Factsheet
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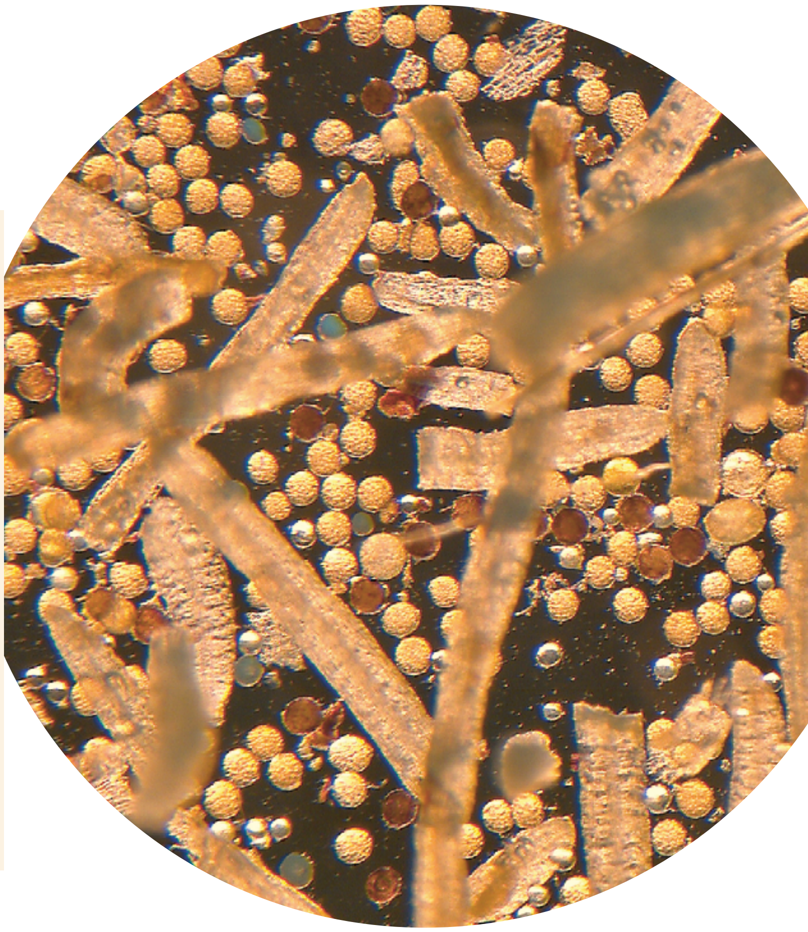
Biofertilisers



In the last years, organic amendments, active natural metabolites or beneficial microbes are discussed as environmentally friendly strategies to reach the goal of a more sustainable crop production. The use of microbial-based inoculants and the exploitation of beneficial interactions with plants has gained increasing interest worldwide. Beneficial microbes can enhance plant growth by increasing their tolerance to adverse soil and environmental conditions or by improving the plants' resource utilisation efficiency. However, developing specific microbial-based inoculants or so called biofertilisers with beneficial effects that are also suitable for agricultural applications under different environmental conditions is challenging. Currently, some commercially available biofertilisers are of low quality or are difficult to apply. This results in a loss of confidence from farmers. However, the quality improvement of microbial-based formulations and the advancements in the understanding of biological mechanisms have continuously helped enhance the efficiency at field level. This fact sheet summarises the latest research findings.

Agriculture and the role of soil microorganisms

The Green Revolution of the 20th century allowed the high increase in global food production. Two main developments characterised it: chemical inputs (such as pesticides, herbicides and chemical fertilisers); and improving crop plants through targeted breeding and genetic manipulations. However, advantages achieved through chemical fertilisation have high environmental costs. In the last few years, there has been a rising demand to reduce the use of chemical products and to develop more sustainable agri-food systems both for environmental and human health. A promising approach to achieve this goal is based on natural inputs with reduced environmental impact, such as the utilisation of microbial-based inoculants and manipulations of the microbial community structure[1].

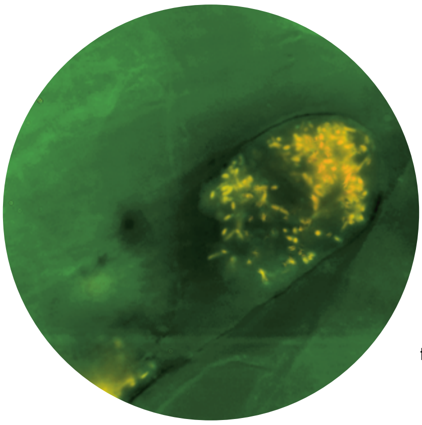
Soil microorganisms are the most abundant organisms on the Earth; there are more microbes in one teaspoon of soil than there are people on Earth. At an area of one square meter to a depth of 15 cm, there can be up to 500 g of bacteria, 500 g of actinomycetes and 1.5 kg of fungi depending on the type of ecosystem[1]. Some of these are essential for decomposing organic matter and recycling of nutrients, while others form relationships with plant roots and provide important nutrients[2]. Their potential was recognised, leading to their

[1] https://ohioline.osu.edu/factsheet/SAG-16

commercialisation. Intensive farming reduces the abundance and activity of soil microbes, but the application of microbial-based inocula might help to restore microbial populations[3]. The use of microbial inoculants has a long history, beginning with broad-scale rhizobial inoculation of legumes for nitrogen (N) fixation in the early 20th century. Recently, strains of *Bacillus, Pseudomonas, Glomus, Azotobacter, Trichoderma* and others have been commercialised due to their abilities to enhance plant production and have been extensively studied and described[1,4]. Within the last decade, the global biofertiliser market size has steadily increased. In 2019, it was valued at USD 1.0 billion and is anticipated to achieve a compound annual growth rate of 12.8 % from 2020 to 2027 [2].

What are biofertilisers?

Microbial inoculants, also known as «soil inoculants» or «bioinoculants» are agricultural amendments containing beneficial rhizospheric or endophytic microbes that promote plant performance. Considering their function, different kinds of microbial inoculants exist. Here we will exclusively focus on biofertilisers.



Kosakonia pseudosacchari, an associative N fixer, colonising tobacco roots.

Biofertilisers are products that contain living or inactive cells of efficient bacteria, fungi or algae alone or in combination. The microbial components are able to colonise the rhizosphere or the interior of the plants. They promote the growth of plants, by improving the acquisition of primary nutrients. Biofertilisers can be applied to soils, seeds and plant surfaces.

Types of biofertilisers and modes of action

Nitrogen fixers

Some bacterial strains and algae are able to fix atmospheric N into plant-available forms like ammonia and nitrate. This process is known as Biological Nitrogen Fixation (BNF)[5].
This mechanism allows the utilisation of some microorganisms as biofertilisers, which may act as a substitute for mineral N fertilisers. This might help to maintain soil N reserves[6]. Nitrogen fixers can be divided into three groups: free-living and associative bacteria like *Azobacter* and *Azospirillium* and symbiotic bacteria like *Rhizobium, Frankia* and *Azolla*. In (sub-) tropical, but less so in temperate climates, the non-symbiontic N-fixers contribute to the N uptake of plants.

Azotobacter: They are free–living and associative N fixing bacteria. Besides BNF, *Azotobacter* strains provide many other beneficial effects that stimulate plant growth and improve plant nutrient uptake, leading to increased growth, yield and quality[7,8].

Azospirillum: Among the associative N fixing bacteria they are one of the earliest discovered and the most well characterised. The plant growth promoting effects exerted by *Azospirillum* have been attributed to several mechanisms including disease resistance and drought tolerance, but especially to BNF[9].

[2]https://www.grandviewresearch.com/industry-analysis/biofertilizers-industry

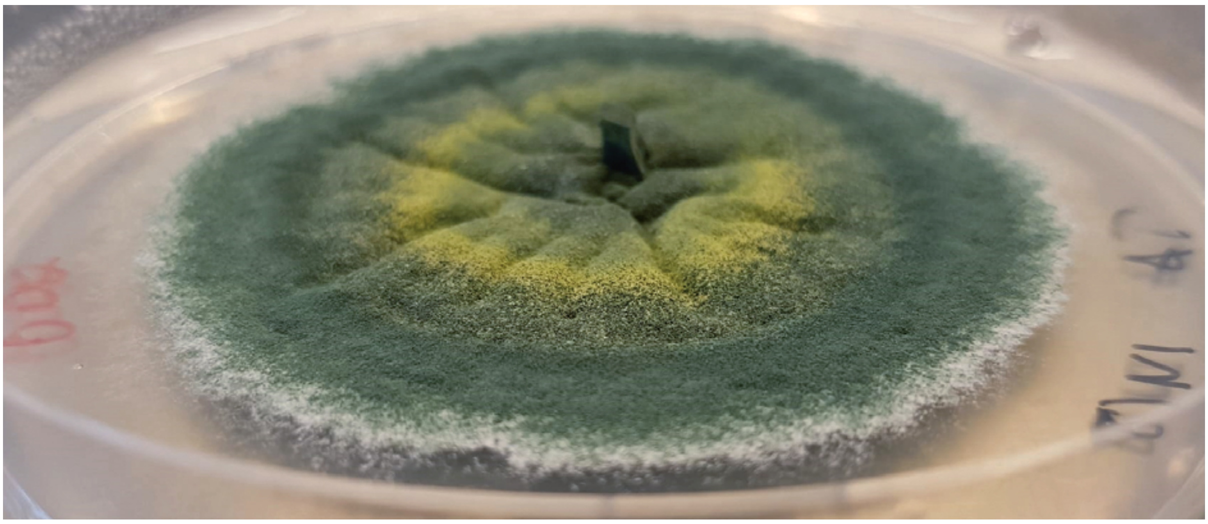
Rhizobia: They are symbiotic N fixing bacteria that induce the formation of nodules with their legume (see picture on the right). The symbiosis contributes to the significant share of N in the biosphere[10]. This interaction is interesting, considering that legumes are among the world's most important crops and fodder plants.

Other N fixers: *Frankia* is well described for its ability to form N fixing root nodule symbioses with specific host plants[11]. *Azolla* is usually called mosquito fern, duckweed fern, fairy moss or water fern, and is a small free-floating aquatic fern[12]. Biofertiliser containing *Azolla* significantly increases the N level in paddy soils. Finally, *Cyanobacteria* can be both free-living and symbionts with lichens, ferns and cycads. Their contribution in total BNF is high, but they are only capable of fixing atmospheric N under N limited conditions[13].

Nodulated soy bean roots

Phosphorus solubilisers

Phosphorus (P) is an essential macronutrient in soil, that is necessary for plant growth and development. It is involved in various fundamental biological functions, but its availability is limited. Thus, P fertilisers have become the second most applied agrochemical in the world following N fertilisers[14]. In soils, P solubilisation is mainly conducted by P solubilising bacteria (PSB) and to a smaller extent by P solubilising fungi (PSF)[15]. It is assumed that 20–25 % of plants' P requirement is fulfilled by bacteria and fungi. Well studied bacteria in soils are *Pseudomonas putida* and *Bacillus megaterium* while the most known fungal genera are *Aspergillus, Penicillium and Trichoderma*. Some actinomycetes are also known for P solubilisation activity, and they are gaining popularity due to their ability to survive in extreme environments[16].



Trichoderma asperelum on agar plate

Potassium and zinc solubilisers

Potassium (K) is an essential macronutrient for plant development. Naturally, soils contain large amounts of K, but only 1 to 2 % of it is available for plant uptake[17]. Bacteria, fungi and actinomycetes can solubilise K in soil, achieved through different chemical reactions[16,18].

Bacillus licheniformis, *Pseudomonas azotoformans* and *Enterobacter hormoechei* are among the most effective K solubilisers, as inoculation studies on rice and cucumber have shown[19,20,21].

Zinc (Zn) can be solubilised by different microbial species like *Bacillus subtilis*, *Thiobacillus thioxidans* and *Saccharomyces sp.*[22]. These microorganisms can be applied as biofertilisers to increase Zn availability for plants.

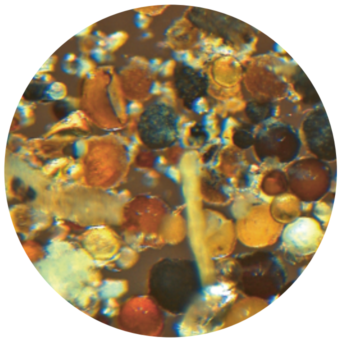
Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts, belonging to the phylum *Glomeromycota*, and form symbiotic associations with the roots of about 80 % of all land plants, including most agricultural crops[23].

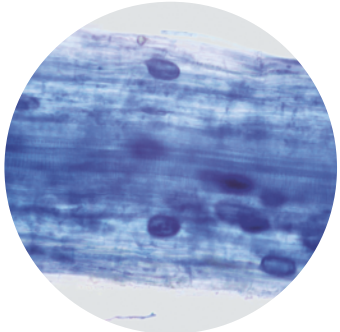
They represent a fundamental link between plants and soil mineral nutrients as they have been shown to increase the uptake of particularly P and Zn, but have also been shown to contribute to N, K, Magnesium, Calcium and Sulfur acquisition[24,25]. In addition, AMFs provide other kinds of benefits to plants, such as an improvement in drought and salinity tolerance and disease resistance[24]. Thus, in the last years, they have received growing interest for use as biofertilisers in agriculture, horticulture, afforestation and reclamation of deserts[25].

Other mycorrhizae

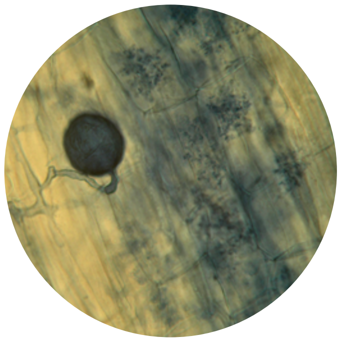
In general, mycorrhiza is the symbiotic association between a fungus and a plant root. Many tree species in worldwide forests depend on *ectomycorrhiza* (ECM). The fungi that form ECM associations taxonomically belong to *basidiomycetes* and to a lesser extent, *ascomycetes*[26]. These fungi improve the nutrition of trees by mobilising nutrients from organic compounds. At the same time, they also contribute to the carbon supply of soils and are thus responsible for carbon flows within forests[26]. *Ericoid mycorrhiza* is an association among plants of the order *Ericales* and soil fungi[27], while *orchid mycorrhiza* are formed between plants of the family *Orchidaceae* and soil fungi[28]. The latter is decisive during germination for the delivery of carbon to the seedling[29].



Spores of different AMF species extracted from an agricultural soil.



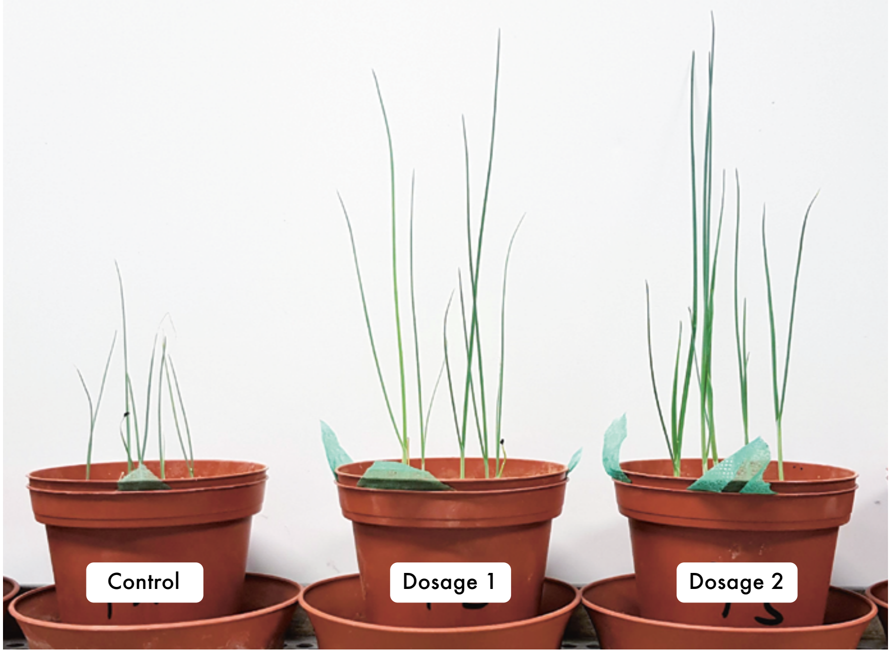
AMF colonising the roots of sorghum.



AMF colonising maize roots by hyphae, arbuscles and vesicles.

Microbial consortia

Combinations of microbial strains such as rhizobacteria and fungi, present a good strategy to develop biofertiliser products for sustainable agriculture[30]. Besides their potential multifunctionality due to the complementarity of their traits, it has been suggested that products containing consortia might better survive in various environments compared to single strain biofertilisers. This is due to their mutual stimulation via communication and differentiation[31]. Also, they were observed to efficiently enhance plant growth and performance under abiotic stresses (extreme temperature, pH, salinity, drought, heavy metal and pesticide pollution)[30,32]. Well-known products in this category are effective microorganisms (EM), which represent a big market. However, some studies revealed that the observed effects of EM were not due to the living microorganisms themselves. But that the nutrients in the substrate that contains the microorganisms promote plant growth[33,34,35].



Leek growing in the absence (control) and presence of low (Dosage1) and high (Dosage 2) dosages of a microbial consortia in a phosphorus deficient soil.

Areas of application

Use in horticulture

Biofertilisers are widely applied in horticulture[36,37,38] for the following reasons:

- the production of high value and high yielding crops
- the use of growth substrates lacking native microorganisms
- the controlled environmental conditions (unlike the open field)
- simplified modes of application via irrigation[36]
- the high specialisation of crops and the use of intensive cultivation practices in horticulture causing losses of soil fertility.

In these contexts, biofertilisers may contribute to plant growth and replenish microbial populations in soil[36]. Biofertilisers used alone or in combination with other inputs are usually applied in different horticultural fields including vegetable production, floriculture, arboriculture and hobby gardening[37,38].

Applications of biofertilisers containing *Azotobacter* alone or in combination with *Glomus* strains in apples and bananas increased plant growth and fruit quality[38,39]. In collaboration with partners, FiBL showed the growth-promoting effect of native and commercial AMF in date palms and naranjilla when inoculated under nursery conditions[40,41,42]. Also in floriculture, the combined application of biofertilisers and natural or chemical inputs increased crop productivity and product quality[43]. Combined application of farmyard manure and P solubilising bacteria significantly improved yield and nutrition in marigold leaf[44] and inoculation of *Azotobacter* increased growth of tulips[45].

Use in arable farming

Biofertilisers use is not limited to horticulture and is becoming increasingly important in arable farming. Rhizobial inoculants already have a long tradition in the cultivation of legumes. Because of rhizobia's host specificity, crop and environment-specific inoculants are of major importance in the cultivation of non-local legumes such as soybean, or in soils with a low population of effective rhizobia[46]. The picture on page 6 showing successful applications of rhizobia products in a field trial with lupins performed by FiBL in collaboration with partners. Other FiBL trials revealed that the choice of effective commercial inoculants is prerequisite for stable yields and protein contents in soybean cultivation[47]. Furthermore, biofertilisers are gaining importance in other arable crops. Inoculation of *Azospirillum* in combination with other microbial strains showed positive results in different crops: with *Pseudomonas*, it increased the grain yield of maize and cotton plants[48]; with *Azotobacter* the yields of pearl millet, sorghum wheat and rice[49]; and with *Arthrobacter* and a P solubilising bacteria strain the grain yield of barley[50]. Studies performed in a collaborative ISCB project, by FiBL with partners in Switzerland and India, revealed that inoculation of AMF and rhizobacteria:

- help reduce the mineral fertiliser needs of pigeon pea and finger millet by 50% while maintaining the same yield as with full mineral fertilisation[51].
- increase crop yield, mineral nutrition and P use efficiency and, at the same time, improve soil quality[52].
- is more effective at sites with low inherent soil fertility and when applied as consortia of mycorrhiza and rhizobacteria strains.

Use for restoration

Another area, which greatly benefits from the application of biofertilisers, in particular AMF, is ecosystem restoration[53]. Human activities such as clear-cutting and mining, but also natural factors

Lupines inoculated (left) or not inoculated (right) with the product HiStick.

such as fires or geomorphic processes, may affect the stability of natural ecosystems. This might lead to the degradation of the soil and strongly limit the spontaneous recovery of the vegetation. The successful application of AMF has been demonstrated in several studies such as revegetation of Mediterranean soils affected by desertification[54] and restoration of mining-impacted soils[55].

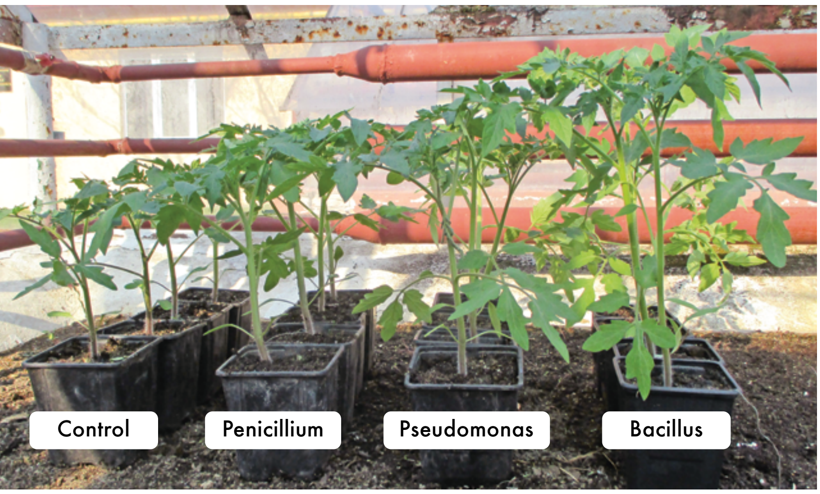
Effectiveness of biofertilisers

Scientific communities across the globe have extensively studied the effectivity of biofertilisers on many different crops in all kinds of ecosystems. This results in a large number of publications summarising the benefits of different biofertiliser types such as AMF, P solubilisers and N fixers. In contrast, when applied in practice by farmers, effects often do not occur. The reasons for this lack of growth improvement are manifold and mostly result from an incompatible combination of environmental factors especially soil conditions, biofertiliser type and crop/genotype[56]. So far, specific recommendations for the use of certain products can hardly be made with few exceptions concerning the use of brady-/rhizobia products for cultivation of non-regional legumes.

However, a recent global analysis revealed some overall patterns to predict biofertiliser effectivity in relation to local soil conditions, climatic conditions, crop type and biofertiliser type[56]. The study highlighted that the effectivity of biofertilisers strongly depends on the soil conditions and that the conditions triggering best performance differ depending on the type of biofertilisers applied:

- AMF perform best under low organic carbon and at low plant-available soil P levels (10–25 kg P/ha)
- P solubilisers also perform better under low organic carbon contents but slightly higher plant-available soil P levels (25–35 kg P/ha).
- N fixers perform best under increasing soil organic carbon contents and plant-available P soil levels of more than 45 kg P/ha.

Besides soil conditions, crop type also affects biofertiliser effectivity. Legumes and vegetables are more responsive to inoculation than root crops and cereals, which might result from their increased needs for nutrients.



Tomatoes growing in the presence of biofertiliser products containing Penicilium, Pseudomonas or Bacillus or without biofertilisers (control).

Another factor driving biofertiliser effectivity is the climate. Biofertilisers were shown to be more effective in dry regions, followed by tropical and continental climates. The main reason is the lower soil fertility with low soil organic matter, N and P contents typically observed in dry regions. **Low soil fertility also means a lower abundance and activity of native soil microbes, which consequently makes the application of microbial inoculants more effective.** In addition, crops growing in dry climates are more prone to stress including heat, drought and salinity. Microbes can produce a number of molecules such as plant hormones, enzymes and secondary compounds, which help to reduce stress in plants, thus stabilising their yields.

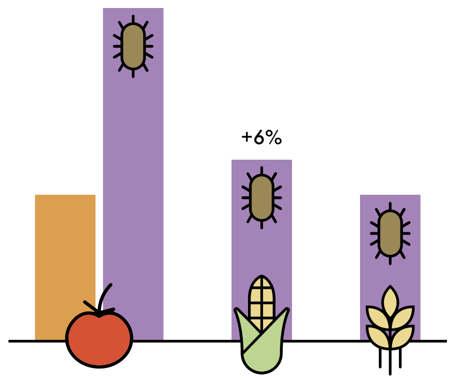
Effectiveness in temperate climates

In collaboration with twenty European partners, FiBL recently terminated the FP7 project BIOFECTOR[3], which aimed to reduce mineral fertiliser input in European agriculture through the use of commercially available and newly developed biofertiliser products. About 150 experiments with more than 1100 experimental variants were conducted to elucidate, which biofertilisers are best suited to improve the cultivation of maize, wheat and tomatoes and which are the driving factors determining successful biofertiliser applications.

A comparative study analysing all pot and field experiments revealed that culture/cropping system and fertiliser type are the major factors determining biofertilisers' efficacy. The study has shown that tomatoes grown in greenhouse culture or open field culture with previous greenhouse nursery responded best to inoculation with growth increases of up to 27 %, followed by maize with 6 % and no growth increase in wheat (Figure 1).

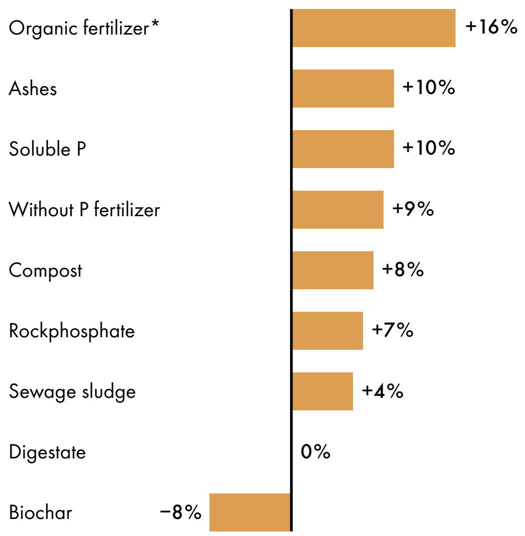
The study further revealed that biofertilisers especially increased growth under low organic matter and soil P levels, which is also in accordance with the results reported before. In addition, P fertiliser types were also shown to affect the efficacy of biofertiliser applications (Figure 2). Biofertilisers combined with manure were most effective at increasing crop growth, followed by ashes, soluble P, compost and rock P. At FiBL, we performed five pot experiments using soil from two locations and six field experiments performed at two locations using maize as the test crop. Under Swiss organic conditions, none of the tested biofertilisers were able to significantly increase the growth of maize.

Figure 1: Effect of biofertiliser on different crops



Tomatoes grown in greenhouse and/or subsequent open field culture responded best to inoculation with growth increases of up to 27%, followed by maize with 6 % and no growth increase in wheat

Figure 2: Effect of biofertiliser in dependence of P fertiliser



Biofertilisers combined with * organic fertilisers such as animal manures, meat/feather/bone meal best increased crop growth, followed by ashes, soluble P, compost and rock P.

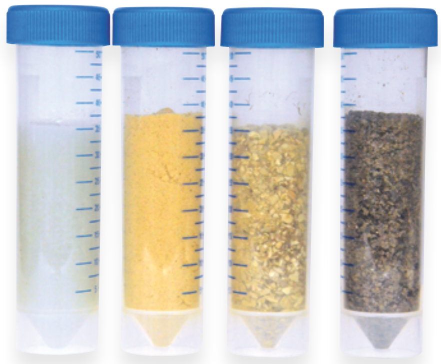
Especially in the field, no stable results could be observed. All experiments were performed in organically managed soils high in organic matter, which might have caused the lack in growth response.

[3]https://www.biofector.info/index.html

Biofertiliser formulations

Laboratory isolation and screening based on plant growth-promoting traits of new microbial strains are the first fundamental steps to develop a new microbial inoculant[1]. These phases are followed by *in vitro*, greenhouse and/or field experiments, including a range of crops to evaluate the microbial effectiveness and persistence in the soil[57]. This is necessary because even if interesting laboratory evidence is obtained, this does not always result in plant growth promotion under field conditions.

Finally, the microbes must be multiplied and formulated in a product that meets various requirements: a high concentration of vital microbial cells and a shelf-life of at least six months. For this, the choice of an appropriate formulation that best preserves the vitality of the microbes, from their production until their application, is of major importance[1].



Different types of biofertiliser formulations: liquid, freeze-dried powder and carrier-based formulations.

Solid formulations

The carriers used in solid (or carrier-based) formulations can consist of organic, inorganic, or synthetic, low-cost materials and are easy to process and sterilise. They should provide a short-time protective niche for the microbes in the soil, either by physical protection or by providing specific nutrients[58,59]. There are two kinds of solid formulations, peat and granule-based formulations. Peat is an inhomogeneous and complex material, which variably affects microbial cell growth and survival during multiplication[60]. In addition, toxic compounds might be released during sterilisation processes resulting in a reduction in microbial growth and survival, which might further hamper microbial efficiency[58,61]. Granule-based formulations are made of peat prill, calcite, vermiculite, or silica grains coated or impregnated with the selected microbial strains[1]. The application procedures for solid biofertilisers can be easily controlled; in fact, they can be placed near to the seeds to facilitate the microbial interaction with the rhizosphere[58]. However, there are some general disadvantages in their use. For instance, their voluminous size results in high costs of transport and storage. The microbial concentrations quickly decrease in solid formulation due to the absence of nutrients or protectors for microbial cells; as a consequence, the rate of application has to be increased to achieve desired results[1]. A special and more stable type of solid formulations are **freeze-dried powders**, obtained by direct freeze-drying of target cells together with a substance such as pure glucose or milk powder to protect the cells against freezing damage[62].

Table 1: Advantages and disadvantages of different biofertiliser formulations

		Advantages	Disadvantages
Solid bio-fertilisers	Carrier-based biofertilisers	• Cheap • Easy to produce • Less investment	• Low shelf-life • Temperature-sensitive • Contamination prone • Low cell counts
	Freeze-dried powders	• Longer shelf life • High cell counts • Contamination-free (100 % sterile)	• Very high cost • Higher investments for production unit
Liquid biofertilisers		• Longer shelf life • Temperature tolerant • High cell counts • More effective • Contamination-free (100 % sterile)	• High cost • Higher investments for production unit

Liquid formulations

Besides the microbial cells, liquid formulations can also contain nutrients, special cell protectants or chemical substances that promote the formation of resting spores or cysts to increase the products' shelf-life and the microbes' stress tolerance[63]. Liquid formulations are the solution to many challenges associated with solid formulations. They have a higher **shelf-life** of up to two years compared to the six months for solid formulations, and they are more tolerant to high temperatures of up to 55 °C (Table 1). Liquid formulations also have higher population densities of up to 10^9 colony forming units (cfu) ml^{-1} instead of only 10^8 cfu g^{-1} found for solid media. In addition, they can be more easily applied using irrigation systems.

Risks and constraints in biofertiliser production and use

Previous sections described limitations regarding biofertilisers' effectiveness, which depends on environmental factors, antagonism/competition with soil microorganism. Also inappropriate handling, transport and storage could result in a lower performance of biofertiliser products. Further problems related to the production and use of biofertilisers and potential solutions are summarised in Table 2.

Table 2: Problems related to the production and use of biofertilisers and potential solutions

Problem	Solution
Introduction of invasive microbes[64]	Selection of suitable and competitive strains for specific climatic regions, crops and soils[22]
Inefficient products[22]	
Low quality products (lack of vital cells)[64]	Major quality assurance and research by producers
Mutations of microbial cells during multiplication in fermenters[22]	
High investment costs[65]	Research in the field of alternative growth media such as industrial by-products[66]
Poor understanding of the importance of microbes for below-ground processes	Raising farmers' awareness of the benefits of biofertilisers by increased communication through specialised journals

Alternatives: management of native soil microbes

An effective long-term alternative to the use of biofertilisers is the propagation of native microbial populations inhabiting the soil in order to improve soil processes and consequently promote plant growth and performance. Studies performed at FiBL revealed that the implementation of a range of management practices typically found in organic agriculture, promote native microorganisms in soils. Those management practices are crop rotation, reduced tillage, integration of legumes and cover crops in the rotation and the application of organic amendments such as compost. Some of these practices can be easily integrated into any existing farming system in order to increase the size and activity of microbial communities[66]. Several studies conducted at FiBL have highlighted the positive effects of compost on soil health and soil fertility caused, among other things, by the microorganisms inhabiting the compost[67,68]. Other popular practices such as the use of compost teas provide so far only little scientific evidence of effects on plants and soil. A study performed in Germany observed that compost tea increased drought stress resistance in maize[69]. However, further studies are needed to confirm their beneficial effect.

Application of compost is a good alternative to promote native soil microorganisms.

Grass-clover ley in the crop rotation

Applications in organic farming

Microorganisms have traditionally been used in organic farming and there is no objection to their application. In European organic farming, the use of microorganisms is authorised by Art. 3(4) of Regulation (EC) No 889/2008, but not explicitly mentioned in Annex I of that regulation. At the time of writing, a new legislation is in preparation, but the authors expect no changes regarding the use of biofertilisers. The following criteria must be met:

- Microbes must not be genetically modified
- Products need to meet the biosafety rules i.e. application of microorganisms must follow the product recommendations and they need to be harmless for humans, environment, crops and animals.
- Special care must be taken in case of strains imported from overseas. In order to meet these requirements, the identity (species and strain) of the microorganism in the product must be known.

Organic farmers must check these aspects before applying a biofertiliser. In case of doubt, they should consult their certification bodies. In countries where a national input list is available, it provides guidance on acceptable biofertiliser products.

In the EU, the «Common Agricultural Policy» promotes the use of biofertilisers along with organic farming. They consider it as a sustainable agricultural practice and provide up to 30% of their budget as green payment to complying farmers. Due to such favourable regulatory scenarios, for example found in North America and Europe, the biofertiliser market is expected to further expand in the coming years. In any case, the choice of products has to be in line with the EU and national legislation on organic farming, as well as rules on fertilisers and on biosafety.

What does the future hold?

Several studies successfully showed the potential of biofertilisers in increasing yield and quality of various crops. However, market prices of low value crops usually make biofertiliser application unprofitable. Considering that biofertilisers' effectiveness depends both on plant and environmental factors, products should be carefully selected and applications should accurately follow the producers' instructions. Biofertilisers represent a valuable tool for sustainable farming in dry areas where crops are challenged by abiotic stresses and low soil fertility. Considering that in the future global dryland areas are expected to increase, biofertilisers will become increasingly important. Moreover, biofertilisers can (partially) replace the use of chemical fertilisers, thus reducing the risks associated with soil pollution and human health.

In general, it is recommended that before broad application of any product, a pre-test should be conducted on a smaller area in order to identify effective products and to avoid wasting money.

«Rhizosphere engineering» is becoming increasingly important in agriculture as the sector begins to recognise the importance of microbes for resilient farming systems. This approach proposes the addition of efficient microbial inoculants, selected farming practices and crop genotypes that effectively stimulate functional and beneficial microbial groups in the rhizosphere, which are positively linked to soil fertility[32,14]. Currently and in the coming years, research is focused on these aspects and further results, which will help choose the most effective farming practices.

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