The use of genus *Bacillus* bacteria in agriculture*

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INTRODUCTION

The agricultural sector is facing concomitant challenges of increasing the crop productivity to feed the growing global populations and increasing the resource use efficiency, while reducing the environmental impact on the ecosystems and human health. In fact, fertilizers and pesticides play a crucial role in agriculture, representing a powerful tool for growers to increase yield and guarantee continuous productivity throughout the seasons under both optimal and suboptimal conditions. In the last three decades, several technological innovations have been proposed to enhance the sustainability of agricultural production systems, through its significant reduction in use of synthetic agrochemicals like pesticides and fertilizers. A promising and environmental-friendly innovation would be the use of natural plant stimulating microorganisms that enhance flowering, plant growth, fruit setting, crop productivity, and nutrient use efficiency, and which are able to improve the tolerance against a wide range of abiotic and biotic stressors.

This has promoted increased research interest in biopesticides and biostimulants in both the public and the industrial sectors (1, 2). Promotions for the use of such microorganisms in sustainable agricultural systems with reduced impact on climate are ongoing, and a number of products are now on or on their way to the market. Microorganisms useful for these purposes include virus, bacteria and fungi. This whitepaper focuses on bacteria and specifically bacteria from the genus *Bacillus*.

Bacteria are microscopic organisms, which can be found everywhere on the earth in every kind of environments e.g., from the cold surface of glaciers in Greenland to boiling springs in USA and from deep sea sediments to the driest sand deserts in Sahara and connected to every kind of macro-organisms including plant, insects and humans. In agricultural fields, bacteria are very abundant (often more than ten billion per gram) and they are very diverse with thousands of species. The rhizosphere, including the root surface and the soil closest to the roots, also harbours numerous bacteria from diverse bacterial groups. The root influences activity and composition of the bacteria due to deposition of organic compounds.

On the other hand, the bacteria play very important roles as one of the main drivers of the mineralization and fixation of nutrients for plant growth, they produce hormones that spur growth, they stimulate the plant immune system and they trigger or dampen stress responses; thus influencing plant growth and productivity. Further, they might be pathogens of plants and animals.

During the last 100 years, bacteria have further been used in agriculture as biopesticides and biostimulants for protecting plants against pests and diseases and stimulating plant growth. This area has been of increasing importance during the last twenty years due to the increasing problems realized by the use of chemical pesticides. An important group of bacteria for these two purposes is species within the genus *Bacillus*.

THE GENUS *BACILLUS*

The genus *Bacillus* is a very diverse group of bacteria encompassing species with a widespread impact on human life. The first species of *Bacillus* subtilis was described in 1872. Today the genus contains approximately 300 named species, which can vary from being extremely pathogenic (*Bacillus* anthracis; the causative agent of anthrax) and *Bacillus* cereus (causing different kind of food infections) to bacteria important for humans as they are used in the industrial production of antibiotics and enzymes, as human probiotics and as biopesticides, biostimulants and feed additives. *B. subtilis*, the type species of the genus, is one of the best-studied prokaryotic organisms and is widely used as a model organism for Gram-positive bacteria. Most *Bacillus* species are rod-shaped, Gram-positive, endospore-forming, aerobic or facultative anaerobic bacteria, and are primarily isolated from soil, water, food or clinical specimens (24).

However, this definition fails to describe the genus as a whole since *Bacillus* species display a wide array of phenotypic properties and there is no phenotypic or molecular characteristic, which is exclusive to all *Bacillus* species. For this reason, this classical view of the genus *Bacillus* has changed during the last decade, and recent research, based on whole genome sequencing, has moved about 240 species into about 30 new genera (3). Therefore, the genus *Bacillus* now exist of about 100 valid species. While the changes appear extensive, there is still no reclassification of the two largest groups of *Bacilli* with which many are familiar – the species closely related to *Bacillus subtilis* and *Bacillus cereus* (3) (Examples stated in Table 1)

BACILLUS AS PLANT BIOLOGICALS

Table 1 displays eleven *Bacillus* species commonly used in products that are used in agriculture.

SPECIES	POSSIBLE FUNCTION	PRODUCTS (EXAMPLES)
B. amyloliquefaciens	Biocontrol of pathogens Plant growth promotion	Several e.g. Integral (EU), Amylo-X (USA)
B. cereus	Biocontrol of pathogens Plant growth promotion Probiotic	Xian Mi (CH), Toyocerin (EU)
B. coagulans[4]	Probiotic	Thorne (USA), Sunny Green (USA)
B. firmus[1]	Biocontrol of pathogens Plant growth promotion	Flocter (EU), Bionem (ISR), Votivo (USA)
B. licheniformis[5]	Biocontrol of pathogens Plant growth promotion	Ecoguard (USA), Quartzo (Br), Presence (BR), Accudo (EU, KOR,)
	Probiotic Protection against nematodes	Pangoon USA), Bacillus Megaterium (USA), Attis (USA)
B. megaterium[2]	Biocontrol of pathogens Plant growth promotion Solubilisation of nutrients	
B. polymyxa[3]	Biocontrol of pathogens Plant growth promotion Solubilisation of nutrients Protection against nematodes Nitrogen fixation	Topsid (Kor), Paenibacillus polymyxa powder (CH)

B. pumillus	Biocontrol of pathogens Plant growth promotion	Yield Shield (USA)
B. subtilis	Biocontrol of pathogens Plant growth promotion Solubilisation of nutrients Protection against nematodes Protection against environmental stresses Probiotic	Several e.g. Serenade (EU), Cease (USA), Subtilex (USA), Quartzo (BR), Presence (BR)
B. thuringiensis	Protection against insect larvae pests and nematodes	Several e.g. Dipel (EU), Vectobac (EU), Turex (EU)
B. velezensis	Biocontrol of pathogens Plant growth promotion	Kodiak (USA), Taegro (USA)
B. licheniformis[5]	Biocontrol of pathogens Plant growth promotion	Ecoguard (USA), Quartzo (Br), Presence (BR), Accudo (EU, KOR,)

Table 1: Examples of *Bacillus* species used as plant biologicals.
Their possible function and examples of products based on the species are mentioned.
Recent corrected nomenclature (year for correction stated) [1]Cytobacillus firmus (2020), [2]Priestia megateriumn (2020), [3]Paenibacillus polymyxa (1993), [4]Weizmannia coagulans (2020), [5]divided into two species B. licheniformis and B. paralicheniformis (2015).

It appears from the table that they are used for biocontrol of pathogens, plant growth promotion, solubilisation of nutrients, protection against environmental stresses and for protection against insect larvae pest and against nematodes. Some probiotics are also mentioned, as some of these have been mentioned for their potential use in agriculture. The table is not complete, and minor products based on other *Bacillus* species evidently exist, and additionally further species have been investigated in research. It appears from the table that numerous products exist; the most successful products on the market are evidently products based on *B. thuringiensis*, which might account for more than 80% of the world market for biopesticides, although the actual number of products is relatively low.

The history of the development of these biopesticides illustrate the background for *Bacillus*-based biopesticides and biostimulants. *B. thuringiensis* was first isolated from infected silkworms in Japan in 1901, however it was first when it was rediscovered from flour moths in Germany ten years later it was described and named *B. thuringiensis* after the German province where the infected moths were found. Experimental uses of the bacterium for protection of different vegetables for lepidopteran larvae started in late 1920´s in a number of countries and the first product appeared ten years later in France; however further progress in the development of products based on *B. thuringiensis* were halted by the discovery of DDT and the outbreak of world War II. During the 1950 and 1960´s some products appeared on the market worldwide, but still they only remained a minor component of pest management against lepidopteran larvae. In 1970, a *B. thuringiensis* strain 2 to 200 times more toxic to major lepidopteran pests were isolated and became the basis of products produced and used worldwide even today (4).

This product was competitive with chemical pesticides in performance and costs. This started an expansion in the use of products based on *B. thuringiensis* during the following many years, mainly due to the development of methods of large-scale fermentation, and increased efficiency in production and quality control, so that formulations with high activity could be developed. The commercial interest in such products also grew rapidly when it became apparent that some chemical pesticides often were harming the environment, while few effects on the environment have been observed and recorded by the use of *B. thuringiensis*. Further isolation of *B. thuringiensis* strains active against dipteran and coleopteran larvae opened for the development of new products against new targets. Notably, products with dipteran active strains are and have been used in products for dipteran pests and vector control, particularly of black flies and mosquitoes, providing both medical and environmental benefits (4).

More recently, strains active against nematode plant pests have been isolated and described, which opens for products for control of this important group of pests.

MODE OF ACTION

The mode of action of the *Bacillus* strains used in products for the very different purposes varies indeed significantly, and the knowledge about the details of the mechanisms involved for different *Bacillus* strains are very unequal.

The mode of action known in most details is that of the *B. thuringiensis* strains used for control of insect larvae. These bacteria produce so-called cry-toxins as crystals, which upon consumption by the insect larvae are solubilized to protoxins due to the conditions existing in the gut. The protoxins are then degraded by gut enzymes to toxins, which binds to specific receptors at the surface of the gut resulting in pore formation, affecting the osmotic regulation, and causing death (4).

The mode of action in relation to control of other pathogens than insect larvae vary between the *Bacillus* strains used in different products and are often affected by a number of different factors.

These factors might include:
- antibiosis, most often affected by specific lipopeptides, directly inhibiting the pathogen
- enzymes degrading the cell-walls and plasma-membranes of the pathogens
- boosting of the plants own defences by eliciting induced systemic resistance
- direct competition for resources such as nutrients and space with the pathogen

Plant growth promotion also vary between the different strains used in the products. The promotion might be affected by improved availability of nutrients such as nitrogen, phosphorous, potassium and iron, which might exist in soil in inaccessible forms for plants and therefore needs to be fixed or mobilized to be taken up by plants. Some *Bacillus* strains are able to fix atmospheric nitrogen, others to solubilize phosphorous and iron from the soil. Another mechanism involved in plant growth promotion is the ability of some *Bacillus* strains to produce or induce production of plant growth hormones. Furthermore, some *Bacillus* strains are able to reduce abiotic stresses limiting plant growth, including salt stress and drought (5, 6, 7).

BACILLUS ECOLOGY

As mentioned above, Bacilli are common in most ecosystems around the world, including indeed agricultural fields and crops. Generally speaking, *Bacillus spp*. are one of the predominant culturable soil bacteria in the majority of soils, where they often constitute 106 to 108 bacteria per gram of soil. These numbers are composed of several species, often dominated by *B. subtilis* and *B. cereus* and species closely related to these two species, however it is very likely that every species mentioned in table 1 are present in any soil. The numbers and species vary indeed between different soils, dependent on soil type, climate and other environmental conditions and plant growth. Most *Bacillus* found in soil are present as endospores, which are survival structures able to survive many different kinds of stress, as desiccation, starvation, high temperatures, acidic and basic conditions etc. Bacillus spp. are also found on every type of crops, where they can be isolated from leaves as epiphytes, from the rhizosphere of the roots and even as endophytes within the plants. In numbers, they vary between few to 106 per gram of leaf and in the rhizosphere in numbers as found in soils. The endophytic *Bacillus* are not present in all plant species and they vary widely in numbers in the same way as the numbers for epiphytes.

The species connected to crops most often reflects the species found in the soil (24). However, almost nothing is known about the mechanisms that modulate germination, growth and sporulation of *Bacillus* in the environment.

However, most members of the genus are saprophytes that are able to degrade a range of different kinds of organic matter, so it is generally assumed that vegetative growing cells notably are associated to organic matter or the rhizosphere (27).

ENVIRONMENTAL RISK ASSESSMENT

Environmental risk assessment (ERA) is an important tool for evaluating risks of the agricultural use of microorganisms for the environment. ERA is a structured, systematic knowledge-based process, which assess the likelihood of an inappropriate effect on the microorganisms in the environment (8). The assessment exists of four steps:

- Hazard identification: a process based on knowledge about the specific microorganism identifies hazards connected to its presence and use
- Hazard characterization: assessment of the identified hazards based on the likelihood of exposure and their consequences

- Assessment of the exposure: assessment of the actual exposure related to the specific use, and possible growth and dispersal related to this use
- Risk characterization: the extent of the risk for the exposed is estimated, allowing for risk management based on the real risk in preference to the potential risk

This systematic assessment corresponds to the method used for chemicals; however, three basic conditions differentiate the microorganisms from the chemicals. First, microorganisms are living organisms, which might be active and might divide and grow at the right conditions, thus increasing in numbers and biomass; at specific uses it is often what is intended to happen. Secondly, microorganisms respond actively to local environmental conditions, so they change their characteristics as the conditions change, Thirdly, most microorganisms are naturally occurring and widespread in the environment, so their usages in the environment do not in most cases add anything new to the environment; however, uses might influence their numbers affecting exposure (8).

ERA of products based on *Bacillus* for agricultural uses must be case by case, as for all other products. Hazard identification in relation to the environment of specific *Bacillus* strains must be based on general knowledge of the organism, its mode of action and the specific use. Because of this, it is not possible to point at general points of concern related to *Bacillus*. However, such general points might be related to some of the different uses of the products, as exemplified in Table 2

EXAMPLE OF *BACILLUS* SPECIES	FUNCTION AS PLANT BIOLOGICAL	POTENTIAL ENVIRONMENTAL HAZARDS
B. thuringiensis	Control of target invertebrate pests	Effects on non-target organisms. Spread to non-agricultural environments effecting target and non-target organisms
B. subtilis	Control of plant diseases by production of antibiotic/toxic compounds	Disturbance of the native microbiota that might cause effects on significant processes driven by microorganisms. Development and spread of antibiotic resistance. Release of toxins to ground water.
B. amyloliquefaciens	Plant growth promotion by production of hormones that promote growth and yield	Effects on plant growth of other plants than the crops
B. subtilis	Protection of crops against environmental stresses	Spread to non-agricultural environments affecting plant diversity
B. polymyxa	Crop growth promotion by improved availability or fixation of nutrients	Increased release of nutrients to the environment

Table 2: Potential environmental hazards related to the use of bacteria from the genus Bacillus in agriculture

In relation to exposure, some information is available in relation to the fate of *Bacillus* after application to crops in agricultural fields. This information is mainly based on studies with *B. thuringiensis*. These studies show that *Bacillus* applied to crops survive for a relatively short period, rarely more than one month with the decrease in numbers notably happening during the first two weeks after the application. This fast decrease in numbers is also evidenced by the fact that applications have to be repeated quite often to keep the protection against the pest. In soil, the survival is much higher and approximately ten percent of the applied bacteria might survive for a year and has been documented after 13 years. In the rhizosphere the fate of the applied bacteria, most often include germination and proliferation, followed by a decrease in numbers during ripening and after crop harvest.

In this context, it is important to note that survival of the applied *Bacillus* does not per se constitute a hazard, as they are already present in the environment. The major factor responsible for the reduction on crop is UV-light; while in the soil, it is biotic interactions and environmental stressors (9, 10, 11, 12).

Risk management in relation to agricultural uses of *Bacillus* could include specified periods between the application of the product and harvest or specific agricultural management procedures to decrease prolonged survival.

Given the supposed often transitory survival of bacterial inoculants many scientist and practitioners assume that inoculants used in agriculture will have negligible effects on the environment. However, such general conclusions are not evidenced by experimental research, as very few comprehensive field-based studies exist. An example of long-lasting changes in the microbial composition in a soil by a strain of *B. amyloliquefaciens* has been described. However, it remained unclear whether the measured changes were due to direct effects from the inoculant or indirect effects (13, 14).

EU REGULATIONS

The use of pesticides in EU is regulated by regulation 1107/2009 (25) concerning the placing of plant protection products on the market. This regulation applies to substances, including microorganisms having general or specific action against harmful organisms or on plants, parts of plants or plant products, referred to as ‘active substances’. This implies that use of microorganisms for control of every kind of harmful organisms on crops by general or specific actions are regulated by this regulation. In the regulation, microorganisms “means any microbiological entity, including lower fungi and viruses, cellular or non-cellular, capable of replication or of transferring genetic material”. According to the regulation, the registration is a procedure in two steps:

- The so-called active substance, here the microorganism, is approved at EU level
- Formulated products are authorized at member state level

The basic criteria for authorization are that they should be safe for the human public, and the environment and be efficacious. For authorization, a number of information is demanded in the regulation. These include identity, biology, mode of action of the microorganism, production of toxins by the organism, and results of a number of standardized tests. In relation to health and effects on non-target organisms, and information in relation to survival and possible effects in the environment. This information is useful, but not all are always necessary, for the risk assessment of the microorganism.

In the EU, biostimulants are going to be regulated by regulation 2019/1009 laying down rules on making available on the market of EU fertilising products from July 16, 2022 (25). According to this regulation is biostimulants defined as *“A plant biostimulant shall be an EU fertilising product the function of which is to stimulate plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits or availability of confined nutrients in the soil or rhizosphere”. These biostimulants “may contain micro-organisms, including dead or empty-cell micro-organisms and non- harmful residual elements of the media on which they were produced, which have undergone no other processing than drying or freeze-drying, and are listed in the following table Azotobacter spp., mycorrhizal fungi, Rhizobium spp., Azospirillum spp”.*

The list is most likely compiled in the basis phenotypic characteristics and function of these groups which allow then, for example, to fix atmospheric nitrogen or solubilize phosphorous compounds. For microorganisms not belonging to any of the four groups on the list, there will be a case-by-case approach for certification according to standardized criteria, which need to be developed (21). As Bacillus is not mentioned in this list, this group cannot immediately be part of a product for biostimulation.

BACILLUS AND HUMAN HEALTH

Microorganisms used for the protection of crops are most likely to be in contact with humans as residues on crops used for food. In such cases, it is likely that these microorganisms are going to be consumed by humans. It is therefore important that these microorganisms do not cause disease. Most *Bacillus* species are considered non-pathogenic, however notably one lineage within *Bacillus* are an important exception from that general assumption. This group is known as the *Bacillus cereus* group. Two important species within this group are *B. anthracis* and *B. cereus*.

B. anthracis causes anthrax, a severe and fatal disease of ungulates. It might also infect humans and cause death. It is also known as a possible agent for biological weapons. It is known worldwide, but infections are very unusual in the northern parts of Europe. The disease is caused by the anthrax toxin existing of three proteins secreted by virulent strains of the bacterium. The genes coding for these three proteins are only known from *B. anthracis* and seems not generally to be transferred to other species. It is easy to differentiate between *B. anthracis* and other *Bacillus* species by phenotypic and molecular methods (16).

B. cereus are especially known to cause two kinds of gastrointestinal diseases, emetic and diarrheal disease. Emetic disease is caused by a toxin, known as cereulide, which are formed in the food and causes emesis and sometimes diarrhoea within few hours after consumption. Most often, diseased persons have recovered within one to two days, however more severe, and fatal cases are known. The genes involved in the production of the toxin are known and seems not to be spread easily between *Bacillus* species. However, they are only known from a limited number of strains within the *B. cereus* group. They are easily differentiated from strains not possessing these genes by molecular methods. The diarrheal disease is caused by enterotoxins, which are produced by the bacterium after germination in the gut. The enterotoxins cause diarrhoea, and sometimes emesis, eight to ten hours after consumption.

Most people have recovered two to three days after the infection; however, more severe and fatal cases are also known. The three enterotoxins are very well known, and it is easy to analyse by molecular methods whether *Bacillus* strains possess the ability to produce the enterotoxins. However, from this information it is only possible to conclude that the strain has the potential to cause the diarrheal disease, as it is unknown why some strains possessing enterotoxins are able to cause diarrheal disease, while others also possessing enterotoxins do not seem to be able to cause the disease. Other species in the *B. cereus* group, such as *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides* and *B. toyoyensis*, all possess the ability to produce enterotoxins, but it is still unclear whether they are able to cause the diarrheal disease. Bacteria from the *B. cereus* group are very common in soil, so the ability to produce enterotoxins is a very common trait in the environment. The total numbers of bacteria to be presumed to cause both diseases vary most likely between 105 and 108 (17, 18, 19).

Microorganisms are classified into four Risk Groups (22):

Risk Group 1:
- Low individual and low community risk. These microorganisms are unlikely to cause disease

Risk Group 2:
- Moderate individual risk, limited community risk. These microorganisms are unlikely to be a significant risk to laboratory workers or the environment, but exposure may cause infection.

Risk Group 3:
- High individual risk, limited/moderate community risk. These microorganisms usually cause serious disease and may present a significant risk to laboratory workers, but may only present a moderate risk of spreading amongst a community.

Risk Group 4:
- High individual and high community risk. These microorganisms usually cause life-threatening disease and may be readily transmissible. Effective treatments are not usually available.

All *Bacillus* species with the exception of some species in the *B. cereus* group are classified as Risk Group 1 microorganisms.

The exceptions are constituted by *B. anthracis* (Risk group 3), *B. cereus* and *B. mycoides* (Risk group 2), while *B. thuringiensis*, *B. psedomycoides* and *B. toyoyensis* are classified as Group 1.

The European Food Safety Organisation (EFSA) has developed a system where microorganisms can be granted a qualified presumption of safety (QPS) status. Microorganisms with this status are considered safe in relation to use in food and feed. Seventeen species of *Bacillus* have granted this status, including seven of the species in table 1. The *Bacillus* species that have not granted the status are *B. cereus*, *B. firmus*, *B. megaterium*, *B. polymyxa* and *B. thuringiensis*. As *B. cereus* might possess the ability to produce the emetic toxin and/or enterotoxins, this species is not included on the list. Also *B. thuringiensis* often possess the ability to produce enterotoxins while the remaining three might have not been evaluated for the status. Specifically for B. velezensis the status is restricted to strains with absence of toxigenic potential and absence of aminoglycoside production (19).

In conclusion, most *Bacillus* species are non-pathogenic; only some species within the *B. cereus* group are considered to be pathogenic and some even very severe. It is quite easy to differentiate between non-pathogenic and pathogenic, and only a few species within the *B. cereus* group is as mentioned above hard to assess in relation to pathogenicity.

AUTHORIZATION PROCESS

According to EU´s Farm to Fork strategy (23). *"The Commission will take additional action to reduce the overall use and risk of chemical pesticides by 50% and the use of more hazardous pesticides13 by 50% by 2030. To pave the way to alternatives and maintain farmers' incomes, the Commission will take a number of steps. It will revise the Sustainable Use of Pesticides Directive, enhance provisions on integrated pest management (IPM) and promote greater use of safe alternative ways of protecting harvests from pests and diseases. IPM will encourage the use of alternative control techniques, such as crop rotation and mechanical weeding, and will be one of the main tools in reducing the use of, and dependency on, chemical pesticides in general, and the use of more hazardous pesticides in particular.*
Agricultural practices that reduce the use of pesticides through the CAP will be of paramount importance and the Strategic Plans should reflect this transition and promote access to advice. The Commission will also facilitate the placing on the market of pesticides containing biological active substances and reinforce the environmental risk assessment of pesticides. The Commission will take additional action to reduce the overall use and risk of chemical pesticides by 50% and the use of more hazardous pesticides by 50% by 2030. To pave the way to alternatives and maintain farmers' incomes, the Commission will take a number of steps. It will revise the Sustainable Use of Pesticides Directive, enhance provisions on integrated pest management (IPM) and promote greater use of safe alternative ways of protecting harvests from pests and diseases. IPM will encourage the use of alternative control techniques, such as crop rotation and mechanical weeding, and will be one of the main tools in reducing the use of, and dependency on, chemical pesticides in general, and the use of more hazardous pesticides in particular. Agricultural practices that reduce the use of pesticides through the common agricultural policy will be of paramount importance and the Strategic Plans should reflect this transition and promote access to advice. The Commission will also facilitate the placing on the market of pesticides containing biological active substances and reinforce the environmental risk assessment of pesticides". However, the authorization of microorganisms to be used for pest control and for biostimulation in EU has for many years of several stakeholders been considered as inefficient and slow compared to other comparable jurisdiction. A recent study tried to identify reasons for this (20).

The main conclusions of this study are: *"although the EU´s regulatory processes have strong scientific foundations, the most appropriate scientific concepts, knowledge and expertise have not been applied in the safety assessment of microorganisms and biological control. Tradition and conceptual legacies from assessments of conventional chemical pesticides have likely contributed to this by steering the evaluations of microorganisms in less appropriate directions. According to our investigation, the current framework for microbial plant protection products complies poorly with the principles that legislation should have legal predictability, proportionality, and that it should be non-discriminative, for instance in comparison to corresponding regulations in comparable jurisdictions. We also found that existing possibilities to take non-safety and ethical considerations into account can probably be used more. To rationalize the EU´s authorization of microbial control products, both the basic legislation and the evaluations of agents and products need stronger rooting in fundamental microbiological science".* A stronger rooting in fundamental microbiological science of the authorization of microbial plant biologicals would make the process more understandable, relevant and reliable.

REFERENCES

1. Rouphael, Y. and Colla, G. (2020) Editorial: Biostimulants in Agriculture, Front. Plant Sci., 04 February 2020 |DOI: https://doi.org/10.3389/fpls.2020.00040

2. Paliwoda, D. and Mikiciuk, G. (2020) Use of Rhizosphere Microorganisms in Plant Production – a Review Study. J. Ecol. Eng. 2020; 21(8):292–310, DOI: https://doi.org/10.12911/22998993/126597

3. Patel, S. and Gupta, R.S. (2020) A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus Bacillus: Proposal for six new genera of Bacillus species, Peribacillus gen. nov., Cytobacillus gen. nov., Mesobacillus gen. nov., Neobacillus gen. nov., Metabacillus gen. nov. and Alkalihalobacillus gen. nov. Int. J. Syst. Evol. Microbiol. 2020; 70:406–438, DOI 10.1099/ijsem.0.003775

4. Sanchis, V. (2011) From microbial sprays to insect-resistant transgenic plants: history of the biospesticide Bacillus thuringiensis. A review. Agron. Sustain. Dev. (2011) 31:217–231. DOI: 10.1051/agro/2010027

5. Blake, C., Nordgaard Christensen, M, and Kovacs, A.T. (2021) Molecular Aspects of Plant Growth Promotion and Protection by Bacillus subtilis. MPMI 34, 1, 2021, 15–25. DOI https://doi.org/10.1094/MPMI-08-20-0225-CR

6. Fira, D, Dimkić, I., Berić, T., Lozo, J., Stanković, S. (2018) Biological control of plant pathogens by Bacillus species. Journal of Biotechnology 285, 10 , 44-55. https://doi.org/10.1016/j.jbiotec.2018.07.044

7. Shafi, J., Tian, H. Ji, M. (2017) *Bacillus* species as versatile weapons for plant pathogens: a review, Biotechnology & Biotechnological Equipment, 31, 3, 446-459, DOI: 10.1080/13102818.2017.1286950

8. Sanderson, H. (ed.) (2021) Miljørisiko analyse. Fremsyn, klarsyn og oplyst miljøbeslutning. Miljøbiblioteket 8, Aarhus Universitetsforlag.

9. Pedersen, J.C., Hansen, B.M., Damgaard, P.H., and Eilenberg, J. (1995) Dispersal of Bacillus thuringiensis var. kurstaki in an experimental cabbage field. Canadian Journal of Microbiology 41, 2, https://doi- org.ez.statsbiblioteket.dk:12048/10.1139/m95-016

10. Hendriksen, N.B. and Hansen, B.M. (2002) Long-term survival and germination of Bacillus thuringiensis var. kurstaki in a field Canadian Journal of Microbiology 48, 3, https://doi-org.ez.statsbiblioteket.dk:12048/10.1139/w02-009

11. Hendriksen, N.B. and Carstensen, J. (2013) Long-term survival of Bacillus thuringiensis subsp. kurstaki in a field trial. Canadian Journal of Microbiology, 24, https://doi-org.ez.statsbiblioteket.dk:12048/10.1139/cjm-2012-0380

12. EFSA Panel on Biological Hazards (BIOHAZ) (2016) Risks for public health related to the presence of Bacillus cereus and other Bacillus spp. Including Bacillus thuringiensis in foodstuffs. EFSA Journal. doi: 10.2903/j.efsa.2016.4524

13. Manfredini, A., Malusà, E., Costa, C. , Pallottino, F. , Mocali, S. , Pinzari, F. and Canfora, L. (2021) Current Methods, Common Practices, and Perspectives in Tracking and Monitoring Bioinoculants in Soil. Front. Microbiol.,12 | https://doi.org/10.3389/fmicb.2021.698491

14. Fu, L., Penton, C. R., Ruan, Y., Shen, Z., Xue, C., Li, R. and Shen, Q. (2017) Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. Soil. Biol. Biochem. 104, 39-48. https://doi.org/10.1016/j.soilbio.2016.10.008

15. Moayeri, M., Leppla, S., Vrentas, C., Pomerantsev, A., and Liu, S. (2015) Anthrax Pathogenesis. Annual Review of Microbiology 69, 185-208. https://doi.org/10.1146/annurev-micro-091014-104523

16. Stenfors Arnesen, L.P., Annette Fagerlund, A., Granum P.E. (2008) From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiology Reviews 32, 4, 579–606. https://doi.org/10.1111/j.1574-6976.2008.00112.x

17. Jessberger, N., Dietrich, R., Granum, P.E. and Märtlbauer, E. (2020) The Bacillus cereus Food Infection as Multifactorial Process. Toxins 12, 11, 701; https://doi.org/10.3390/toxins12110701

18. Dietrich R., Jessberger, N., Ehling-Schulz, M., Märtlbauer, E., and Granum, P.E. (2021) The Food Poisoning Toxins of Bacillus cereus. Toxins 2021, 13(2), 98; https://doi.org/10.3390/toxins13020098.

19. EFSA, Qualified presumption of safety (QPS), https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps

20. Sundh, I. and, Eilenberg, J: (2020) Why has the authorization of microbial biological control agents been slower in the EU than in comparable jurisdictions? Pest Management Science; https://doi.org/10.1002/ps.6177

21. Sundh, I.; Del Giudice, T.; Cembalo, L. (2021) Reaping the Benefits of Microorganisms in Cropping Systems: Is the Regulatory Policy Adequate? Microorganisms 2021, 9, 1437. https://doi.org/10.3390/microorganisms9071437.

22. World Health Organization. Laboratory biosafety manual. – 3rd ed. (2004). ISBN 92 4 154650 6

23. https://ec.europa.eu/food/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf

24. Bergeys Manual of Systematic Bacteriology, Second edition. Vol. 3 (2009) The Firmicutes. Genus I. Bacillus (Logan, N.A. and DeVos, P.) 21-128.

25. REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. https://eur-lex.europa.eu/legal- content/EN/TXT/PDF/?uri=CELEX:32009R1107&from=EN

26. REGULATION (EU) 2019/1009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. https://eur- lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32019R1009&from=EN

27. Mandic-Mulec, I., Stefanic P. and Van Elsas, J.D. (20159 Ecology of Bacillaceae. Microbiology Spectrum 3: TBS-0017-2013. https://10.1128./microbiolspec.TBS-0016-2013.