



## OPINION

# Rooting for success: Evolutionary enhancement of *Bacillus* for superior plant colonization

Vincent Charron-Lamoureux<sup>1,2</sup> | Sandrine Lebel-Beaucage<sup>3</sup> |  
Maude Pomerleau<sup>4</sup>  | Pascale B. Beauregard<sup>3</sup> 

<sup>1</sup>Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, California, USA

<sup>2</sup>Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, California, USA

<sup>3</sup>Département de Biologie, Université de Sherbrooke, Sherbrooke, Quebec, Canada

<sup>4</sup>INRS Centre Eau Terre Environnement, Québec, Quebec, Canada

## Correspondence

Pascale B. Beauregard, Département de Biologie, Université de Sherbrooke, Sherbrooke, Quebec, Canada.  
Email: [pascale.b.beauregard@usherbrooke.ca](mailto:pascale.b.beauregard@usherbrooke.ca)

## Funding information

Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: RGPIN-2020-07057; J.A. DeSève Fellowship (Université de Sherbrooke)

## Abstract

Many strains from the *Bacillus subtilis* species complex exert strong plant growth-promoting activities. However, their efficacy in relevant conditions is variable, due in part to their inability to establish a strong interaction with roots in stressful environmental conditions. Adaptive laboratory evolution (ALE) is a powerful tool to generate novel strains with traits of interest. Many *Bacillus* evolved isolates, stemming from ALE performed with plants, possess a stronger root colonization capacity. An in-depth analysis of these isolates also allowed the identification of key features influencing the interaction with plant roots. However, many variables can influence the outcome of these assays, and thus, caution should be taken when designing ALE destined to generate better root colonizers.

## INTRODUCTION

With the increasing world population and the impact of global changes, agriculture is under pressure. One major concern is that global change is predicted to reduce the pool of beneficial microorganisms with which plants can interact (Custer, 2024). In parallel, the traditional use of chemicals to sustain crop growth and protection poses challenges for both the health of the ecosystems and the population (Good & Beatty, 2011; Lini et al., 2024). For many years, plant-beneficial bacteria including diverse *Bacillus* strains have been sold as sustainable biocontrol agents and fertilizers (Glare

et al., 2012; Santos et al., 2019). The interest in *Bacillus* stem from their capacity to secrete high amount of diverse secondary metabolites such as antimicrobial molecules, and their spore-forming ability which ensures long shelf-life (Blake, Christensen, & Kovács, 2021; Caulier et al., 2019; Fira et al., 2018; Zhang et al., 2023). Recent meta-analysis demonstrated that microbial inoculants significantly enhance plant productivity and reduce microbial-mediated disease severity in a wide variety of plants (Li et al., 2022; Serrão et al., 2024). However, in unfavourable conditions, the beneficial effect of inoculated bacteria is often incomplete or absent, which constitutes a barrier to adoption for the farmers

Vincent Charron-Lamoureux, Sandrine Lebel-Beaucage and Maude Pomerleau contributed equally to this article.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2024 The Author(s). *Microbial Biotechnology* published by John Wiley & Sons Ltd.

who rely on reliable results to combat pests (Moser et al., 2008; O'Callaghan et al., 2022).

The inconsistent efficacy of bioinoculants in the field might be explained, at least partially, by a weak association between the bacteria and the plant (Gange & Gadhave, 2018; Martínez-Viveros et al., 2010; O'Callaghan et al., 2022). The colonization capacity of bacteria with strong plant-beneficial activities is only occasionally examined, yet many reports demonstrate that robust plant colonization is a prerequisite for the bacteria to provide plant-beneficial activities (Bais et al., 2004; Chen et al., 2013; Liu et al., 2024; Santoyo et al., 2021). For the *Bacillus subtilis* species complex, robust plant colonization results first from attraction of the bacteria to the roots via chemotaxis, a directed motility towards increasing concentration of attracting molecules secreted by the plant (Allard-Massicotte et al., 2016; Feng et al., 2019; Gao et al., 2023; Jiao et al., 2022; Perea-Molina et al., 2022). Formation of a biofilm on the root surface, that is, a multicellular community engulfed in a self-secreted matrix, then provides a durable association with the plant (Beauregard et al., 2013; Chen et al., 2012; Li et al., 2024; Xu et al., 2019). In addition to these two active roles, other conditions will impact the success of colonization such as fitness towards the pre-existing community and metabolic adaptation to the abiotic conditions prevailing on the root (Carlström et al., 2019; Debray et al., 2022; Fukami, 2015). To artificially improve plant root colonization, many of these phenotypic traits could be genetically manipulated. However, the wide adoption of a bio-inoculant formulated using a genetically modified organism (GMO) might elicit from various groups a similar outcry than for GMO plants. Genome-wide association studies between plants and their microbiome suggest a co-evolution process, but the nature of the beneficial bacteria's natural adaptations to the roots have never been examined (Trivedi et al., 2020). A better understanding of this process would allow us to make more informed choices about the bacterial species and traits that will promote efficient root colonization in diverse conditions.

## ADAPTIVE LABORATORY EVOLUTION OF *BACILLUS* ON PLANTS

Adaptive laboratory evolution (ALE) are experiments in which an organism is cultured under a specific condition for many generations, allowing the emergence and selection of evolved strains with an increased fitness (Dragosits & Mattanovich, 2013). The mutations responsible for the improved phenotype can be determined by whole-genome sequencing, allowing the identification of the genetic drivers for adaptation. ALE has been extensively used with model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* to gain mechanistic

insights into specific processes and to develop strains with improved traits for biotechnology purposes (Dragosits & Mattanovich, 2013; Mavrommati et al., 2022; Sandberg et al., 2019). In this perspective, ALE was used in recent years to develop *B. subtilis* and other *Bacilli* strains with an increased root colonization capacity, and to understand the cellular adaptations underlying this phenotype.

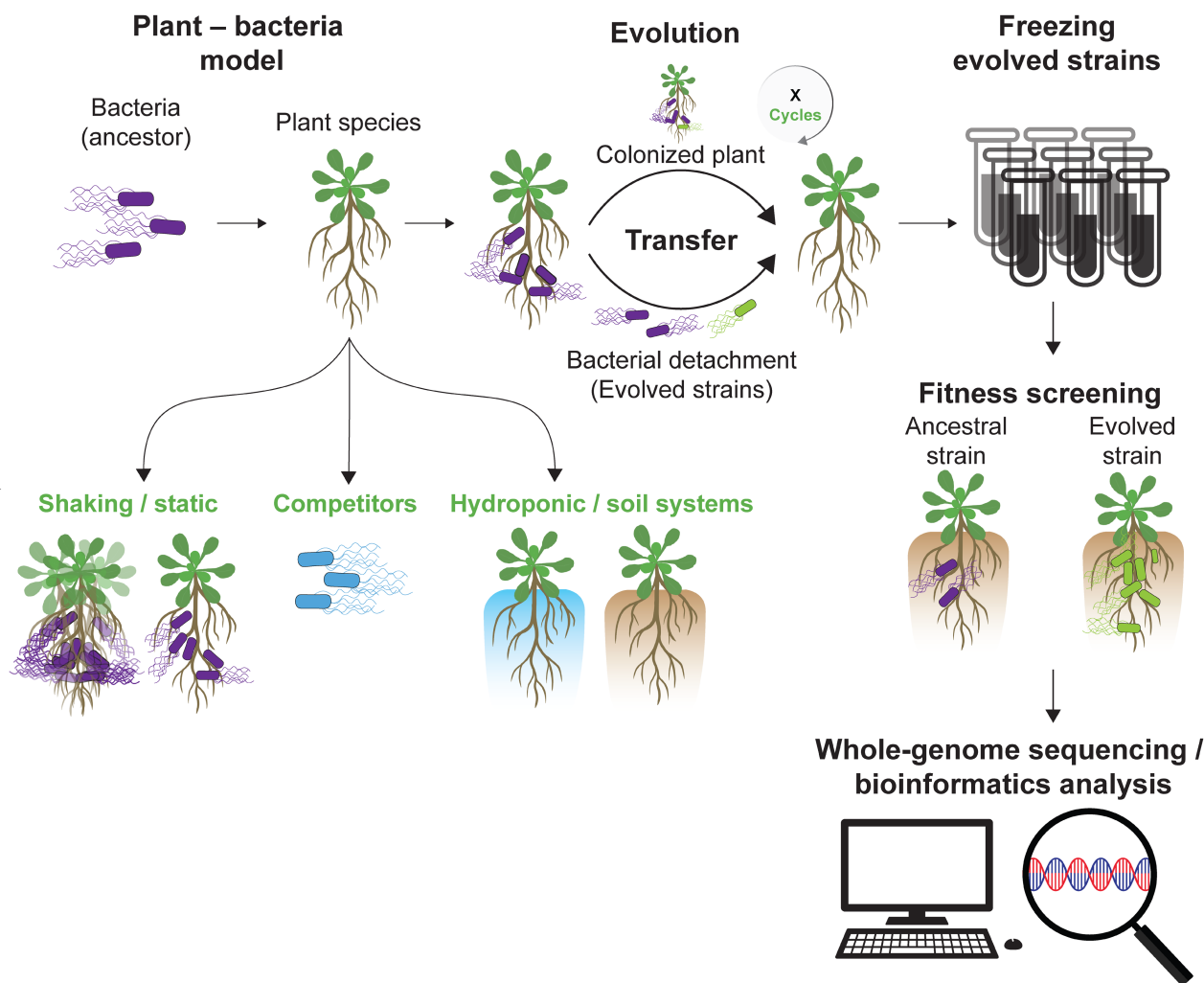
Blake, Nordgaard, et al. (2021) evolved *B. subtilis* on *Arabidopsis thaliana* seedlings in a minimal medium with a little amount of glycerol (Blake, Nordgaard, et al., 2021). Cycles were performed by transferring the colonized plant in a new growth medium containing a sterile seedling. This ALE led to the emergence of three morphotypes, with different plant colonization capacity. Many evolved isolates had enhanced colonization capacity on *A. thaliana* roots, but not on tomato seedlings (*Solanum lycopersicum*), showing a plant-specific adaptation. Inoculation of *A. thaliana* with all three morphotypes simultaneously provided stronger root colonization than predicted, suggesting a complementarity between the strains. The ALE performed by Nordgaard et al. (2022) had a similar set up, but was performed in mildly shaking conditions. Rapid adaptation to the root was also observed, resulting in increased colonization and emergence of different morphotypes. Multiple evolved isolates displayed increased individual root colonization, lower motility and stronger biofilm formation in presence of the plant polysaccharide xylan. Whole-genome sequencing revealed mutations in genes related to motility, cell wall metabolism and biofilm formation, suggesting that these cellular pathways are interconnected with root colonization efficacy. One evolved isolate also showed better root establishment compared to the ancestor in the presence of a soil-derived synthetic community. Finally, Lin et al. (2021) also obtained Cry<sup>-</sup> *Bacillus thuringiensis* strains with stronger plant colonization on *A. thaliana*. Whole genome sequencing revealed that a nonsense mutation in *rho*, present in certain evolved isolates, led to reduced motility and sporulation efficiency, and increased plant polysaccharide usage, leading to increased aggregation and establishment on plants.

Plant-specific adaptation of *B. subtilis* was further examined by Hu et al. (2023), using ALE on *A. thaliana* and *S. lycopersicum*. Mutations from evolution regimen with both plants in alternance had more similarity with mutations accumulated in ALE performed with *S. lycopersicum*, suggesting that this plant species exerted a stronger selection pressure. Pomerleau et al. (2024) also evolved *B. subtilis* on *S. lycopersicum*, in presence or not of a competing *Pseudomonas fluorescens*, another plant-beneficial bacterium. One key difference to the previously described ALEs was the usage of sonication to remove the bacteria from the colonized roots before re-inoculation on a sterile seedling. This approach, combined with shaking conditions, likely diminished the selective pressure for motility traits. Despite these differences, rapid root adaptation also emerged in that study. Many evolved isolates displayed enhanced root

colonization on both *S. lycopersicum* and *A. thaliana*, with or without the presence of the competitor. These evolved strains all possessed loss-of-function or mutations in genes encoding biofilm transcriptional repressors, which caused a moderate increase in biofilm formation.

All these *Bacillus* ALE on plants revealed that increased biofilm formation, either constitutively or in presence of plant-specific carbon source, might be one of most important traits for increased root colonization (Blake, Nordgaard, et al., 2021; Hu et al., 2023; Lin et al., 2021; Pomerleau et al., 2024). Biofilm was also correlated with increased secondary metabolite secretion, many of which promote plant health and antagonize pathogens (Pisithkul et al., 2019; Schoenborn et al., 2021). Thus, a general strategy to increase *Bacillus* root colonization could be to positively affect biofilm productivity. However, a motility versus biofilm trade-off was observed in most evolved isolates. This is not surprising, since biofilm formation in *B. subtilis* was

shown to be mutually exclusive with motility (Vlamakis et al., 2008). Additionally, many ALEs were performed in mildly shaking conditions, which might have rendered motility and chemotaxis superfluous since stochastic contact triggered by agitation is likely sufficient for initial attachment to the root. The rhizosphere fitness of these strains in a natural substrate would need to be investigated to examine the real impact of motility loss. Direct inoculation of the bacteria on the root by drenching or seed coating might bypass the issue, but it could become problematic when bacteria need to reach the root before colonizing, such as in soil applications. Another parameter closely linked with biofilm formation is sporulation, since both are controlled by the same genetic regulation pathway. Sporulation was shown to be induced rapidly in the rhizosphere (Charron-Lamoureux et al., 2020; Charron-Lamoureux & Beaugard, 2019), and while it can provide persistence and stress tolerance, the extent of the beneficial activities provided by that dormant form is likely



**FIGURE 1** The many parameters of adaptive laboratory experiments. The various parameters chosen in an ALE protocol (in bold green) can influence the outcome of the evolution, leading to adaptations with various fitness to the natural rhizosphere. Subsequent steps should include validation of the colonization efficacy and whole-genome sequencing to determine the driver of adaptations.

drastically diminished. Indeed, no secondary metabolites or nutrients solubilization can be performed by an endospore. In *B. thuringiensis*, the increase in biofilm formation was associated with a delay in sporulation for certain isolates (Lin et al., 2021). Thus, it would be of interest to examine if increased biofilm formation on root also affects the dynamic of spore formation in other *Bacillus*.

## THE POTENTIAL USAGE OF *BACILLUS* ADAPTIVE EVOLUTION TO GENERATE NEW, EFFECTIVE STRAINS

The site of origin of most bacterial strains used to formulate biofertilizers is unclear (Mehnaz, 2016). Thus, we can speculate that many of them were probably not isolated from the plant they are inoculated on, and this is particularly true for biofertilizers with a wide array of usage. As revealed by ALE experiments, certain plants exert stronger selection pressure than others on *Bacillus*, which might impair colonization by an ill-adapted biofertilizer strain. Therefore, a well-designed ALE with the plant of interest could provide a powerful tool to naturally adapt those strains to the plant they are used on, and to promote their colonization efficacy.

Experimental conditions in which ALE are performed, including factors such as agitation, nutritional conditions, growth substrate or process of selection, can apply selective pressure that may predominantly drive adaptation towards a specific laboratory condition rather than to root colonization (Figure 1). At the same time, reproducing the complex rhizosphere environment to obtain stable evolved isolates that are adapted to the different, varying conditions may pose significant challenges. One potential approach could be to determine the fitness of different evolved isolates in natural settings to evaluate which evolution conditions yield strains better adapted to the constraints of the rhizosphere. Such strains would present stronger colonization levels and higher persistence in the rhizosphere despite the presence of a pre-existing microbiota. If no single strains show improvement, an alternative would be to combine various, phenotypically different isolates stemming from the same ALE. Indeed, it was reported that such a consortium can exhibit stronger colonization capacities compared to individual isolates, and thus could show better fitness in the natural rhizosphere (Blake, Nordgaard, et al., 2021). This strategy would ensure that all the beneficial tasks provided by the original *Bacillus* strain are accomplished by one or another member of the consortium. Importantly, the capacity of these evolved isolates to better promote plant growth and antagonize pathogens would still need to be validated, similarly to what was performed with *Pseudomonas protegens* (Li et al., 2021). In an

eco-evolution perspective, the impact of the evolved isolates on the rhizosphere microbial community, including fungi, protist, archaea and bacteria, should also be examined since the evolved strain might impact its ecosystem differently. Thus, while ALE could become a powerful approach to generate new, more efficient plant growth-promoting *Bacillus*, certain parameters need to be examined before bringing these strains in the fields.

## AUTHOR CONTRIBUTIONS

PBB conceived and wrote the draft. VCL draw Figure 1. VCL, SLB and MP wrote specific subsections, and revised and bonified the draft.

## ACKNOWLEDGEMENTS

We thank Julie Beaudin for the critical reading of the manuscript, Andréa Marini for contribution to Figure 1, and members of the Beaugard laboratory for their helpful discussions. This work was supported by the NSERC discovery grant RGPIN-2020-07057 to PBB and a J.A. DeSève fellowship (Université de Sherbrooke) to SL-B.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## ORCID

Maude Pomerleau  <https://orcid.org/0009-0009-9885-1615>

Pascale B. Beaugard  <https://orcid.org/0000-0003-2947-0500>

## REFERENCES

- Allard-Massicotte, R., Tessier, L., Lécuyer, F., Lakshmanan, V., Lucier, J.-F., Garneau, D. et al. (2016) *Bacillus subtilis* early colonization of *Arabidopsis thaliana* roots involves multiple chemotaxis receptors. *MBio*, 7, e01664-16.
- Bais, H.P., Fall, R. & Vivanco, J.M. (2004) Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiology*, 134, 307–319.
- Beaugard, P.B., Chai, Y., Vlamakis, H., Losick, R. & Kolter, R. (2013) *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proceedings of the National Academy of Sciences of the United States of America*, 110, E1621–E1630.
- Blake, C., Christensen, M.N. & Kovács, Á.T. (2021) Molecular aspects of plant growth promotion and protection by *Bacillus subtilis*. *Molecular Plant-Microbe Interactions*, 34, 15–25.
- Blake, C., Nordgaard, M., Maróti, G. & Kovács, Á.T. (2021) Diversification of *Bacillus subtilis* during experimental evolution on *Arabidopsis thaliana* and the complementarity in root colonization of evolved subpopulations. *Environmental Microbiology*, 23, 6122–6136.
- Carlström, C.I., Field, C.M., Bortfeld-miller, M. & Müller, B. (2019) Synthetic microbiota reveal priority effects and keystone strains



- impact bacterial community structure in the *Arabidopsis thaliana* phyllosphere. *Nature Ecology & Evolution*, 3(10), 1445–1454.
- Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C. & Mahillon, J. (2019) Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in Microbiology*, 10, 302.
- Charron-Lamoureux, V. & Beaugregard, P.B. (2019) *Arabidopsis thaliana* seedlings influence *Bacillus subtilis* spore formation. *Molecular Plant-Microbe Interactions*, 32, 1188–1195.
- Charron-Lamoureux, V., Thérien, M., Konk, A. & Beaugregard, P.B. (2020) *Bacillus subtilis* and *Bacillus velezensis* population dynamics and quantification of spores after inoculation on ornamental plants. *Canadian Journal of Microbiology*, 66, 664–669.
- Chen, Y., Cao, S., Chai, Y., Clardy, J., Kolter, R., Guo, J. et al. (2012) A *Bacillus subtilis* sensor kinase involved in triggering biofilm formation on the roots of tomato plants. *Molecular Microbiology*, 85, 418–430.
- Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R. et al. (2013) Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environmental Microbiology*, 15, 848–864.
- Custer, G.F. (2024) Unearthing opportunity amid declining plant-beneficial bacteria. *Trends in Plant Science*, 29, 834–836.
- Debray, R., Herbert, R.A., Jaffe, A.L., Crits-Christoph, A., Power, M.E. & Koskella, B. (2022) Priority effects in microbiome assembly. *Nature Reviews. Microbiology*, 20, 109–121.
- Dragosits, M. & Mattanovich, D. (2013) Adaptive laboratory evolution – principles and applications for biotechnology. *Microbial Cell Factories*, 12, 64.
- Feng, H., Zhang, N., Fu, R., Liu, Y., Krell, T., Du, W. et al. (2019) Recognition of dominant attractants by key chemoreceptors mediates recruitment of plant growth-promoting rhizobacteria. *Environmental Microbiology*, 21, 402–415.
- Fira, D., Dimkić, I., Berić, T., Lozo, J. & Stanković, S. (2018) Biological control of plant pathogens by *Bacillus* species. *Journal of Biotechnology*, 285, 44–55.
- Fukami, T. (2015) Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics*, 46, 1–23.
- Gange, A.C. & Gadhave, K.R. (2018) Plant growth-promoting rhizobacteria promote plant size inequality. *Scientific Reports*, 8, 13828.
- Gao, T., Wang, X., Qin, Y., Ren, Z. & Zhao, X. (2023) Watermelon root exudates enhance root colonization of *Bacillus amyloliquifaciens* TR2. *Current Microbiology*, 80, 110.
- Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Köhl, J. et al. (2012) Have biopesticides come of age? *Trends in Biotechnology*, 30, 250–258.
- Good, A.G. & Beatty, P.H. (2011) Fertilizing nature: a tragedy of excess in the commons. *PLoS Biology*, 9, e1001124.
- Hu, G., Wang, Y., Blake, C., Nordgaard, M., Liu, X., Wang, B. et al. (2023) Parallel genetic adaptation of *Bacillus subtilis* to different plant species. *Microbial Genomics*, 9, mgen001064.
- Jiao, H., Xu, W., Hu, Y., Tian, R. & Wang, Z. (2022) Citric acid in Rice root exudates enhanced the colonization and plant growth-promoting ability of *Bacillus altitudinis* LZP02. *Microbiology Spectrum*, 10, e01002-22.
- Li, E., de Jonge, R., Liu, C., Jiang, H., Friman, V.-P., Pieterse, C.M.J. et al. (2021) Rapid evolution of bacterial mutualism in the plant rhizosphere. *Nature Communications*, 12, 3829.
- Li, J., Wang, J., Liu, H., Macdonald, C.A. & Singh, B.K. (2022) Application of microbial inoculants significantly enhances crop productivity: a meta-analysis of studies from 2010 to 2020. *Journal of Sustainable Agriculture and Environment*, 1, 216–225.
- Li, Y., Narayanan, M., Shi, X., Chen, X., Li, Z. & Ma, Y. (2024) Biofilms formation in plant growth-promoting bacteria for alleviating agro-environmental stress. *Science of the Total Environment*, 907, 167774.
- Lin, Y., Alstrup, M., Pang, J.K.Y., Maróti, G., Er-Rafik, M., Tourasse, N. et al. (2021) Adaptation of *Bacillus thuringiensis* to plant colonization affects differentiation and toxicity. *mSystems*, 6, e0086421.
- Lini, R.S., Scanferla, D.T.P., de Oliveira, N.G., Aguera, R.G., Santos, T.S., Teixeira, J.J.V. et al. (2024) Fungicides as a risk factor for the development of neurological diseases and disorders in humans: a systematic review. *Critical Reviews in Toxicology*, 54, 35–54.
- Liu, Y., Xu, Z., Chen, L., Xun, W., Shu, X., Chen, Y. et al. (2024) Root colonization by beneficial rhizobacteria. *FEMS Microbiology Reviews*, 48, fuad066.
- Martínez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G. & Mora, M.L. (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of Soil Science and Plant Nutrition*, 10, 293–319.
- Mavrommati, M., Daskalaki, A., Papanikolaou, S. & Aggelis, G. (2022) Adaptive laboratory evolution principles and applications in industrial biotechnology. *Biotechnology Advances*, 54, 107795.
- Mehnaz, S. (2016) An overview of globally available bioformulations. In: Arora, N.K., Mehnaz, S. & Balestrini, R. (Eds.) *Bioformulations: for sustainable agriculture*. Springer India: New Delhi, pp. 267–281.
- Moser, R., Pertot, I., Elad, Y. & Raffaelli, R. (2008) Farmers' attitudes toward the use of biocontrol agents in IPM strawberry production in three countries. *Biological Control*, 47, 125–132.
- Nordgaard, M., Blake, C., Maróti, G., Hu, G., Wang, Y., Strube, M.L. et al. (2022) Experimental evolution of *Bacillus subtilis* on *Arabidopsis thaliana* roots reveals fast adaptation and improved root colonization. *iScience*, 25, 104406.
- O'Callaghan, M., Ballard, R.A. & Wright, D. (2022) Soil microbial inoculants for sustainable agriculture: limitations and opportunities. *Soil Use and Management*, 38, 1340–1369.
- Perea-Molina, P.A., Pedraza-Herrera, L.A., Beaugregard, P.B. & Uribe-Vélez, D. (2022) A biocontrol *Bacillus velezensis* strain decreases pathogen *Burkholderia glumae* population and occupies a similar niche in rice plants. *Biological Control*, 176, 105067.
- Pisithkul, T., Schroeder, J.W., Trujillo, E.A., Yeasin, P., Stevenson, D.M., Chaiamarit, T. et al. (2019) Metabolic remodeling during biofilm development of *Bacillus subtilis*. *MBio*, 10, e00623-19.
- Pomerleau, M., Charron-Lamoureux, V., Léonard, L., Grenier, F., Rodrigue, S. & Beaugregard, P.B. (2024) Adaptive laboratory evolution reveals regulators involved in repressing biofilm development as key players in *Bacillus subtilis* root colonization. *mSystems*, 9, e0084323.
- Sandberg, T.E., Salazar, M.J., Weng, L.L., Palsson, B.O. & Feist, A.M. (2019) The emergence of adaptive laboratory evolution as an efficient tool for biological discovery and industrial biotechnology. *Metabolic Engineering*, 56, 1–16.
- Santos, M.S., Nogueira, M.A. & Hungria, M. (2019) Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Express*, 9(1), 205.
- Santoyo, G., Urtis-Flores, C.A., Loeza-Lara, P.D., Orozco-Mosqueda, M.D.C. & Glick, B.R. (2021) Rhizosphere colonization determinants by plant growth-promoting Rhizobacteria (PGPR). *Biological*, 10, 475.
- Schoenborn, A.A., Yannarell, S.M., Wallace, E.D., Clapper, H., Weinstein, I.C. & Shank, E.A. (2021) Defining the expression, production, and signaling roles of specialized metabolites during *Bacillus subtilis* differentiation. *Journal of Bacteriology*, 203(22), e0033721.
- Serrão, C.P., Ortega, J.C.G., Rodrigues, P.C. & de Souza, C.R.B. (2024) *Bacillus* species as tools for biocontrol of plant diseases: a meta-analysis of twenty-two years of research, 2000–2021. *World Journal of Microbiology and Biotechnology*, 40, 110.

- Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T. & Singh, B.K. (2020) Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews. Microbiology*, 18, 607–621.
- Vlamakis, H., Aguilar, C., Losick, R. & Kolter, R. (2008) Control of cell fate by the formation of an architecturally complex bacterial community. *Genes & Development*, 22, 945–953.
- Xu, Z., Zhang, H., Sun, X., Lui, Y., Yan, W., Xun, W. et al. (2019) *Bacillus velezensis* wall teichoic acids are required for biofilm. *Applied and Environmental Microbiology*, 85, 1–14.
- Zhang, N., Wang, Z., Shao, J., Xu, Z., Liu, Y., Xun, W. et al. (2023) Biocontrol mechanisms of *Bacillus*: improving the efficiency of green agriculture. *Microbial Biotechnology*, 16(12), 2250–2263.

**How to cite this article:** Charron-Lamoureux, V., Lebel-Beaucage, S., Pomerleau, M. & Beauregard, P.B. (2024) Rooting for success: Evolutionary enhancement of *Bacillus* for superior plant colonization. *Microbial Biotechnology*, 17, e70001. Available from: <https://doi.org/10.1111/1751-7915.70001>