QUORUM SENSING IN RHIZOBIA ISOLATED FROM THE SPORES OF THE MYCORRHIZAL SYMBIONT *RHIZOPHAGUS INTRARADICES*

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RUNNING HEAD: Quorum sensing in AMF sporosphere rhizobia

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ABSTRACT

Most beneficial services provided by arbuscular mycorrhizal fungi (AMF), encompassing improved crop performance and soil resource availability, are mediated by AMF-associated bacteria, showing key plant growth promoting (PGP) traits, *i.e.* the production of indole acetic acid, siderophores and antibiotics, and activities increasing the availability of plant nutrients by nitrogen fixation and phosphate mobilization. Such functions may be affected by the ability of AMF-associated bacteria to communicate through the production and secretion of extracellular small diffusible chemical signals, N-acyl homoserine lactone signal molecules (AHLs), that regulate bacterial behaviour at the community level (quorum sensing, QS). This work investigated the occurrence and extent of QS among rhizobia isolated from AMF spores, using two different QS reporter strains, *Agrobacterium tumefaciens* NTL4 pZRL4 and *Chromobacterium violaceum* CV026. We also assessed the quorum quenching (QQ) activity among *Bacillus* isolated from the same AMF spores. Most rhizobia were found to be quorum-signalling positive, including six isolates producing very high levels of AHLs. The results were confirmed by microtiter plate assay, which detected 65% of the tested bacteria as medium/high AHL producers. A 16S rDNA sequence analysis grouped the rhizobia into two clusters, consistent with the QS phenotype. None of the tested bacteria showed QQ activity able to disrupt the QS signalling, suggesting the absence of antagonism among bacteria living in AMF sporosphere. Our results provide the first evidence of the ability of AMF-associated

rhizobia to communicate through QS, suggesting further studies on the potential importance of such a behaviour in association with key-plant growth-promoting functions.

KEYWORDS**:** arbuscular mycorrhizal fungi; mycorrhizospheric bacteria; *Sinorhizobium meliloti*; N-acyl homoserine lactones production; quorum quenching; reporter strains.

INTRODUCTION

The agroecosystem services and beneficial activities provided by arbuscular mycorrhizal (AM) fungi (AMF) can be mediated by a third component of the symbiosis, the microbiota living intimately associated with fungal structures, such as spores, sporocarps and extraradical hyphae (Azcon-Aguilar and Barea 2015; Barea et al. 2002; Rouphael et al. 2015). Several molecular studies, utilizing PCR-denaturing gradient gel electrophoresis (DGGE), have revealed the complexity and diversity of bacterial communities associated with the spores of different AM fungal species (Long et al. 2008; Roesti et al. 2005). PCR-DGGE analyses have also revealed the occurrence of specific and diverse microbial communities tightly associated with the spores of six different AMF isolates, consisting of bacteria belonging to Actinomycetales, Bacillales, Rhizobiales, Pseudomonadales, Burkholderiales, and endobacteria related to the Mollicutes (Mre) (Agnolucci et al. 2015). Other studies, focused on the isolation and characterization of AMF-associated microorganisms, have reported the occurrence of dense and active bacterial communities able to promote mycorrhizal establishment and biological control of soilborne pathogens, fix nitrogen, and provide nutrients and growth factors (Alonso et al. 2008; Azcon-Aguilar and Barea 1996, 2015; Barea et al. 2002). Recent studies have reported the isolation of bacteria associated with the spores of the AMF species *Rhizophagus intraradices*, showing multiple functional Plant Growth Promoting (PGP) traits, such as siderophore and indole acetic acid (IAA) production, phosphorus (P) solubilization from inorganic and organic sources and nitrogen fixation (Battini et al. 2016b) and producing large increases in the uptake and translocation of P from the soil to the host plant (Battini et al. 2017). Some of them were able to improve the biosynthesis of plant health-promoting secondary metabolites and to affect the expression levels of transcripts encoding for key enzymes involved in their biosynthetic pathways (Battini et al. 2016a).

So far, nothing is known about the ability of AMF-associated bacteria belonging to the same species to communicate with one another through the production and secretion of extracellular small diffusible chemical signals, called autoinducers, that regulate their behaviour at the community level, *i.e.* quorum sensing (QS) (Fuqua et al. 1994). Such signalling molecules are represented by N-acyl homoserine lactones (AHLs) in Gram-negative bacteria, by a family of small oligopeptides in Gram-positive bacteria and by other molecules (autoinducer-2) in both Gram-positive and negative bacteria (Miller and Bassler 2001). QS can control the expression of many genes responsible for different bacterial activities and functional traits, such as swarming and motility, biofilm formation, bioluminescence, plasmid conjugal transfer and virulence, metabolite production, as well as the production of antibiotics, siderophores and exoenzymes, and symbiotic interactions (Hartmann and Schikora 2012). As AMF-associated bacteria are able to carry out many of the described activities, some of which are necessary for the optimal performance of mycorrhizal plants, investigations able to reveal their QS phenotype are important and highly relevant.

Studies on the diversity of AHL-producing bacteria have focused on many diverse habitats, but only a few have investigated the occurrence and diversity of AHL-producing bacteria in the soil environment and in particular in the rhizosphere (Chan et al. 2011). So far, the details of QS processes taking place in the diverse bacterial species and strains isolated from the mycorrhizosphere, and in particular from AMF spores, remain to be unravelled.

The aim of the present study was to assess the occurrence and extent of QS among bacteria living associated with AMF spores. To this aim, we screened 28 Gram-negative bacteria previously isolated from the spores of *Rhizophagus intraradices* IMA6, for AHL production, using two different QS reporter strains, *Agrobacterium tumefaciens* NTL4 pZRL4 (sensitive to medium, long chain AHLs) and *Chromobacterium violaceum* CV026 (sensitive to short chain AHLs). In addition, using the latter reporter, we evaluated the ability of 9 Gram-positive bacteria isolated from the same ecological niche, to interfere with QS, a process generally described as ‘quorum quenching’ (QQ), by assessing their ability to degrade AHLs.

MATERIALS AND METHODS

Bacteria

The bacteria utilized in this work were previously isolated from the *Rhizophagus intraradices* IMA6 sporosphere (Battini et al. 2016b) and maintained in the collection of the Microbiology Labs of the Department of Agricultural, Food and Environment, University of Pisa, Italy (International Microbial Archives, IMA). Among the heterotrophic isolates showing the mucoid morphotype and originating from TSA medium (tryptic soy agar) 28 Gram-negative bacteria were screened for AHL production. For AHL degradation, 9 Gram-positive bacteria morphologically ascribed to Bacillaceae were selected. All the bacterial isolates were molecularly analysed by 16S rRNA gene sequencing (Supplementary material, SM, Tables 1 and 2), except four of them (TSA3, TSA26, TSA41, TSA50), which had been previously identified (Battini et al. 2016b).

Extraction of total DNA, PCR analysis and sequencing

Genomic DNA was extracted from bacterial liquid cultures grown overnight at 28 °C using "MasterPureTM Yeast DNA Purification Kit" (Epicentre®) following the manufacturer's protocols. Bacterial isolates were identified based on 16S rDNA sequencing, as reported by Battini et al. (2016b).

Nucleotide sequence accession numbers

The sequences of 16S rRNA genes were submitted to the European Nucleotide Archive (ENA) under the accession numbers from LT984816 to LT984840 and from LT984844 to LT984851.

Screening for AHL-producing bacteria

The 28 Gram-negative bacteria were screened for AHL production using the AHL reporter strains *Agrobacterium tumefaciens* NTL4 pZRL4 and *Chromobacterium violaceum* CV026 by microtiter plate assays (McClean et al. 1997; Shaw et al. 1997; Trovato et al. 2014). Bacteria were grown at 28 °C overnight with continuous shaking (120 rpm) on LB broth until the exponential growth phase was reached and then centrifuged at 7,500 rpm for 10 min. A volume of 1 mL of supernatant was transferred to a 1.5 mL Eppendorf tube and stored at -20 °C. Two mL of *A. tumefaciens* NTL4 preculture were inoculated in a 50 mL tube containing 18 mL of AB liquid medium (3 g/L K_2HPO_4, 1 g/L NaH_2PO_4, 1 g/L NH_4Cl, 0.3 g/L $MgSO_4$, 0.15 g/L KCl, 0.01 g/L $CaCl_2$, 2.5 m g/L $FeSO_4$, 0.5% glucose) supplemented with gentamycin (30 µg/mL) and incubated at 28 °C with continuous shaking (120 rpm) for 24 h. A volume of 16.75 mL of bacterial culture was mixed with AB agar (33.25 mL, 0.7% Agarose I; Euroclone) containing 5-Bromo-4-chloro-3-indolyl b-d-galactopyranoside (X-Gal; 20 mg/mL) and gentamycin (30 µg/mL) previously melted and cooled at 43 °C. Aliquots of 200 µL of *A. tumefaciens*/AB agar mixture were poured in each of the wells of a sterile 96-well microtiter plate (Cellstar, Greiner bio-one, Kremsmuenster, Austria). Upon solidification, 10 µL of overnight culture supernatant grown and harvested as described above, were dispensed over the agar in the wells and incubated for 24 h at 30 °C. Negative control wells contained 10 µL of sterile LB growth medium, while the medium amended with 10 µL of a 10 ng/µL solution of N-octanoyl-L-homoserine lactone (OHL; Fluka Chemie GmbH Buchs, Switzerland) and subsequent fivefold stepwise dilutions, was used as positive control. Bacteria able to produce AHL could be identified by the activation of the reporter strains through blue coloration. Digital images of the results were acquired directly on an Epson Perfection 1240U flatbed digital scanner. The bacterial isolates were further screened for the production of AHL by plate assay on TSA agar using the bioreporter strain *C. violaceum* CV026. Briefly, the isolates were streaked against the reporter strain in a perpendicular manner, incubated at 28 °C for 24 h and observed for violet colouration due to the induction of violacein pigment in the reporter strain. *C. violaceum* CV026 streaked against itself was used as a negative control. Isolates testing positive to the qualitative tests were subsequently analyzed for semi-quantitative AHL production by the same microtiter assay upon performing serial 10:1 serial dilutions of the supernatants.

AHL degradation assay

The QQ activity was assessed in solid plate assays carried out with the AHL biosensor *C. violaceum* CV026 (McClean et al. 1997; Romero et al. 2011). The 9 bacterial isolates were grown in 1 mL of TSA at 28 °C and 200 rpm for 24 h. A volume of 40 µL of a stock solution (50 µg/mL) of N-octanoyl-L-homoserine lactone (OHL; Fluka Chemie GmbH Buchs, Switzerland) were added to achieve a final concentration of 2 µg/mL, and incubated for further 24 h. In order to detect the inhibition of OHL activity, 50 µL of the supernatants were spotted in wells made in TSA plates overlaid with 5 mL of a 1:100 dilution of an overnight culture of *C. violaceum* CV026 in soft TSA (0.8% agar). Sterile water, sterile TSA growth medium and OHL were used as negative controls. The formation of halo zones around wells indicated the capacity of the bacteria to degrade OHLs after 24 h, eliminating the violacein production.

RESULTS

Identification of bacteria

Among the 37 bacteria analysed, 33 of them were 16S-sequenced and affiliated to genus and species using BLAST, together with the four bacteria previously sequenced (Battini et al. 2016b). The 25 Gram-negative bacteria belonged to *Sinorhizobium meliloti* (Table S1), while the 8 Gram-positive ones were affiliated to *Bacillus* and *Fictibacillus* spp. (Table S2).

Screening for AHL-producing bacteria (Quorum sensing)

Most of the isolates were found to be quorum-sensing positive in one or both AHL assays, but they showed different response patterns to the two AHL reporter strains. Specifically, *A. tumefaciens* NTL4 detected 23 out 28 bacteria (82%) as AHL producers, while *C. violaceum* CV026 detected 14 out of 28 bacteria (50%) as AHL producers. The bacteria unable to produce AHL (*S. meliloti* TSA 22, 95, 99, 107, 137), as revealed by both reporter strains, were not further investigated. According to the colour pattern observed in the semi-quantitative bioassay performed with *A. tumefaciens* NTL4, 15 out of 23 (65%) and 8 out of 23 (35%) of the tested bacteria showed medium/high and low production of signal molecules, respectively (Table 1). The semi-quantitative test did not reveal short signal molecules for 9 (39%) of the rhizobia tested, consistently with the qualitative assay (Table 1). It is interesting to note that 16S rDNA sequence analysis detected two clusters, corresponding with the level of AHL production (high versus low producers). Accordingly, the non-short chain AHL producing strains, as assessed by *C. violaceum* CV026, grouped together in the second cluster (Fig.

1). The sequences of the tested bacteria were aligned, revealing two base changes, C→T at positions 1026 and 1146, referring to the 16S rRNA gene of *Escherichia coli*.

AHL degradation assay (Quorum quenching)

The reporter strain AHL biosensor *C. violaceum* CV026, producing violacein in response to the presence of short-chain AHLs, revealed that none of the tested Gram-positive bacteria was capable of interfering with AHL activity in the plate bioassay.

DISCUSSION

This work provides the first evidence of the ability of rhizobia living associated with AMF spores to communicate through the production of extracellular small diffusible signalling molecules, able to regulate their behaviour at the community level, as most of the tested isolates showed the quorum sensing phenotype.

The 28 tested bacteria utilized in this work, previously isolated from AMF spores, belonged to *S. meliloti*. Their occurrence in association with AMF may be ascribed to their ability to produce exopolysaccharides and to form biofilms, thus allowing an efficient colonization of roots and mycorrhizal hyphae (Toljander et al. 2006). It is important to note that, beyond biological nitrogen fixation, rhizobia produce phytohormones, improving plant nutritional status and biocontrolling phytopathogens (Chandra et al. 2007). In particular, some of the *S. meliloti* isolates tested here - TSA3, TSA26 and TSA41 - show multiple functional PGP traits, such as siderophore and IAA production, P solubilization and phytate mineralization (Battini et al. 2016b).

A high percentage of isolates showed the QS phenotype (82%) and 6 produced very high levels of the signal molecules AHLs, revealing the large extent of the phenomenon within the rhizobia isolated from AMF spores. Our data on the abundance of AHL-producing bacteria are higher than those reported by previous studies performed on bacteria isolated from the rhizosphere of wheat (8%), tomato (12%), tobacco (ca. 20%) and ginger (12%) (Chan et al. 2011; D'Angelo-Picard et al. 2004; Pierson et al. 1998; Steidle et al. 2001). This can be explained taking into account that the rhizobia tested here were isolated from a peculiar and specialised ecological niche - AMF spores - where the QS phenotype may represent an important factor for their establishment and maintenance. Moreover, such isolates belong to a species, *S. meliloti*, that has been reported to produce seven compounds with N-acyl-L-homoserine lactone signalling activity (Cha et al. 1998).

Here, we report for the first time that the bacteria showing different QS activity differed in their 16S rDNA sequences, a trait to be further investigated in order to develop a possible molecular marker for the rapid identification of high AHL-producers.

The potential importance of the QS phenotype in the modulation of key PGP functions may be suggested by the P solubilisation and phytate mineralization ability of some of the tested strains, one of which, *S. meliloti* TSA26, improved root P content in maize plants (Battini et al. 2017). Indeed, diverse rhizobial strains have been reported to secrete organic anions chelating cations bound to phosphate and to produce phytase/phosphatase enzymes (Owen et al. 2015).

Here, none of the tested Gram-positive bacteria was capable of interfering with AHL activity, i.e. of disrupting the QS signalling, suggesting the absence of antagonism towards the rhizobia living in the same ecological niche, the AMF sporosphere. This is an interesting finding, as the bacteria tested belonged to the species *Bacillus*, widely studied for its efficient QQ activity (Grandclément et al. 2015); actually, the QQ trait is a strain characteristic and it is feasible that the coexistence of bacteria showing QS and QQ activities in the same niche, entails the exclusion of competitive strains, in favour of commensal/neutral ones.

In conclusion, our work provides the first evidence of AHL production in rhizobia living associated with AMF spores. As the secretion of such compounds, able to regulate the QS behaviour, occurred in most of the bacteria analyzed, we propose that it may represent an important mechanism allowing them to become established in the mycorrhizosphere, where they may be functionally complementary to AMF, in the promotion of plant nutrition and health. Further investigations will reveal whether the bacteria producing the highest AHL levels show also the best functional traits, in order to select the appropriate AMF/bacteria consortia to be utilized in sustainable and innovative food production systems, where soil biological fertility and natural biogeochemical cycles are protected and maintained.

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REFERENCES

Agnolucci M, Battini F, Cristani C, Giovannetti M (2015) Diverse bacterial communities are recruited on spores of different arbuscular mycorrhizal fungal isolates. Biol Fertil Soils 51:379–389. https://doi.org/10.1007/s00374-014-0989-5

Alonso ML., Kleiner D, Ortega E (2008). Spores of the mycorrhizal fungus *Glomus mosseae* host yeasts that solubilize phosphate and accumulate polyphosphates. Mycorrhiza 18:197-204. https://link.springer.com/article/10.1007/s00572-008-0172-7

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. Mycorrhiza 6:457–464. https://doi.org/10.1007/s005720050

Azcón-Aguilar C, Barea JM (2015) Nutrient cycling in the mycorrhizosphere. J Soil Sci Plant Nutr 25:372-396. https://dx.doi.org/10.4067/S0718-95162015005000035

Barea JM, Azcón R, Azcón-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van Leeuwenhoek 81:343–351. https://doi.org/10.1023/A:1020588701325

Battini F, Bernardi R, Turrini A, Agnolucci M, Giovannetti M (2016a) *Rhizophagus intraradices* or its associated bacteria affect gene expression of key enzymes involved in the rosmarinic acid biosynthetic pathway of basil. Mycorrhiza 26:699-707. https://doi.org/10.1007/s00572-016-0707-2

Battini F, Cristani C, Giovannetti M, Agnolucci M (2016b) Multifunctionality and diversity of culturable bacterial communities strictly associated with spores of the plant beneficial symbiont *Rhizophagus intraradices*. Microbiol Res 183:68–79. https://doi.org/10.1016/j.micres.2015.11.012

Battini F, Grønlund M, Agnolucci M, Giovannetti M, Jakobsen I (2017) Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. Sci Rep. https://doi.org/10.1038/s41598-017-04959-0

Cha C, Gao P, Chen Y-C, Shaw PD, Farrand SK (1998) Production of Acyl-Homoserine Lactone Quorum-Sensing Signals by Gram-Negative Plant-Associated Bacteria. Mol Plant-Microbe Interact 11:1119–1129. https://doi.org/10.1094/MPMI.1998.11.11.1119

Chan KG, Atkinson S, Mathee K, Sam CK, Chhabra SR, Cmara M, Koh CL, Williams P (2011) Characterization of N-acylhomoserine lactone-degrading bacteria associated with the *Zingiber officinale* (ginger) rhizosphere: Co-existence of quorum quenching and quorum sensing in *Acinetobacter* and *Burkholderia*. BMC Microbiol. https://doi.org/10.1186/1471-2180-11-51

Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). Brazilian J Microbiol 38:124–130. https://doi.org/10.1590/S1517-83822007000100026

D’Angelo-Picard C, Faure D, Carlier A, Uroz S, Raffoux A, Fray R, Dessaux Y (2004) Bacterial populations in the rhizosphere of tobacco plants producing the quorum-sensing signals hexanoyl-homoserine lactone and 3-oxo-hexanoyl-homoserine lactone. FEMS Microbiol Ecol 51:19–29. https://doi.org/10.1016/j.femsec.2004.07.008

Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: The LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176:269–275. https://doi.org/10.1128/jb.176.2.269-275.1994

Grandclément C, Tannières M, Moréra S, Dessaux Y, Faure D (2015) Quorum quenching: Role in nature and applied developments. FEMS Microbiol Rev 40:86–116. https://doi.org/10.1093/femsre/fuv038

Hartmann A, Schikora A (2012) Quorum Sensing of Bacteria and Trans-Kingdom Interactions of N-Acyl Homoserine Lactones with Eukaryotes. J Chem Ecol 38:704–713. https://doi.org/10.1007/s10886-012-0141-7

Long L, Zhu H, Yao Q, Ai Y (2008) Analysis of bacterial communities associated with spores of *Gigaspora margarita* and *Gigaspora rosea*. Plant Soil. https://doi.org/10.1007/s11104-008-9611-7

McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, Daykin M, Lamb JH, Swift S, Bycroft BW, Stewart GSAB, Williams P (1997) Quorum sensing and *Chromobacterium violaceum*: Exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiology 143:3703–3711. https://doi.org/10.1099/00221287-143-12-3703

Miller MB, Bassler BL (2001) Quorum Sensing in Bacteria. Annu Rev Microbiol 55:165–199. https://doi.org/10.1146/annurev.micro.55.1.165

Owen D, Williams AP, Griffith GW, Withers PJA (2015) Use of commercial bio-inoculants to increase agricultural production through improved phosphrous acquisition. Appl Soil Ecol 86:41–54. https://doi.org/10.1016/j.apsoil.2014.09.012

Pierson Ea, Wood DW, Cannon Ja, Blachere FM, Pierson LS (1998) Interpopulation Signaling via N-Acyl-Homoserine Lactones among Bacteria in the Wheat Rhizosphere. Mol Plant-Microbe Interact 11:1078–1084. https://doi.org/10.1094/MPMI.1998.11.11.1078

Roesti D, Ineichen K, Braissant O, Redecker D, Wiemken A, Aragno M (2005) Bacteria associated with spores of the arbuscular mycorrhizal fungi *Glomus geosporum* and *Glomus constrictum*. Appl Environ Microbiol 71:6673–6679. https://doi.org/10.1128/AEM.71.11.6673-6679.2005

Romero M, Martin-Cuadrado AB, Roca-Rivada A, Cabello AM, Otero A (2011) Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. FEMS Microbiol Ecol 75:205–217. https://doi.org/10.1111/j.1574-6941.2010.01011.x

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Pascale S, De Bonini P, Colla G (2015) Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci Hortic 196:91–108. https://doi.org/10.1016/j.scienta.2015.09.002

Shaw PD, Ping G, Daly SL, Cha C, Cronan JE, Rinehart KL, Farrand SK (1997) Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. Proc Natl Acad Sci 94:6036–6041. https://doi.org/10.1073/pnas.94.12.6036

Steidle A, Sigl K, Schuhegger R, Ihring A, Schmid M, Gantner S, Stoffels M, Riedel K, Givskov M, Hartmann A, Langebartels C, Eberl L (2001) Visualization of N-Acylhomoserine Lactone-Mediated Cell-Cell Communication between Bacteria Colonizing the Tomato Rhizosphere. Appl Environ Microbiol 67:5761–5770. https://doi.org/10.1128/AEM.67.12.5761-5770.2001

Toljander JF, Artursson V, Paul LR, Jansson JK, Finlay RD (2006) Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. FEMS Microbiol Lett 254:34–40. https://doi.org/10.1111/j.1574-6968.2005.00003.x

Trovato A, Seno F, Zanardo M, Alberghini S, Tondello A, Squartini A (2014) Quorum vs. diffusion sensing: A quantitative analysis of the relevance of absorbing or reflecting boundaries. FEMS Microbiol Lett 352:198–203. https://doi.org/10.1111/1574-6968.12394

TABLES

Table 1 Semi-quantitative AHL production in rhizobia isolated from *Rhizophagus intraradices* spores, as assessed by the microtiter assay upon performing 10:1 serial dilutions of the supernatants, using the reporter strains *Agrobacterium tumefaciens* NTL4 and *C. violaceum* (CV026).

Isolates	Reporter strain	
	NTL4	CV026
S. meliloti TSA1	++++	++
S. meliloti TSA3	+++	++
S. meliloti TSA6	+++	++
S. meliloti TSA10	++++	+/-
S. meliloti TSA11	+++	+/-
S. meliloti TSA24	++++	++
S. meliloti TSA26	+++	++
S. meliloti TSA27	+++	++
S. meliloti TSA28	++++	++
S. meliloti TSA29	+++	++
S. meliloti TSA41	+++	+
S. meliloti TSA42	+++	+/-
S. meliloti TSA45	++++	++
S. meliloti TSA91	+	-
S. meliloti TSA94	+	-
S. meliloti TSA96	+	-
S. meliloti TSA98	+	-
S. meliloti TSA100	+	-
S. meliloti TSA101	+	-
S. meliloti TSA102	++	-
S. meliloti TSA105	+	-
S. meliloti TSA106	+	-
S. meliloti TSA139	++++	++

NTL4: *A. tumefaciens* NTL4 (pZLR4); CV026: *C. violaceum* CV026.

- no production; +/− = scarce production; + = low production; ++ = medium production; +++/++++ = high production.

FIGURE LEGEND

Fig. 1 Affiliation of the sequences of the bacteria isolated from spores of *Rhizophagus intraradices* IMA6 with the existing 16S rRNA gene sequences, using Neighbor-Joining method based on the kimura 2-parameter method. Bootstrap (1000 replicates) values below 50 are not shown. Evolutionary analyses were conducted in MEGA6. The DNA sequences retrieved in this work are indicated by their isolate code and accession numbers.

Figure

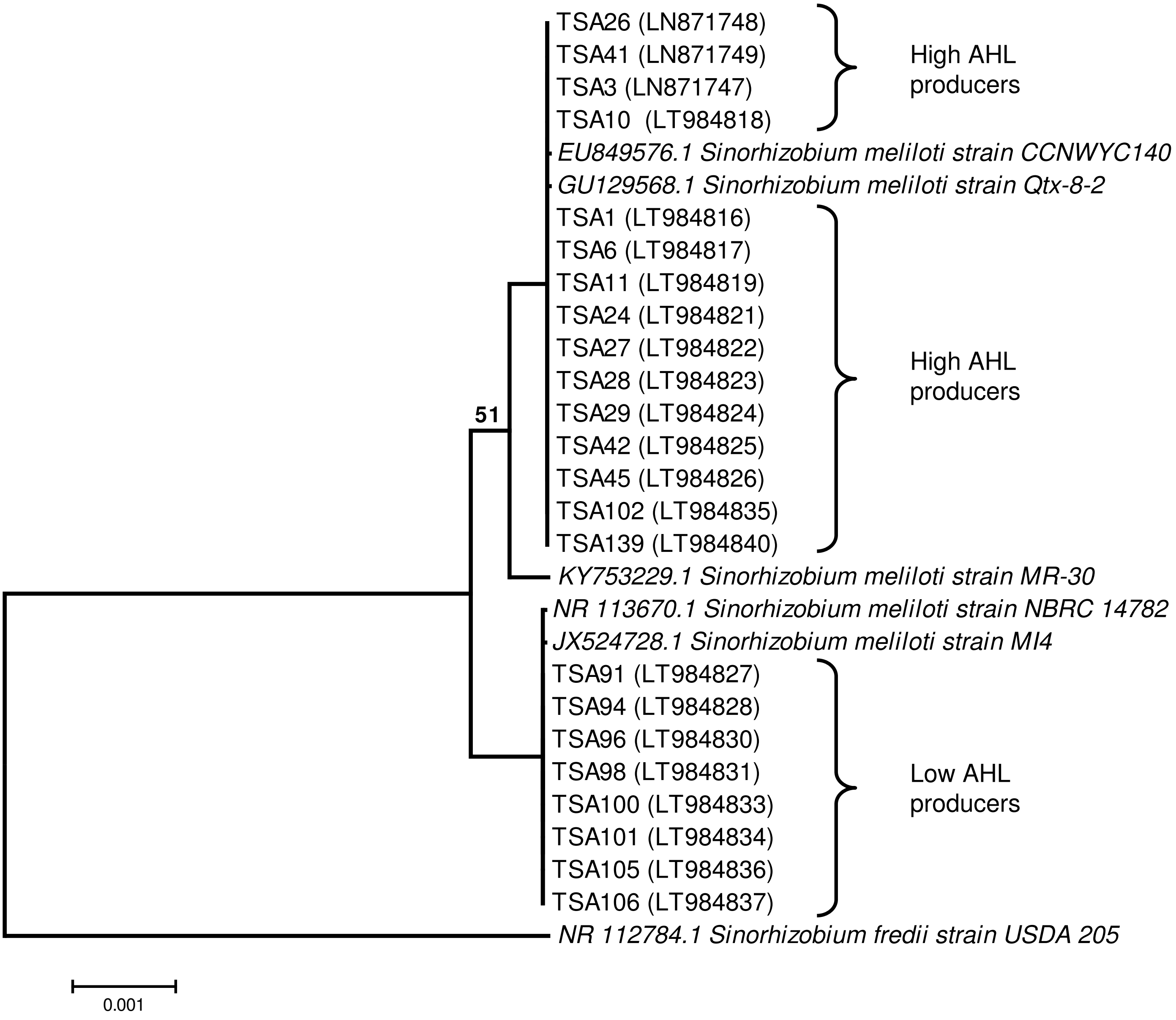
TSA26 (LN871748)
TSA41 (LN871749)
TSA3 (LN871747)
TSA10 (LT984818)
High AHL producers
EU849576.1 Sinorhizobium meliloti strain CCNWYC140
GU129568.1 Sinorhizobium meliloti strain Qtx-8-2
TSA1 (LT984816)
TSA6 (LT984817)
TSA11 (LT984819)
TSA24 (LT984821)
TSA27 (LT984822)
TSA28 (LT984823)
TSA29 (LT984824)
TSA42 (LT984825)
TSA45 (LT984826)
TSA102 (LT984835)
TSA139 (LT984840)
High AHL producers
51
KY753229.1 Sinorhizobium meliloti strain MR-30
NR 113670.1 Sinorhizobium meliloti strain NBRC 14782
JX524728.1 Sinorhizobium meliloti strain MI4
TSA91 (LT984827)
TSA94 (LT984828)
TSA96 (LT984830)
TSA98 (LT984831)
TSA100 (LT984833)
TSA101 (LT984834)
TSA105 (LT984836)
TSA106 (LT984837)
Low AHL producers
NR 112784.1 Sinorhizobium fredii strain USDA 205
0.001

Fig. 1