropical origin such as maize, soybean or tomato. Apart from water limitation or temperature extremes per se, all stress factors are additionally associated with limitations in plant nutrient acquisition.

Therefore, the general goal of WP04 is to assess functions and benefits of bio-effectors in stress environments in order to provide specific guidelines for their effective use in resource-limited agriculture. The specific objectives of WP04 are: characterisation of bio-effectors able to improve nutrient acquisition under conditions of limited nutrient availability, low root zone temperatures, drought stress and high salt concentrations. Although, salinity is not a major stress factor in European agriculture, high salt concentrations may cause problems for the effectiveness of bio-effector application in association with local fertiliser placement strategies. Output of WP04 will further support the activities in WP05 (Organic Farming), WP06 (Recycling Fertilisers) and WP07 (Fertiliser Placement).

Participants: UNINA, UHOH, UCPH, AFBI, BIOAT, ARO, AGRIGES

WP05: Organic Farming

Organic farming is the most widespread cropping system using alternatives to mineral fertilisation with continuously increasing significance in European agriculture. In organic farming systems, mineral nutrients are largely bound in complex organic compounds and exceptionally also in sparingly soluble mineral fertilisers such as rock phosphate. As compared with mineral fertilisation, this requires a more intense expression of mechanisms for nutrient mobilisation and nutrient acquisition, both, in plants and soil microorganisms.

WP05 investigates perspectives for the use of bio-effectors in organic farming and low-input systems for improvement of plant nutrient supply via an extended rooting with more root branching and root hairs and the stimulation of fungal symbionts (mycorrhiza). Moreover, various bio-effectors have the potential to mobilize sparingly available sources of mineral nutrients, such as phosphate and micronutrients and liberate bound forms of P, N and other essential elements from organic fertilisers. WP05 will unravel the capacity of candidate bio-effectors from WP01-04 to improve plant growth and nutrient uptake in greenhouse and small scale field experiments and addresses also the efficiency of bio-effectors in combination with organic amendments based on recycling products (WP06), green manure, and mineral recycling fertilizers (WP06) to promote plant growth and nutrient uptake in various soils of different European regions used for organic agriculture.

Participants:FIBL, UHOH, JKI, CULS, CUB, UNINA, UCPH, AFBI, BIOAT, AUAS

WP06: Recycling Fertilisers

WP06 addresses perspectives for bio-effector applications to improve plant nutrient supply from fertilisers based on recycling products. The recycled waste products to be investigated comprise sewage sludge, solid fibre, fractions of separated animal slurry, biogas digestates, municipal household waste and incineration slags. These wastes are abundant in Europe and are currently not widely used as nutrient sources for agricultural production.

Research is focused on:

(i) Increased bioavailability and minimised losses of essential plant nutrients in organic wastes by additions of bio-effectors during storage or processing.

(ii) Increased fertiliser value of organic and mineral wastes added to agricultural soils by combination with bio-effectors and placed application close to the plant roots (link to WP07).

(iii) Increased survival and (root) colonisation of bio-effectors by using organic waste substrates for soil application.

(iv) Quantifying effects of bio-effectors in combination with organic wastes on native soil and soil-microbial nutrient pools.

Participants: UCPH, UHOH, CULS, FIBL, HKKalke, Kom Tek

WP07: Fertiliser Placement

Fertiliser placement and application of fertilizers and plant protectants by drip irrigation (fertigation) are approaches with the aim to reduce inputs of fertilisers, water, pesticides and labour by localised application of fertilisers close to the root system of crops. Several mineral nutrients, such as phosphate, nitrate and ammonium are able to stimulate root growth for exploitation of nutrient-rich patches. Therefore, fertiliser placement close to crop roots is expected to reduce nutrient competition with other plants. However, under real agricultural production conditions, intense localized root proliferation is not frequently observed. This may be attributed to the absence of steep nutrient concentration gradients in many agricultural soils due comparatively high background concentrations of nutrients even outside the local patch of fertilisers. Therefore, WP07 investigates perspectives to increase the efficiency of crops to exploit fertilizer patches by application of bio-effectors with the ability to stimulate root growth. Additionally also the possibility to use fertilizers based on organic or inorganic waste recycling products for fertilizer placement will be tested including options to integrate bio-effectors for improved nutrient solubilisation within the fertilizer patches (link to WP06). Fertiliser depots are frequently characterized by high local salt concentrations and pH extremes to be tolerated by microbial bio-effectors selected for application in fertilizer placement strategies (link to WP04).

Participants: ARO, UHOH, JKI, CULS, DLO, UCPH

WP08: International Field Testing Network

The general objective of WP08 is validation of the agronomic effectiveness and the economic value of innovative bio-effector-based plant nutrition strategies developed within WP04-07. This requires field testing under the different geographical situations and crop management systems, representing the current practice in European agriculture. Plant performance, yield and product quality obtained within the novel fertilisation strategies will be compared with standard fertilization regimes in farmers practice. The international field testing network comprises field sites in Northern Ireland, Denmark, Central and South Germany, Switzerland, Czech Republic, Hungary, Romania, Southern Italy and Israel to cover the different climatic and geographical conditions in Europe. Although, the different characteristics, stress factors and agricultural practices in the different regions of Europe are considered, setup and evaluation of the field trials follows a standardised protocol to ensure comparability of the results for the final economic evaluation (link to WP09). Another important task of WP08 is the organisation of demonstration trials to promote implementation of the novel fertilisation strategies into agricultural practice.

Participants: UHOH, CULS, BUAS, CUB, DLO, UNINA, UCPH, AFBI, AUAS, FIBL

Distribution of field experimental sites within the BIOFECTOR Field Testing Network covering characteristic geo-climatic conditions for Northern-(blue), Central- (green) , and Southern Europe (red)

WP09: Economic Evaluation

The overall objective of this work package is the economic analysis of new plant nutrition strategies in comparison with current approaches. This implies the investigation of economic viability and sustainability of proposed alternative plant nutrition approaches. Thus, standardised field experiments considering different plant nutrition strategies under different climatic and soil conditions characteristic for European agriculture have to be conducted (link to WP08) and analysed to allow a comparative cost benefit analysis between new and conventional strategies. 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. The economic results will support WP10 in terms of implementing the results into marketing, training and public dissemination strategies.

Participant: UHOH

WP10: Training and Public Dissemination

All aspects concerning the dissemination of project results and activities are covered by WP10, starting with installation and regular updating of an open project homepage, press releases, information and networking with relevant target groups and potential users. Promising approaches identified in WP08 and WP09 will be presented by organisation and contributions to public field days. Perspectives for patenting, registration, and international marketing of novel BIOFECTOR products in different countries are investigated and developed in close cooperation of all contributing project partners. Training activities comprise organisation training of courses on application technology for BIOFECTOR products for extension service as well as student workshops on bio-effector research. Apart from developing public dissemination structures for the project, one of the first activities is the installation of a public web-based data base; collecting the current knowledge on perspectives for application of bio-effector products in agricultural practice as an information guide for farmers and scientists and a platform for producers of bio-effectors to present products with a proven record of Efficiency.

Participants: MADORA, UHOH, FIBL-Projekte, CMAST

WP11: Project Management

The major tasks of this work package comprise the administrative management of the project and supporting the coordinator and participants in all aspects regarding the scientific management of the project according to the EC regulations, including reporting, contractual duties, management of financial resources, publication plan, capture and protection of intellectual property. The WP will provide an efficient web-based communication infrastructure to foster the integrative activities within the consortium and to facilitate reporting and administrative processes. Linked with WP10, an additional field of activities is the dissemination of knowledge produced within the project to the relevant target Groups.

Participants: UHOH, CMAST

BIOFECTOR CONSORTIUM

General Meeting Czech University of Agriculture Prague 2016

An international consortium of scientists, led by the University of Hohenheim, Germany, has launched a large, EU-funded, integrated project for the development of alternative fertilization strategies by the use of so-called "bio-effectors". "Resource Preservation by Application of BIOefFECTORs in European Crop Production" (BIOFECTOR) is a unique project in European Union's 7th Framework Programme, bringing together top scientists from academic institutions and small and medium Enterprises with a wide range of topic Expertise.

Participant 01 a:

University of Hohenheim
Institute of Crop Sciences
Nutritional Crop Physiology (UHOHa)

BIOFECTOR Project coordinator:
Prof.Dr. GünterNeumann Stuttgart-Hohenheim, Germany

The research lines covered by this group comprise molecular characterisation of nutrient uptake and regulatory aspects of root growth and root physiology. The rhizosphere research team lead by Prof. Dr. Günter Neumann has an outstanding reputation in the characterisation of root secretions and adaptive root-induced modifications of the root-soil interface (rhizosphere) as key factors for plant nutrient acquisition, with special emphasis on phosphate and micronutrients. Competence in bio-effector research is reflected by a long-lasting 20-years history of industrial research co-operations, with focus on the fate and function of bio-effectors to reduce fertilizer input.

Tasks: Scientific coordination and management of the project as a whole; leadership of WP08 (international field testing network); focus on measurements of root growth, rhizosphere chemistry and root physiology.

Team members: Uwe Ludewig, Torsten Müller, Klara Bradakova, Narges Moradtalab, Isaac Mpanga, Nino Weber, Markus Weinmann

Participant 01 b:

University of Hohenheim
Institute of Crop Sciences
Fertilisation and Soil Matter Dynamics (UHOHb)

Chair: Prof. Dr. Torsten Müller
Stuttgart-Hohenheim, Germany

The main research focus of the group is the optimisation of mineral and organic fertiliser application to reduce environmental risks in and to increase the economical benefit of sustainable agricultural production systems. Within this context, optimisation of fertilisation strategies to reduce fertilizer losses by leaching and greenhouse gas emissions, fertiliser placement close to the roots, the use of alternative fertilizers based on recycling products and the perspectives for integration of bio-effectors to improve nutrient use efficiency are investigated on the field scale and in on-farm research approaches. Experiments under controlled environmental conditions are conducted to understand underlying mechanisms. Another focus is on soil humus management and turnover of soil organic matter including modelling of related processes under different framework conditions including climate change.

Tasks: Involvement in WP06 (use bio-effectors in combination with recycling fetilisers for fertilizer placement), WP07 (use of bio-effectors to optimize placement strategies with mineral fertilisers) and WP08 (contribution to the international field testing network).

Participant 01 c:

University of Hohenheim Institute of Soil Science and Land Evaluation
Soil Biology (UHOHc)

Chair: Prof. Dr. Ellen Kandeler, Stuttgart-Hohenheim

The research group of Prof. Ellen Kandeler covers a wide range of topics in soil biology, environmental microbiology and microbial ecology. Research focus is the influence of soil microorganisms on cycling of soil carbon and mineral nutrients on different scales, starting with the molecular level and single organisms up to microbial communities and whole Habitats.

Tasks: The group will particularly contribute to the functional characterization of plant-associated microbial communities upon the introduction of bio-effectors by application biochemical, microbiological and molecular techniques. The available expertise will also facilitate up-scaling of results to the field level. Involvement in WP03, 05, 06 and 07.

Partcipant 01 d:

University of Hohenheim
Institute of Farm Management, Division: Farm Management (410B) (UHOHd)

Chair: Prof. Dr. Enno Bahrs, Stuttgart-Hohenheim

The Institute of Farm Management is part of the Faculty of Agricultural Sciences at the University of Hohenheim. The Farm Management Division focuses its research fields on: microeconomic evaluation of agricultural and environmental measures using farm and regional models and analyses and evaluation of the potential of agricultural supply for bio-energy usage. During the recent years, the emphasis of research work has shifted particularly towards resolving the dichotomy between ecology and economy. The group has comprehensive experience in modeling production costs with regard to agricultural commodities, considering ecological aspects such as carbon footprints and carbon mitigation costs. It also has experience in national and international accounting systems, as well as taxation and valuation of agricultural goods.

Tasks: Leader of WP09 and responsible for the economic evaluation of bio-effector-based fertilization strategies developed within the project with special emphasis on relationships between ecology and economy.

Participant 02:

Julius Kuehn-Institute Federal Research Centre for Cultivated Plants (JKI); Institute for Epidemiology and Pathogen Diagnostics

Chair: Prof. Dr. Kornelia Smalla, Braunschweig, Germany

The “Julius Kuehn-Institute (JKI)” is an independent research institution subordinated to the “Federal Ministry of Food, Agriculture and Consumer Protection”. Its main task is to advise the German Federal Government concerning issues of crop production, soil science, plant breeding, plant protection, plant health and biological safety.

The group has long-term expertise in the field of microbial ecology of agro-ecosystems and pioneered DNA-based methods for studying complex interactions between plants and microorganisms in the root-soil interface (rhizosphere). These novel tools were applied to study the diversity of microorganisms depending on the soil type, the plant species and cultivar, agricultural practice and their response to bio-effectors and pathogens.

Tasks: Main contribution to WP01 and WP03 by characterizing the composition of microbial communities in the rhizosphere, influenced by the application of bio-effectors.

Participant 03:

Czech University of Life Sciences (CULS)
Faculty of Agrobiology, Food and Natural Resources

Chair: Prof. Dr. Pavel Tlustos,
Prague, Czech Republic

The Czech University of Life Sciences in Prague (CULS) is the largest Life Science University in the Czech Republic.

Tasks:The major field of expertise of the working group headed by Prof. Dr. Pavel Tlustos is the production and testing of plant-growth promoting bio-effectors based on compost extracts and humic acids as a contribution to WP01 (product development), as well as compost production technology. In WP06 the respective composts will be tested as recycling fertilisers based on organic waste products, including in combination with microbial and non-microbial bio-effector products, to increase the fertiliser quality of the composts and plant nutrient availability. The application potential of the newly developed bio-effector-recycling fertiliser combinations will be tested in organic farming Systems (WP05) and also for fertiliser placement close to the roots to increase their utilisation Efficiency (WP07). The working group is also partner of the BIOFCTOR International Field Testing Network (WP08) providing field sites for standardised testing of the most promising bio-effector products developed within the Project.

Participant 04:

Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara (BUAS)

Chair: Assoc. Prof. Dr. Gheorghe Posta Timisoara, Romania

Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara is a state higher education institution that has the didactic mission to train specialists for scientific research in agricultural sciences and consultancy through the university extension. The second mission of our institution for experimental research in agricultural sciences and veterinary medicine. The research infrastructure of the group comprises experimental field sites as well as large scale protected cultural areas (greenhouses and solariums), agricultural machines and equipment for testing bio-effector products under realistic conditions for agricultural production.

Tasks: The working group is a main partner of the International BIOFECTOR Field testing Network (WP08).

Participant 05:

Corvinus University Budapest, Hungary

Chair: Prof. Dr. Borbala Biro, Budapest, Hungary.

The Corvinus University of Budapest, Faculty of Horticulture, Departments of Ecological and Sustainable Farming Systems and Soil Sciences and Water Management has its main activities in research in national and international projects on organic and sustainable agriculture and are currently studying the role of soil-properties and the various management/soil tillage practices on the growth of tomato, green bean and several other horticultural crops.

Tasks: The CUB will provide combination products based on bacteria, plant extracts and humic acids, suggested and available for the organic agricultural practice in Hungary, to the consortium Partners (WP01). In own research activities, the group will test these bio-effector combinations for applications in organic tomato production in model and field experiments (WP02, WP05) with special emphasis on elucidation of mechanisms behind interactive effects (WP03). Moreover, the group contributes to the International Field Testing Network" (WP08), providing field sites for commercial tomato production in organic farming systems.

Participant 06:

WUR Plant Research International (DLO)

Chair: Dr. Leonard S. van Overbeek, Wageningen, The Netherlands

Plant Research International is part of the Wageningen University and Research Centre. Participating research group is member of the business unit "Bio-interactions and Plant Health". In this business unit, the interactions between arthropods, nematodes, fungi, bacteria and viruses with plants are investigated.

Tasks: Leadership of WP03, and specifically investigating the functional mechanisms behind bio-effector-plant interactions in relation to plant-associated (endophytic) microbial populations. Research activities comprise the development of novel tools to measure endophyte densities in bio-effector-treated plants and to monitor the fate of microbial bio-effectors inside plants (WP03), as well as synergistic effects on endophytic communities upon application of combined bio-effectors to plants (WP02). The group also contributes to WP08 by technical support and sample analysis in the "International Field Testing Network" for final evaluation of BE products (WP08).

Participant 07a:

<u>University of Naples, Department of Agricultural Engineering and Agronomy (UNINA7a)</u>

Chair: Dr. Albino Maggio; Naples

The Università of Napoli Federico II is the second largest University in Italy, involved in all disciplines of scientific research. The BIOFECTOR team consists of two research groups, focused respectively on plant responses to environmental stresses and agricultural microbiology, belonging to the Department of Agricultural-Science. The research groups operate as true interdisciplinary teams to address complex issues, such as sustainable crop production under stress environments.

<u>Tasks:</u> Leadership of <u>WP04</u>, investigating perspectives or bio-effector applications to increase crop tolerance against environmental stress factors, such as limited nutrient availability, low soil temperature during early growth, drought or high salt concentrations. As a contribution to WP01 (Product Development) the group provides screening and production of bio-effectors adapted for salt-stress environments, based on microorganisms and plant extracts in close cooperation with the SME partners AGRIGES and BIOATLANTIS. Products and product combinations are tested in model experiments and small greenhouse trials (<u>WP02</u>), including also the investigation of functional mechanisms (<u>WP03</u>). Within the International Field Testing Network (<u>WP08</u>), the group provides field sites for validation and demonstration of the agronomic effectiveness and economic value of bio-effector strategies under real production conditions, characteristic for the Mediterranean agriculture.

Participant 07b:

The Università of Napoli Federico II (UNINA7b)

Chair: Prof. Dr. Alessandro Piccolo, Naples

The research Center "CERMANU" is an Inter-Departmental Institution of applied NMR spectroscopy, aimed at studying complex biological systems and their bioactivities. It is located in the Department of Agriculture in Portici near Napoli and gathers scientists from 8 different Departments carrying out research in the fields of agricultural chemistry, environment, agro-food and new materials.

Tasks: The group contributes to WP01 for the molecular characterization of microbial bioeffectors by NMR, for provision of compost extracts, humic acids, organic recycling fertilizers (WP06) and formulation of natural extraction products, partly enriched with beneficial microbes (WP02). Special emphasis is placed on adaptation of bio-effectors for improved availability of mineral nutrients in low fertilizer input (WP04) and organic farming systems (WP05). Moreover, the group provides analytical facilities (NMR spectroscopy) for the chemical characterisation of complex organic fertilizers, based on recycling products (WP06).

Participant 08:

University of Copenhagen (UCPH)

Chair: Prof. Dr. Andreas de Neergaard; Copenhagen.

The Plant and Soil Science research group at UCPH has a strong tradition of research on biological soil fertility and the influence of organic matter decomposition processes on nutrient turnover in temperate and tropical agro-ecosystems. Particular focus areas have been the interactions at the root-soil interface, the role of soil microorganisms in determining nutrient availability and nutrient release from various agricultural and urban wastes.

Tasks: With their outstanding expertise in fertilisers based on organic waste products. the team provides the leadership of WP06. The main focus of the research activities is the investigation of bio-effector-recycling fertilizer combinations to increase the plant nutrient availability of the recycling products in pot and small field trials for the development of fertilizer strategies adapted to the requirements of low-input systems (WP04), organic farming (WP05) and fertiliser placement techniques close to the plant roots. For the International Field Testing Network (WP08) the group provides field sites with contrasting properties concerning phosphate levels and organic matter content.

Participant 09:

Agri-Food Biosciences Institute (AFBI)

Chair: Prof. Dr. Jr Rao, Belfast, Northern Ireland, UK.

AFBI is a non-departmental public institution. More than 800 experienced and internationally trained scientists are engaged in basic, applied and strategic research on plant and animal sciences with focus on low-input and organic arable crop production to minimise the use of mineral fertilizer and crop protection agents.

Tasks : The core competencies of the research group led by Prof. Sharma are rapid compositional analyses of plant materials and monitoring of signalling factors (WP03) bio-stimulant formulation efficiency testing of the products (WP01). Special emphasis is placed on the development and function of combination products based on seaweed extracts and microbial bio-effectors for improving cold tolerance of wheat (WP02 , WP04) in close cooperation with the SME partners BIOATLANTIS, AGRIGES and ABITEP. As a member of the International Field Testing Network (WP08), the group provides field testing sites for; assessing the agronomic effectiveness and economic value of the developed bio-effector strategies under the climatic conditions of Northern Europe.

Participant 10:

Chair: John T. O'Sullivan

Bioatlantis Ltd. (BIOAT)

Kerry Technology Park, Tralee, Co. Kerry, Ireland

BIOATLANTIS is a biotechnology company specializing in the provision of sustainable solutions to problems caused by stress in crops, both Abiotic and Biotic, and those prevalent in systems of intensive monogastric animal production.

BioAtlantis Ltd. work in close collaboration with universities across the world and have developed a range of extracts derived from raw materials including *Ascophyllum nodosum* and *Laminaria* sp. The company markets its products in over 30 countries, providing products for use in enhancing yield and marketable grade in major commercial crops. In addition to BioFector, BioAtlantis also act as the coordinators of an EU-wide FP-7 consortium, which aims to validate the functionality and effectiveness of naturally-derived products for use in solving problems facing modern systems of monogastric animal production (THRIVE-RITE, Grant Agreement n°315198; http://www.thriverite.eu/).

Tasks: Responsibilities include development and delivery of bio-effector products, based on seaweed, large-scale production and adaptation of bio-effectors to improve stress resistance of crops and acquisition of mineral nutrients. In turn, this will provide a means of increasing biomass and yield in crops. (WP01, WP04).

Participant 11**:**

<u>Anhalt University of Applied Sciences</u> (AUAS)

Chair: Dr. Jörg Geistlinger, Bernburg, Germany

AUAS offers degree courses in Agriculture, Ecotrophology, Farm Management, Food- and Agribusiness, Landscape Architecture and Plant Biotechnology and is the domicile of the German Agronomical Society (DLG).

The research team of Dr. Joerg Geistlinger provides expertise in production and characterization of bio-effectors based on plant and fungal extracts with potential to stimulate plant growth and root colonization with symbiontic microorganisms. Geistlinger is the leader of <u>WP02</u>

PD Dr. Helmut Baltruschat is an outstanding specialist for Sebacinales, a novel group of ubiquitous, potentially plant-growth promoting fungi.

<u>Tasks</u>: AUAS is the leader of <u>WP02</u> (product combinations). the group investigates the effects and the function of combined bio-effector products, based on natural extraction products and various bacteria and fungi as well as the potential of sebacinales as bio-effctors (WP02, <u>WP03</u>). successful product combinations are delivered to other project partners for further testing (<u>WP01</u>). as a member of the international field testing network (<u>WP08</u>), auas provides field testing sides for validation of bio-effector products on fertile soils in central germany, supported by a small unmanned aircraft system for remote sensing of soil quality, especially phosphate content and plant population development.

Participant 12:

Research Institute of Organic Farming (FiBL)

Chair: Dr. Paul Mäder, Frick, Switzerland

FIBL is one of the world's leading organic farming research centers, dedicated to sustainable agriculture. Two of its divisions (Soil Sciences and Crop Protection & Biodiversity) combine research and farm-scale dissemination activities with focus on nutrient use efficiency, bio-effector research and assessment of soil microbial community structures.

Tasks: Adaptation and testing of bio-effectors at various scales with special emphasis on applications in low-input and organic farming systems (WP05); developing molecular tools to trace inoculated *Pseudomonas* strains (WP03); testing the P solubilising effects of bioeffectors in combination with P recycling fertilizers (WP 06); investigation of the ecology of microbial bio-effector strains and their interactions with the native soil microflora, particularly with fungal plant-symbionts (mycorrhiza). As a contribution to the International Field Testing network (WP08), FIBL has access to fields with various soil conditions. The unique long-term DOK system comparison experiment will serve as a reference for bio-effector testing.

Participant 13:

DR.RAUPP E.K. & madora gmbh (madora)

Chair: Prof. Dr. Manfred G. Raupp; Stutensee & Loerrach, Germany

Madora manages international trading with Eastern Europe and central Asia as trader and consultant, organising projects and creating marketing and staff training plans. Madora scientifically tests the ability of liposomes, microorganisms, plant extracts and natural minerals to induce plant resistance to disease and pests, as well as researching plant activators for their ecological and yield potential.

Tasks: Leader of WP10 with Expertise in bio-effector patent and marketing issues. Responsibilities are training and public dissemination of BIOFECTOR outputs; collection, exchange, arrangement, linkage and dissemination of new information from current BIOFECTOR research activities and the general web-presentation of the project; development of new channels for public dissemination and marketing of BIOFECTOR project outcomes. Installation of an open information data-base on application fields and effectiveness of bio-effectors in agricultural production in close cooperation with FIBL Projekte.

Participant 14:

ABiTEP GmbH (ABI)

Chair: Dr. Helmut Junge, Berlin, Germany

ABI is a private company, producing agents for plant strengthening and growth promotion, based on naturally occurring soil bacteria. It also offers a sound service of production technology. ABI owns research laboratories and bioreactors and is well equipped for extraction and formulation of a wide range of bio-formulations. The company provides a large collection of defined Bacillus mutants and Bacillus strains of different ecological origin.

Tasks: As leader of WP01, ABI will develop and deliver bio-effector products, with special emphasis on collection, fermentation and formulation of *Bacillus* strains. Application fields comprise soils with limited nutrient availability, tolerance to low temperature, drought and high salt concentrations (WP04). Based on the experiences in WP02, the company will provide also production and development of combination products with synergistic interactions.

Participant 15: **Arbeitsgemeinschaft Huettenkalk e. V. (HKKalke)**

Chair: Dr. Martin Rex Duisburg, Germany

The "Association for Liming Fertilisers from Iron and Steel Slags" encompasses all fertiliser-producing steelworks in Germany and Austria. It works on research, development and testing of fertilizers, based on recycling products from steel production and has long experience in vegetation experiments for nutrient research with special emphasis on phosphate and liming fertilisers. Moreover, HKKalke has been engaged in the development of technologies for phosphate recovery from residues and wastes from bone meal and sewage sludge incineration to produce fertilisers with high phosphate efficiency.

Tasks: Responsible for production and delivery of fertilizers, based on industrial waste products (WP01). Contributes to WP05 (Organic Farming) and WP06 (Recycling Fertilizers), by testing the respective fertilizers with respect to nutrient efficiency and environmental characteristics. In WP06, strategies to improve plant nutrient supply from industrial recycling fertilizers by application of bio-effectors are developed, including the investigation of perspectives for fertiliser placement (WP07).

Participant 17:

Bayer Crop Science Biologics GmbH former Prophyta Biologischer Pfanzenschutz GmbH (PROPH)

Chair: Dr. Arite Wolf, Malchow, Germany

Prophyta is a German-based bio-control company which develops, produces and markets biological products, processes and services for integrated crop protection. The products are based on living fungal microorganisms and the company has developed a patented solid state technology for the mass production of filamentous fungi. Prophyta was acquired by Bayer CropScience in January 2013.

Tasks: As contribution to WP01, PROPH will develop and deliver bio-effector products, based on collection, fermentation and formulation of filamentous fungi. Moreover, compatibility studies on the effects of single and multiple combinations of promising bio-effectors on model plants will be conducted as a contribution to WP02 with the final goal to produce combination products with synergistic properties.

Participant 18:

Sourcon Padena GmbH & Co. KG (SP)

Chair: Karin Mai, Tuebingen, Germany

Sourcon Padena is a medium-sized biotechnology company as a spin-off from the Universities Hohenheim and Tuebingen. The company develops and produces biological plant protection products. Over the past few years the company has collected valuable experience in the field of biological plant protectors, especially bacterial pathogen antagonists based on Pseudomonades (resp. non spore forming bacteria) with a wide range of additional plant growth promoting properties.

Tasks: Contribution to WP01, especially production of bacterial bio-effector products based on pseudomonas strains with a unique fermentation and formulation technology.

Participant 19:

FIBLProjekte GmbH (FIBL-Projekte)

Chair: Rolf Maeder, Frankfurt, Germany

FIBL-Projekte provides scientific services to organic agriculture at the interface between research and agricultural practice. From this mandate it derives the four pillars of its work for organic agriculture and the organic food industry: Knowledge transfer, drawing up concepts to strengthen organic agriculture, scientific support for actors in the field and promotion of actor networks. Key activities are the co-ordination of actors within the organic movement as well as the development and distribution of information about organic agriculture to a broad range of players from science, consultancy and practice.

Tasks: FIBL-Projekte will contribute to the public dissemination of BIOFECTOR project results (WP10), create a public data-base on applications of bio-effectors for information of farmers and agricultural advisors across Europe, and apply their experience of organizing workshops, field days, conferences, symposia and exhibitions.

Participant 20:

The Agricultural Research Organisation of Israel – the Volcani Centre (ARO)

Chair: Dr. Asher Bar-Tal Bet Dagan, Israel

ARO is the research institute of the ministry of agriculture and rural development of Israel and is responsible for research and development in the areas of agriculture and the environment, to further the prosperity of the country and the well-being of the public.. Focuses are basic and applied research with special emphasis on arid zone agriculture, enabling Israel - a country short of all the resources required for agriculture - to achieve among the highest levels of agricultural output in the world.

Tasks: Leading WP07 (Utilization of bio-effectors (BEs) in combination with localised fertilizer application technologies), the centre will adapt bio-effectors for integration into strategies of fertilizer placement or combined application of water and fertilisers by drip irrigation close to the target plants. The final goal is a more efficient use of the applied fertilisers and of water (particularly under Mediterranean conditions) and assessing functions and benefits of bio-effectors under abiotic stress (WP04). The site-specific adaptation of bio-effectors combined with placed fertilization and drip fertigation will be tested under real production conditions as a contribution to the International Field Testing network (WP08).

BIOFECTOR

Participant 21:

Agriges s.r.l (AGRIGES)

Chair: Patrizia Ambrosino, San Salvatore Telesino, Italy
patrizia.ambrosino@agriges.com

AGRIGES is a company, operating in the area of biological and integrated crop nutrition with strong interest in developing new products for sustainable agriculture. Currently, the company produces a broad range of fertilizers including algal derivatives, containing various organic compounds with plant growth regulator activity.

Tasks: As contribution to WP01, the company will develop and deliver fertilisers and bio-effector products based on seaweed extracts, including combination products and synergistic mixtures (WP02) and will assess functions and benefits of bio-effectors under abiotic stress conditions (WP04).

Participant: 26

CMAST-Modis (CMAST)

Chair Project Management BIOFECTOR:
Kathrin Prebeck

CMAST-Modis delivers Project Management, Consulting and Business Solutions services to its life science clients, with >50 experienced project managers and consultants, specialized in 4 business areas. With our Strategic Collaborations business unit / team, services range from facilitating multi-stakeholder collaboration platforms to advisory services on funding opportunities and on-site project management of large multi-stakeholder or consortia projects. Our recent project engagements summarize as follows:

* IMI projects (ND4BB, Predict TB, EBOVAC, EMIF, RADAR, EPAD, PHAGO)
* 3 large consortium H2020/FP7 projects (MSCA-IAPP, SPIRE)
* Other funding agencies (BARDA, NIH, BMGF, WHO, BioAster, VLAIO, IWT)

With thorough understanding of academic as well as industrial Life Sciences research environments, CMAST is able to form a neutral "bridge" between the various stakeholders' needs and facilitate the joint effort towards high quality project outcomes.

Tasks: As leader of WP11, CMAST-Modis has the general task of administrative project management to support the coordinator to accomplish all tasks except scientific coordination and management of the scientific activities. To organise communication within the project and to ensure punctual and error-free submission of reports and financial statements, CMAST provides a specifically developed web-based project management tool solely for the use of FP6/7 consortia.

BIOFECTOR - Data Base

As one of the first activities, **BIOFECTOR** starts the installation of a public data base, collecting information on commercially available bio-effector (BE) products, as an information guide for farmers and scientists and a platform for producers to present their products, including also independent evaluations concerning the efficiency of the respective BEs.

However, the ambitious goal of a complete and reliable database can only be achieved in close cooperation with producers and distributors of bio-effector products. Therefore, we would kindly like to invite all producers and distributors to participate in this project. More information and a questionaire required for the registration of BE products for the data base.

https://www.biofector-database.eu/en/biofectors-homepage.html

http://www.biofector.info/mediapool/137/1375686/data/Biofector_Questionnaire_2016.pdf

Summery of the Scientific Results

Grant Agreement number: 312117 Project acronym: BIOFECTOR Project title: Resource Preservation by Application of BIOefFECTORs in European Crop Production Funding Scheme: FP7 collaborative project Date of latest version of Annex I against which the assessment will be made: 13 June 2017 Period covered: from 1 September 2012 to 31 August 2017 Name, title and organisation of the scientific representative of the project's coordinator1: Prof. Dr. Günter Neumann, University of Hohenheim Tel: +49 711 459-24273 Fax: +49 711 459-23295 E-mail: guenter.neumann@uni-hohenheim.de Project website2 address: www.biofector.info

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.

Final Report: Section 1 – Final Publishable Summary Report

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 bioeffectors (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.

Final Report: Section 1 – Final Publishable Summary Report

• 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.

Final Report: Section 1 – Final Publishable Summary Report

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 bioeffectors (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.

Final Report: Section 1 – Final Publishable Summary Report

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 smallscale 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

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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 growthpromoting potential of the inoculants. This was related with synergistic effects on ammoniuminduced 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 Metaanalysis (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 noninoculated 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 (CombiFectB, 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

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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

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.

Bio-effector Reported effects Application mode Stage of Marketing Bacillus atrophaeus, B. simplex strains (Combi product ABI)

B. amyloliquefaciens FZB42 + Trichoderma harzianum OMG16 +Zn/Mn (AUAS/ABI)

Trichoderma harzianum OMG16 (AUAS)

Pseudomonas jessenii RU47 (JKI, SP)

Azotobacter chroococcum A76 (UNINAa)

Algavyt ZnMn. Seaweed extract combi product (AGRIGES)

Manek: Plant extract combi product (AGRIGES)

Superfifty (BIOAT) Seaweed extract

DMPP-Ammonium + microbial BEs (UHOHa EurochemAgro)

DMPP-Ammonium + non-microbial BEs (UHOHa)

Plant growth promotion, stress resistance

Plant growth promotion Cold/drought stress resistance

Plant growth promotion Stress resistance

Plant growth promotion, P mobilization, Biocontrol

Salinity/drought resistance

Cold/drought resistance

P mobilization Plant growth promotion# Cold/drought stress resistance

Plant growth promotion Cold/drought stress resistance

Seed dressing, soil incorporation, placement

Soil incorporation, placement

Soil incorporation, placement

Seed dressing, Soil incorporation

Soil incorporation

Soil incorporation, foliar spray

Foliar spray

Foliar spray

Soil incorporation, placement

Soil incorporation, placement foliar spray Patented

Ready for patenting

Ready for patenting

Ready for large scale production

Ready for large scale production

Commercial product. Novel application perspectives in agriculture

Commercial product: Novel application perspectives in agriculture

Commercial product: Improved formulation Patented

Ready for large scale production

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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.

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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 growthpromoting 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.

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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 nonmicrobial BEs and target plants detected in the framework of WP03 activities.

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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; SuperFifty), plant-(Manek) and compost extracts (compost tea) was able to reduce drought stressinduced 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.

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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

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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 metaanalysis, 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.

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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).

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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 nonmicrobial 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)

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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.

Country Crops Bio-Effectors Fertilizers Soil P level Plant growth results Germany Wheat NH4+ + Microbial Min. / org. Low Tendential increases Germany Maize NH4+ + Microbial Min. / org. Moderate but not limiting Improved early growth Italy Maize NH4+ + Microbial+ Algae +Humic acids Mineral very low Improved early growth and yield Italy Maize Microbial Organic Very low Improved early growth Czech Republic Maize NH4+ + 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 NH4+ + Microbial Mineral Low Increased biomass and yield

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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, plantmicrobial 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 welldocumented 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

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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 nonmicrobial 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 FIBLProjekte, 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/biofectorshomepage.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 peerreviewed 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.

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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 bioeconomy) 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).

Final Report: Section 2 – Use and dissemination of foreground

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)

Final Report: Section 3 – Report on societal implications

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

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