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Mesorhizobium australicum sp. nov. and *Mesorhizobium opportunistum* sp. nov., isolated from *Biserrula pelecinus* L. in Australia

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Abstract

Biserrula pelecinus L. is a pasture legume that was introduced to Australia from the Mediterranean basin in 1993. Although the native rhizobial population could not nodulate *B. pelecinus* at the time of its introduction, recent research has shown the emergence of a diversity of strains (novel isolates) that are able to do so. Three novel isolates, WSM2073[T], WSM2074 and WSM2076, had nearly identical 16S rRNA gene sequences, and clustered separately with all recognized species of the genus *Mesorhizobium*. Conversely, the novel isolate WSM2075[T] had >23 nt mismatches with the above three isolates. All four novel isolates shared 97–99% 16S rRNA gene sequence similarity with the type strains of all recognized *Mesorhizobium* species. However, strains WSM2073[T], WSM2074 and WSM2076 showed <95.2% *dnaK* gene sequence similarity to the type strains of recognized *Mesorhizobium* species, and <92.9% to WSM2075[T] (which also shared <95.5% *dnaK* gene sequence similarity to the type strains of recognized *Mesorhizobium* species). Results for *GSII* gene sequencing were consistent with those for the *dnaK* gene. The fatty acid profiles of the novel isolates were diagnostic of root-nodule bacteria, but did not match those of recognized bacterial species. Strain WSM2075[T] had a significantly different fatty acid profile from the other three isolates. The above results indicated that strains WSM2073[T], WSM2074 and WSM2076 represent the same species. Strain WSM2073[T] showed <45% DNA–DNA relatedness and WSM2075[T] <50% DNA–DNA relatedness with the type strains of recognized *Mesorhizobium* species; these two novel isolates shared 59% DNA–DNA relatedness. Collectively, these data indicate that strains WSM2073[T], WSM2074 and WSM2076, and strain WSM2075[T] belong to two novel species of the genus *Mesorhizobium*, for which the names *Mesorhizobium australicum* sp. nov. and *Mesorhizobium opportunistum* sp. nov. are proposed, respectively. The type strain of *Mesorhizobium australicum* sp. nov. is WSM2073[T] (=LMG 24608[T]=HAMBI 3006[T]) and the type strain of *Mesorhizobium opportunistum* sp. nov. is WSM2075[T] (=LMG 24607[T]=HAMBI 3007[T]).

Neighbour-joining phylogenetic trees based on *dnaK* and *GSII* gene sequences showing the positions of the novel isolates WSM2073[T], WSM2074, WSM2075[T] and WSM2076, *Mesorhizobium ciceri* bv. *biserrulae* isolated from the Mediterranean basin and other root-nodule bacteria in the *Alphaproteobacteria*, together with tables detailing differential phenotypic characteristics of the novel species described here and recognized species of the genus *Mesorhizobium*, and levels of 16S rRNA gene sequence similarity between strains WSM2073[T] and WSM2075[T] and type strains of *Mesorhizobium* species are available as supplementary material with the online version of this paper.

Biserrula pelecinus L. is an important pasture species in sustainable farming systems in Australia because it is one of few deep-rooted, acid-tolerant forage legume species with the potential to reduce the development of dry-land salinity (Howieson *et al.*, 2000; Loi *et al.*, 2005). We have reported recently that root nodule isolates from *B. pelecinus* growing in the Mediterranean basin, the centre of origin for this species (Allen & Allen, 1981), belong to *Mesorhizobium ciceri* biovar *biserrulae* (Nandasena *et al.*, 2007b). *B. pelecinus* was introduced to Australia in 1993, in regions where resident rhizobia failed to nodulate with it. However, we have recently shown the rapid evolution of nodulating bacterial strains for *B. pelecinus* in Australia via the *in situ* lateral transfer of a symbiosis island (a genomic island carrying genes required for nodulation and nitrogen fixation). The symbiosis island was transferred from the original inoculant strain *Mesorhizobium ciceri* bv. *biserrulae* WSM1271 to other soil bacteria (Nandasena *et al.*, 2006). The recipient strains of the symbiosis island are hereafter referred to as novel isolates. This manuscript reports on the taxonomic status of four of these novel isolates (WSM2073^T=N17^T, WSM2074=N18, WSM2075^T=N45^T and WSM2076=N87).

The novel isolates WSM2073^T, WSM2074 and WSM2076 formed finger-like, indeterminate nodules on *B. pelecinus* that fixed N$_2$ poorly (Nandasena *et al.*, 2007a). The symbiotic genes of these three isolates were located on a mobile symbiosis island, providing the ability to nodulate *B. pelecinus*, *Astragalus membranaceus* and *Macroptilium atropurpureum*. The three isolates had nearly identical sequences for a 1440 bp internal region of the 16S rRNA gene (DNA amplification for the 16S rRNA gene and sequencing methods were as described by Nandasena *et al.*, 2007a), with only 1 nt mismatch among them. In the phylogenetic tree based on 16S rRNA gene sequences the novel isolates clustered together within the genus *Mesorhizobium* but were separate from all recognized species of this genus (Fig. 1). The three isolates were most closely related to *Mesorhizobium ciceri* bv. *biserrulae*, having 6 nt mismatches (99.5% 16S rRNA gene sequence similarity) and were most distantly related to *Mesorhizobium thiogangeticum* SJT^T (97.1%) (Table 1).

A fourth novel isolate, WSM2075^T, formed small white nodules on *B. pelecinus* that were completely ineffective at fixing N$_2$. The symbiotic genes of this organism were also located on a mobile symbiosis island, but provided a broader range of hosts for nodulation, including *B. pelecinus*, *Astragalus adsurgens*, *A. membranaceus*, *Lotus peregrinus* and *Macroptilium atropurpureum*. Strain WSM2075^T had a markedly different 16S rRNA gene sequence, with >23 nt mismatches with the other three novel isolates (Nandasena *et al.*, 2007a). Strain WSM2075^T clustered with *Mesorhizobium huakuii* MAFF303099 (99.8% 16S rRNA gene sequence similarity; Table 1, Fig. 1).

All four novel isolates shared 97–99% 16S rRNA gene sequence similarity with the type strains of recognized species of the genus *Mesorhizobium* (Table 1). Brenner *et al.* (2005) stated that a '16S rRNA gene sequence similarity of less than 97% between strains indicates that they represent different species, but at 97% or higher 16S rRNA gene sequence similarity, DNA–DNA relatedness must be used to determine whether strains
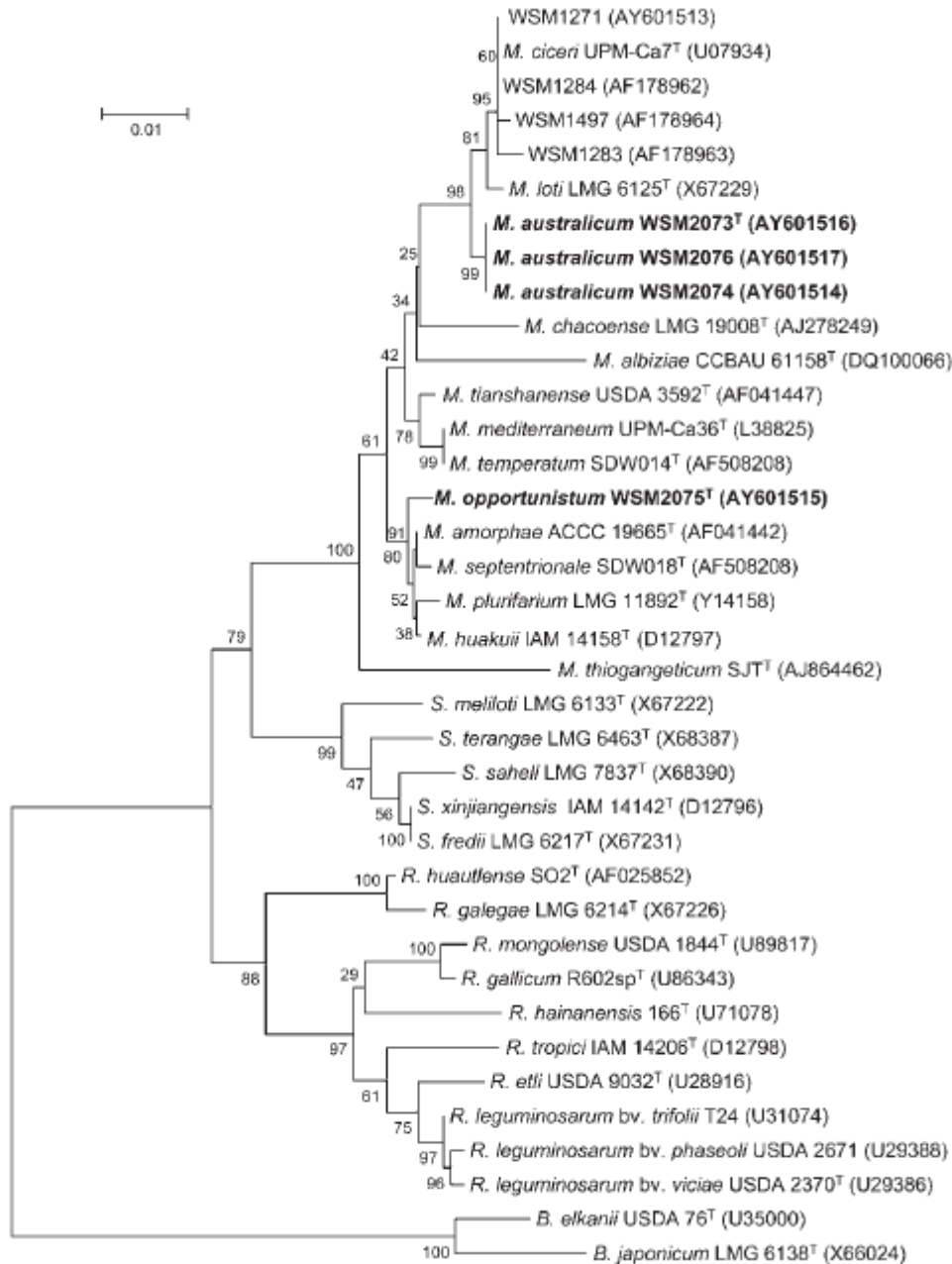
Fig. 1. Molecular phylogeny based on 16S rRNA gene sequences obtained from novel isolates WSM2073[T], WSM2074, WSM2075[T] and WSM2076, and *Mesorhizobium ciceri* bv. *biserrulae* WSM1271, WSM1283, WSM1284 and WSM1497, and other root-nodule bacteria in the *Alphaproteobacteria*. A sequence of 1440 bp was analysed. All sites were informative and there were no gap-containing sites. Sequences were aligned by using the clustal w program in the Wisconsin package of the Genetics Computer Group (Madison, WI, USA) and a phylogenetic analysis was conducted by using mega version 3.1 (Kumar *et al.*, 2004). Kimura two-parameter distances were derived from aligned sequences (Kimura, 1980) and bootstrap analysis (Felsenstein, 1985) was undertaken with 500 replicates to construct consensus unrooted trees via the neighbour-joining method (Saitou & Nei, 1987). Abbreviations: *B*, *Bradyrhizobium*; *M*, *Mesorhizobium*; *R*, *Rhizobium*; *S*, *Sinorhizobium*. Bar, 0.01 changes per nucleotide position.

Table 1. Levels of 16S rRNA and *dnaK* gene sequence similarity, DNA G+C content and levels of DNA–DNA relatedness for strains WSM2073^T and WSM2075^T and recognized species of the genus *Mesorhizobium*

NA, Data not available.

Strain	DNA G+C content (mol%)	WSM2073^T			WSM2075^T			Reference
		16S rRNA gene sequence similarity (%)	*dnaK* gene sequence similarity (%)	DNA–DNA relatedness (%)	16S rRNA gene sequence similarity (%)	*dnaK* gene sequence similarity (%)	DNA–DNA relatedness (%)	
WSM2073^T	62.96	–	–	100	98.4	93.2	59	This study
WSM2075^T	63.22	98.4	93.2	59	–	–	100	This study
M. ciceri bv. Biserrulae WSM1271	62.70	99.5	89.6	NA	98.3	91.0	NA	Nandasena et al. (2007b)
M. amorphae LMG 18977^T	63.37	98.4	91.1	37	99.6	91.9	40	Wang et al. (1999)
M. chacoense LMG 19008^T	62.04	98.0	88.4	16	98.3	89.0	17	Velázquez et al. (2001)
M. ciceri LMG 14989^T	64.34	99.3	89.9	35	98.1	91.4	36	Nour et al. (1994b, 1995)
M. huakuii LMG 14107^T	63.49	98.1	95.2	45	99.4	95.4	49	Chen et al. (1991)
M. huakuii MAFF303099	NA	98.7	91.4	NA	99.8	93.9	NA	Kaneko et al. (2000)
M. loti LMG 6125^T	62.70	99.3	91.4	45	98.1	93.5	50	Jarvis et al. (1982)
M. mediterraneum LMG 17148^T	62.45	98.3	85.4	36	99.3	88.9	35	Nour et al. (1994b, 1995)
M. plurifarium LMG 11892^T	62.83	98.0	86.8	42	99.3	88.2	47	de Lajudie et al. (1998)
M. tianshanense LMG 18976^T	62.51	98.4	85.7	30	99.2	88.6	32	Jarvis et al. (1997)
M. septentrionale LMG 23930^T	63.12	98.4	90.9	36	99.6	92.5	40	Gao et al. (2004)
M. temperatum LMG 23931^T	62.22	98.4	91.6	29	99.4	93.6	28	Gao et al. (2004)
M. thiogangeticum LMG 22697^T	60.72	97.1	86.9	11	98.1	87.2	11	Ghosh & Roy (2006)
M. albiziae LMG 23507^T	59.00	98.1	80.0	NA	98.5	82.9	NA	Wang et al. (2007)

belong to different species'. Therefore, two housekeeping genes, *dnaK* (encoding a highly conserved chaperone protein with multiple cellular functions; Stepkowski *et al.*, 2003) and *GSII* (encoding glutamine synthetase; Turner & Young, 2000), were sequenced for the four novel isolates. Levels of DNA–DNA relatedness between strains WSM2073^T and WSM2075^T and the type strains of recognized *Mesorhizobium* species were determined. DNA amplification and sequencing methods for the above two genes were as described by Nandasena *et al.* (2007a).

The novel isolates WSM2073^T, WSM2074 and WSM2076 shared >97.7% sequence similarity (<8 nt mismatches) with each other for a 300 bp intragenic fragment of the *dnaK* gene, but <95.2% sequence similarity to the type strains of recognized

Mesorhizobium species. Although these three strains were most closely related to *Mesorhizobium ciceri* bv. *biserrulae* based on their 16S rRNA gene, they showed highest *dnaK* gene sequence similarity with the type strain of *Mesorhizobium huakuii* (Table 1) and clustered close to this species in the phylogenetic tree developed based on Kimura two-parameter distance values (Kimura, 1980) (Supplementary Fig. S1 in IJSEM Online). Interestingly, the *dnaK* gene sequence of strain WSM2075[T] was <92.9% similar to those of the other three novel isolates, and this isolate was <95.5% similar to the type strains of all *Mesorhizobium* species tested based on *dnaK* gene sequences. Strain WSM2075[T] was also phylogenetically most closely related to *Mesorhizobium huakuii*, a result consistent with that obtained for 16S rRNA gene sequence phylogeny (Table 1 and Supplementary Fig. S1 available in IJSEM Online).

The novel isolates WSM2073[T], WSM2074 and WSM2076 shared >98.7% sequence similarity (<9 nt mismatches) with each other for a 600 bp intragenic fragment of the *GSII* gene (Nandasena *et al.*, 2007a) but showed <90.7% *GSII* gene sequence similarity to strain WSM2075[T]. In the phylogenetic tree based on *GSII* gene sequences with Kimura two-parameter distance values (Kimura, 1980) strains WSM2073[T], WSM2074 and WSM2076 clustered together but were distant and separate from other sequenced species of root-nodule bacteria (Supplementary Fig. S2, in IJSEM Online). These three strains were phylogenetically most closely related to *Rhizobium etli* whereas strain WSM2075[T] clustered close to *Mesorhizobium loti* (Supplementary Fig. S2). Turner & Young (2000) have reported the possible lateral transfer of the *GSII* gene amongst genera of root-nodule bacteria. Therefore, the above clustering pattern of the novel isolates is not surprising. Although *GSII* gene sequences may not be definitive for determining phylogenetic relationships of root-nodule bacteria, the above results indicate that the novel isolates belong to two separate species that have gained their *GSII* gene independently from different bacterial genera.

Cellular fatty acid profiles can provide a definitive tool in the identification of root-nodule bacteria and this analysis was undertaken for all four novel isolates as described by Tighe *et al.* (2000). Growth conditions, harvesting and extraction procedures were identical for all strains according to methods described by Sasser (1990). The fatty acids of these isolates included straight-chain saturated fatty acids, unsaturated fatty acids, 11-methyl branched unsaturated fatty acids and 3-hydroxy fatty acids (Table 2). These isolates also synthesized terminally iso-branched fatty acids in higher amounts than *Mesorhizobium ciceri* bv. *biserrulae*, but less of the 10-methyl branched fatty acids (Table 2). Although the fatty acid patterns of the novel isolates were diagnostic of root-nodule bacteria in general, these isolates could not be assigned to any of the recognized root-nodule bacterial species when their fatty acid patterns were compared with all the bacterial strains available in the Microbial Identification System fatty acid database. Furthermore, strain WSM2075[T] had a significantly different fatty acid profile to the other three novel isolates (Table 2).

The carbon source utilization patterns for 14 compounds (*N*-acetylglucosamine, arabinose, arbutin, dulcitol, *β*-gentiobiose, lactose, maltose, melibiose, raffinose, sucrose, l-sorbose, d-tagatose, trehalose and turanose), antibiotic resistance [ampicillin (50 μg ml^{-1}), chloramphenicol (40 μg ml^{-1}), gentamicin (40 μg ml^{-1}), kanamycin (50 μg ml^{-1}), nalidixic acid (50 μg ml^{-1}), spectinomycin (50 μg ml^{-1}), streptomycin (100 μg ml^{-1}) and tetracycline (20 μg ml^{-1})] and pH range for growth for the novel isolates were reported previously (Nandasena *et al.*, 2007a). These phenotypic characteristics were compared with the data available for all recognized *Mesorhizobium* species (Supplementary Table S1, in IJSEM Online), and comparisons between the species that are phylogenetically most closely related to the novel isolates based on molecular methods are given in Table 3. Differences in carbon source utilization were observed for raffinose, dulcitol and melibiose for strains WSM2073^{T} and WSM2075^{T} and the type strains of *Mesorhizobium ciceri*, *Mesorhizobium ciceri* bv. *biserrulae*, *Mesorhizobium huakuii* and *Mesorhizobium loti* (Table 3). Differences were also observed for resistance to gentamicin amongst the above strains (Table 3).

The results obtained for 16S rRNA, *dnaK* and *GSII* gene sequencing and cellular fatty acid profiles strongly suggest that strains WSM2073^{T}, WSM2074 and WSM2076 belong to one novel species of the genus *Mesorhizobium*, and that WSM2075^{T} may belong to another separate species within this genus. DNA–DNA hybridization experiments were performed [microplate method described by Ezaki *et al.* (1989) as modified by Goris *et al.* (1998)] with the type strains of 11 nodulating *Mesorhizobium* species to verify the above hypotheses (Table 1). Levels of DNA–DNA relatedness between WSM2073^{T} and WSM2075^{T} and the type strains of recognized *Mesorhizobium* species were <45 and <50%, respectively (Table 1). Strains WSM2073^{T} and WSM2075^{T} shared 59% DNA–DNA relatedness. These data clearly indicate that these two isolates belong to two separate novel species (Brenner *et al.*, 2005).

At the time of writing, the genus *Mesorhizobium* comprised 12 recognized species, 11 of which (*Mesorhizobium albiziae*, *M. amorphae*, *M. chacoense*, *M. ciceri*, *M. huakuii*, *M. loti*, *M. mediterraneum*, *M. plurifarium*, *M. septentrionale*, *M. temperatum* and *M. tianshanense*) have been shown to form nitrogen-fixing symbiotic associations with leguminous plants (Wang *et al.*, 2007; Gao *et al.*, 2004; Garrity, 2005; Ghosh & Roy, 2006). The type strains of the above 11 species share >97.8% 16S rRNA gene sequence similarity (Supplementary Table S2, in IJSEM Online). Levels of *dnaK* gene sequence similarity are significantly lower and cover a wider range (80.0–96.2%). However, the type strains of two pairs of these species shared even higher *dnaK* gene sequence similarity: *Mesorhizobium mediterraneum* and *Mesorhizobium temperatum* (98.2%) and *Mesorhizobium amorphae* and *Mesorhizobium septentrionale* (98.1%). Consistent with previous reports (Stepkowski *et al.*, 2003; Ormeño-Orrillo *et al.*, 2006), our results indicate that the *dnaK* gene is a more discriminating marker for species distinction in the genus *Mesorhizobium* than is the 16S rRNA gene.

Table 2. Fatty acid profiles of the novel isolates and recognized species of the genus *Mesorhizobium*

Taxa: 1, *M. ciceri*; 2, *M. huakuii*; 3, *M. mediterraneum*; 4, *M. loti*; 5, *M. tianshanense*; 6, *M. plurifarium*; 7, *M. ciceri* bv. *biserrulae*; 8, strain WSM 2073^T; 9, strain WSM2074; 10, strain WSM2076; 11, strain WSM2075^T. Values are percentages of the total fatty acids. Values in parentheses indicate standard deviation. Bold type indicates differences in fatty acids between the novel isolates and recognized species of *Mesorhizobium*. nd, Not detected.

Fatty acid	1*	2*	3*	4*	5*	6*	7	8	9	10	11
$C_{12:0}$ 3-OH	0.27 (0.22)	0.23 (0.31)	0.11 (0.16)	0.34 (0.36)	0.09 (0.25)	0.20 (0.25)	0.53	0.46	0.52	0.25	0.35
iso-$C_{13:0}$ 3-OH	0.24 (0.23)	1.00 (0.26)	0.35 (0.28)	1.23 (0.35)	0.27 (0.38)	0.84 (0.43)	0.45	0.78	0.70	0.54	0.9314
Unknown (ECL 14.780)	0.16 (0.25)	ND	0.04 (0.15)	ND	ND	0.01 (0.06)	ND	ND	ND	ND	ND
$C_{15:0}$	0.02 (0.06)	ND	0.04 (0.13)	ND	ND	ND	0.10	**0.39**	**0.48**	**0.28**	ND
anteiso-$C_{15:0}$	ND	ND	ND	0.12 (0.51)	ND	ND	ND	ND	ND	ND	ND
iso-$C_{15:0}$	0.18 (0.23)	ND	0.13 (0.20)	0.13 (0.25)	0.16 (0.4)	ND	0.21	0.62	0.73	0.77	0.20
$C_{15:1}\omega8c$	ND	ND	ND	ND	ND	ND	0.21	ND	ND	ND	ND
$C_{16:0}$	13.41 (2.11)	15.83 (0.98)	10.29 (1.39)	14.51 (2.15)	12.25 (2.26)	11.60 (1.53)	12.56	11.7	11.62	9.73	11.95
$C_{17:0}$	1.29 (0.95)	0.42 (0.66)	1.71 (1.77)	0.50 (0.59)	0.83 (1.02)	0.69 (0.51)	2.14	1.98	2.23	1.74	1.61
iso-$C_{17:0}$	4.16 (0.69)	5.88 (1.13)	4.19 (1.14)	8.17 (1.93)	3.34 (0.68)	6.26 (0.77)	4.18	3.67	3.22	4.86	4.98
$C_{17:0}$ cyclo	0.84 (0.38)	0.70 (0.52)	0.85 (0.82)	0.69 (0.51)	0.16 (0.3)	0.12 (0.23)	0.58	**1.32**	**0.86**	**0.59**	ND
$C_{17:1}\omega6c$	ND	ND	ND	ND	0.11 (0.32)	0.09 (0.26)	ND	ND	ND	ND	**0.41**
$C_{17:1}\omega8c$	0.15 (0.23)	0.25 (0.40)	0.51 (0.65)	0.12 (0.31)	0.52 (0.77)	0.71 (0.50)	0.62	1.81	1.64	1.23	1.56
Unknown (ECL 17.875)	0.17 (0.52)	ND	0.50 (0.99)	ND	ND	ND	ND	ND	ND	ND	ND
$C_{18:0}$	3.25 (1.25)	4.10 (0.48)	4.21 (1.59)	4.50 (1.22)	4.99 (0.72)	2.99 (1.22)	2.65	1.85	2.36	2.47	2.04
11-methyl $C_{18:1}\omega7c$	11.92 (3.61)	4.82 (2.77)	5.57 (4.33)	11.14 (2.48)	9.99 (1.38)	ND	15.03	3.04	7.02	2.60	7.34
$C_{18:1}$ 2-OH	ND	0.11 (0.32)	ND	0.47 (0.62)	ND	ND	ND	**0.30**	**0.54**	**0.68**	ND
$C_{18:1}\omega5c$	ND	ND	0.06 (0.21)	ND	ND	ND	ND	ND	ND	ND	ND
$C_{18:1}\omega7c$	ND	ND	ND	ND	ND	ND	ND	**53.75**	**58.43**	**62.34**	**61.74**
$C_{18:1}\omega9c$	0.12 (0.15)	ND	ND	0.34 (0.42)	ND	ND	0.38	ND	**0.28**	**0.28**	ND
Unknown (ECL 18.794)	0.21	ND	0.32 (0.49)	0.08 (0.18)	0.11 (0.31)	0.25 (0.35)	ND	ND	ND	ND	ND
$C_{19:0}$	ND	ND	ND	ND	ND	ND	0.23	**0.41**	**0.54**	**0.66**	ND
iso-$C_{19:0}$	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0.22**	ND
$C_{19:0}$ cyclo $\omega8c$	37.27 (8.48)	22.96 (6.05)	33.35 (11.05)	21.93 (8.17)	12.37 (4.31)	11.33 (5.67)	35.29	14.66	8.99	7.29	4.10
10-methyl $C_{19:0}$	0.18 (0.28)	1.16 (0.45)	0.08 (0.19)	1.23 (0.45)	0.16 (0.44)	1.28 (0.56)	0.81	1.03	0.80	0.66	0.99
$C_{20:0}$	0.05 (0.11)	0.04 (0.15)	ND	0.07 (0.19)	ND	0.04 (0.10)	0.27	0.39	0.56	1.24	0.37
$C_{20:1}\omega9t$	0.14 (0.22)	ND	0.46 (0.40)	0.15 (0.26)	ND	0.10 (0.16)	ND	ND	ND	ND	ND
$C_{20:2}\omega6,9c$	0.95 (0.68)	0.04 (0.15)	0.77 (0.44)	0.05 (0.15)	ND	ND	0.87	**0.40**	**0.45**	ND	ND
$C_{20:1}\omega7c$	ND	ND	ND	ND	ND	ND	ND	**0.51**	**0.52**	**0.66**	**0.38**

Table 3. Differential phenotypic characteristics between the novel species described herein and closely related species of the genus *Mesorhizobium*

Species: 1, *M. ciceri* (data from Nour *et al.*, 1994a, b; Velázquez *et al.*, 2001); 2, *M. huakuii* (Chen *et al.*, 1991; Velázquez *et al.*, 2001); 3, *M. loti* (Jarvis *et al.*, 1982; Velázquez *et al.*, 2001); 4, *M. ciceri* bv. *biserrulae* (Nandasena *et al.*, 2001, 2007a, b); 5, *M. australicum* sp. nov.; 6, *M. opportunistum* sp. nov. d, Differences observed between different strains of the same species; nd, not determined.

Characteristic	1	2	3	4	5	6
Monotrichous flagellation	ND	+	+	+	ND	ND
Colony diameter (mm) (incubation time)	2–4 (3–5 days)	2–4 (5–6 days)	1 (7 days)	2–4 (4–5 days)	2–4 (3–4 days)	2–4 (3–4 days)
Generation time (h)	<6	4–6	ND	4–6	4–6	4–6
Maximum growth temperature (°C)	40	37	39	ND	ND	ND
Maximum NaCl tolerance for growth (%, w/v)	2	1	2	1.5	ND	ND
pH range for growth	5–10	5–9.5	4–10	5.5–8	5.5–9	5.5–9
DNA G+C content (mol%) (T_m)	63–64	59–64	59–64	62.7	62.9	63.2
Arbutin	ND	ND	ND	+	d	+
DL-Arabinose	+	+	+	+	+	+
Raffinose	−	+	d	d	d	−
D-Tagatose	ND	ND	ND	+	d	+
Turanose	+	ND	ND	d	d	+
Dulcitol	ND	−	ND	+	−	−
Lactose	+	+	+	d	d	+
Maltose	+	+	+	d	d	+
Melibiose	+	−	+	+	+	+
N-Acetylglucosamine	+	ND	ND	+	d	+
Sorbose	ND	ND	ND	−	−	−
Sucrose	+	+	+	d	d	+
Trehalose	+	+	+	d	d	+
β-Gentiobiose	ND	ND	ND	+	+	+
Ampicillin	−	−	−	−	−	−
Chloramphenicol	+	ND	ND	−	−	−
Gentamicin	+	−	−	+	+	+
Kanamycin	ND	ND	ND	+	+	+
Nalidixic acid	+	ND	ND	+	+	+

The considerable differences observed for the phenotypic and genotypic characteristics between the two groups of novel isolates suggest that they represent two novel species of the genus *Mesorhizobium*. We propose the name *Mesorhizobium australicum* sp. nov. for strains WSM2073[T], WSM2074 and WSM2076, and the name *Mesorhizobium opportunistum* sp. nov. for strain WSM2075[T].

Description of *Mesorhizobium australicum* sp. nov.

Mesorhizobium australicum (au.stra.li′cum. N.L. neut. adj. *australicum* pertaining to Australia, from where these bacteria were isolated).

Cells are Gram-negative rods. Moderately fast-growing, forming colonies of 2–4 mm in diameter within 3–4 days on half-strength lupin agar (½LA; Howieson *et al.*, 1988) and have a mean generation time of 4–6 h when grown in ½LA broth at 28 °C. Colonies on ½LA are white, opaque, slightly domed, moderately mucoid, with smooth margins. Able

to tolerate a pH range between 5.5 and 9.0. Utilizes arabinose, β-gentiobiose and melibiose as sole source of carbon. With the exception of strain WSM2074, is also able to utilize maltose, sucrose and trehalose but not dulcitol or l-sorbose. Sensitive to ampicillin ($50\,\mu g\,ml^{-1}$), chloramphenicol ($40\,\mu g\,ml^{-1}$), spectinomycin ($50\,\mu g\,ml^{-1}$), streptomycin ($100\,\mu g\,ml^{-1}$) and tetracycline ($20\,\mu g\,ml^{-1}$), but resistant to gentamicin ($40\,\mu g\,ml^{-1}$), kanamycin ($50\,\mu g\,ml^{-1}$) and nalidixic acid ($50\,\mu g\,ml^{-1}$). Synthesizes the following fatty acids: $C_{12:0}$ 3-OH, iso-$C_{13:0}$ 3-OH, $C_{15:0}$, iso-$C_{15:0}$, $C_{16:0}$, $C_{17:0}$, iso-$C_{17:0}$, $C_{17:0}$ cyclo, $C_{17:1}\omega 8c$, $C_{18:0}$, 11-methyl $C_{18:1}\omega 7c$, $C_{18:1}$ 2-OH, $C_{18:1}\omega 7c$, $C_{19:0}$, $C_{19:0}$ cyclo $\omega 8c$, 10-methyl $C_{19:0}$, $C_{20:0}$ and $C_{20:1}\omega 7c$. Some strains contain a non-symbiotic plasmid ($\sim 500\,kb$). The DNA G+C content of the type strain is 62.96 mol% (HPLC).

The type strain, WSM2073[T] (=LMG 24608[T]=HAMBI 3006[T]), was isolated from nodules on *Biserrula pelecinus* L.

Description of *Mesorhizobium opportunistum* sp. nov.

Mesorhizobium opportunistum (op.por.tu.nis′tum. L. neut. adj. *opportunistum* after the opportunistic behaviour of the organism in obtaining symbiotic genes from other root-nodule bacteria and its ability to nodulate a broad range of legume hosts).

Cells are Gram-negative rods. Moderately fast-growing, forming colonies of 2–4 mm in diameter within 3–4 days on ½LA and have a mean generation time of 4–6 h when grown in ½LA broth at 28 °C. Colonies on ½LA are white, opaque, slightly domed, moderately mucoid, with smooth margins. Able to tolerate a pH range between 5.5 and 9.0. Utilizes *N*-acetylglucosamine, arabinose, arbutin, β-gentiobiose, lactose, maltose, melibiose, sucrose, d-tagatose, trehalose and turanose as sole source of carbon, but not dulcitol, raffinose or l-sorbose. Sensitive to ampicillin ($50\,\mu g\,ml^{-1}$), chloramphenicol ($40\,\mu g\,ml^{-1}$), spectinomycin ($50\,\mu g\,ml^{-1}$), streptomycin ($100\,\mu g\,ml^{-1}$) and tetracycline ($20\,\mu g\,ml^{-1}$), but resistant to gentamicin ($40\,\mu g\,ml^{-1}$), kanamycin ($50\,\mu g\,ml^{-1}$) and nalidixic acid ($50\,\mu g\,ml^{-1}$). Synthesizes the following fatty acids: $C_{12:0}$ 3-OH, iso-$C_{13:0}$ 3-OH, iso-$C_{15:0}$, $C_{16:0}$, $C_{17:0}$, iso-$C_{17:0}$, $C_{17:1}\omega 8c$, $C_{18:0}$, 11-methyl $C_{18:1}\omega 7c$, $C_{18:1}\omega 7c$, $C_{19:0}$ cyclo $\omega 8c$, 10-methyl $C_{19:0}$, $C_{20:0}$ and $C_{20:1}\omega 7c$. Does not contain any plasmids. The DNA G+C content of the type strain is 63.22 mol% (HPLC).

The type strain, WSM2075[T] (=LMG 24607[T]=HAMBI 3007[T]), was isolated from nodules on *Biserrula pelecinus* L.

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