Phylogenetic and population structure of the *nod*-free but nodulating *Bradyrhizobium* phylogroup

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Key words: *Bradyrhizobium*, *Aeschynomene*, nodulation, population genomics

Abstract

Bradyrhizobium is a main rhizobial lineage of which the majority of its members nodulate legume plants using Nod factors (NFs) encoded by the *nod* genes. However, the "photosynthetic" phylogroup within *Bradyrhizobium* (PB) does not contain *nod* genes but are still capable of establishing a nitrogen-fixing symbiosis with some tropical legumes of the *Aeschynomene* genus. Furthermore, members of this phylogroup colonize rice roots and promote plant growth. Despite this unique combination of ecological features, genomes of only 13 PB members are sequenced, and almost all are isolated from *Aeschynomene* spp. nodules. Here, we sequenced 208 new PB members predominantly associated with rice. The extended PB phylogroup comprises three main clades: a basal clade with significant expansion of its diversity, followed by the diversification of a singleton clade from a new clade exclusively represented by our strains. Although the PB strains universally lack the canonical *nod* genes, all 27 assayed strains representing the broad diversity of these clades induced nodules on *Aeschynomene indica*. Interestingly, significant differences in the efficiency of symbiosis between the main clades were observed and the singleton clade showed an intermediate symbiotic phenotype aligning well with its intermediate phylogenetic position. Our strain collection expands the phylogenetic and ecological diversity of the *nod*-free but nodulating *Bradyrhizobium* and shows that the NF-independent nodulation of *Aeschynomene* is a common trait of this phylogroup, in contrast to the photosynthetic trait originally thought as its unifying feature.

Introduction

Bradyrhizobium is one of the largest and most diverse rhizobial genera and the primary symbiont of a wide range of legumes [1, 2]. The genus *Bradyrhizobium* encompasses seven phylogenetic supergroups (phylogroups) [3, 4], among which the "photosynthetic" *Bradyrhizobium* (PB) supergroup is one of the most special. Members belonging to this supergroup engage in a mutualistic relationship with some tropical semi-aquatic species of the *Aeschynomene* genus [5], and they form nodules not only on roots but also on stems [6, 7]. Further, none of the completely sequenced PB members carry *nodABC* genes encoding the enzymes synthesizing the core structure of the nodulation factors (NF), the only exception being *Bradyrhizobium* sp. ORS285, which can use both a *nod*-dependent and -independent pathway reliant to the *Aeschynomene* host plant [8, 9]. This indicates that the PB phylogroup uses a novel mechanism of interaction with legume hosts that differs from the traditional universal NF-based pathway [10, 11]. Moreover, all known PB strains carry photosynthetic genes except for four deep-branching ones isolated from French Guiana as represented by *Bradyrhizobium* sp. STM3843 [7, 12].

Despite these unique features, the research on photosynthetic *Bradyrhizobium* has been very limited. Currently, there are only 13 PB strains that have their genomes sequenced, largely limiting comparative genomics analysis on this important *Bradyrhizobium* phylogroup. Although most PB strains were isolated from *Aeschynomene* spp. nodules, the PB phylogroup was also detected in paddy soil, rice roots as well as in lake water [13-16], suggesting that its members have much wider ecological niches than previously thought. Here, we cultured and genome-sequenced 208 isolates (Fig. 1) predominantly from three niches of rice (within root, rhizosphere, bulk soil) and conducted genomic analyses and physiological assays to reveal the phylogenetic

and population structure of the photosynthetic *Bradyrhizobium* supergroup.

Results and discussion

The new genomes expand the phylogenetic diversity of the photosynthetic supergroup of *Bradyrhizobium*

With the 208 newly isolated members, the phylogeny of the PB supergroup is split into three deep-branching clades (see Fig. S1 for their phylogenetic position in the species tree of the entire *Bradyrhizobium* genus). Clade 1 represents the evolutionarily basal lineage. It initially comprised only 12 strains, but it now has its diversity expanded by having 68 new strains representing several new lineages delineated as distinct genetically isolated populations (Fig. 1, Datasets S1 and S2; also discussed in the next section). Clade 2 is a singleton clade with only one strain (*Bradyrhizobium* sp. STM3843) available and represents a phylogenetic intermediate among the three clades. Clade 3 is new and consists exclusively of 140 new strains. The evolutionary branching order of the three clades is verified with two outgroup-independent methods (Fig. 1, Fig. S7A) and the outgroup-dependent method (Fig. S1). Collectively, our new strains appreciably increase the existing phylogenetic diversity of the PB supergroup. Comparative genomic analysis shows that except for *Bradyrhizobium* sp. ORS285, which is known to use both a *nod*-dependent and -independent strategy for nodulation, all PB supergroup members lack the *nod* genes.

Fine-scale population structure of the photosynthetic supergroup of *Bradyrhizobium*

Apart from the broad diversity of the PB supergroup, we further asked whether fine-scale phylogenetic differentiation correlates with the ecological niches where the PB supergroup

members were found. Of the 208 new PB supergroup strains, 205 were collected from three rice plants located in the same rice field with each plant 5-10 m apart from the others. The remaining three strains were isolated from grassland and forest. Bacterial isolation was performed from within the root, rhizosphere, and bulk soil for each plant, collectively giving rise to 145, 23 and 40 PB strains, respectively (Dataset S1). To facilitate the correlative analysis between fine-scale phylogenetic groups and the niches, we applied the population genomics software PopCOGenT [17], which defines the boundaries of populations (reported as "main cluster" or MC) between which gene flow is limited, distinguishes recent gene flow from historical events, and detects subpopulations (reported as 'subclusters') under ongoing differentiation within a population.

Using this approach, we showed that all strains in Clade 3 share the membership of a single population (MC1), whereas strains from Clade 1 fall into many populations. In Clade 1, MC2, MC3, MC4, MC5 and MC6 are each exclusively comprised of the new strains, whereas MC7 and the remaining unassigned isolates are from published resources. In Clade 1, the within- and between-population similarity was above and below 95% ANI (Fig. S2), respectively, consistent with the operational species threshold of 95% ANI [18], whereas the 16S rRNA gene shows little divergence with the between-population similarity generally above 99% (Fig. S2, Dataset S3). Based on the available samples, intra-population subdivision occurred within MC1, MC2 and MC4. We found that each population and subpopulation have members sampled from different plants and niches (Fig. 1), suggesting that the PB populations sampled from the rice field are not separated or subdivided according to the physical separation between plant individuals and the three typical niches (root, rhizosphere, and bulk soil).

Rather, we found an interesting association between important metabolic pathways and population identity. Remarkably, although the photosynthetic supergroup was named by the

presence of the photosynthesis genes [3], these genes are exclusively and universally found in MC2, MC3, MC6 and MC7 but completely missing from MC1, MC4 and MC5 (Fig. 1, Dataset S4). Our result indicates that photosynthesis is not a characteristic trait that defines the PB phylogroup.

Also interesting is the prevalence of a unique Type III secretion system (T3SS) in MC2, MC4 and MC5 but completely missing in other populations (Fig. 1, Fig. S3B and S3C, Dataset S4). It is one of the six T3SS subtypes identified from known *Bradyrhizobium* phylotypes [19]. It was previously identified only in the PB strain *Bradyrhizobium oligotrophicum* S58 (thus named 'S58-T3SS' subtype) and its function remains unknown [19]. Since the main function of T3SS is to translocate effector proteins into host cells that modulate the host immune response [20], it cannot be excluded that this T3SS type specifically identified in PB members plays an important role during their interaction with their host plants (*Aeschynomene* spp. and rice).

We also found that *mxa* gene sets participating in methanol metabolism were specific to MC1 (Dataset S4), which suggests that these MC1 strains may form intimate associations with plants considering that methanol is a waste product formed during plant-cell wall degradation [21]. Our methanol metabolism experiment showed that MC1 strains could not use methanol as a sole C source (Fig. S4), hinting that the expression of *mxa* genes in these MC1 strains might be under regulation of unknown mechanisms.

<u>The ability to nodulate *Aeschynomene indica* is a conserved trait shared by all PBs but differs between the three major PB clades</u>

Since the new PB strains were isolated from non-legume environments, we thought that it would be very interesting to investigate (i) whether they are capable of performing nitrogen

fixation under free-living and symbiotic states, (ii) whether they are capable of nodulating *Aeschynomene indica*, the typical host plant of the previously isolated PB strains, and (iii) whether there is a difference in nodulation ability across the clades and the populations.

All PB supergroup members have a *nif* gene cluster necessary for nitrogenase fixation. Interestingly, duplication of the *nifH* gene which encodes one of the structural proteins of the nitrogenase enzyme complex was observed in all PB strains (Fig. S3A), although the functional consequence is not known. Across the PB supergroup members, an important difference was observed regarding how the *nif* genes are structured. In most populations, all *nif* genes are co-located, along with other genes potentially involved in nitrogen fixation. This is not the case for a few members such as some basal lineages (e.g., *Bradyrhizobium* sp LMG 8443) and some members assigned to several populations (e.g., SZCCHNR1015 in MC3 and *Bradyrhizobium* sp. 83002 in MC7), where the *nif* genes were found in two contigs. This is likely due to structural rearrangement, but a DNA assembly artefact cannot be ruled out.

Another original feature observed of the PB *nif* cluster is the universal presence of the *nifV* gene encoding for a homocitrate synthase that is involved in the biosynthesis of the nitrogenase cofactor (FeMo-Co), and which is absent in most other rhizobial lineages [22, 23]. An acetylene reduction assay on 27 representative PB strains covering all three major clades, all newly identified populations (MC1 to MC6) within Clade 1 and Clade 3, and most of the identified subpopulations within each population (with the model strain ORS278 as control) confirmed their ability to fix nitrogen under free-living conditions (Fig. S5A), a trait absent in most rhizobia [23].

The plant inoculation experiments showed that all 27 representative strains were also able to induce nodules on *A. indica* (Fig. 2B, 2C). However, a significant difference in symbiosis

efficiency was observed between the major clades. Clade 1 strains had the same symbiotic properties as the model strain ORS278, inducing a large number of nitrogen-fixing nodules that stimulated the growth of the plants (Fig. 2A, 2B, Fig. S5B and Fig. S6A). In contrast, Clade 3 strains formed a much smaller number of nodules compared to Clade 1 strains and the measured nitrogenase enzyme activity was lower under the symbiotic condition (Fig. 2B, Fig. S5B). Microscopic analysis showed that the nodules elicited by Clade 3 strains displayed multiple aberrant phenotypes: i) some nodules contained necrotic areas, ii) in others the central tissue was completely digested, and finally, iii) the nodules that displayed less drastic symptoms contained mainly dead bacteria (Fig. 2C and 2F). These observations indicate that besides inducing fewer nodules, Clade 3 strains are unable to maintain an effective chronic infection, which explains why they have no beneficial effect on plant growth (Fig. 2A, 2C and 2F). Interestingly, the singleton Clade 2 represented by strain STM3843 which is phylogenetically positioned between Clade 1 and Clade 3, showed an intermediate phenotype. The number and phenotype of the nodules is comparable to those of Clade 1 strains but stimulation of plant growth is less (Fig. 2A, 2B and 2E).

The symbiotic differences observed between the major clades may result from an inability of the Clade 3 strains to cope with the plant's immune response, and/or an over-induction of the plant's defense mechanisms due to the absence or not properly recognized symbiotic signal(s). However, it is remarkable that all strains from the PB phylogroup, which come from different origins and geographical locations, are capable of nodulating *A. indica*. The ability to develop a NF-independent nodulation with *Aeschynomene* thus appears to be a common trait of PB supergroup members. Thus, unlike the *nod* genes of other rhizobial lineages which are accessory genes acquired by horizontal transfer, the genes governing NF-independent

symbiosis could be essential genes belonging to the core genome of the PB phylogroup.

Concluding remarks

By large-scale isolation and genome sequencing of the 'photosynthetic' *Bradyrhizobium* supergroup, we discovered a novel major clade, thereby greatly expanding the phylogenetic diversity of the cultured members within the PB supergroup. The branching order of the three major clades correlates nicely with their symbiosis efficiency, with the clade taking the intermediate phylogenetic position showing the symbiosis efficiency in between. It is therefore likely that the ability to establish symbiosis with *Aeschynomene* spp. is a key factor that shapes the broad diversity of the PB supergroup. At the population level, no difference in symbiosis efficiency was observed, suggesting that other traits, such as photosynthesis and T3SS, that are uniquely associated with some but not all populations, might be among the important drivers of population differentiation. Collectively, these results suggest that only deep divergence within the PB supergroup can impact significantly on the ability to nodulate *Aeschynomene* spp.

Acknowledgements

This work was supported by the Hong Kong Research Grants Council Area of Excellence Scheme (AoE/M-403/16).

Data availability

The genomic sequences and raw reads of the 208 newly sequenced *Bradyrhizobium* isolates are in the process of uploading to NCBI (Project ID: PRJNA983111) and will be released in the next version of the manuscript.

Competing interests

The authors declare no competing interests in relation to this work.

Reference

1. Teulet A, Busset N, Fardoux J, Gully D, Chaintreuil C, Cartieaux F, et al. The rhizobial type III effector ErnA confers the ability to form nodules in legumes. *Proc Natl Acad Sci U S A* 2019; **116:** 21758-21768.
2. Spaink HP. The molecular basis of infection and nodulation by rhizobia: the ins and outs of sympathogenesis. *Annu Rev Phytopathol* 1995; **33:** 345-368.
3. Avontuur JR, Palmer M, Beukes CW, Chan WY, Coetzee MPA, Blom J, et al. Genome-informed Bradyrhizobium taxonomy: where to from here? *Syst Appl Microbiol* 2019; **42:** 427-439.
4. Ormeno-Orrillo E, Martinez-Romero E. A Genomotaxonomy View of the Bradyrhizobium Genus. *Front Microbiol* 2019; **10:** 1334.
5. Giraud E, Fleischman D. Nitrogen-fixing symbiosis between photosynthetic bacteria and legumes. *Photosynthesis Research* 2004; **82:** 115-130.
6. Chaintreuil C, Arrighi JF, Giraud E, Miche L, Moulin L, Dreyfus B, et al. Evolution of symbiosis in the legume genus Aeschynomene. *New Phytol* 2013; **200:** 1247-1259.
7. Mornico D, Miche L, Bena G, Nouwen N, Vermeglio A, Vallenet D, et al. Comparative genomics of aeschynomene symbionts: insights into the ecological lifestyle of nod-independent photosynthetic bradyrhizobia. *Genes (Basel)* 2011; **3:** 35-61.
8. Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, Avarre JC, et al. Legumes symbioses:

absence of Nod genes in photosynthetic Bradyrhizobia. *Science* 2007; **316:** 1307-1312.

9. Bonaldi K, Gargani D, Prin Y, Fardoux J, Gully D, Nouwen N, et al. Nodulation of Aeschynomene afraspera and A. indica by photosynthetic Bradyrhizobium Sp. strain ORS285: the nod-dependent versus the nod-independent symbiotic interaction. *Mol Plant Microbe Interact* 2011; **24:** 1359-1371.

10. Lerouge P, Roche P, Faucher C, Maillet F, Truchet G, Prome JC, et al. Symbiotic host-specificity of Rhizobium meliloti is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature* 1990; **344:** 781-784.

11. Oldroyd GED. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* 2013; **11:** 252-263.

12. Miché L, Moulin L, Chaintreuil C, Contreras-Jimenez JL, Munive-Hernández JA, Del Carmen Villegas-Hernandez M, et al. Diversity analyses of Aeschynomene symbionts in Tropical Africa and Central America reveal that nod-independent stem nodulation is not restricted to photosynthetic bradyrhizobia. *Environ Microbiol* 2010; **12:** 2152-2164.

13. Chaintreuil C, Giraud E, Prin Y, Lorquin J, Ba A, Gillis M, et al. Photosynthetic bradyrhizobia are natural endophytes of the African wild rice Oryza breviligulata. *Appl Environ Microbiol* 2000; **66:** 5437-5447.

14. Piromyou P, Greetatorn T, Teamtisong K, Okubo T, Shinoda R, Nuntakij A, et al. Preferential association of endophytic bradyrhizobia with different rice cultivars and its implications for rice endophyte evolution. *Appl Environ Microbiol* 2015; **81:** 3049-3061.

15. Van Berkum P, Eardly BD. The aquatic budding bacterium Blastobacter denitrificans is a nitrogen-fixing symbiont of Aeschynomene indica. *Appl Environ Microbiol* 2002; **68:** 1132-1136.

16. Okubo T, Fukushima S, Itakura M, Oshima K, Longtonglang A, Teaumroong N, et al. Genome analysis suggests that the soil oligotrophic bacterium Agromonas oligotrophica (Bradyrhizobium oligotrophicum) is a nitrogen-fixing symbiont of Aeschynomene indica. *Appl Environ Microbiol* 2013; **79:** 2542-2551.

17. Arevalo P, VanInsberghe D, Elsherbini J, Gore J, Polz MF. A Reverse Ecology Approach Based on a Biological Definition of Microbial Populations. *Cell* 2019; **178:** 820-834 e814.

18. Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A* 2005; **102:** 2567-2572.

19. Teulet A, Gully D, Rouy Z, Camuel A, Koebnik R, Giraud E, et al. Phylogenetic distribution and evolutionary dynamics of nod and T3SS genes in the genus Bradyrhizobium. *Microb Genom* 2020; **6**.

20. Teulet A, Camuel A, Perret X, Giraud E. The Versatile Roles of Type III Secretion Systems in Rhizobium-Legume Symbioses. *Annu Rev Microbiol* 2022; **76:** 45-65.

21. Nemecek-Marshall M, MacDonald RC, Franzen JJ, Wojciechowski CL, Fall R. Methanol Emission from Leaves (Enzymatic Detection of Gas-Phase Methanol and Relation of Methanol Fluxes to Stomatal Conductance and Leaf Development). *Plant Physiol* 1995; **108:** 1359-1368.

22. Hakoyama T, Niimi K, Watanabe H, Tabata R, Matsubara J, Sato S, et al. Host plant genome overcomes the lack of a bacterial gene for symbiotic nitrogen fixation. *Nature* 2009; **462:** 514-517.

23. Nouwen N, Arrighi J-F, Cartieaux F, Chaintreuil C, Gully D, Klopp C, et al. The role of rhizobial (NifV) and plant (FEN1) homocitrate synthases in Aeschynomene/photosynthetic Bradyrhizobium symbiosis. *Scientific Reports* 2017; **7:** 448.

24. Tao J, Wang S, Liao T, Luo H. Evolutionary origin and ecological implication of a unique nif

island in free-living Bradyrhizobium lineages. *ISME J* 2021; **15:** 3195-3206.

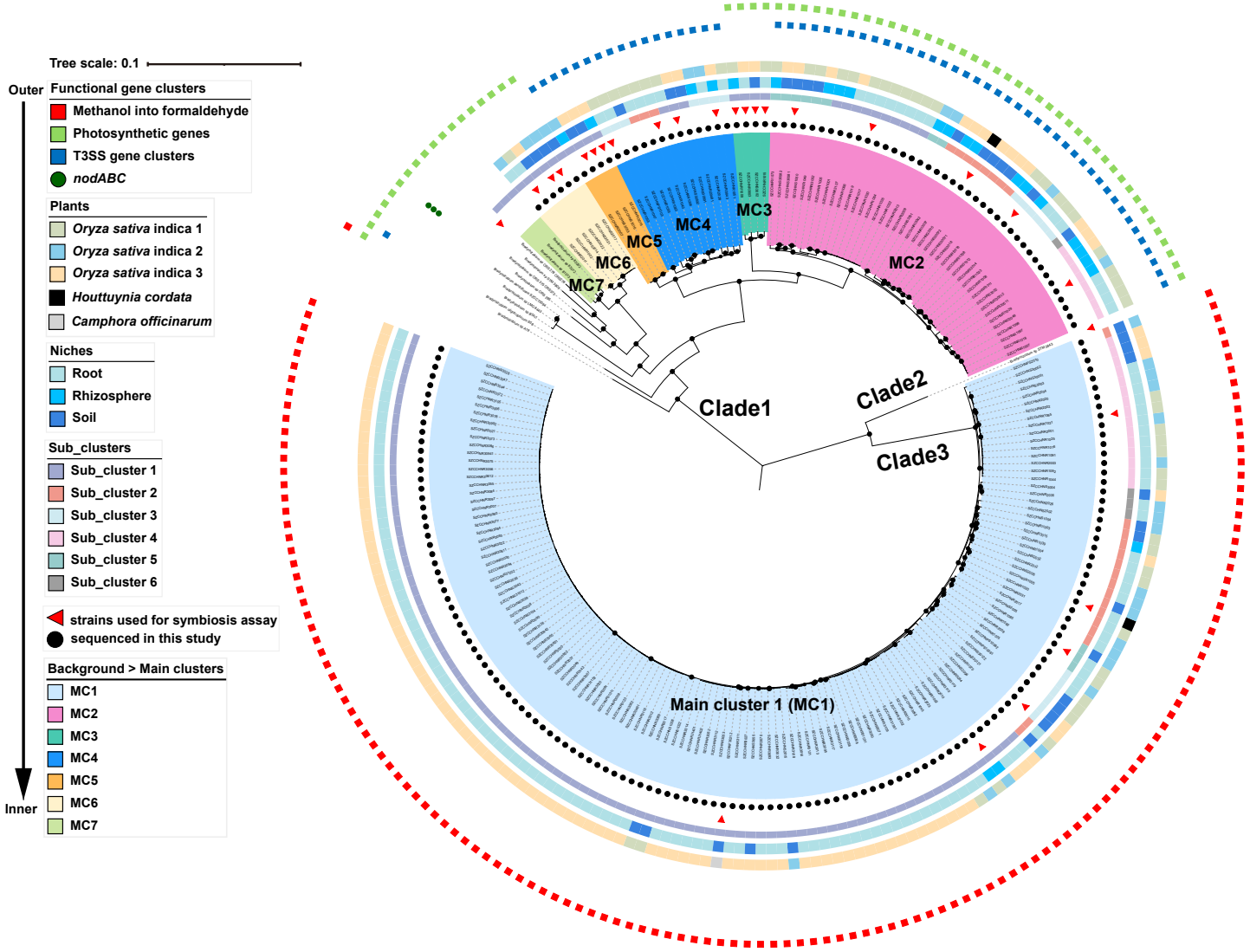
25. Zulkower V, Rosser S. DNA Features Viewer: a sequence annotation formatting and plotting library for Python. *Bioinformatics* 2020; **36:** 4350-4352.

Figure legends

Figure 1. The phylogenomic tree and population delineation of the "photosynthetic" *Bradyrhizobium* (PB) supergroup. The phylogenomic tree was rooted using the minimum variance (MV) method. The 208 PB strains sequenced in the present study are indicated by black dots in the innermost layer surrounding the phylogeny. The ultrafast bootstrap values higher than or equal to 95% calculated by IQ-Tree were labeled on the nodes with black circles.

Figure 2. Symbiotic properties of several representative strains of the main populations identified in the PB supergroup. (A) Comparison of growth of the *A. indica* plants (leaf phenotype) non-inoculated (NI) or inoculated with different representative strains of PB. The six MC1 representative strains are presented in the following order: SZCCHNR1021, SZCCHNR1085, SZCCHNS30121, SZCCHNGR1005, SZCCHNRI2010 and SZCCHNF30212. For the other MCs, only one representative strain is shown: SZCCHNRI2049 (MC2), SZCCHNS3002 (MC3), SZCCHNS30592 (MC4), SZCCHNRI3016 (MC5) and SZCCHNS2021 (MC6), Photos were taken 17 days after inoculation. (B) Nodule number on *A. indica* plants induced by different representative strains of PB at 17 days after inoculation. Box plots show the results of one of the two experiments performed independently (4 plants each). (C) Symbiotic phenotypes of some representative strains tested on *A. indica*. Column 1 and 2: photo of roots and nodules. Scale bars: column 1, 0.5 cm; column 2, 0.2 cm. Column 3: Micro-sections of nodules observed using light microscopy. Scale bars: 250 μm. (D) Confocal microscopy images of micro-sections of nodules elicited by Clade 1 strains. The nodules formed by SZCCHNRI3016 from MC4 and ORS278 are shown as examples. The central nodule tissue is intracellularly infected with live spherical bacteroids stained green by SYTO9. (E) Confocal

microscopy images of micro-section of nodules elicited by Clade 2 strain STM3843. The nodules look normal, but a mix of live and dead bacteria (stained red by propidium iodide) can be noticed. (F) Confocal microscopy images of micro-section of nodules elicited by Clade 3 strains displaying different phenotypes: nodule containing mainly dead bacteria (SZCCHNS30121), nodule with a completely digested central tissue (SZCCHNRI2010); and nodule with a necrotic area and a digested zone (SZCCHNGR1005). Scale bars for (D), (E) and (F): column 1, 200 µm; column 2, 10 µm.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Outer
Functional gene clusters
Methanol into formaldehyde
Photosynthetic genes
T3SS gene clusters
nodABC
Plants
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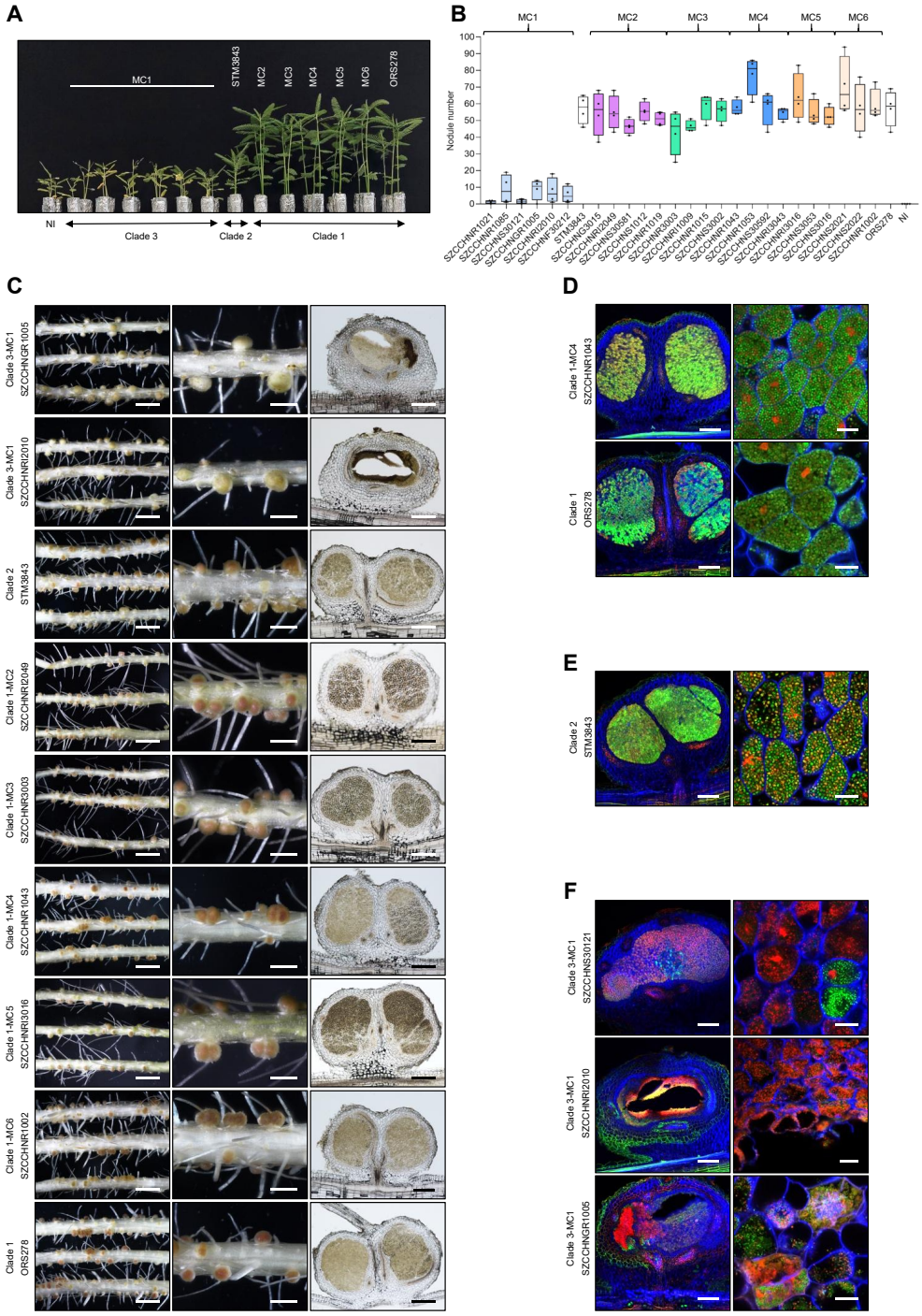
Oryza sativa indica 2
Oryza sativa indica 3
Houttuynia cordata
Camphora officinarum
Niches
Root
Rhizosphere
Soil
Sub_clusters
Sub_cluster 1
Sub_cluster 2
Sub_cluster 3
Sub_cluster 4
Sub_cluster 5
Sub_cluster 6
strains used for symbiosis assay
sequenced in this study
Background > Main clusters
MC1
MC2
MC3
MC4
MC5
MC6
MC7
Inner
MC7
MC6
MC5
MC4
MC3
MC2
Clade1
Clade2
Clade3
Main cluster 1 (MC1)


A
MC1
STM3843
MC2
MC3
MC4
MC5
MC6
ORS278
NI
Clade 3
Clade 2
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B
MC1
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MC3
MC4
MC5
MC6
Nodule number
SZCCHNR1021
SZCCHNR1085
SZCCHNS3121
SZCCHNGR1005
SZCCHNR2010
SZCCHNF3012
STM3843
SZCCHNS3015
SZCCHNR2049
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SZCCHNR3016
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SZCCHNS3021
SZCCHNS2022
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Clade 3-MC1 SZCCHNGR1005
Clade 3-MC1 SZCCHNR2010
Clade 2 STM3843
Clade 1-MC2 SZCCHNR2049
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