OPEN ACCESS

Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) |ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Production of Mychorrhiza Inoculum Enriched by Using Mychorrizal Helper Bacteria

Betty Natalie Fitriatin[1*], Reginawanti Hindersah[1], Iis Nur Asyiah[2], Rita Harni[3], Dwi Suci Rahayu[4]

[1]Department of Soil Sciences and Land Resouces Management, Agriculture Faculty, Universitas Padjadjaran – Jatinangor 45363-West Java, Indonesia
[2]Biology Education Program, Faculty of Teacher and Education Training, University of Jember Jalan Kalimantan No. 37, Jember Indonesia
[3]Center for Estate Crops Research and Development, Jalan Raya Pakuwon Km. 2 Parungkuda, Sukabumi 43357
[4]Indonesian Coffee and Cocao Research Institute (ICCRI) Jl. PB, Sudirman 90, Jember, Indonesia

DOI: 10.36348/sjls.2021.v06i11.001 | **Received:** 26.09.2021 | **Accepted:** 02.11.2021 | **Published:** 06.11.2021

***Corresponding author:** Betty Natalie Fitriatin

Abstract

Arbuscular Mycorrhizal Fungi (AMF) is a type of mycorrhizal that is in symbiosis with most plants. AMF symbiotic association provides many benefits for plants such as to help the nutrient absorption, especially phosphor, survival in drought conditions. Bacteria that can increase the growth and development of mycorrhizae is known as Mycorrhiza Helper Bacteria (MHB). The aim of pot experiment was to analyze the quality of the inoculum on mycorrhizal propagation enriched with the addition of MHB. The experiment used a Randomized Block Design to test three treatments of MHB liquid formula in the AMF propagation. The MHB liquid formula consisted of *Pseudomonas diminuta*, *Bacillus subtilis*, and a mixture of both isolates and controls (sterilized distilled water). The results revealed that mycorrhizal propagation using MHB increased the colonization of maize root and population of *P. diminuta* and *B. subtilis* in the mycorrhizal inoculum. Significant sporulation of *Glomus* spp. and bacterial population were increased by MHB inculation were also observed.

Keywords: Bacterial population, germination, root colonization, spores.

INTRODUCTION

Mycorrhiza is a symbiosis between the roots of higher plants and fungi [1]. Based on the mode of infection of the host plant, mycorrhizae can be grouped into three groups, namely ectomycorrhizae, endomycorrhizae and ectendomycorrhiza [2]. The ectomycorrhizal fungus is commonly found in forest plants [3]. This fungus can be seen directly in the field. The infected root will enlarge / swell and branch dichotomous, and the root surface is covered with mycelia commonly called the mantle [4].

The presence of infection by endomycorrhizae can be characterized by the formation of vesicles and arbuscules, so it is known as AMF (arbuscular mycorrhizal fungi) [5]. This type of mycorrhizal infection occurs inside the cell. Examples of AMF genera: *Glomus*, *Scelerocystis*, *Acaulospora*, *Entroshospora*, and *Gigaspora*. This endomycorrhizal fungus infects most food crops and horticulture. In general, the important roles of this mycorrhizal fungus include improving nutrition and increasing plant growth, as biological protection, increasing plant resistance to drought and synergizing with other microorganisms [6]. AMF can be associated with plant roots in almost 90% of plants, including vegetable crops [7].

Mycorrhizal symbiosis does not only involve two parties (fungi and plant roots) but also involves other organisms. The organisms associated with these mycorrhizae are known to influence each other. Bacteria that are able to increase the development of mycorrhizae are known as Mycorrhiza Helper Bacteria (MHB) [8]. Some researchers have found that bacteria isolated from mycorrhizal fungi can stimulate mycorrhizal infection, spore production and also resistance to plant pathogens [9, 10]. MHB promote the AMF establishment and to enhance the efficiency of the mycorrhizal effect on the plant growth [11].

MHB is widely used to assist the role of AMF in colonizing and infecting plant roots so that the ability of roots to increase nutrients up take [12]. There are five main roles of MHB in assisting mycorrhizal performance, including in preference for root acceptance of mycorrhizae, in introducing mycorrhizae with plant roots, in increasing mycorrhizal growth, in mobilizing nutrients in the rhizosphere, and in increasing mycorrhizal propagules germination [13].

Each type of AMF has associations with different bacteria. Each AMF has a different effect on bacterial and fungal populations [14]. This relates to specific competition for growth substrates. Therefore, research is needed to examine the quality of the inoculum on mycorrhizal propagation due to the MHB inoculation.

MATERIALS AND METHODS

Propagation and formulation

Mycorrhizae are obligate fungi with host plants, therefore mycorrhizal propagation is generally by using plants [15]. Mycorrhizal propagation was carried out using spores collected from the Faculty of Agriculture, Gajah Mada University, grown on zeolite media with a size of 2 - 3 cm sterile which had been saturated with NaCl solution (5000 ppm) and had been planted with corn seeds aged 7-10 days.

The first fertilization was carried out a week after planting with 30 ml of Hyponex (25-5-20) fertilizer with a concentration of 1 g/L. Subsequent fertilization with the same dose is done twice a week. At the time of three months after planting, stressing was carried out to stimulate spore formation by gradually reducing watering up to 10 ml, until not watered (1 week approximately). Spores were harvested at 70 days.

The Experimental Design

The experiment used a Randomized Block Design to test three treatments of MHB liquid formula in the AMF propagation. The MHB liquid formula consisted of *Pseudomonas diminuta* (collected from Soil BiologyLaboratory, Faculty of Agriculture, Universitas Padjadjaran) and *Bacillus subtilis* (collected from University of Jember) and a mixture of both isolates and controls (distilled water).

Inoculation and plant maintenance

AMF inoculation was carried out twice, the first when the maize seeds were germinated (pre-inoculation) and at the time of transplanting into plastic tranparant pots containing 300 g of zeolite. A week after transplanting, a liquid formula was applied as much as 300 mL per pot. For each treatment, the concentration of the liquid formula was 1mL L^{-1} while the density of MHB was 10^8 cfu mL^{-1}. Plants were kept in a greenhouse for 3 months. Watering was stopped when the plants were 2 months old to stimulate spore formation and root colonization by hyphae.

Parameters Observation

At harvest time the plant roots were separated from the crown and the degree of root infection (%) was determined by the acid fuchsin staining method [16]. The concentration of MHB bacteria in the growing media was determined by the Plate Dilution method [17]. Observations of the bacteria growth on the surface of mycorrhizal spores were also carried out using the modified method.

Mycorrhizal Formulations and Mychorrizal Helper Bacteria

The mycorrhizal propagation enriched with MHB was carried out, consisting of 6 treatments. The MHB inoculants used were a consortium of *P. diminuta* and *B. subtilis* with a ratio of 2:3 aged 3 days. Figure 1 showed MHB-enriched microzae propagation with maize host in zeolite media and mycorrhizal hyphae on maize roots on one month after treatment.

Fig-1: (a) Mycorrhizal propagation with maize as host plant; (b) Mycorrhizal hyphae on maize roots

RESULTS AND DISCUSSION

The application of MHB increased the root colonization on one month after treatments about 81,33-94,67%. The percentage of root infection is high as a biofertilizer because root colonization is above 50%. Root colonization was classified according to the criteria of [18] ie. not colonized (0%) , low (< 10%), moderate (10-30%), and high (> 30%). The experiments showed that the addition of MHB with densities of 10^6 to 10^9 increased root colonization up to 21.37% (Fig. 2).

Mycorrhizal propagation enriched with molasses increased root colonization lower than addition with MHB. This indicates that MHB is able to increase the growth and activity of mycorrhizal fungi in infecting roots in AMF propagation. MHB is able to increase mycorrhizal fungi in colonizing roots [19]. The mechanisms of action in the MHB, among others a stimulation of the pre-symbiotic growth of the mycelium, a stimulation of the root-mycelium recognition and a modification of the physico-chemical properties that support mycorrhiza formation [20]. This experiment showed that the increased density of MHB given further increased root colonization. The addition of MHB with a density of 10^9 gave the highest increase in root colonization (21.37%), while the lowest root colonization was in the treatment of mycorrhizal propagation with the addition of water.

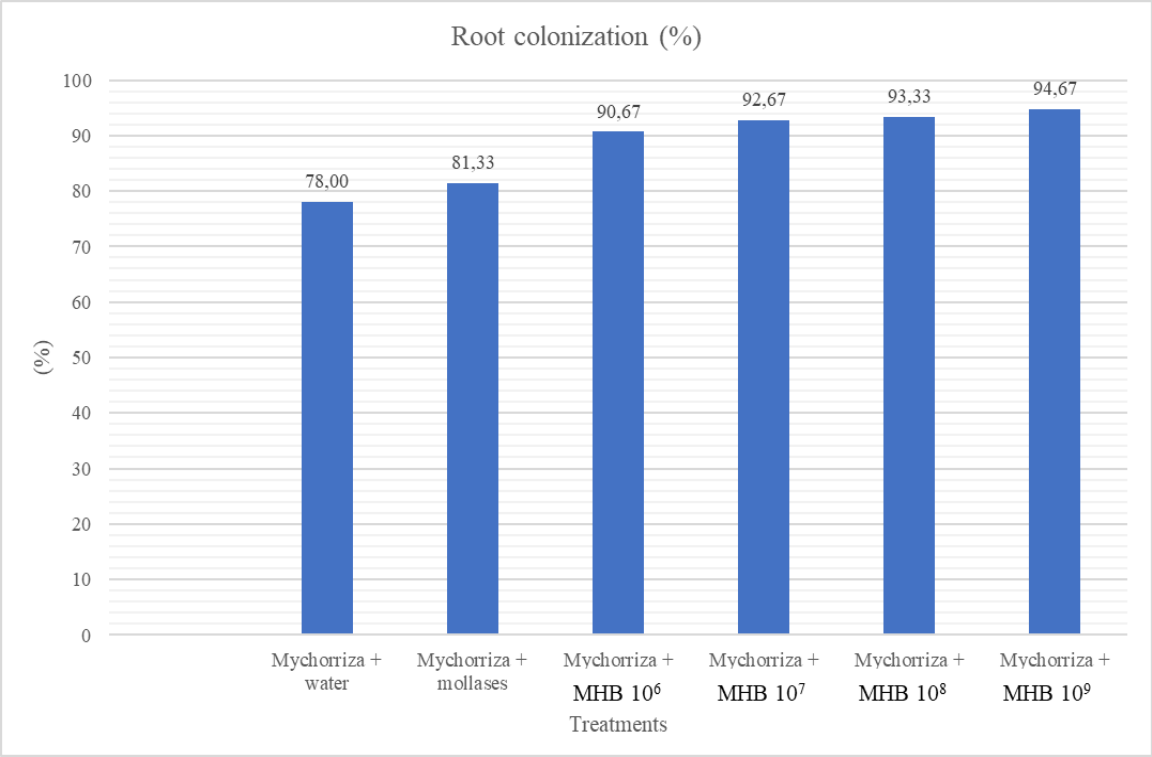

Fig-2: Percentage of mycorrhizal infections

The results showed that MHB was able to grow in zeolite media after 3 days of application, i.e. 57.33 x 10^7 cfu ml^{-1} for *B. subtilis* and 27.67 x 10^7 cfu ml^{-1} for *P. diminuta*. This indicates that MHB can grow in zeolite media.

In general, the population of *P. dimunita* and *B. subtilis* at one month after treatment increased about 12,33-102,33 x 10^7 cfu g^{-1} (Fig. 3). Mycorrhizal propagation by giving MHB at a density of 10^9 was able to increase the highest population of *B subtilis* up to 740,8%. This indicated that the MHB consisting of *P. diminuta* and *B. sublitis* added to myccorhiza inculum propagation was able to grow optimally. In this experiment, the density of bacteria in the mycorrhizal inoculum qualifies as biofertilizer, which was above 10^7 cfu g^{-1}.

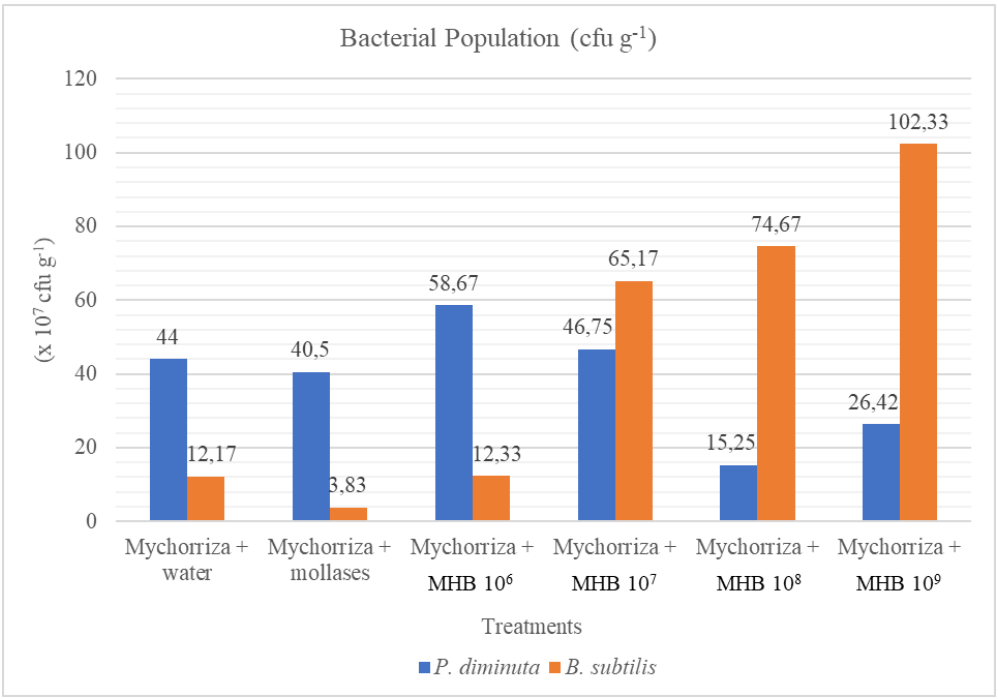

Fig-3: Population of *P. diminuta* and *B. subtilis* (1 month after treatment)

Spores number of *Glomus* spp. were the most in the treatment of *Glomus* spp. enriched with MHB by a density of 10^8 (Fig.4). The number of *Glomus* spp. spores. from all treatments *Glomus* spp. with MHB more than 50 spores g^{-1} dry weight of the sample. This amount qualifies as biofertilizer. This indicates that MHB indicated mycorrhizal spores germination. MHB had a strong positive effect on spore germination and growth of presymbiotic fungi in solutions contaminated with heavy metals [21].

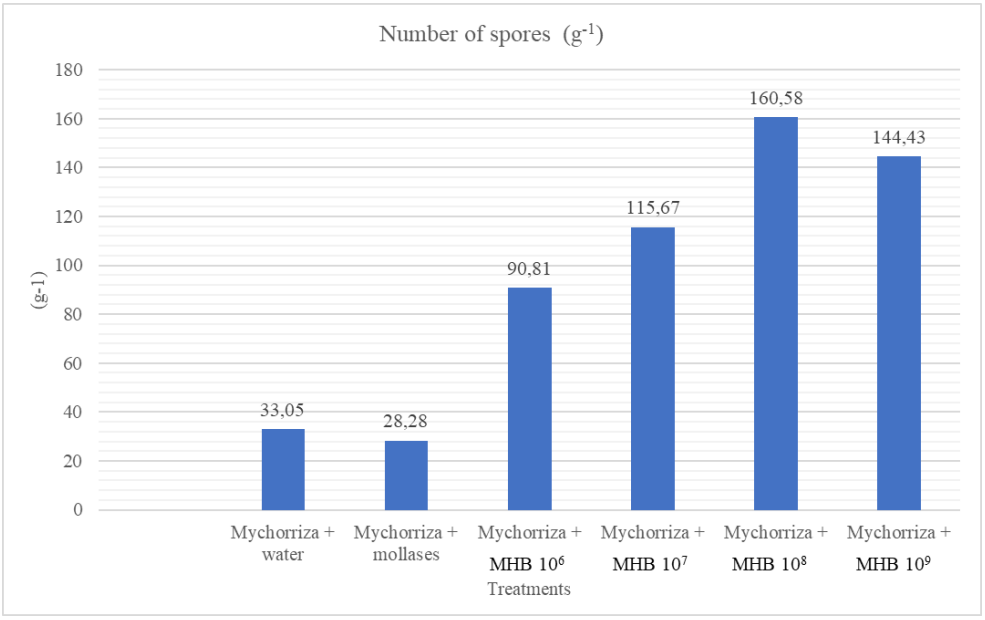

Fig-4: The number of *Glomus* spp. spores

The most bacteria from the cell wall of AMF spores were able to increase the germination of *G. clarum* spores when there was direct contact between spores and bacteria, while some bacterial isolates inhibited spore germination by producing antagonistic volatiles [22]. Furthermore, MHB affects the concentration of antagonistic compounds produced by mycorrhizal fungi [23]. The bacteria were able to detoxify the liquid medium of inhibitory fungal metabolites. MHB bacteria may also be able to suppress the production of toxic compounds by soil microbes [24].

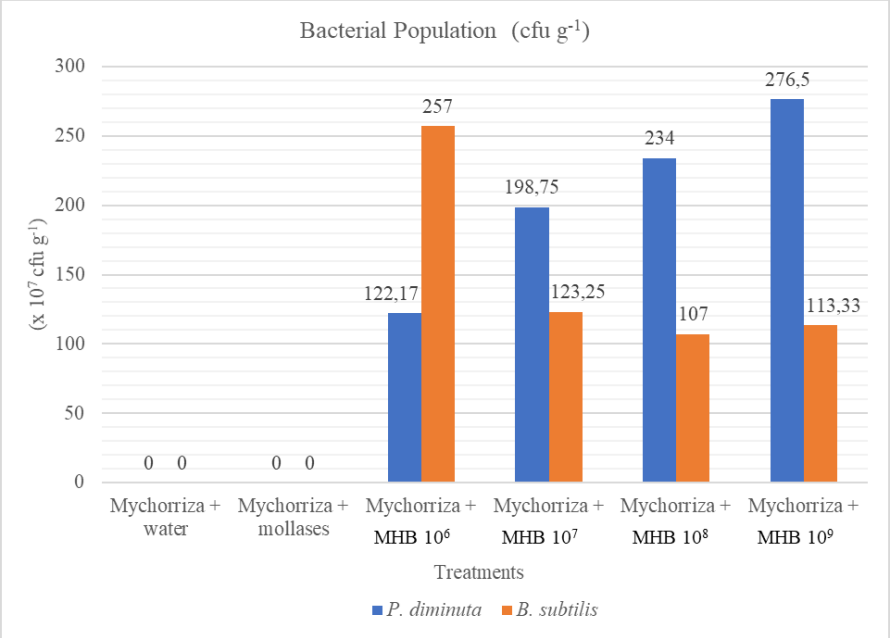

Fig-5: Population of *P. diminuta* and *B. subtilis*

The results of the bacterial counting at harvest showed that the population of *P. dimunita* and *B. subtilis* increased about 107-276,5 x 10^7 cfu g^{-1} (Fig. 5). On the other hand, mycorrhizal propagation with the addition of water and molasses does not show any growth of these bacteria. This shows that the applied MHB is able to grow on the propagation medium until harvest time. Furthermore, when compared to this bacterial population in one month, the bacterial population at harvest time was higher than one month after treatment.

CONCLUSIONS

In this study, we probed the effect of mycorrhiza helper bacteria (*P. diminuta* and *B. sublitis*) on mycorrhizal propagation. The result showed that MHB increased the colonization of maize root and population of *P. diminuta* and *B. subtilis* in the mycorrhizal inoculum. Spores germination of *Glomus* spp. and bacterial population were increased by MHB inoculation.

ACKNOWLEDGEMENT

The research was funded by Ministry of Agriculture of Indonesia. Thanks to staff Laboratory of Soil Biology, Faculty of Agriculture, Universitas Padjadjaran and staff of Biology Education Program, Faculty of Teacher and Education Training, University of Jember for their cooperation.

REFERENCES

1. Alizadeh, O. (2011). Mycorrhizal Symbiosis. *Advanced Studies in Biology*. 3 (6) : 273 – 281
2. Brundrett, M. (2004). Diversity and classification of mycorrhizal associations. *Biol Rev Camb Philos Soc*, *79*(3); 473-95. doi: 10.1017/s1464793103006316.
3. Policelli, N., Thomas R. Horton, TR ., Hudon, AT., Taylor R. Patterson, TR., & Bhatnagar, J.M. (2020). Back to roots: The role of ectomycorrhizal fungi in boreal and temperate forest restoration. *Front. For. Glob. Change*, *3*(97). https://doi.org/10.3389/ffgc.2020.00097
4. Münzenberger, B., Schneider, B., Nilsson, R.H., Bubner, B., Larsson, KH., & Hüttl, R.F. (2012). Morphology, anatomy, and molecular studies of the ectomycorrhiza formed axenically by the fungus Sistotrema sp. (Basidiomycota). Mycological, 11; 817–826
5. Sharma, S., Sharma, S., Aggarwal, A., Sharma, V., Singh, M. J., & Kaushik, S. (2017). Mass multiplication of arbuscular mycorrhizal fungi. *Mycorrhizal Fungi*. Januari; 157–174.
6. Diagne, N., Ngom, M., Djighaly, PI., Fall, D., Hocher, V., & Svistoonoff, S. (2020). Roles of arbuscular mycorrhizal fungi on plant growth and performance: importance in biotic and abiotic stressed regulation. *Diversity*, *12*; 370; doi:10.3390/d12100370
7. Altuntas, O. (2021). Production of vegetable crops by using arbuscular mycorrhizae. DOI: http://dx.doi.org/10.5772/intechopen.97552
8. Garbaye, J. (1994). Mycorrhiza helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytologist*, 128, 197–210.
9. Von, A.H., Lindermann, A., Schonbeck, F. (1993). Stimulation of vesicular arbuscular mycorrhiza fungi by fungicide or rhizospere bacteria. *Mycorrhizae*. 2; 167173.

10. Barea, J.M., Pozo, M.J., Azcon, R., Azcon-Aguilar, C. (2005). Microbial co-operation in the rhizosphere. *J Exp Bot*, 56;1761-1778.
11. Lies, A., Delteil, A., Prin, Y., & Duponnois, R. (2018). Using Mycorrhiza Helper Microorganisms (MHM) to Improve the Mycorrhizal Efficiency on Plant Growth. In book: *Role of Rhizospheric Microbes in Soil*, 277-298. DOI:10.1007/978-981-10-8402-7_11
12. Dhawi, F. (2016). Mycorrhiza, bacteria and plant an organized model of rhizoshere
Interaction. *International Journal of Scientific & Engineering Research*, *7*(2); 61-77
13. Rigamonte, T. A., Pylro, V. S., & Duarte, G. F. (2010). The role of Mycorrhization helper bacteria in the establishment and action of ectomycorrhizae associations. *Brazilian Journal of Microbiology*, *41*(4); 832–840. https://doi.org/10.1590/S1517-83822010000400002
14. Marschner, P., Crowley, D.E. (1996). Root colonization of mycorrhizal and non-mycorrhizal pepper (Capsicum annuum) by Pseudomonas fluorescens 2-79RL. *New Phytol*. 134:115-122.
15. Selvakumar, G., Shagol, C. C., Kang, Y., Chung, B. N., Han, S. G., & Sa, T. M. (2018). Arbuscular mycorrhizal fungi spore propagation using single spore as starter inoculum and a plant host. *Journal of applied microbiology*, *124*(6), 1556-1565.
16. Kormanik, P.P., & A. C. Mc. Grow. (1982). Quantification of VAM in Plants Roots, Method and principles of Mycorrhizal Research, *Amer Phytophatol. Minnesota*, 37-38.
17. Schinner, F., Oninger, R., Kandeler, E., & Margesin, R. (1996). Methods in Soil Biology. Springer-Verlag. Berlin Heidelberg-Jerman.
18. O'Connor, P. J., Smith, S. E., & Smith, F. A. (2001). Arbuscular mycorrhizal associations in the southern Simpson Desert. *Australian Journal of Botany*, *49*(4), 493-499.
19. Martin, F. (Ed.). (2016). *Molecular mycorrhizal symbiosis*. John Wiley & Sons.
20. Garbaye, J. (1994). Helper bacteria– a new dimension to the mycorrhizal symbiosis. *New Phytologist*, *128*; 197–210.
21. Vivas, A., Barea, J. M., & Azcón, R. (2005). Brevibacillus brevis isolated from cadmium-or zinc-contaminated soils improves in vitro spore germination and growth of Glomus mosseae under high Cd or Zn concentrations. *Microbial ecology*, *49*(3), 416-424.
22. Xavier, L. J., & Germida, J. J. (2003). Bacteria associated with Glomus clarum spores influence mycorrhizal activity. *Soil biology and biochemistry*, *35*(3), 471-478.
23. Frey-Klett, P., & Garbaye, J. (2005). Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal–bacterial interactions. *New Phytologist*, *168*(1), 4-8.
24. Duponnois, R., & Garbaye, J. (1990). Some mechanisms involved in growth stimulation of ectomycorrhizal fungi by bacteria. *Canadian Journal of Botany*, *68*(10), 2148-2152.