

Date:	27 November 2023
Author:	EBIC
Status:	Final
Distribution:	Public
Classification:	Public

POSITION PAPER

Rhizobia in microbial plant biostimulants

Following a request for clarification from the Forum of Notified Bodies under the FPR, the European Biostimulants Industry Council (EBIC) would like to suggest replacing *Rhizobium* spp. under CMC 7 in the FPR by the term “rhizobia”.

This change is supported by decades of research on the use of nitrogen-fixing bacteria to promote nutrient use efficiency in crop production and it would avoid confusion caused by taxonomic reclassification of rhizobial species in recent years due to the advancement of genome sequencing technologies.

Introduction

Despite the widespread use of microbial plant biostimulants in the European Union and across the world, the Fertilising Products Regulation (FPR) [Regulation (EU) 2019/1009] only allows four types of microorganisms to be used in microbial plant biostimulants (PFC 6 A).

Component Material Category (CMC) 7 lists the microorganisms that can be used in PFC 6 A: *Azotobacter* spp., mycorrhizal fungi, *Rhizobium* spp. and *Azospirillum* spp.

EBIC would like to point out the inconsistency of using such a broad term as “mycorrhizal fungi”, which includes hundreds of species, at the same level as *Rhizobium* spp., *Azotobacter* spp. and *Azospirillum* spp., which strictly speaking would only include species within those genera; and in particular to the problem of referring to the genus *Rhizobium*, which has undergone extensive taxonomic reclassification in recent years, as explained in this document.

Following a request for clarification from the Notified Bodies under the FPR about the scope of the *Rhizobium* spp. entry in CMC 7, EBIC would like to suggest replacing *Rhizobium* spp. by the term “rhizobia”, which would be more consistent with other entries in CMC 7 such as “mycorrhizal fungi”, and less problematic from a taxonomical point of view. The rationale for this proposal is explained in the following sections of this document.

The term “rhizobia”

“Rhizobia” is the term used to refer to non-pathogenic soil and rhizospheric bacteria that form nitrogen-fixing symbioses with legumes (Ormeño-Orrillo et al. 2015; Poole et al. 2018). These diazotrophic bacteria induce the formation of specialized plant organs (i.e., nodules) in which they differentiate into bacteroids and fix atmospheric dinitrogen (N₂) to ammonia (NH₃) (Avontuur et al. 2019; Poole et al. 2018).

Thus, ‘rhizobia’ is a general term used to cover all bacterial genera and species known to occupy root (and occasionally stem) nodule tissues, where they reduce N₂ to NH₃ (Sprent et al. 2017). It comprises different genera in the *Alphaproteobacteria* and *Betaproteobacteria*

classes of bacteria (Figure 1) that are ubiquitous in nature (Sprent et al. 2017), and have been exploited for decades to promote nutrient use efficiency in legume crops (Alves et al. 2003).

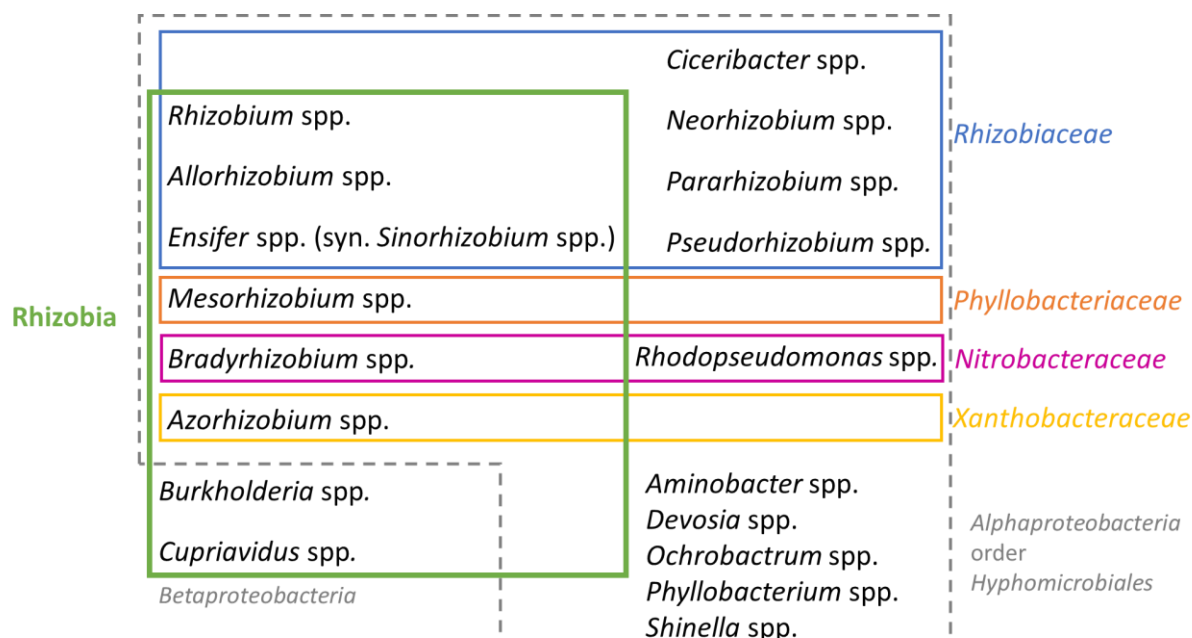


Figure 1: Diagram representing the classification of rhizobia

Consequently, using the term “rhizobia” for CMC 7 in the FPR would be equivalent to using the term “mycorrhizal fungi”. It is a well-defined term that would be understood by a wide range of stakeholders, and it would avoid problems related to taxonomical reclassification.

The taxonomy of rhizobia

Traditionally, most rhizobia have been allocated to six genera in the *Alphaproteobacteria* class of Gram-negative bacteria: the fast- to moderately fast-growing genera *Rhizobium*, *Allorhizobium*, *Ensifer* (syn. *Sinorhizobium*) and *Mesorhizobium*, the slow-growing genus *Bradyrhizobium*, and the stem-nodulating genus *Azorhizobium* (De Meyer et al. 2011; Sprent et al. 2017).

A few other non-classical rhizobia (*Burkholderia* spp., *Cupriavidus* spp.) belong to the class *Betaproteobacteria* (Figure 2). In addition, recent studies have reported some rhizobia belonging to *Gammaproteobacteria* and *Actinobacteria*, but these have not been confirmed (De Meyer et al. 2011; Sprent et al. 2017).

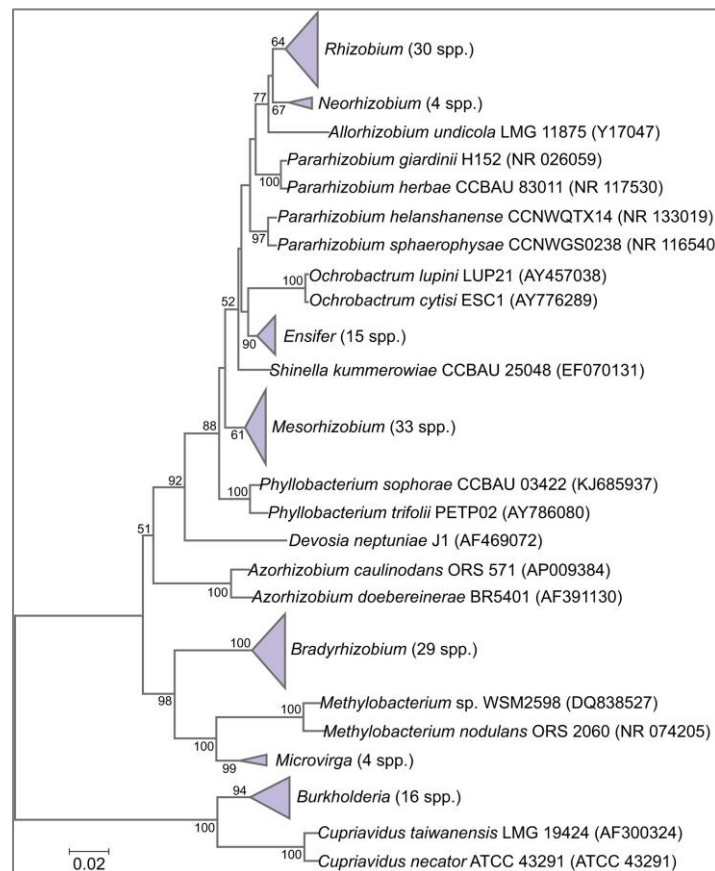


Figure 2: Phylogenetic tree showing the relationships of currently described genera and species of Alpha- and Beta-rhizobia, based on aligned sequences of the 16S rRNA gene (1341-bp internal region) (Sprenst et al. 2017).

All of the “traditional” or “classical” rhizobia belong to the bacterial order *Hyphomicrobiales* within the class *Alphaproteobacteria*. The order *Hyphomicrobiales*, (previously known as *Rhizobiales*) was recently renamed and rearranged after a study by Hördt et al. (2020). It currently comprises 38 families with a validly published and correct name, including *Rhizobiaceae*, *Nitrobacteraceae* (formerly known as *Bradyrhizobiaceae*), *Phyllobacteriaceae* and *Xanthobacteraceae* (ICSP 2023k).

The family *Rhizobiaceae*

The family *Rhizobiaceae* was proposed in 1938 and has since undergone numerous taxonomic revisions (Kuzmanović et al. 2022). Bacteria of the family *Rhizobiaceae* are usually soil-borne and found in association with plant roots, where they mostly rely on a saprophytic lifestyle degrading soil organic compounds and plant exudates (Lassalle et al. 2021). Some members of this taxon can engage in a symbiotic (*Rhizobium* spp.) or pathogenic (*Agrobacterium* spp., *Liberibacter* spp.) relationship with a specific host plant, and the ability to switch to these lifestyles is determined by the presence of certain plasmids in the bacterium (Lassalle et al. 2021).

The agricultural and ecological significance of the family *Rhizobiaceae* has prompted the isolation and whole genome sequencing of hundreds of strains, at a rate that has often outpaced taxonomic refinement of the family (Kuzmanović et al. 2022). As a result, this highly

diverse family currently comprises 181 species classified into 19 genera, including *Allorhizobium*, *Ciceribacter*, *Ensifer* (syn. *Sinorhizobium*), *Neorhizobium*, *Pararhizobium*, *Pseudorhizobium* and *Rhizobium*, among others (ICSP 2023c). Most genera in this family are delineated based on genomic relatedness and phylogenetic relationships, but some historically named genera show inconsistent distribution and phylogenetic breadth (Kuzmanović et al. 2022).

In 2022, a new genomic metric with a threshold of ~86% was proposed to define the genera in the family *Rhizobiaceae*, and several new genera and combinations were proposed (Kuzmanović et al., 2022). Following this metric, Ma et al. (2023) used genome sequences from 138 of the 181 validly published *Rhizobiaceae* species, as well as 18 not validly published species, to confirm the taxonomic status of all species within the family *Rhizobiaceae*. This analysis resulted in the proposal of eight novel genera, one novel species, and 22 novel combinations (Ma et al. 2023), illustrating how much the taxonomy of these bacterial species is changing as new genomes become available and analytical techniques are refined.

The genus *Rhizobium* currently comprises 94 species with a validly published and correct name (ICSP 2023i). From a taxonomic perspective, *Rhizobium* is considered to be the most problematic genus within the *Rhizobiaceae* family, because it is highly paraphyletic (i.e., composed of a common ancestor and some, but not all, of the descendants), and many newly described species in the *Rhizobiaceae* family are assigned to this genus without consideration of their proximity to existing genera, or the need to create novel genera (Kuzmanović et al. 2022).

The genus *Ensifer* currently contains 20 species with a validly published and correct name (ICSP 2023g). Until very recently, *Ensifer* was considered synonymous with *Sinorhizobium*, but the new genomic metric mentioned above has resulted in a proposal to separate both genera (Kuzmanović et al. 2022).

The family *Nitrobacteraceae*

The family *Nitrobacteraceae* (formerly known as *Bradyrhizobiaceae*) currently comprises 11 genera, including *Bradyrhizobium*, *Nitrobacter* and *Rhodopseudomonas* (ICSP 2023a)

The genus *Bradyrhizobium* was introduced in the early 1980s to accommodate the so-called slow-growing rhizobia (i.e., bacteria capable of nodulating and fixing atmospheric nitrogen in symbiosis with legumes) (Avontuur et al. 2019). The first validly described species was *B. japonicum* in 1982, followed by the description of *Bradyrhizobium elkanii* in 1992 and *Bradyrhizobium liaoningense* in 1995 (Avontuur et al. 2019). Since then, descriptions of new *Bradyrhizobium* species have increased drastically, and the genus currently comprises 70 species that have a validly published and correct name (ICSP 2023f).

As currently described, the genus *Bradyrhizobium* includes bacteria with diverse lifestyles, which can live symbiotically or endophytically with legume and non-legume hosts, and some are photosynthetic (Avontuur et al. 2019). *Bradyrhizobium* species associated with legumes in the subfamilies Papilionoideae and Caesalpinioideae have a broad geographic distribution, especially in tropical and subtropical areas (Avontuur et al. 2019). They can establish symbiosis with many agriculturally important legumes, including native crops used by subsistence farmers in India (Ojha et al. 2017) and Africa (Jaiswal and Dakora 2019), or row crops such as soybean, which is routinely inoculated with *Bradyrhizobium* strains (Alves et al. 2003). The photosynthetic species (e.g. *B. oligotrophicum* and *B. denitrificans*) are predominantly symbionts of species in the *Aeschynomene* genus of aquatic legumes (Avontuur et al. 2023).

The family *Phyllobacteriaceae*

The family *Phyllobacteriaceae* currently comprises 15 genera with a validly published and correct name, including *Mesorhizobium* (ICSP 2023b). In turn, the genus *Mesorhizobium* is currently composed of 62 species with a validly published and correct name (ICSP 2023h).

The family *Xanthobacteraceae*

The family *Xanthobacteraceae* currently comprises 5 genera with a validly published and correct name, including *Azorhizobium* (ICSP 2023d). In turn, the genus *Azorhizobium* is currently composed of 3 species with a validly published and correct name (ICSP 2023e).

Natural occurrence of rhizobia

Rhizobia are saprophytes (i.e., organisms that live on dead and decaying organic matter). They survive in the soil by adopting an oligotrophic lifestyle, using a broad range of carbon sources in a nutritionally limited environment (Poole et al. 2018). However, once they enter their host legume plant, they can change from their free-living saprophyte form into endosymbiotic bacteroids (Poole et al. 2018). This drastic change of lifestyle is associated with some of the largest bacterial genomes known (~5-10 Mb) (Poole et al. 2018).

Most rhizobia have a narrow host range; for example, *Rhizobium leguminosarum* bv. *trifolii* only infects clover (*Trifolium*) species. However, a few rhizobia are promiscuous; for example, *Sinorhizobium fredii* NGR234 infects 112 legume genera (Poole et al. 2018).

The largest rhizobial genus by far is *Bradyrhizobium* (*Bradyrhizobiaceae*), members of which also nodulate the widest range of legume genera, as well as the non-legume *Parasponia* (Sprent et al. 2017). *Bradyrhizobia* are particularly dominant in Australia and in central South America, where they nodulate native legumes growing in the acidic and seasonally dry soils that predominate in these environments (Sprent et al. 2017). *Bradyrhizobia* are also the principal symbiont type isolated from nodulated 'basal' legumes in the old Caesalpinioideae subfamily of legumes, most of which are neotropical woody species (Sprent et al. 2017).

Within the family *Rhizobiaceae*, the genera *Rhizobium* and *Ensifer* (*Sinorhizobium*) are also very widespread, nodulating a large variety of legumes on all continents (Sprent et al. 2017). In terms of environmental preferences, *Rhizobium* strains vary enormously, but *Ensifer* spp. are often found associated with legumes that are native to alkaline and semi-arid/saline soils (Sprent et al. 2017). In fact, although both *Bradyrhizobium* and *Sinorhizobium* establish symbioses with soybean plants, it has repeatedly been found that *Bradyrhizobium* is the dominant genus in neutral and acidic soils, while *Sinorhizobium* is the dominant genus in alkaline soils (Tian et al. 2012; Han et al. 2020).

The third largest group of Alpha-rhizobia is *Mesorhizobium* in the *Phyllobacteriaceae* family. *Mesorhizobia* are very widespread and nodulate a wide range of legumes. They are common symbionts in legumes that prefer acidic soils, but are also found as preferred microsymbionts of chickpea (*Cicer arietinum* L.) growing in alkaline soils in northwest China (Sprent et al. 2017).

The final 'classical' rhizobial genus, *Azorhizobium* in the *Xanthobacteraceae* family, is not so widespread, being quite specifically associated with a few species of the tropical genus *Sesbania*, most notably *Sesbania rostrata*, with which it forms stem nodules (Sprent et al. 2017).

Other ‘nonclassical’ symbiotic rhizobial strains that have been confirmed over the last two decades include *Aminobacter*, *Devosia*, *Ochrobactrum*, *Phyllobacterium* and *Shinella*, which all belong to the order *Hyphomicrobiales*. In most cases they appear to be symbiotic with only one or two legume species that are already known to be associated with ‘classical’ rhizobia (Sprent et al. 2017).

History of safe use of rhizobia

Rhizobia have been used in agriculture for over 120 years (Ormeño-Orrillo et al. 2015). Legume crops are routinely inoculated in agricultural systems to deliver rhizobia to the soil where there are no compatible rhizobia, where the rhizobia numbers are insufficient to maximise biological nitrogen fixation, or where the native rhizobia are ineffective, with the aim of providing high numbers of viable effective rhizobia to the rhizosphere to allow rapid colonisation, nodulation and nitrogen fixation by the selected inoculant strain, in order to maximise legume yield potential (Atieno and Lesueur 2019).

In general, inoculation of legume seed is a more economical way of introducing rhizobia into the rhizosphere, as soil application requires large volumes of inoculant for adequate distribution of rhizobia (Atieno and Lesueur 2019). Most programmes aimed at the development of improved rhizobial inoculants focus on strains with high efficiency of N₂ fixation, competitiveness or wide host-range compatibility (Rodríguez-Navarro et al. 2003).

Soybean (*Glycine max* (L.) Merr.) is the crop that most benefits from the use of rhizobia worldwide (Santos et al. 2019). In 2003, it was estimated that almost all the arable land cropped to soybean in Brazil had been inoculated with *Bradyrhizobium* strains at some time or other (Alves et al. 2003). In 2019, approximately 78% of the soybean cropping area in Brazil, 36.5 million hectares, was estimated to be inoculated yearly (Santos et al. 2019). South American countries (Brazil, Argentina, Uruguay, Paraguay) are the leaders in soybean inoculation, while in the USA only about 15% of the soybean area is estimated to be inoculated (Santos et al. 2019).

Common beans (*Phaseolus vulgaris* L.) or cowpea (*Vigna unguiculata* L. Walp.) are also routinely inoculated with *Rhizobium* spp. (Santos et al. 2019). In countries such as Australia, chickpeas have been inoculated with *Mesorhizobium ciceri* since the 1970s (Bullard et al. 2005).

In Northern Europe, legume crops such as faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) are nodulated by the common soil bacterium *Rhizobium leguminosarum* sv. *viciae* (*Rlv*), which also nodulates several native European legumes in the genera *Lathyrus* and *Vicia* (Maluk et al. 2022). However, continual fallow and cereal cropping may limit nodulation due to low rhizobial populations and/or ineffective rhizobial genotypes. To overcome this, it has been proposed that “elite” strains of *Rlv* tailored to a particular crop could be used to inoculate legume crops, particularly on soils that have been under long periods of fallow and in which low *Rlv* populations have been estimated (Maluk et al. 2022).

Further elements to support EBIC’s proposal

Annex III, Part II in the FPR establishes the specific labelling requirements for microbial plant biostimulants (PFC 6 A), indicating that “all intentionally added micro-organisms shall be indicated. Where the micro-organism has several strains, the intentionally added strains shall be indicated”.

Because of this requirement to identify the strain, the technical specification for the determination of *Rhizobium* spp. in microbial plant biostimulants (CEN/TS 17718:2022) was developed to incorporate a combination of isolation steps through the use of specific growing media and molecular methods to detect the strain in question. Therefore, the methodology to discriminate between different rhizobial strains has already been established.

In addition, rhizobia are part of microbial plant biostimulants already placed on the EU market under national rules, products commercialised in other parts of the world and/or being investigated for their potential plant biostimulant effects.

In December of 2021, and in [April of 2022](#), EBIC submitted to the Commission Expert Group on Fertilising Products a list of microorganisms that have the market potential, safety and agronomic efficiency to justify an amendment of the FPR. This list incorporated many different species of rhizobia, including the genera *Bradyrhizobium*, *Mesorhizobium* or *Sinorhizobium* (*Ensifer*), but also *Nitrobacter*, *Nitrosomonas*, *Ochrobactrum* and *Rhodopseudomonas*, among others.

In addition, EBIC conducted an internal survey among its members in 2022 that showed that strains of the species *Bradyrhizobium elkanii*, *Bradyrhizobium japonicum*, *Nitrobacter winogradskyi*, *Nitrosomonas europaea* or *Rhodopseudomonas palustris* are already part of microbial plant biostimulants lawfully placed on the market in Austria, Spain, France, Germany, Ireland and/or the Netherlands since at least 2011.

Moreover, the presentation from DG GROW at the CEG-FP meeting on October 24th, 2022, entitled "[EU survey on developments of the FPR: Feedback received](#)" showed that strains of *Nitrosomonas* spp., *Nitrobacter* spp., *Rhodopseudomonas* spp., *Ochrobactrum* spp. and *Ensifer* spp. were proposed as new microorganisms to be included in CMC 7.

EBIC's proposal

Considering the arguments presented in this paper, EBIC would like to propose the replacement of "*Rhizobium* spp." in the table under CMC 7 in the FPR by "rhizobia", which is a commonly used term that would avoid taxonomic reclassification problems and would be equivalent to the term "mycorrhizal fungi".

If needed, a definition of "rhizobia" could be given in the FAQ document related to the FPR, specifying that rhizobia are non-pathogenic soil and rhizospheric bacteria known to occupy root (and occasionally stem) nodule tissues, where they reduce N_2 to NH_3 .

EBIC remains available for any clarifications that might be needed considering the content of this document.

For more information about this topic, please contact Sara Garcia Figuera (sara.gfiguera@prospero.ag)

ABOUT EBIC



The European Biostimulants Industry Council (EBIC) promotes the contribution of plant biostimulants to make agriculture more sustainable and resilient and in doing so promotes the growth and development of the European biostimulants industry. Our mission is to ensure biostimulant technologies are valued as integral to sustainable agriculture, while securing an enabling regulatory framework for all of them.

References

- Alves, B. J. R., Boddey, R. M., and Urquiaga, S. 2003. The success of BNF in soybean in Brazil. *Plant Soil*. 252:1–9 Available at: <https://doi.org/10.1023/A:1024191913296>.
- Atieno, M., and Lesueur, D. 2019. Opportunities for improved legume inoculants: enhanced stress tolerance of rhizobia and benefits to agroecosystems. *Symbiosis*. 77:191–205 Available at: <https://doi.org/10.1007/s13199-018-0585-9>.
- Avontuur, J. R., Palmer, M., Beukes, C. W., Chan, W. Y., Coetzee, M. P. A., Blom, J., et al. 2019. Genome-informed Bradyrhizobium taxonomy: where to from here? *Syst. Appl. Microbiol*. 42:427–439 Available at: <https://www.sciencedirect.com/science/article/pii/S0723202019300475>.
- Avontuur, J. R., Wilken, P. M., Palmer, M., Coetzee, M. P. A., Stępkowski, T., Venter, S. N., et al. 2023. Complex evolutionary history of photosynthesis in Bradyrhizobium. *Microb. Genomics*. 9 Available at: <https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.001105>.
- Bullard, G. K., Roughley, R. J., and Pulsford, D. J. 2005. The legume inoculant industry and inoculant quality control in Australia: 1953-2003. *Aust. J. Exp. Agric*. 45:127–140 Available at: <https://doi.org/10.1071/EA03159>.
- De Meyer, S. E., Van Hoorde, K., Vekeman, B., Braeckman, T., and Willems, A. 2011. Genetic diversity of rhizobia associated with indigenous legumes in different regions of Flanders (Belgium). *Soil Biol. Biochem*. 43:2384–2396 Available at: <https://www.sciencedirect.com/science/article/pii/S0038071711003117>.
- Han, Q., Ma, Q., Chen, Y., Tian, B., Xu, L., Bai, Y., et al. 2020. Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean. *ISME J*. 14:1915–1928 Available at: <https://doi.org/10.1038/s41396-020-0648-9>.
- Hördt, A., López, M. G., Meier-Kolthoff, J. P., Schleuning, M., Weinhold, L.-M., Tindall, B. J., et al. 2020. Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria. *Front. Microbiol*. 11 Available at: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.00468>.
- ICSP. 2023a. Family Nitrobacteraceae. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/family/nitrobacteraceae> [Accessed October 4, 2023].

- ICSP. 2023b. Family Phyllobacteriaceae. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/family/phyllobacteriaceae> [Accessed October 5, 2023].
- ICSP. 2023c. Family Rhizobiaceae. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/family/rhizobiaceae> [Accessed October 4, 2023].
- ICSP. 2023d. Family Xanthobacteraceae. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/family/xanthobacteraceae> [Accessed October 5, 2023].
- ICSP. 2023e. Genus Azorhizobium. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/genus/azorhizobium> [Accessed October 5, 2023].
- ICSP. 2023f. Genus Bradyrhizobium. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/genus/bradyrhizobium> [Accessed October 4, 2023].
- ICSP. 2023g. Genus Ensifer. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/genus/ensifer> [Accessed October 5, 2023].
- ICSP. 2023h. Genus Mesorhizobium. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/genus/mesorhizobium> [Accessed October 5, 2023].
- ICSP. 2023i. Genus Rhizobium. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/genus/rhizobium> [Accessed October 4, 2023].
- ICSP. 2023j. Genus Rhodopseudomonas. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/genus/rhodopseudomonas> [Accessed October 4, 2023].
- ICSP. 2023k. Order Hyphomicrobiales. List Prokaryotic Names Standing Nomencl. Available at: <https://lpsn.dsmz.de/order/hyphomicrobiales> [Accessed October 4, 2023].
- Jaiswal, S. K., and Dakora, F. D. 2019. Widespread Distribution of Highly Adapted Bradyrhizobium Species Nodulating Diverse Legumes in Africa. *Front. Microbiol.* 10 Available at: <https://www.frontiersin.org/articles/10.3389/fmicb.2019.00310>.
- Kuzmanović, N., Fagorzi, C., Mengoni, A., Lassalle, F., and diCenzo, G. C. 2022. Taxonomy of Rhizobiaceae revisited: proposal of a new framework for genus delimitation. *Int. J. Syst. Evol. Microbiol.* 72 Available at: <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijsem.0.005243>.
- Larimer, F. W., Chain, P., Hauser, L., Lamerdin, J., Malfatti, S., Do, L., et al. 2004. Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. *Nat. Biotechnol.* 22:55–61 Available at: <https://doi.org/10.1038/nbt923>.
- Lassalle, F., Dastgheib, S. M. M., Zhao, F.-J., Zhang, J., Verburg, S., Frühling, A., et al. 2021. Phylogenomics reveals the basis of adaptation of *Pseudorhizobium* species to extreme environments and supports a taxonomic revision of the genus. *Syst. Appl. Microbiol.* 44:126165 Available at: <https://www.sciencedirect.com/science/article/pii/S072320202030120X>.
- Lee, S.-K., Chiang, M.-S., Hseu, Z.-Y., Kuo, C.-H., and Liu, C.-T. 2022. A photosynthetic bacterial inoculant exerts beneficial effects on the yield and quality of tomato and affects bacterial community structure in an organic field. *Front. Microbiol.* 13 Available at: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.959080>.
- Ma, T., Xue, H., Piao, C., Jiang, N., and Li, Y. 2023. Phylogenomic reappraisal of the family Rhizobiaceae at the genus and species levels, including the description of *Ectorhizobium*

quercum gen. nov., sp. nov. *Front. Microbiol.* 14 Available at: <https://www.frontiersin.org/articles/10.3389/fmicb.2023.1207256>.

Maluk, M., Ferrando-Molina, F., Lopez del Egado, L., Langarica-Fuentes, A., Yohannes, G. G., Young, M. W., et al. 2022. Fields with no recent legume cultivation have sufficient nitrogen-fixing rhizobia for crops of faba bean (*Vicia faba* L.). *Plant Soil.* 472:345–368 Available at: <https://doi.org/10.1007/s11104-021-05246-8>.

Ojha, A., Tak, N., Rathi, S., Chouhan, B., Rao, S. R., Barik, S. K., et al. 2017. Molecular characterization of novel Bradyrhizobium strains nodulating Eriosema chinense and Flemingia vestita, important unexplored native legumes of the sub-Himalayan region (Meghalaya) of India. *Syst. Appl. Microbiol.* 40:334–344 Available at: <https://www.sciencedirect.com/science/article/pii/S0723202017300760>.

Ormeño-Orrillo, E., Servín-Garcidueñas, L. E., Rogel, M. A., González, V., Peralta, H., Mora, J., et al. 2015. Taxonomy of rhizobia and agrobacteria from the Rhizobiaceae family in light of genomics. *Taxon. Age Genomics.* 38:287–291 Available at: <https://www.sciencedirect.com/science/article/pii/S0723202014001908>.

Poole, P., Ramachandran, V., and Terpolilli, J. 2018. Rhizobia: from saprophytes to endosymbionts. *Nat. Rev. Microbiol.* 16:291–303 Available at: <https://doi.org/10.1038/nrmicro.2017.171>.

Rodríguez-Navarro, D. N., Bellogín, R., Camacho, M., Daza, A., Medina, C., Ollero, F. J., et al. 2003. Field assessment and genetic stability of Sinorhizobium fredii strain SMH12 for commercial soybean inoculants. *Eur. J. Agron.* 19:299–309 Available at: <https://www.sciencedirect.com/science/article/pii/S116103010200076X>.

Sakarika, M., Spanoghe, J., Sui, Y., Wambacq, E., Grunert, O., Haesaert, G., et al. 2020. Purple non-sulphur bacteria and plant production: benefits for fertilization, stress resistance and the environment. *Microb. Biotechnol.* 13:1336–1365 Available at: <https://doi.org/10.1111/1751-7915.13474> [Accessed October 4, 2023].

Santos, M. S., Nogueira, M. A., and 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.* 9:205 Available at: <https://doi.org/10.1186/s13568-019-0932-0>.

Sprent, J. I., Ardley, J., and James, E. K. 2017. Biogeography of nodulated legumes and their nitrogen-fixing symbionts. *New Phytol.* 215:40–56 Available at: <https://doi.org/10.1111/nph.14474> [Accessed October 5, 2023].

Tian, C. F., Zhou, Y. J., Zhang, Y. M., Li, Q. Q., Zhang, Y. Z., Li, D. F., et al. 2012. Comparative genomics of rhizobia nodulating soybean suggests extensive recruitment of lineage-specific genes in adaptations. *Proc. Natl. Acad. Sci.* 109:8629–8634 Available at: <https://doi.org/10.1073/pnas.1120436109> [Accessed October 5, 2023].

Tookmanian, E. M., Belin, B. J., Sáenz, J. P., and Newman, D. K. 2021. The role of hopanoids in fortifying rhizobia against a changing climate. *Environ. Microbiol.* 23:2906–2918 Available at: <https://doi.org/10.1111/1462-2920.15594> [Accessed October 4, 2023].

Wong, W.-T., Tseng, C.-H., Hsu, S.-H., Lur, H.-S., Mo, C.-W., Huang, C.-N., et al. 2014. Promoting Effects of a Single Rhodopseudomonas palustris Inoculant on Plant Growth by Brassica rapa chinensis under Low Fertilizer Input. *Microbes Environ.* 29:303–313.