VKM Report 2020: 09

Risk assessment of six commercial mycorrhizal products

Opinion of the Panel on Plant Health of the Norwegian Scientific Committee for Food and Environment

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Scientific opinion of the Panel on Plant Health of the Norwegian Scientific Committee for Food and Environment
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Risk assessment of six commercial mycorrhizal products

Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of three VKM members, one external member and a project manager from the VKM secretariat. Two referees commented on and reviewed the draft opinion. The Committee, by the Panel on Plant Health, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Plant Health.

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Acknowledgment

VKM would like to thank the referees professor Klaus Høiland (University of Oslo) and Dr. Erik Joner (Norwegian Institute for Bioeconomy Research and member of the VKM panel on Genetically Modified Organisms and the panel Microbial Ecology) for reviewing and commenting on the manuscript. VKM emphasises that the referees are not responsible for the content of the final opinion. In accordance with VKM's routines for approval of a risk assessment (VKM, 2018), VKM received the referee comments before evaluation and approval of the manuscript by the Panel on Plant Health, and before the opinion was finalised for publication.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Environment Agency, mycorrhiza.

Mycorrhiza is a beneficial association between plant roots and fungi. This mutualistic symbiosis is essential for plant growth in most natural terrestrial ecosystems and in agriculture. Commercial mycorrhizal products containing fungi and bacteria may promote plant growth, especially on sites without a natural microbial community.

Due to the risk of unintended negative effects, introduction of new species or genetically different isolates of native species should always be considered carefully. This report assesses the risk of establishment and spread of six fungal species and six bacterial species included in different commercial mycorrhizal products, as well as the species' potential impact on Norwegian biodiversity.

Most of the evaluated fungi and bacteria are probably present in Norway, even though presence at present data only exist for two of the six fungal species.

Establishment of the assessed fungi on the plants and sites where they are applied is considered moderately likely, with medium uncertainty, while establishment of the bacterial species is considered to range from very unlikely to very likely depending on the bacterial group, with low uncertainty.

The probability of spread to the wider environment ranges from unlikely (four fungal species), to moderately likely (two fungal species), to very likely (five of the six bacterial species).

However, for all species it is considered unlikely that establishment and spread would have negative effects on other native species, habitats and ecosystems in Norway.

Sammendrag på norsk

Mykorrhiza er en symbiose mellom sopp og planterøtter. Dette samlivet mellom planter og sopp er trolig livsnødvendig for de fleste landlevende planter, inkludert jordbruksplanter. Kommersielle mykorrhizaprodukter som inneholder både sopp og bakterier, markedsføres for å fremme plantevekst, spesielt i jordtyper som ikke har et naturlig mikrobielt miljø.

På grunn av risikoen for utilsiktede skadevirkninger på biologisk mangfold, bør innføring av nye arter eller genetisk forskjellige individer av stedegne arter alltid vurderes nøye. Denne rapporten vurderer risikoen for at seks mykorrhizasopper og seks bakterier som er inkludert i ulike kommersielle produkter, skal etablere seg og spres i norsk natur, og risikoen for at artene skal ha innvirkning på norsk biologisk mangfold.

De fleste av de vurderte soppene og bakteriene antas å være til stede i Norge fra før, selv om norske funndata kun finnes for to av mykorrhizasoppene.

Det anses som moderat sannsynlig at de vurderte mykorrhizasoppene kan etablere seg i områdene hvor produktene blir brukt. Usikkerheten er moderat.

Sannsynligheten for at bakteriene kan etablere seg, varierer fra veldig sannsynlig til veldig usannsynlig, avhengig av bakterietype. Det er lav usikkerhet ved resultatet.

Videre spredning av mykhorrizasopper i norsk natur antas å variere fra lite sannsynlig (fire sopper), til moderat sannsynlig (to sopper). Det er veldig sannsynlig at bakteriene vil spre seg i norsk natur (fem av seks bakterier).

For alle de vurderte artene anses det som lite sannsynlig at etablering og spredning av artene vil ha negative effekter på andre stedegne arter, naturtyper eller økosystemer i Norge.

Abbreviations and glossary

Abbreviations

BISON: Biodiversity Information Serving Our Nation

GBIF: Global Biodiversity Information Facility

iDigBio: Integrated Digitalized Biocollections

NCBI: National Center for Biotechnology Information

Glossary

Table 1. Definition of terms used in this assessment. Most definitions are according to the ISPM No.5 Glossary of phytosanitary terms by FAO (2020)

Term	Definition
Dispersal	The spread of mature individuals, their offspring, or propagules away from the place of birth to another region
Ectomycorrhizae	Fungi living as symbionts with roots of certain plant species with which they form sheaths around the root tips and nets of hyphae surrounding the plant cells
Establishment	The process where a species in a new habitat successfully produces viable offspring with a likelihood of continued survival
Hitchhiking organisms	A pest that is carried by a commodity and, in the case of plants and plant products, does not infest those plants or plant products
Intentional introduction	Deliberate movement of a non-indigenous species into an area, including its release into the environment
Invasive species	A non-indigenous species that spreads rapidly, and whose introduction causes or is likely to cause economic or environmental harm or harm to human health
Mycorrhizal fungi	Fungi that form mutualistic associations with plant roots. The plant provides the fungus with carbon, and the fungus takes up soil nutrients that are in turn provided to the plant.

Term	Definition
Native species	A species occurring naturally in an area and whose presence does not result from human activity. A native species need not be endemic, as it may be found in a broader area than the one under consideration.
Non-indigenous species	A successfully introduced and established species
Occurrence	The presence of a particular species in a particular geographically defined entity
Spread	Expansion of the geographical distribution of a species within an area

Background as provided by the Norwegian Environment Agency

In Norway, it has become increasingly common to add different fungal spores of mycorrhiza-forming fungi to agricultural production agents. The Norwegian Environment Agency is aware that this is happening for several products that are being marketed today.

Several of these products originate from outside Europe. Several recent studies suggest that different biological properties that may be beneficial for increased production, may also increase the possibility for alien organisms to establish and spread, thereby potentially causing adverse impacts on native biodiversity (see literature).

The regulations relating to alien organisms under the Norwegian Nature Diversity Act, which entered into force on 1 January 2016, require permission to import and release products containing alien organisms. This also applies to mycorrhiza-forming fungi.

The Norwegian Environment Agency has received an application on import and release of the mycorrhiza-forming fungi *Entrophospora columbiana*, *Glomus etunicatum*, *G. clarum*, *G. intraradices*, *Pisolithus tinctorius* and *Scleroderma citrinum*.

As a basis for processing of applications, the Norwegian Environment Agency requires a scientific assessment of the risk of unintended consequences for biodiversity concerning import and release of these species as input in agricultural production in Norway. The Norwegian Environment Agency also requires an assessment of what measures, as well as research and development that can be implemented to increase knowledge.

Terms of reference as provided by the Norwegian Environment Agency

The Norwegian Environment Agency asks VKM to assess the risk of adverse impact on biodiversity concerning the import and release of the six species applied for:

1. Describe the natural distribution of the six species.
2. Identify potential hazards associated with the import and release of the species, including:
    a. Genetic changes in local populations / native individuals of the same species
    b. Spread of species beyond the natural range
    c. Effects on other native species, habitats and ecosystems
    d. Introduction and spread of hitchhiking organisms
    e. Other ecological effects

3. Assess the consequences of:
    a. Genetic changes in local populations / native individuals of the same species
    b. Spread of species beyond the natural range
    c. Effects on other native species, habitats and ecosystems
    d. Introduction and spread of hitchhiking organisms
    e. Other ecological effects
4. Genetic changes in local populations / native individuals of the same species
    a. Genetic changes in local populations / native individuals of the same species
    b. Spread of species beyond the natural range
    c. Effects on other native species, habitats and ecosystems
    d. Introduction and spread of hitchhiking organisms
    e. Other ecological effects
5. Assess the probability of:
    a. Genetic changes in local populations / native individuals of the same species
    b. Spread of species beyond the natural range
    c. Effects on other native species, habitats and ecosystems
    d. Introduction and spread of hitchhiking organisms
    e. Other ecological effects
6. Characterize the risk of:
    a. Genetic changes in local populations / native individuals of the same species
    b. Spread of species beyond the natural range
    c. Effects on other native species, habitats and ecosystems
    d. Introduction and spread of hitchhiking organisms
    e. Other ecological effects

In addition, the Norwegian Environment Agency asks VKM to:

7. Identify relevant risk mitigation measures (including mapping tools that can be used to detect relevant species) and evaluate their effectiveness and feasibility. A brief assessment of the possible negative effects of the measures on local biodiversity should be included.

If the introduction of the mycorrhizal-forming fungi may have adverse impacts on ecosystem services, this should be stated in the report, but not included as part of the assessment of the risk of adverse impacts on biodiversity.

The Norwegian Environment Agency requests that the risk of adverse impact for biodiversity be assessed from a 50-year perspective.

Methodology and Data

Data and information gathering

For information on species distributions, we obtained data from different sources including:

- DNA-sequence data from NCBI: https://www.ncbi.nlm.nih.gov/
- Occurrence data from Global Biodiversity Information Facility (GBIF), iNaturalist (iNat), Integrated Digitized Biocollections (iDigBio) and Atlas of Living Australia (ALA)
- Artsdatabanken/artskart: https://artskart.artsdatabanken.no

Literature search and selection

Searches for relevant literature were performed in Medline, Embase, ISI Web of Science, Scopus and CABI. These databases were chosen to ensure comprehensive study retrieval. The comprehensive search strategy is presented in Appendix III. The literature search was performed by senior librarians at the Norwegian Public Institute of Public Health on December 3, 2019.

The literature search resulted in 464 records after duplicates were removed, both automatically and during primary screening of the EndNote bibliography. In the primary screening, titles and abstracts of all the retrieved publications were independently screened against the inclusion and exclusion criteria.

Inclusion and exclusion criteria:

- Inclusion criteria:
  - Publication type – primary research studies, review papers, systematic reviews, editorials and meeting abstracts.
  - Only examples including biodiversity were included.
  - Only species-specific records were included.
  - Only records written in English were included.

- Exclusion criteria:
  - Publication not relevant for answering the questions stated in the terms of references

Articles that did not appear to meet the inclusion criteria were excluded from further analysis. If it was unclear whether the publication was of relevance to the study it was retained for further screening. Full text articles that passed the primary screening were retrieved, compared again against the inclusion criteria and assessed for relevance and quality.

The primary and secondary screenings as well as quality assessment of papers were performed by members of the project group. Any disagreements were solved in the project group. The primary screening resulted in 33 full text articles, of which 10 papers passed the secondary screening and were included in the opinion.

In order to strengthen the data basis of the opinion, additional manual searches for papers and relevant grey literature were also performed. Manual searches included 'snow-balling', i.e. interesting articles that were referred to in papers found in the main literature searches were retrieved via Google, Google Scholar, and PubMed via EndNote. The manual searches resulted in 35 relevant papers and documents included in the opinion (Figure 1).

Ratings of probabilities and uncertainties

The conclusions on probability of entry and establishment of the organisms are presented and rated separately, following a fixed scale: very unlikely, unlikely, moderately likely, likely, very likely. The descriptors for these qualitative ratings are presented in Appendix II Table A2-1.

For the conclusions on entry and establishment, the levels of uncertainty are rated separately, following a fixed scale: low, medium, high. The descriptors for these qualitative ratings of uncertainty are presented in Appendix I Table A2-2.

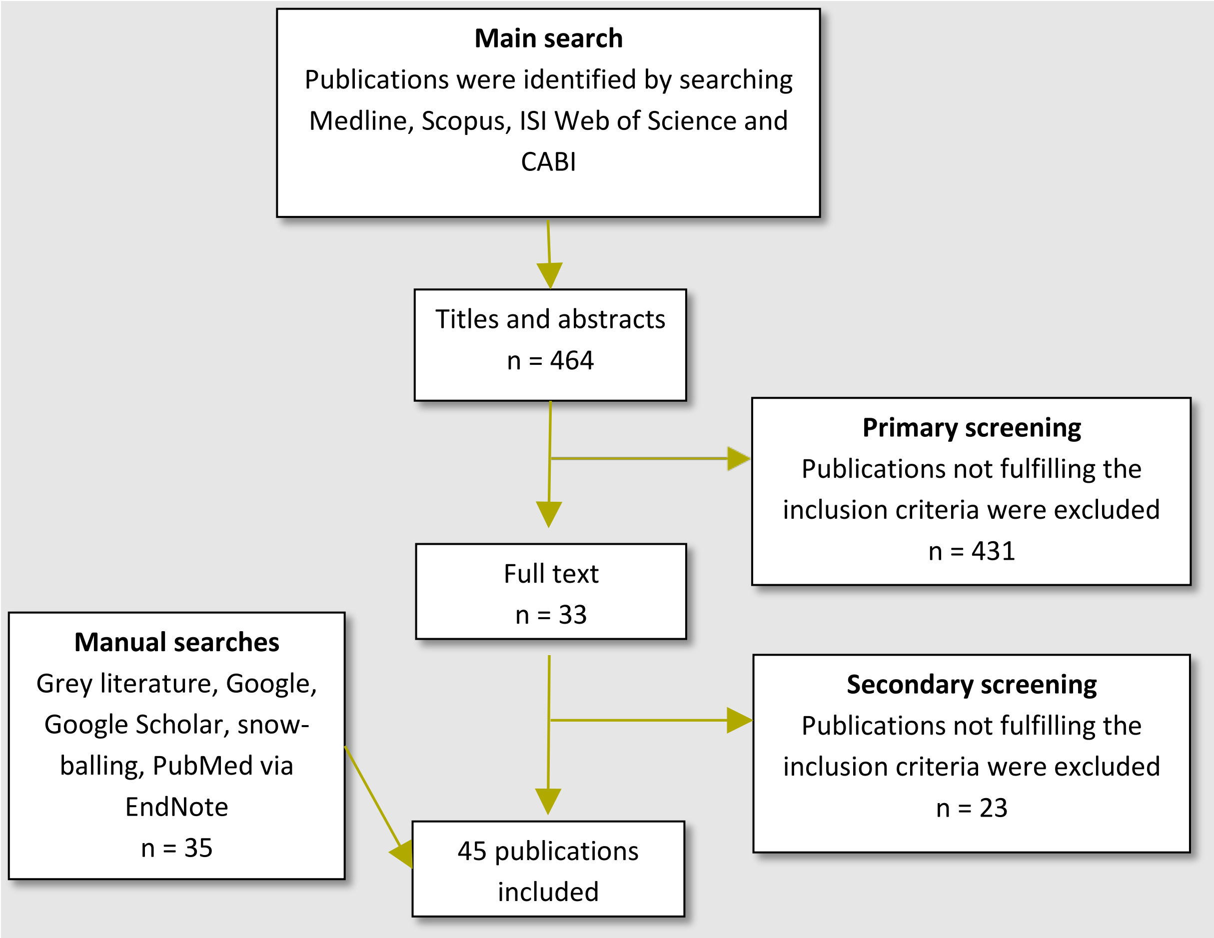

Figure 1. Flowchart for the literature search.

Assessment

1 Introduction

1.1 Background

This document presents a scientific opinion prepared by the VKM Panel on Plant Health, in response to a request from the Norwegian Environmental Agency. The opinion is a risk assessment of six commercial products containing a mixture of mycorrhizal fungi and bacteria, to be used as bio-stimulators for plants. The intended target plants are mainly trees, shrubs and grasses. For trees and shrubs, the main use of the products seems to be in production of plug plants and cuttings, but treatment of established trees is also mentioned in the product data sheets. As requested by the Norwegian Environment Agency this opinion assesses the risk of establishment and spread of the fungi and bacteria included in the products, as well as their potential impact on Norwegian biodiversity. The opinion also identifies and evaluates risk reduction options.

The structure we follow in this opinion differs from that used in the terms of reference provided by the Norwegian Environment Agency. The terms of reference start out by identifying hazards associated with the use of mycorrhizal fungi and moves on to assessing consequences, probabilities and risks. For each step the terms of reference ask for an evaluation of genetic changes in local populations, spread beyond the natural range, introduction and spread of other hitchhiking organisms, and other ecological effects. In the opinion we first briefly summarize the biology of the type of fungi and bacteria evaluated in this opinion, followed by two chapters concerning natural distribution of the fungal and bacterial species included in the products. We then assess the probability that these species will establish and spread beyond the introduced areas and discuss whether this may lead to genetic changes in local populations of the same species or affect other native species, habitats and ecosystems. Finally, we assess the risks of introducing the described species, and identify uncertainties, data gaps and possible risk mitigation measures.

The terms of reference refers to introduction and spread of 'hitchhiking organisms'. We do not consider the bacteria that are intentionally included in the products to be hitchhiking organisms. In the context of the products assessed in this opinion, hitchhiking organisms are any microorganisms that are present by accident. This could for example be microorganisms that contaminate the products during the production process. Such microorganisms are likely to be ubiquitous organisms that enter the production system via e.g. airborne spores, depending on the production process. This opinion focuses on the six fungal and six bacterial species that are intentionally included in the products.

1.2 Mycorrhiza

Mycorrhizal fungi are part of the soil microbial community, together with a wide variety of other organisms. Soil microbial communities include an enormous diversity of archaea, bacteria, fungi, oomycetes and protists that interact with plant roots and soil-living animals. This diverse community delivers key ecosystem services and is crucial for cycling carbon, nitrogen and phosphorous – compounds that are essential for plant growth. Emerging evidence demonstrate the huge impact networks of microbial species have on plant growth and plant health in general. The microbial networks are maintained through interactions between thousands of individual species and are influenced by complex chemical and physical factors in the soil environment. Perhaps as a result of this complexity, soil microbial networks often form resilient ecosystems that rapidly reach new equilibria after smaller disturbances. Only after serious disturbances, such as contamination with toxic waste, are soil microbial communities disrupted or degraded (Jansson and Hofmockel, 2018; Jiao et al., 2019; Rillig et al., 2018).

Mycorrhizal fungi are species that form symbiotic relationships with plants via the plants' fine roots. The fungi provide nutrients and water to the plants, and in return receive carbohydrates. Most plant species form mycorrhizal associations. Mycorrhiza promotes plant growth, and broadly speaking this mutualistic symbiosis may be considered essential for natural terrestrial ecosystems and agriculture. In forests, individual trees are connected via mycorrhiza and shared soil more than via direct root contact, and afforestation of degraded soils often require introduction of both trees and their mycorrhiza partners (Figure 2).

Figure 2. Replanting of areas on Iceland degraded by old deforestation and subsequent erosion, using larch in combination with the mycorrhizal fungus larch bolete (*Suillus grevillei*).

There are several types of mycorrhizal symbioses. One important type is arbuscular mycorrhizal (AM) symbiosis which occur in 75% of all terrestrial plants, including many crop species. In AM symbiosis, hyphae penetrate the root cells to form intracellular structures (arbuscules) where the nutrient exchange happens. Arbuscular mycorrhiza is formed by fungi in the phylum Glomeromycota, which contains about 230 recognized species that are almost exclusively mycorrhiza-forming. The Glomeromycota is an ancient group dating back about 400 million years.

It is uncertain whether AM fungi have sexual reproduction and recombination, as there is conflicting evidence for this in the literature. Earlier, AM fungi were thought to have strictly asexual reproduction and, hence, to disperse only with clonal propagation. However, recent evidence from genome sequencing studies indicate that AM fungi possess the machinery for sexual recombination and may have some form of cryptic sexuality/recombination. Fusion of hyphae from different individuals could be a method for exchanging genetic material (Chagnon, 2014) and has been demonstrated in *Glomus intraradices*, one of the fungi assessed in this opinion (Croll et al., 2009). Still, the degree of genetic recombination in AM fungi is probably low.

The genetic composition of AM fungi is very complex, making it hard to define species based on their DNA. Therefore, AM fungal "species" are mainly morphospecies (i.e. species defined by morphology only) and likely often represent wider species complexes. It is thus unknown whether the currently recognized species are true biological species or not. Further, AM fungi do not form macroscopic fruitbodies but exist solely as hyphae in soil and plant tissues or as spores. This, combined with the poor knowledge on species boundaries, makes it difficult to map the geographical distribution of AM fungi. Occurrence data in public databases must therefore be handled with great care and scepticism, as they may include many misidentifications.

Another important type of mycorrhizal symbiosis is ectomycorrhizal (EM) symbiosis. Although EM symbiosis is only present in about 2% of all plant species, it is very important in temporal, boreal and alpine ecosystems. This includes large parts of Norway, and most of the forest trees in Norway have EM symbiosis. In EM symbiosis fungal hyphae enclose the plant's root tips in a sheath and grow between, but not into, the root cells. Most fungi involved in EM symbiosis are so-called macrofungi, meaning that they produce large, above-ground fruitbodies and numerous airborne spores. Many basidiomycetes, such as boletes and agarics, form EM symbiosis with trees (Figure 1). Most EM fungi have sexual reproduction and recombination. Because of this, and the presence of macroscopic fruitbodies accessible for morphological and DNA analyses, we have a much better overview of species delimitations and species distributions among EM fungi compared to AM fungi.

There are also other types of mycorrhizal symbiosis, including ericoid mycorrhiza and orchid mycorrhiza, but they will not be dealt with in this opinion since none of the assessed species are involved in these types of symbiosis.

The fact that most plant species form beneficial interactions with mycorrhiza has led to the marketing of mycorrhizal products to increase plant growth and crop yield in agriculture, horticulture and forestry. Despite the importance of mycorrhiza in natural systems the use of mycorrhizal products might not always improve growth or yield in cropping systems (Hart et al., 2018). One reason is that mycorrhizal fungi do not compete well under the high phosphorous levels that exist in many agricultural soils. Another reason is that effective mycorrhizal mutualisms tend to be quite specialized and form species-specific associations with certain plant species. Because of this species-specificity the general mycorrhizal species that often are included in commercial products may not provide the intended benefits.

Four of the six fungal species assessed in this opinion are AM fungi (*Entrophospora columbiana*, *Glomus etunicatum*, *G. clarum*, *G. intraradices*) and two are EM fungi (*Pisolithus tinctorius* and *Scleroderma citrinum*). In the presentations below of species distributions and risk assessments we group the fungal species according to the AM/EM classification.

1.3 Bacteria

Like many other mycorrhizal products marketed for plant growth promotion the products assessed in this opinion contain various supplemented bacterial species. Such bacteria may promote plant growth directly, e.g. by supplying nutrients, or indirectly, by reducing plant susceptibility to pests and diseases. Four of the six supplemented bacterial species assessed in this opinion belong to the genus *Bacillus*, and the last two species belongs to *Paenibacillus*.

The genus *Bacillus* consists of a large number of species of rod-shaped, gram-positive bacteria that are able to form heat- and desiccation-resistant endospores. Originally, any rod-shaped, aerobic or facultative anaerobic bacterium that could form endospores was classified as *Bacillus* (Grady et al., 2016). Priest et al. (1988) suggested splitting the genus *Bacillus* into several genera. A study based on 16S rRNA sequences segregated *Bacillus* into at least five distinct clusters, one of which was reassigned to the novel genus *Paenibacillus* in 1993 (Ash et al., 1993). Both *Bacillus* and *Paenibacillus* species can be readily cultured from soils, and usually 10^3 to 10^6 cells per gram can be found in the rhizosphere (i.e. the narrow region of soil that is directly influenced by root secretions) (McSpadden Gardener, 2004).

Bacteria in the genus *Bacillus* are ubiquitous and have been isolated from a broad range of habitats such as freshwater, sea water, soil, plants, animals, and air. Several species can survive high temperatures, high salinity or acidic conditions (Maughan and Van der Auwera, 2011). Some species have been extensively used in industry, such as *B. cereus* and *B. subtilis*, or in agriculture for pest control, such as *B. thuringiensis*. Some *Bacillus* species are highly pathogenic to humans, with the most prominent example being *B. anthracis*, the etiologic agent of anthrax (Fritze, 2004). It should also be noted that some species or strains of *Bacillus*, for example *B. cereus* and *B. thuringiensis*, are able to produce toxins that may be harmful to invertebrates and/or vertebrates (Schoeni and Lee Wong, 2005).

Bacteria belonging to the genus *Paenibacillus* can be isolated from a variety of sources, and several species are associated with humans, animals, plants, and the environment. Species of *Paenibacillus* have been isolated from diverse habitats ranging from arctic to tropic regions in aquatic and terrestrial environments (Grady et al., 2016). Most *Paenibacillus* species, however, are found in soil, often in association with plants roots where some of them are known to promote plant growth. Some *Paenibacillus* species may be honeybee pathogens whilst others may infect humans.

2 Natural distribution of the six mycorrhizal species

As discussed above, the taxonomical classification of many mycorrhiza-forming fungi is uncertain because the biological species concept is not meaningful for AM fungi that mostly reproduce asexually. Also, most mycorrhizal species are defined only by morphological characters and may in fact be species complexes consisting of several genetically distinct cryptic species. Due to these taxonomical challenges, the geographical distribution and national species records of many mycorrhiza fungi are uncertain. With this uncertainty in mind, we describe the known distribution of the six mycorrhiza species listed in the terms of reference.

Our assessment of species distributions is mainly based on records from GBIF and other curated databases that largely include occurrence data based on morphological assessments (Figure 3 to 9), as well as on records from NCBI ("GenBank") that includes DNA sequence data (Supplementary Table 1, Appendix 1). Note that many of the accessions in these databases could be wrong - earlier literature has suggested that up to 20% of DNA data in GenBank have errors in their taxonomic affiliation. Also, note that the maps in Figure 3 to 9 only show records with accurate geographic coordinates.

Most AM fungi appear to have wide geographic distributions, but this could be an artifact of poor species delimitations and cryptic species complexes. On the other hand, some AM species have been little studied, at least taxonomically, and may have broader distributions than currently indicated due to limited occurrence data. In general, there is better data on the distribution of EM fungi than AM fungi, largely because most EM fungi have conspicuous fruitbodies. Still, one should always remember that distribution maps may reflect the presence of mycologists looking for fungi more than true distributions.

2.1 Arbuscular mycorrhizae (AM) species

Entrophospora colombiana

(current name: *Kuklospora colombiana* (Spain and N.C. Schenck) (Sieverding and Oehl, 2006).)

We found 51 records of this fungus in NCBI (Supplementary Table 1) and GBIF/iDigBio (Figure 3). This species appears to have a rather fragmented but widespread distribution in tropical areas; records from NCBI includes 32 specimens from Africa, South/North America and Asia (Supplementary Table 1), while occurrence data from GBIF/iDigBio includes 25 records from Asia and South America (of which only a few South American records are georeferenced; Figure 3). There are no records of *E. colombiana* from Norway. The tropical distribution suggests that this species will not thrive in cold environments.

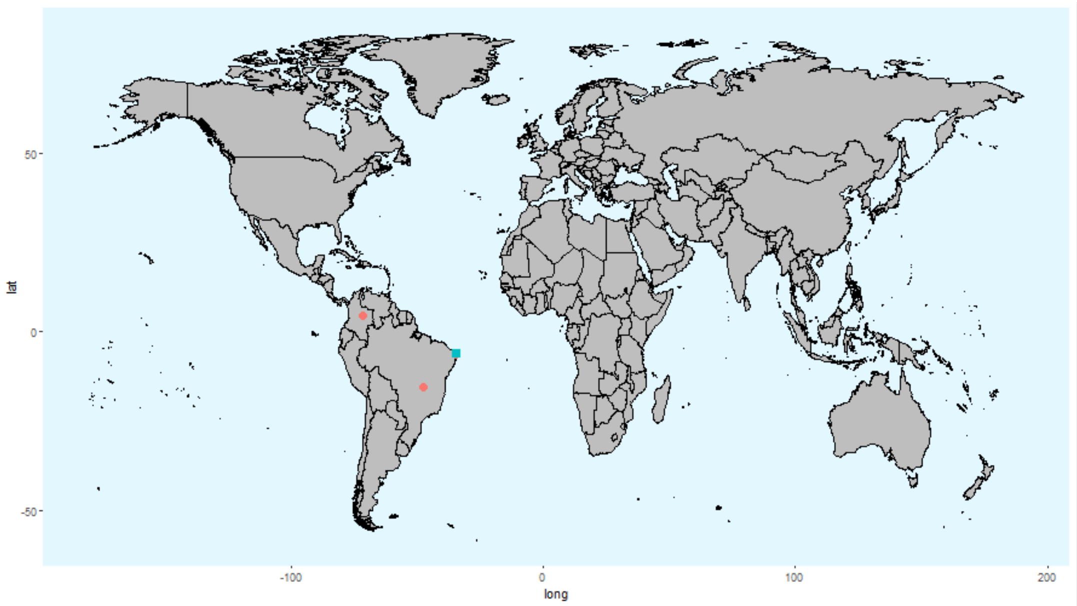

Figure 3. Map of records of *Entrophospora colombiana* according to GBIF (orange symbols) and iDigBio (blue symbols).

Glomus etunicatum

(current name: *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) (Schüßler and Walker, 2010)

This species seems to have a very broad distribution and is considered one of the most commonly occurring AM fungi in the world (Figure 4). There are 650 and 308 records of this fungus in NCBI and GBIF, respectively, from all continents except Antarctica, but only georeferenced records are shown in Figure 4. There are few records of this species in cold environments, but this might largely be due to lack of research. There are no records of *G. etunicatum* from Norway, but the species has been recorded in nearby European countries, including Finland, and might also occur in Norway.

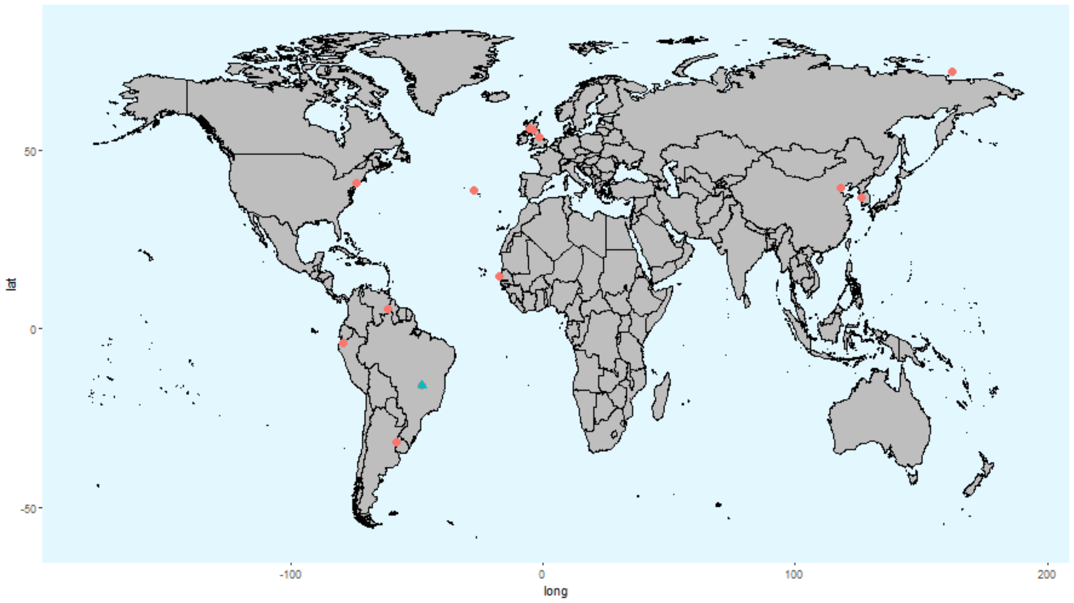

Figure 4. Map of records of *Glomus etunicatum* according to GBIF (orange symbols) and iDigBio (blue symbols).

Glomus clarum

(current name: Rhizophagus clarus (N.C. Schenck & G.S. Sm.) (Schüßler and Walker, 2010)

This species also appears to have a worldwide distribution. It has mainly been recorded in more southern regions, but there are a few records from the UK and Iceland (Figure 5). There are no records from Norway, but the species is likely naturally present in Norway.

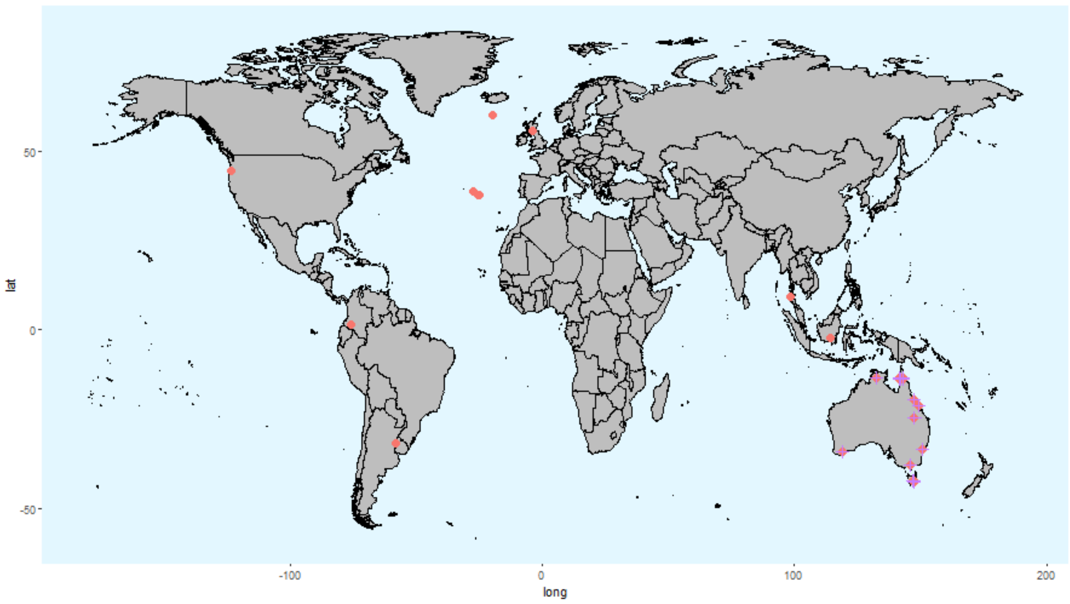

Figure 5. Map of records of *Glomus clarum* according to GBIF (orange symbols) and Atlas of Living Australia (purple symbols).

Glomus intraradices

(current name: *Rhizophagus intraradices* (T.H. Nicolson & N.C. Schenck) (Schüßler and Walker, 2010)

This species has a widespread distribution, including many records from temperate areas. (Figure 6). There are no records from Norway, but the species is likely naturally present in Norway.

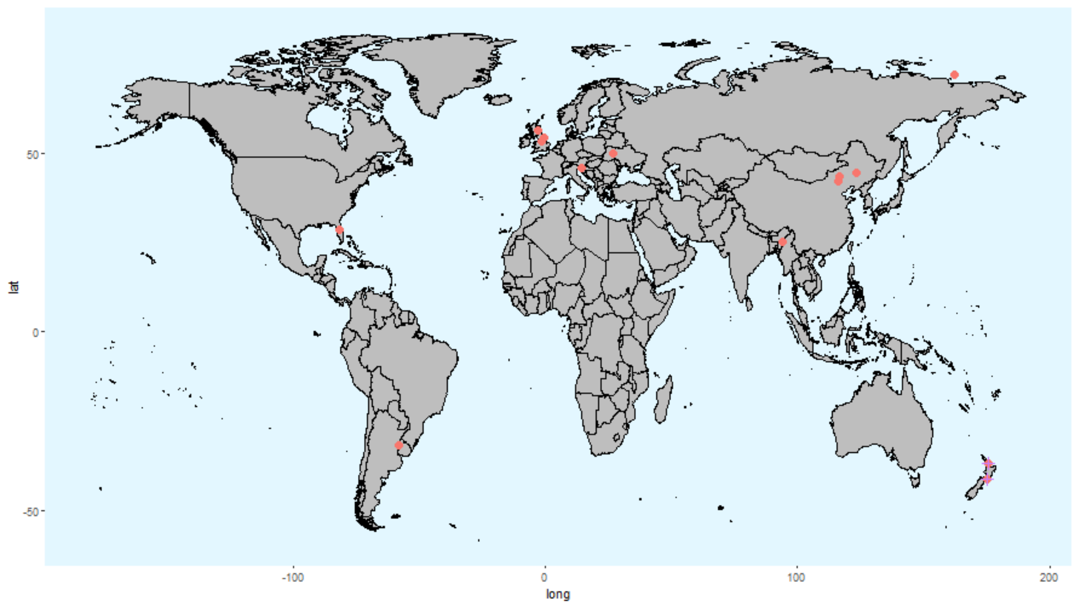

Figure 6. Map of records of *Glomus intraradices* according to GBIF (orange symbols) and Atlas of Living Australia (purple symbols).

2.2 Ectomycorrhizal (EM) species

Pisolithus tinctorius

(current name: *Pisolithus arrhizus* (Scop.) Rauschert; Norwegian name: 'trollrøyksopp').

This species appears to have a worldwide distribution, with numerous records from continental Europe (Figure 7). However, it is unknown whether the taxon name *P. tinctorius* represents one or several biological species. There are currently only four observations of *P. tinctorius* in Norway, and the species is considered to be rare. It was earlier thought to be an introduced species to Norway, but might be naturally present.

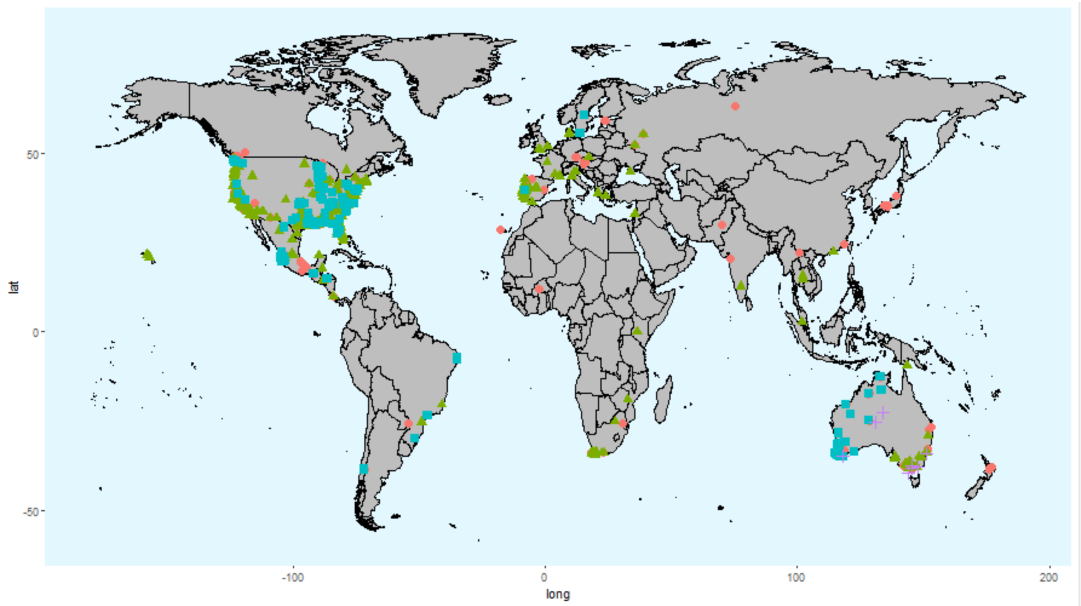

Figure 7. Map of records of *Pisolithus tinctorius* according to GBIF (orange symbols), Atlas of Living Australia (purple symbols), iNaturalist (green symbols) and iDigBio (blue symbols).

Scleroderma citrinum

(Norwegian name: 'gul potetrøyksopp')

This species has a wide distribution in Europe, including Norway (Figure 8). There are 572 observations of the species from Norway, mainly in southern, coastal areas (Figure 9).

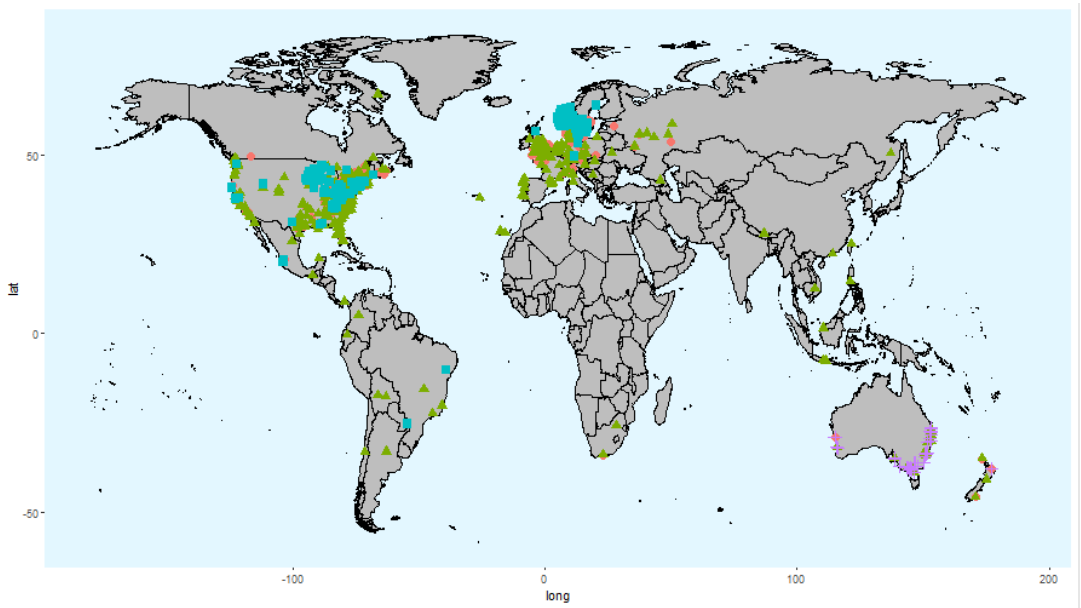

Figure 8. Map of records of *Scleroderma citrinum* according to GBIF (orange symbols), Atlas of Living Australia (purple symbols), iNaturalist (green symbols) and iDigBio (blue symbols).

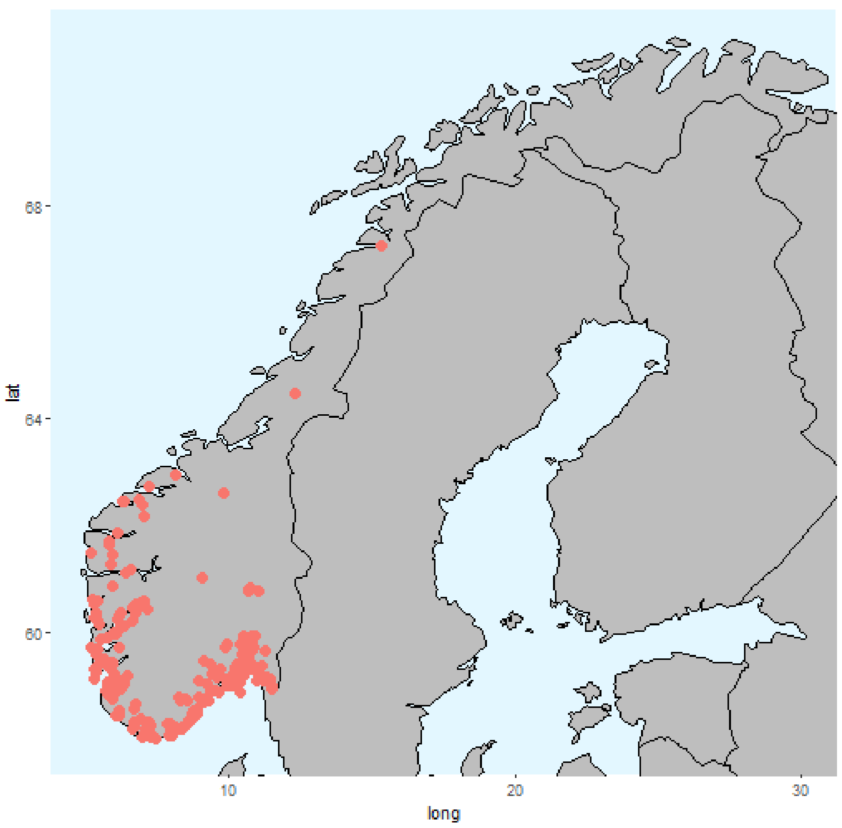

Figure 9. Map of records of *Scleroderma citrinum* in Norway according to Artsdatabanken.

3 Natural distribution of the six bacterial species

As described above many bacteria are ubiquitous and can be found in a broad range of habitats, including soil. This is also true for many members of *Bacillus* and *Paenibacillus,* mainly because their spores can be transported very long distances on e.g. soil particles carried by the wind (Istock et al., 2001).

The following six bacterial species are included in the commercial mycorrhizal products assessed in this opinion:

Bacillus licheniformis is a ubiquitous saprophytic bacterium, that is very widespread (Figure 10), existing predominantly as spores in soil. *Bacillus licheniformis* is closely related to *B. subtilis* and *B. pumulis.* Strains of *B. licheniformis* have been found in Norway (Manachini and Fortina, 1998).

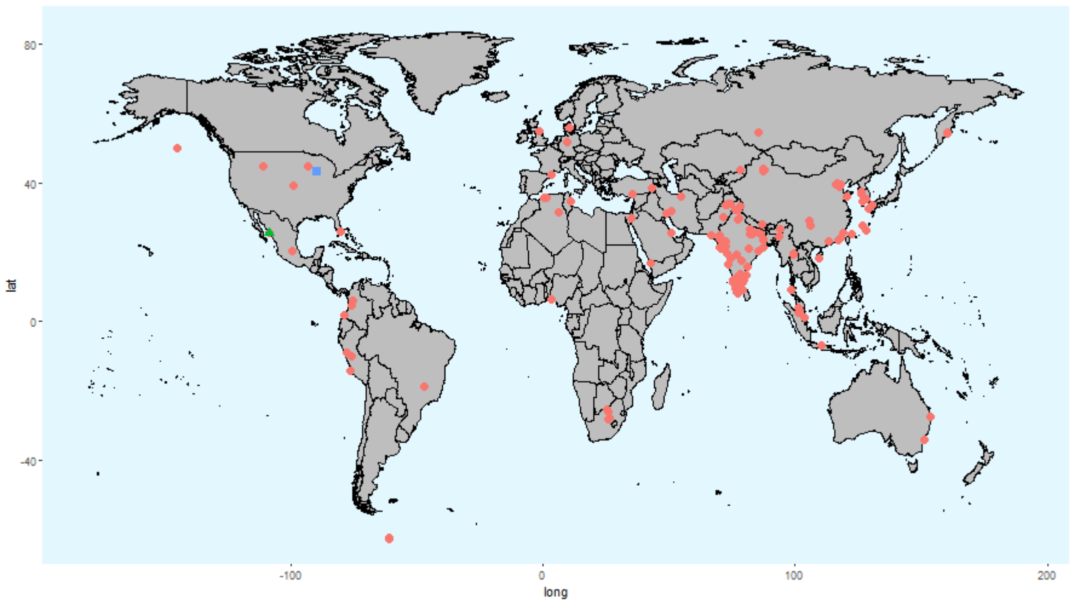

Figure 10. Map of records of *Bacillus licheniformis* according to GBIF (orange symbols), iNaturalist (blue symbols) and iDigBio (green symbols).

Bacillus megaterium is also ubiquitous (Figure 11) and can be found in diverse habitats such as soil, sea water, rice paddies, honey, fish and dried food. The bacterium is widely used to produce various industrial compounds, for instance after genetic modification (Vary et al., 2007).

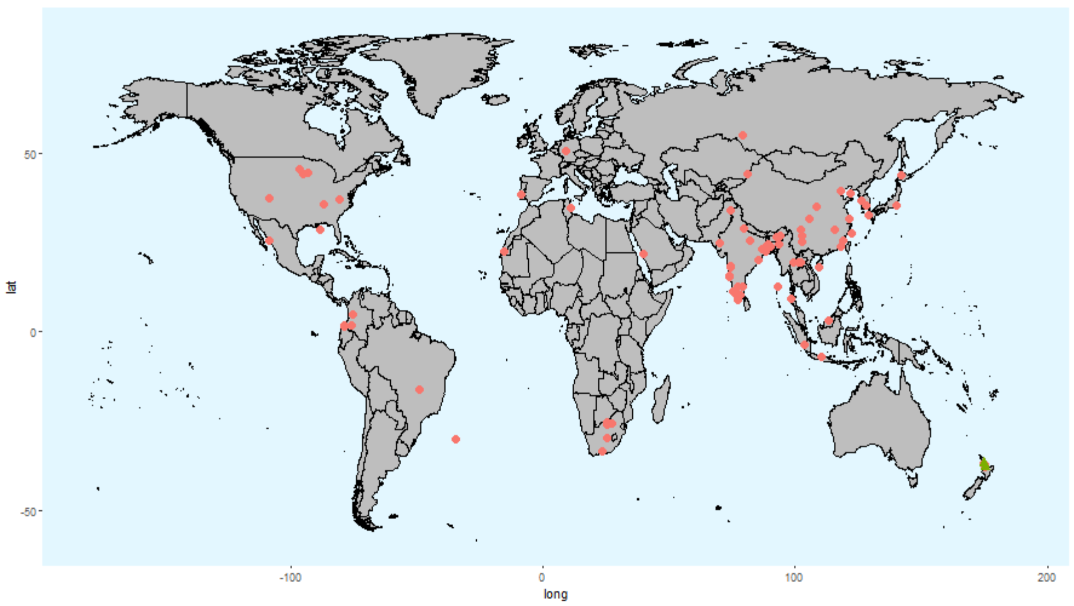

Figure 11. Map of records of *Bacillus megaterium* according to GBIF (orange symbols) and iNaturalist (green symbols).

Bacillus polymyxa has been renamed *Paenibacillus polymyxa*, but will be referred to as *Bacillus polymyxa* in this report. The bacterium is mostly isolated from the soil and rhizosphere. It has been used as an antagonist against plant pathogenic oomycetes, fungi and other bacteria (Jeong et al., 2019). The bacterium has been isolated from a wide variety of sources and geographical areas (Padda et al., 2017), including Denmark (Nielsen and Sørensen, 1997) (Figure 12).

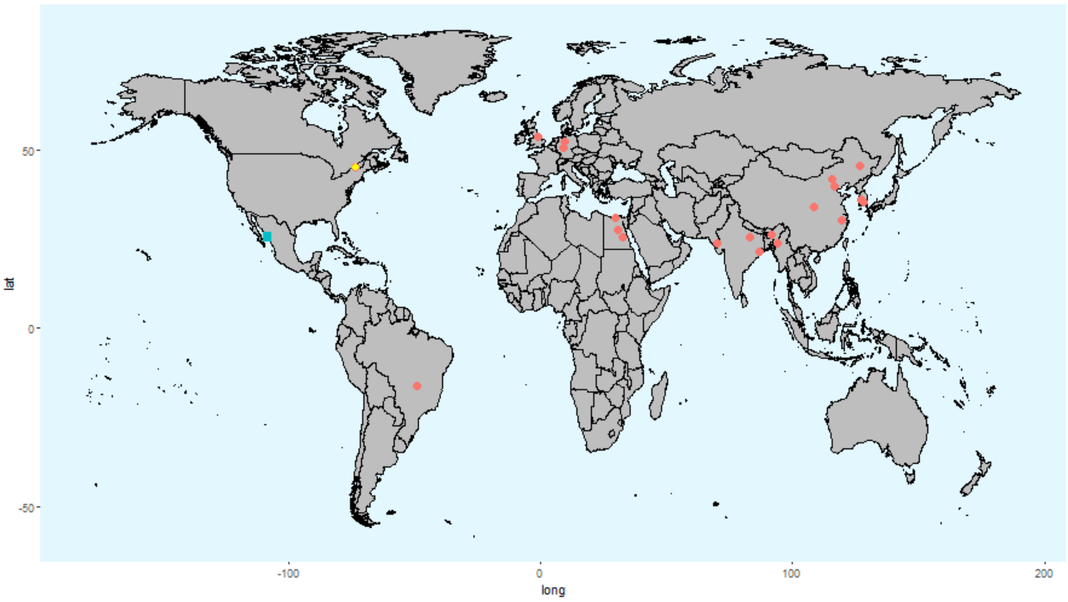

Figure 12. Map of records of *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) according to GBIF (orange symbols), BISON (yellow symbols) and iDigBio (blue symbols).

Bacillus pumilus is another ubiquitous species that has been implicated in food spoilage. It has, however, also been found on plant roots. It has been recorded in both terrestrial and marine environments (Branquinho et al., 2014) in diverse geographical regions including Norway (From et al., 2005) and Denmark (Nielsen and Sørensen, 1997) (Figure 13).

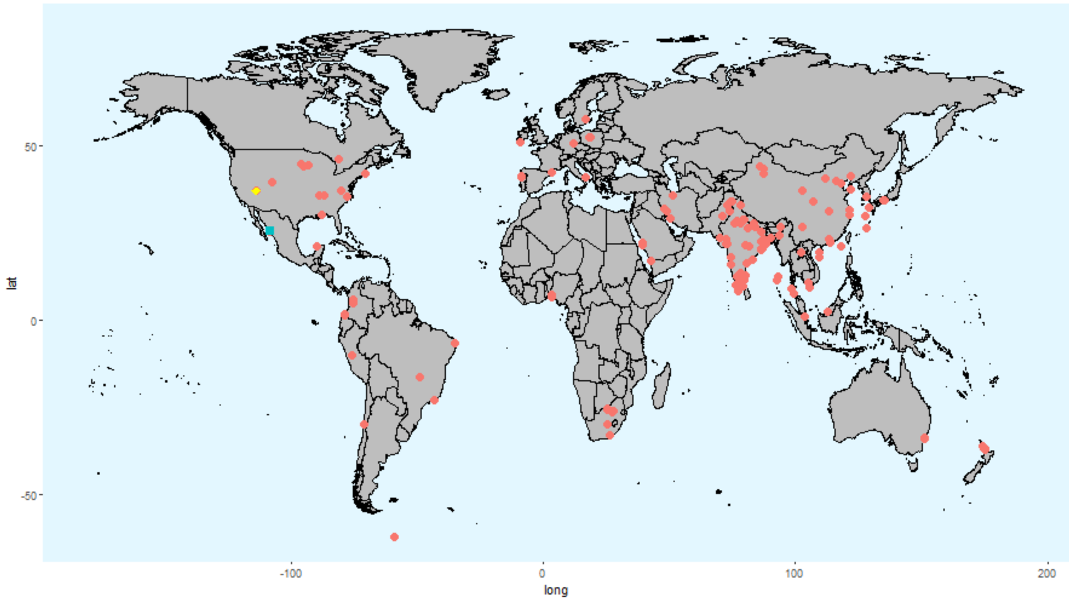

Figure 13. Map of records of *Bacillus pumilus* according to GBIF (orange symbols), BISON (yellow symbols) and iDigBio (blue symbols).

Bacillus subtilis is another ubiquitous species (Figure 14) that can be isolated from numerous environments, ranging from terrestrial to aquatic environments. Like all members of the genus *Bacillus, B. subtilis* can form highly resistant spores. These spores may be airborne and thus spread long distances by wind (Earl et al., 2008). *Bacillus subtilis* has been recorded from Norwegian surface waters (Østensvik et al., 2004).

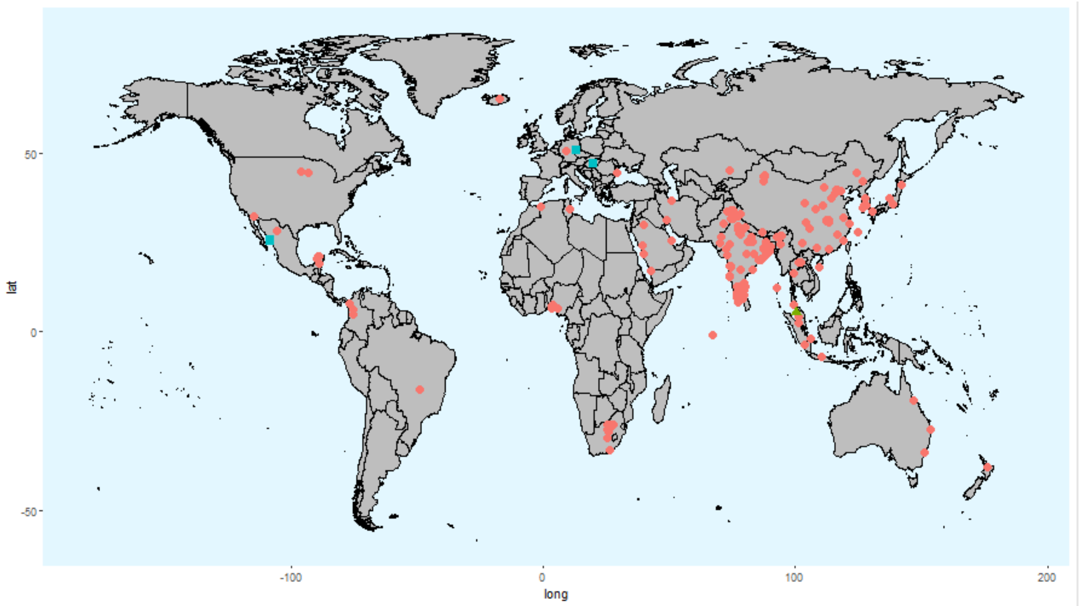

Figure 14. Map of records of *Bacillus subtilis* according to GBIF (orange symbols), iNaturalist (green symbols) and iDigBio (blue symbols).

Paenibacillus azotofixans (formerly *Bacillus azotofixans,* now *Paenibacillus durus*) is a nitrogen-fixing soil bacterium that is often found in the rhizosphere of maize, sugarcane, wheat and forage grasses (Rosado et al., 1996). According to Berge et al. (2002), *P. azotofixans* has only been found in Brazilian and Hawaiian soils (Rosado et al., 1998; Seldin et al., 1984) and never in soils from temperate zones (L. Seldin, personal communication to Rosado). The fact that the bacterium has not been isolated from soils in temperate zones indicates that it may not survive in colder climates such as those found in Norway.

Note: there are no records of *P. azotofixans* in GBIF or other databases.

4 Establishment and spread

Introduction of new species or genetically different isolates of native species should always be considered carefully, because introduced species or isolates may spread and cause unintended consequences in their new area. Most damage is caused by the unintended introduction of pests and pathogens, but there are also examples of harmful effects from intentional introduction of organisms for useful or beneficial purposes. One such introduction, which later backfired was the use of the harlequin ladybeetle (*Harmonia axyridis*) for aphid control (Roy and Brown, 2015). There are no documented examples yet of damage caused by intended or unintended introduction of mycorrhizal fungi, but there are examples of both mycorrhizal species and saprophytic fungi spreading into new environments.

The descriptors for the qualitative ratings used are presented in appendix II.

4.1 Establishment of mycorrhizal fungi in the introduced range

Rating of probability of establishment of the mycorrhizal fungi in the introduced range: Moderately likely

Rating of uncertainty: Medium

In order for introduced species to become a problem, they must successfully establish, persist and spread in their new environment ('introduced range') (Hart et al., 2018). Most experiments and studies of species assessed in this opinion were carried out in closed greenhouse systems and have limited applicability to field conditions. The few field trials that exist have mostly provided inconclusive results on the long-term persistence of the species, although some have shown that the species may have an impact on their host plants (Akyol et al., 2019) or on damaged soils (Maltz et al., 2019).

Successful establishment of mycorrhizal fungi on plants may also depend on the inoculation method used. Plants can be inoculated under controlled conditions, e.g. in greenhouses, and subsequently transplanted to their intended growth place with the mycorrhizal fungi already present in the root system. This method would be used on so-called plug plants where the fungi are inoculated during plant production. Another method would be to inoculate the soil during sowing, seed germination or planting. Finally, the mycorrhizal product could be introduced during the growth phase to become established on new roots. This method is used on grassy areas such as golf courses, or for trees that suffer growth stress due to poor root conditions.

With all inoculation methods, the mycorrhizal fungi have to compete for access to the plants' fine roots of the plants with other fungi that already are established in the soil. In addition, many other members of the diverse soil microbial community will interact or compete with the inoculated species. This competitive environment may reduce the likelihood for successful establishment of the six assessed mycorrhizal species. On the other hand, these species are generalists, which can form symbiosis with several plant species and that probably are adapted to living in Norway (although in the case of *Pisolithus tinctorius,* with limited distribution). This may increase the probability that the species will be able to join the soil microbial community, but is no guarantee for successful establishment.

Most of the available documentation concerning the six mycorrhizal fungi assessed in this opinion shows variable inoculation success, with low long-term persistence. Establishment and persistence of the species can be good in closed systems with more or less sterile soils (Janouskova et al., 2017). Inoculation success can also be fairly high in heavily degraded soils which lack microbial communities. *Pisolithus tinctorius* has for example been found to flourish on acidic soils left after strip mining in the USA (Medve and Gill, 1982) and was therefore used to promote establishment of pine trees on such sites (Mullins et al., 1989). The species has also been shown to enhance fungal communities and plant growth on acidified soil (Maltz et al., 2019). However, the specific field conditions that might prevent natural establishment of mycorrhizal species in the first place, such as disturbed agricultural and urban soils, are also likely to affect the inoculated fungi. This is especially true if the inoculated fungi are not introduced together with their host plants, but are inoculated later.

Even if we assume that soil conditions and inoculation methods favour establishment of the mycorrhizal fungi, it is uncertain whether this would translate into long-term establishment in the environment (see below). We thus consider it moderately likely that the mycorrhizal fungi will establish in the environment, with medium uncertainty.

4.2 Establishment of bacteria in the introduced range

Rating of probability of establishment of the bacteria in the introduced range (based on names used in the products):

Very likely (*Bacillus*), Very unlikely (*Paenibacillus*)

Rating of uncertainty: Low

As most of the assessed *Bacillus* species are ubiquitous and thus probably occur naturally in Norwegian soils, we consider it very likely, with low uncertainty, that the introduced strains of *Bacillus* will be able to establish in Norway following inoculation. *Paenibacillus azotofixans*, on the other hand, seems to be adapted to tropical soils and we consider it very unlikely, again with low uncertainty, that it will be able to establish in Norwegian soils.

4.3 Spread of mycorrhizal fungi to the wider environment

Rating of probability of spread to the wider environment: Unlikely (AM fungi), moderately likely (EM fungi)

Rating of uncertainty: High

Some introduced species do not remain in the area and on the hosts where they were initially introduced, but spread to the surroundings or to other hosts. Such spread can have unintended consequences if for example introduced mycorrhizal species compete with or replace local species in the soil microbial community (Engelmoer et al., 2014; Tiunov and Scheu, 2005). Introduced mycorrhizal fungi could also facilitate the spread of their plant hosts into the natural environment, or they could form new symbiotic relationships with local plant hosts and increase or decrease their fitness (Akyol et al., 2019).

The risk of spread will likely differ between species groups, such as EM and AM fungi. In general, AM fungi are thought to be less able to spread locally since they do not form above-ground fruitbodies. However, they may be spread by animals (including birds), water or movement of soil. Introduction of EM fungi, with their above-ground fruitbodies and great spore production, is much more likely to lead to local and more long-distance spread, provided that the fungi are able to persist after introduction.

The likelihood and impact of establishment and introduction of a mycorrhizal fungus depend on whether the species already is present in the introduced range or not. If an introduced species is new to the country or region, or is very rare, commercial inoculation would equal a deliberate introduction of the fungus. If the species is already present in the country or region, commercial inoculations are more likely to succeed and the ecological impact is likely to be lower, although this depends on the natural range of the fungus. *Pisolithus tinctorius* has, for example, only been found in one area of Norway. If this reflects a true distribution, the commercial inoculation of this fungus could increase its range considerably and make it much more common. In contrast, *Scleroderma citrinum* is widespread and abundant in Norway and would probably be less affected by commercial inoculation.

None of the four AM fungi assessed in this opinion are registered as present in Norway today, but this may be because AM fungi have been little studied in Norway. However, as described in Chapter 2 we consider it likely that the three assessed *Glomus* species (*G. etunicatum, G. clarum* and *G. intraradices*) are already present in Norway. Establishment and spread of these fungi are therefore possible, but the impact of introduction is probably low.

4.4 Spread of bacteria to the wider environment

Rating of probability of spread to the wider environment: Very likely

Rating of uncertainty: Low

The biological importance of spread is likely to be smaller if the imported species already are present and widespread in the country where they are introduced. Most of the assessed bacteria are cosmopolites that are spread by animals (including birds), water or transport of soil and are already present almost everywhere (Chapter 3). Thus, with a few exceptions their distribution is unlikely to be affected by commercial import and use.

5 Potential negative impact

Fungal species used in mycorrhizal products are often host generalists and produce copious spores in culture. The very same characteristics that make them useful as bio-stimulators may also be the factors that give them the potential to become invasive (Thomsen and Hart, 2018). The invasive potential of a species may not manifest itself immediately and some invasive species in Europe were present for decades before they became problematic. This includes invasive plant species such as giant hogweed (*Heracleum mantegazzianum*, 'kjempebjørnekjeks' in Norwegian) or Canadian goldenrod (*Solidago canadensis*, 'kanadagullris'). Such examples illustrate that it is hard to predict if an introduced species will become a problematic, invasive species, e.g. after they have reached a certain population threshold or following environmental change. However, since most of the fungal and bacterial species assessed in this opinion probably are native to Norway, we consider it unlikely that they will cause a negative impact in the wider environment. Still, their introduction may impact local populations of the same species or the wider environment through competition or effects on ecosystem functions.

5.1 Genetic changes in local populations / native individuals of the same species

Rating of probability of genetic changes in local populations: Unlikely

Rating of uncertainty: High

There are no studies of genetic changes caused by introduction of any of the mycorrhizal fungi assessed in this opinion. However, the decay fungus *Phlebiopsis gigantea* provides a relevant example of how artificial introduction of fungal spores may affect the genetics of local populations of the same species. The basidiomycete *P. gigantea* is used as a biocontrol agent for stump treatment against the root rot pathogen *Heterobasidion annosum*. Rotstop®, made from vegetative spores of *P. gigantea*, has been used in Scandinavian forestry since the early 1990s. Concerns about the potential impact of Rotstop on native populations of *P. gigantea* led to research on the population structure and spread of the inoculated strain. Several studies concluded that the introduced strain generally remained restricted to treated forest plots and had no impact on other wood-inhabiting fungi, including local populations of *P. gigantea* (Samils et al., 2009; Vainio, 2008; Vainio et al., 2001).

When it comes to the assessed AM fungi, we do not know for sure whether they already are present in Norway or not, but we assume that the three *Glomus* species are present. However, it is uncertain if any introduced strains will recombine with local populations. The basic biology of these fungi is unclear and it is unknown if they undergo sexual recombination at all. However, since anastomosis between hyphae is a common in fungi, this type of recombination is possible for all the assessed AM fungi.

As discussed in Chapter 3, the two EM fungi assessed in this opinion are already present in Norway. Recombination with introduced strains is likely for *Scleroderma citrinum,* which is widely spread and abundant, but is less likely for *Pisolithus tinctorius*, unless inoculations are made in the very restricted area near Kongsberg where this fungus has been found.

We do not know the geographic origin of the commercial inoculum and whether it differs genetically from native Norwegian populations. However, many species of fungi show little genetic differentiation across wide geographical ranges, probably due to the high dispersal capacity and corresponding high gene flow of many species. Hence, for the species that already are present in Norway, the introduced genetic material may not differ significantly from local populations. However, this obviously depends on the geographic origin of the introduced material. If the isolates originate from other continents, commercial inoculum will likely introduce new and different genetic material that even could represent unknown cryptic species.

Finally, we do not know whether the introduced material will survive under temperate or boreal conditions. Several of the AM fungi and at least one of the bacteria have a more southern/tropical distribution and might not be able to survive in our climates.

5.2 Effects on other native species, habitats and ecosystems

Rating of probability of effects on other native species, habitats and ecosystems: Unlikely

Rating of uncertainty: Medium

There are few documented examples where imported mycorrhizal fungi have negatively affected local biodiversity. However, this may be because there are very few studies and little data on this topic. Possible adverse impacts of introductions of mycorrhizal fungi on biodiversity include undesirable direct consequences for host plants in managed systems, direct and indirect negative consequences for biodiversity, and negative consequences for ecosystem function (Schwartz et al., 2006).

AM fungi have been shown to compete with each other in greenhouse experiments, and inoculated strains could outcompete native AM fungi (Engelmoer et al., 2014; Janouskova et al., 2017; Tiunov and Scheu, 2005). One potential hazard associated with the introduction of AM fungi could thus be loss of genetic diversity and of locally adapted genotypes, if the introduced species replace or suppress indigenous species of fungi and bacteria. One recent field trial with a *Glomus* species shows an impact on indigenous AM fungic(Akyol et al., 2019).

Species of *Amanita* (fly agaric) that are involved in EM symbiosis have been introduced to the southern hemisphere and to North America. Some *Amanita* species have persisted in planted forests and have in some cases spread to the wider environment, but no negative impacts have been demonstrated thus far (Pringle et al., 2009; Sawyer et al., 2001; Schwartz et al., 2006). Species of saprophytic fungi, both pathogens and true decomposers, has been introduced to Europe from other continents, and vice versa. While pathogens often have devastating impacts, more benign decomposers can also spread in the environment and affect local biodiversity. Often, the distribution of such decomposers is initially restricted and they are considered rare and harmless. Only later, when they become widespread and compete with native fungi, are they seen as a problem. Examples from Norway include *Phaeolepiota aurea* (golden bootleg, in Norwegian 'gullskjellsopp') and *Mutinus ravenelii* (red stinkhorn, 'hagestinksopp'). *Phaeolepiota aurea* was introduced from North America to Europe more than a century ago, but has become more widespread in Norway in the last few decades (Artsdatabanken.no). *Mutinus ravenelii* has been known from Norway for more than 50 years and is mainly found in gardens and parks, but locally also in the wider environment (Artsdatabanken.no). While neither of these species have known ecological impacts, they still serve as examples of introduced species with potential negative effects.

Another potential negative effect might be that mycorrhizal fungi enable crop species or closely related wild species to be more successful in spreading and establishing in areas where they would not normally grow. Examples of this are introduction of tree species, such as *Pinus radiata* (Monterey pine) and various eucalypt species, together with their associated EM fungi. The fungi could potentially help the host to spread outside plantations (Schwartz et al., 2006), as the case for eucalypt plantations in Spain (Díez, 2005).

The total microbial diversity in soil is generally high and the introduction of a few extra bacterial and fungal species into such a complex system is not likely to make much difference. For example, a single plant species can form symbiosis with tens (or hundreds) of EM fungi at the same time, and different AM and EM fungi appears to have overlapping ecological roles and largely fulfil the same ecosystem functions. So even if one host plant recruits some new AM or EM fungi, it is unlikely that ecosystem functions are afected. Provided the introduced fungi are not extremely competitive and aggressively replaces native fungi, we expect no or small consequences on native species, habitats and ecosystems. We have not found any data showing that the six assessed fungi are particularly aggressive in establishing and spreading.

Most of the *Bacillus* species described above are considered to be ubiquitous and thus present in most soils worldwide. The maps of records shown in Figures 10, 11, 13 and 14 illustrates this, and most species are present in diverse climatic regions including temperate regions. Thus, release of strains of the assessed *Bacillus* species in Norwegian soils will probably take place into a pool of pre-existing strains of the same species. However, it is unknown whether the introduced strains will have properties that are not already present in the native strains.

Concerning the unintended presence of hitchhiking fungi or bacteria in the products, the main hazard would be the inadvertent introduction of plant pathogens, which could subsequently spread and damage crops or native species. Based on the description of the production system for the assessed mycorrhizal products provided by the manufactures, it seems unlikely that the products will contain such contaminants.

6 Conclusion of the risk assessment

We consider the establishment of AM and EM fungi in their introduced range in Norway to be possible, but with some uncertainty. The probability of spread is unlikely for the four assessed AM fungi, and moderately likely for the two assessed EM fungi. The likelihood of negative impacts is considered to be unlikely for all six fungi, since most of the assessed species are present or likely present in Norway already. None of the fungi has been reported as problematic anywhere in the world.

We consider it unlikely that introduction of the assessed mycorrhizal fungi will lead to dramatic genetic changes in native populations, even though it is unknown to what degree the introduced material is genetically different from native populations.

There is no clear evidence that the assessed mycorrhizal species have negatively impacted ecosystems in other countries. Also, there is no evidence of the six fungi being able to spread aggressively into the wider environment or displace native fungal species. We therefore consider it unlikely that they will have negative effects on other native species, habitats and ecosystems in Norway. The few examples found in literature concerning unintended spread of EM fungi do not relate to the two species assessed in this opinion (*Pisolithus tinctorius* and *Scleroderma citrinum*). Likewise, we have found no examples of negative ecosystem effects caused by the four assessed AM species. Considering that mycorrhizal fungi also may be introduced with plants for planting, especially burlap trees which include considerable amounts of soil from the production areas, the extra risk caused by using the commercial products assessed here seems low. However, one should always bear in mind that many introduced species of plants, insects or fungi may start out as rare with little impact on the environment, but may show their full potential as invasive species several decades later.

We consider it very likely that the assessed strains of *Bacillus* will be able to survive and spread in Norway. However, release of the six assessed species of *Bacillus* and *Paenibacillus* is not considered to have significant negative impacts or consequences for natural ecosystems. This is because the species probably already are present in Norway, or, in the case of *Paenibacillus azotofixans*, not will be able to survive in temperate regions. There are no indications that the assessed strains have biological properties that are not already present in Norwegian soils, and therefore they do probably not represent a threat to native ecosystems.

7 Risk reduction options

Since we conclude that the potential negative impacts and consequences of using the assessed products in Norway are limited, there is little need for specific risk reduction measures. A possible mitigation measure could be to use the products in a manner that prevents the included fungi and bacteria from being introduced accidentally in areas where they are not meant to be introduced.

8 Uncertainties

8.1 Summary of uncertainties

Generally, there is very little published information about genetic effects on native populations, potential invasiveness, or wider impacts on the environment for the species assessed in this opinion. This is true for both fungal and bacterial species. Most of the available studies focus on how the species affect host plants or are carried out in controlled environments such as greenhouses.

The taxonomical classification of many mycorrhiza-forming fungi is uncertain. Firstly, the biological species concept is not meaningful for AM fungi, since these species are not known to reproduce sexually. Secondly, most species are defined based on morphological characters and these morphological species may include several genetically distinct cryptic species. For these reasons, the geographical distribution and national species records of mycorrhizal fungi are uncertain.

9 Conclusions (with answers to the terms of reference)

9.1 Natural distribution of the six species (ToR #1)

The natural distribution of the six mycorrhizal species is presented in Chapter 2.

Our assessment of species distributions is mainly based on records from GBIF and other curated databases that largely include occurrence data based on morphological assessments (Figure 3 to 9), as well as on records from NCBI ("GenBank") that includes DNA sequence data (Supplementary Table 1, Appendix 1). Note that many of the accessions in these databases could be wrong - earlier literature has suggested that up to 20% of DNA data in GenBank have errors in their taxonomic affiliation. Also, note that the maps in Figure 3 to 9 only show records with accurate geographic coordinates.

Most AM fungi appear to have wide geographic distributions, but this could be an artifact of poor species delimitations and cryptic species complexes. On the other hand, some AM species have been little studied and may have broader distributions than currently indicated due to limited occurrence data. In general, there is better data on the distribution of EM fungi than AM fungi, largely because most EM fungi have conspicuous fruitbodies.

9.2 The likelihood of spread and potential establishment (ToR #2-5)

Introduction of new species or genetically different isolates of native species should always be considered carefully, because introduced species or isolates may spread and cause unintended consequences in their new area. There are no documented examples yet of damage caused by intended or unintended introduction of mycorrhizal fungi, but there are examples of mycorrhizal species spreading into new environments.

The Panel assesses the probability of establishment of the assessed mycorrhizal fungi in the introduced range to be moderately likely, with a medium level of uncertainty. This is described in more detail in Chapter 4.1. Furthermore, the Panel assesses the mycorrhizal species to have a low potential for unintended spread following establishment, with a high level of uncertainty. This is described in more detail in Chapter 4.3

The panel assesses that establishment in the introduced range is very likely for the species called *Bacillus* included in the mycorrhizal products, and very unlikely for the named *Paenibacillus* species, with a low level of uncertainty for both groups. This is described in more detail in Chapter 4.2. Furthermore, the panel assesses that the included bacteria have a low potential for unintended spread after establishment, with a high level of uncertainty. This is described in more detail in Chapter 4.4.

9.3 Potential negative impact (ToR #2-5)

Five of the six fungal species, as well as most of the bacterial species, are considered to be already present in Norway. Therefore, the probability that they will become invasive in the wider environment is considered to be very low. However, the species may still have an impact on local populations of the same species or on the wider environment via competition or impacts on ecosystem functions (see Chapter 5).

9.3.1 Genetic changes in local populations / native individuals of the same species

The Panel assesses the probability of genetic changes in local populations to be low, with a high level of uncertainty (Chapter 5.1).

There are no studies of genetic changes caused by introduction of any of the mycorrhizal fungi covered in this opinion. We do not know the geographic origin of the imported inoculum or whether the imported isolates differ genetically from the populations that are already present in Norway. However, many species of fungi show little genetic differentiation across wide geographical ranges, probably due to high dispersal capacity of many species and high gene flow. Hence, for those species that are already present in Norway the introduced genetic material may not differ significantly from what is already present.

For some of the assessed species it is uncertain whether the introduced material will survive under temperate/boreal conditions. Several of the AM fungi and at least one of the bacteria have a more southern/tropical distribution and are probably not adapted to a Norwegian climate (Chapter 5.1).

9.3.2 Effects on other native species, habitats and ecosystems

The Panel assesses the probability of effects on other native species, habitats and ecosystems to be unlikely, with a medium level of uncertainty (Chapter 5.2).

There are few documented cases where imported mycorrhizal fungi have negatively affected local biodiversity. However, this may be because there are very few studies and little data on this topic. Possible adverse impacts on biodiversity include undesirable direct consequences for host plants in managed systems, direct and indirect negative consequences to biodiversity, and negative consequences to ecosystem function. One potential hazard could be loss of species diversity and of locally adapted genotypes, if the introduces species replace or suppress indigenous fungi and bacteria, but no such negative impacts have been documented so far (see Chapter 5.2).

The total microbial diversity in soil is generally high, and the introduction of a few extra species into such complex systems will likely not have significant consequences. There is no data indicating that the six assessed fungi are particularly aggressive competitors that quickly establish and spread in new environments.

Concerning the unintended presence of other fungi or bacteria in the products ("hitchhiking organisms"), the greatest hazard would be the inadvertent introduction of plant pathogens, which could subsequently spread and damage crops or wild plants. Based on the description by the manufacturers of the production system for the assessed products, it seems unlikely that the products will contain problematic hitchhiking organisms.

9.4 The overall risk of negative impact following spread and potential establishment of the six mycorrhizal products in Norway (ToR #6).

The probability of negative impacts is considered to be unlikely for all six assessed fungi, since most of the species are present or likely present in Norway already.

The panel considers the risk for adverse impacts for biodiversity to be about the same in a 50-year perspective as it is today. This is because most of the assessed species have an almost global distribution and likely are present in Norway already.

We consider it unlikely that introduction of the assessed species will lead to dramatic genetic changes in native populations. However, it should be noted that we do not know to what degree the introduced material is genetically different from native populations.

For more details see Chapter 6.

9.5 Assessment of various mitigation measures (ToR #7).

Since the potential negative impacts and consequences of using the assessed products likely are limited, there is little need for specific risk reduction measures. One mitigation measure could be to use the products in a manner that prevents the fungi and bacteria from being accidentally introduced in areas where they are not meant to be present.

10 Data gaps

There are no records of the four AM fungi from Norway. We assume that three of them are present, due to their general global distribution, but the lack of data introduces some uncertainty. There is limited information about the survival and spread of mycorrhizal species in nature. This is true both for the six species assessed in this opinion and for mycorrhizal species in general. If more information had been available, we could have presented our probability ratings with less uncertainty.

More knowledge about the establishment and spread of mycorrhizal species in nature could be obtained by using molecular methods to detect the species in soil samples from both inoculated areas and from the wider environment. This would probably require sampling both before and after inoculation of mycorrhizal fungi.

11 References

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Appendix I

Supplementary Table 1. Occurrence data based on DNA sequences deposited in GenBank.

Country/ Organism*	*E. columbiana*	*G. etunicatum*	*G. clarum*	*G. intraradices*	*P. tinctorius*	*S. citrinum*
Africa						
Cameroon	6	0	0	0	0	0
Kenya	0	43	0	3	0	0
Libya	0	7	0	1	0	0
Madagascar	0	11	0	0	0	0
Morocco	0	75	0	22	0	0
Namibia	0	28	4	4	0	0
Senegal	0	0	0	1	0	0
Asia						
China	0	10	0	48	0	1
India	1	1	0	2	0	0
Indonesia	7	0	6	0	0	0
Iran	0	0	0	13	0	0
Japan	1	13	3811	3	1	0
South Korea	0	0	0	0	1	0
Thailand	0	0	0	2	1	1
Europe						
Czech Republic	0	0	0	16	0	3
France	0	0	0	8	0	0
Germany	0	0	1	47	0	1
Italy	0	0	0	0	4	0
Macedonia	0	0	0	0	4	0
Montenegro	0	0	0	0	1	0
Netherlands	0	0	0	3	0	1
Poland	0	29	0	0	0	2
Russia	0	0	0	0	0	1
Spain	0	68	1	192	2	6
Switzerland	0	0	0	52	0	0
United Kingdom	0	46	0	1	0	3

Latin America						
Argentina	0	0	0	1	0	0
Bolivia	0	0	0	1	0	0
Brazil	0	31	7	0	0	2
Colombia	1	40	2	0	0	0
Costa Rica	0	0	0	3	0	0
Cuba	0	27	10	0	0	0
Mexico	0	32	0	0	0	0
Venezuela	0	13	0	0	0	0
North America						
Canada	2	2	0	75	0	0
USA	0	1095	1	72	4	12
Oceania						
Australia	0	52	0	3	0	0

* E. = *Entrophospora*; G. = *Glomus*; P. = *Pisolithus*; S. = *Scleroderma*

Appendix II

Descriptors for qualitative ratings

Ratings and descriptors are based on Appendix 2 in VKMs Risk Assessment of cockspur grass (*Echinochloa crus-galli*).

Table A2-1: Rating of the probability of establishment and spread.

Rating	Descriptors
Very unlikely	The likelihood would be very low because of: • the absence or very limited availability of suitable habitat/crop; • the unsuitable environmental conditions; • and the occurrence of other considerable obstacles preventing establishment
Unlikely	The likelihood would be low because of: • the limited availability of suitable habitat/crop; • the unsuitable environmental conditions over the majority of the risk assessment area; • the occurrence of other obstacles preventing establishment
Moderately likely	The likelihood would be moderate because: • suitable habitats/crops are abundant in a few areas of the risk assessment area; • environmental conditions are suitable in a few areas of the risk assessment area; • no obstacles to establishment occur
Likely	The likelihood of would be high because: • suitable habitats/crops are widely distributed in some areas of the risk assessment area; • environmental conditions are suitable in some areas of the risk assessment area; • no obstacles to establishment occur • Alternatively, the pest has already established in some areas of the risk assessment area
Very likely	The likelihood would be very high because: • hosts plants are widely distributed; • environmental conditions are suitable over the majority of the risk assessment area; • no obstacles to establishment occur. • Alternatively, the pest has already established in the risk assessment area

Table A1-2: Ratings used for describing the level of uncertainty.

Rating	Descriptors
Low	No or little information is missing or no or few data are missing, incomplete, inconsistent or conflicting. No subjective judgement is introduced. No unpublished data are used.
Medium	Some information is missing or some data are missing, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used.
High	Most information is missing or most data are missing, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used.

Appendix III

VURDERING AV RISIKO FOR UHELDIGE FØLGER FOR BIOLOGISK MANGFOLD VED INNFØRSEL OG UTSETTING AV MYKORRHIZA-DANNENDE SOPPARTER

Kontaktperson:	Micael Wendell/FHI
Søk:	Astrid Nøstberg
Fagfelle:	Nataliya Byelyey
Dublettsjekk i EndNote:	Før dublettkontroll: 736 Etter dublettkontroll: 464

12 Pico-skjema:

Hva er spørsmålet som litteratursøket er ment å besvare?	Spørsmålet i PICO format				Kjente relevante studier
	Population (pasient)	Intervensjon (tiltak)	Comparison (sammenlignin g)	Outcome (utfall)	
Er innføring og utsetting av mykorrhiza-dannende sopparter en risiko for biologisk mangfold?	Biologisk mangfold	Mykorrhiza-dannende sopparter (entrophospora colombiana, entrophospora columbiana, glomus etunicatum, glomus clarum, glomus intraradices, rhizophagus irregularis, pisolithus tinctorius, scleroderma citrinum)			

Database: **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to November 27, 2019>**

Date: 03.12.2019

No. hits: 71

#	Searches	Results
1	"entrophospora col#mbiana".tw,kf.	2
2	"glomus etunicatum".tw,kf.	84
3	"glomus clarum".tw,kf.	29

4	"glomus intraradices".tw,kf.	518
5	"rhizophagus irregularis".tw,kf.	327
6	"pisolithus tinctorius".tw,kf.	114
7	"scleroderma citrinum".tw,kf.	23
8	or/1-7	1055
9	exp Biodiversity/	69045
10	(biodiversit* or "bio diversit*" or ((biological or microbial or microbe or microorganism or "micro-organism") adj diversit*)).tw,kf.	31892
11	(biota or biocenose* or "bio-cenose*" or microbiome* or "micro-biome*" or microbiota* or "micro-biota*" or ((biotic or biological or ecological or microbial or fungal) adj communit*)).tw,kf.	84995
12	("enteric bacteria*" or ((gastrointestinal or intestinal or gut) adj (flora or microflora or "micro flora"))).tw,kf.	12449
13	("microbial consorti*" or "micro-bial consorti*" or mycobiome* or "myco-biome*" or periphyton* or "peri-phyton*" or ecotype* or "eco-type*").tw,kf.	6406
14	((endanger* or threaten* or introduc* or invasive) adj3 species).tw,kf.	15698
15	or/9-14	164749
16	8 and 15	71

Database: **Embase 1974 to 2019 November 27**
Dato: 03.12.2019
Antall treff: 21

#	Searches	Results
1	"entrophospora col#mbiana".tw,kw.	1
2	"glomus etunicatum".tw,kw.	92
3	"glomus clarum".tw,kw.	37
4	"glomus intraradices".tw,kw.	571
5	"rhizophagus irregularis".tw,kw.	283
6	"pisolithus tinctorius".tw,kw.	118
7	"scleroderma citrinum".tw,kw.	26

8	or/1-7	1079
9	exp biodiversity/	53271
10	(biodiversit* or "bio diversit*" or ((biological or microbial or microbe or microorganism or "micro-organism") adj diversit*)).tw,kw.	34055
11	(biota or biocenose* or "bio-cenose*" or microbiome* or "micro-biome*" or microbiota* or "micro-biota*" or ((biotic or biological or ecological or microbial or fungal) adj communit*)).tw,kw.	103162
12	("enteric bacteria*" or ((gastrointestinal or intestinal or gut) adj (flora or microflora or "micro flora"))).tw,kw.	15141
13	("microbial consorti*" or "micro-bial consorti*" or mycobiome* or "myco-biome*" or periphyton* or "peri-phyton*" or ecotype* or "eco-type*").tw,kw.	6838
14	((endanger* or threaten* or introduc* or invasive) adj3 species).tw,kw.	15983
15	or/9-14	185815
16	8 and 15	68
17	limit 16 to (conference abstracts or embase)	21

Database: **Scopus**
Dato: 03.12.2019
Antall treff: 253

Set	Search	Results
8	#1 AND #7	253
7	#2 OR #3 OR #4 OR #5 OR #6	440,927
6	TITLE-ABS-KEY ((endanger* OR threaten* OR introduc* OR invasive) W/2 species)	97,556
5	TITLE-ABS-KEY ("microbial consorti*" OR "micro-bial consorti*" OR mycobiome* OR "myco-biome*" OR periphyton* OR "peri-phyton*" OR ecotype* OR "eco-type*")	21,179
4	TITLE-ABS-KEY ("enteric bacteria*" OR ((gastrointestinal OR intestinal OR gut) PRE/0 (flora OR microflora OR "micro flora")))	17,027
3	TITLE-ABS-KEY (biota OR biocenose* OR "bio-cenose*" OR microbiome* OR "micro-biome*" OR microbiota* OR "micro-biota*" OR ((biotic OR biological OR ecological OR microbial OR fungal) PRE/0 communit*))	164,370
2	TITLE-ABS-KEY (biodiversit* OR "bio diversit*" OR ((biological OR microbial OR microbe OR microorganism OR "micro-organism") PRE/0 diversit*))	191,034
1	TITLE-ABS-KEY ("entrophospora colombiana" OR "entrophospora columbiana" OR "glomus etunicatum" OR "glomus clarum" OR "glomus intraradices" OR "rhizophagus irregularis" OR "pisolithus tinctorius" OR "scleroderma citrinum")	3,346

Database: **Web of Science**
Dato: 03.12.2019
Antall treff: 358

Set	Results	Search
# 15	**358**	#14 AND #8
# 14	**338,276**	#13 OR #12 OR #11 OR #10 OR #9
# 13	**58,877**	TS=(("endanger*" or "threaten*" or "introduc*" or "invasive") NEAR/2 "species")
# 12	**16,149**	TS=("microbial consorti*" or "micro-bial consorti*" or "mycobiome*" or "myco-biome*" or "periphyton*" or "peri-phyton*" or "ecotype*" or "eco-type*")
# 11	**13,761**	TS=("enteric bacteria*" or (("gastrointestinal" or "intestinal" or "gut") NEAR/0 ("flora" or "microflora" or "micro flora")))
# 10	**146,393**	TS=("biota" or "biocenose*" or "bio-cenose*" or "microbiome*" or "micro-biome*" or "microbiota*" or "micro-biota*" or (("biotic" or "biological" or "ecological" or "microbial" or "fungal") NEAR/0 "communit*"))
# 9	**134,062**	TS=("biodiversit*" or "bio diversit*" or (("biological" or "microbial" or "microbe" or "microorganism" or "micro-organism") NEAR/0 "diversit*"))
# 8	**3,572**	#7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1
# 7	**57**	TS=("scleroderma citrinum")
# 6	**640**	TS=("pisolithus tinctorius")
# 5	**504**	TS=("rhizophagus irregularis")
# 4	**2,103**	TS=("glomus intraradices")
# 3	**199**	TS=("glomus clarum")
# 2	**356**	TS=("glomus etunicatum")
# 1	**29**	TS=(("entrophospora colombiana" or "entrophospora columbiana"))

Database: **Crop Protection Compendium (CABI)**
Dato: 03.12.2019
Antall treff: 33 + 6 Datasheets
Kommentar: De 6 "datasheetsene" er ikke importert til EndNote, men limt inn nedenfor.

Søk: ("entrophospora colombiana" or "entrophospora columbiana" or "glomus etunicatum" or "glomus clarum" or "glomus intraradices" or "rhizophagus irregularis" or "pisolithus tinctorius" or "scleroderma citrinum") AND (mycorrhiza* OR ectomycorrhiza* OR endomycorrhiza*) AND (biodiversit* or "bio diversit*" or "biological diversit*" or "microbial diversit*" or "microbe diversit*" or "microorganism diversit*" or biota or biocenose* or microbiome* or microbiota* or "biotic communit*" or "biological communit*" or "ecological communit*" or "microbial communit*" or "fungal communit*" or "enteric bacteria*" or "gastrointestinal flora" or "intestinal flora" or "gut flora" or "gastrointestinal microflora" or "intestinal microflora" or "gut microflora" or "microbial consorti*" or mycobiome* or periphyton* or ecotype* or "endangered species" or "threatened species" or "introduced species" or "invasive species"