Labrenzia salina sp. nov., isolated from the rhizosphere of the halophyte *Arthrocnemum macrostachyum*

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Running title: *Labrenzia salina* sp. nov.

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ABSTRACT

A novel, halophilic, motile rod, Gram-negative and non-endospore forming bacterium, designated Cs25^T, was isolated in a tidal flat from the rhizosphere of the halophyte *Arthrocnemum macrostachyum*. Strain Cs25^T was observed to be catalase negative and oxidase positive, and to hydrolyze hipoxantine. Growth occurred from 15-40 ºC, at pH 7.0-10.0 and with 1-11 % (w/v) NaCl. Q-10 was identified as the dominant ubiquinone and the major cellular fatty acids were $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{20:1}\omega7c$ and $C_{18:0.}$ The polar lipids comprised phosphatidylmonomethylethanolamine, phosphatidylcholine, sulphoquinovosyldiacylglyceride, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The 16S rRNA gene showed 99.19 %, 98.6 % and 98.59 % sequence identity with *L. alba* DSM18320^T, *L. aggregata* DSM13394^T and *L. marina* DSM17023^T, respectively. Based on the phenotypic and molecular features and DNA-DNA hybridization data, it is concluded that strain Cs25^T represents a novel species for which the name of *Labrenzia salina* is proposed. The type strain is Cs25^T (= DSM 29163^T = CECT 8816^T)

Keywords *Labrenzia,* rhizosphere, taxonomy.

The genus *Labrenzia,* whose type species is *L. alexandrii* (Biebl *et al*., 2007), comprises five salt-dependent validly-named species isolated from marine habitats being found as free-living microorganisms as associated to marine dinoflagellates, oysters and halophytes. The members of the genus are characterised by the presence of the glycolipid sulphoquinovosyldiacylglyceride (SQDG), their ability to synthesize the photosynthetic pigment bacterochlorophyll a (bchl a; Biebl *et al.,* 2007), although Bibi *et al.* (2014) described that both features may be variable among species, and the presence of the gene encoding carbon monoxide (CO) dehydrogenase (Biebl *et al.,* 2007). Chemotaxonomic profiles and molecular phylogenetic studies enabled the taxonomic reclassification of three of species in the genus: *Agrobacterium aggregatum* described by Ahrens (1968), later on reclassified as *Stappia aggregata* (Uchino *et al*., 1998) and finally transferred to the genus as *L. aggregata* by Biebl *et al.* (2007), *S. alba* originally described by Pujalte *et al.* (2005) and then reclassified as *L. alba* (Biebl *et al*., 2007) and *S. marina* (Kim *et al*., 2006) reclassified as *L.*

marina (Biebl *et al*., 2007). In addition, *L. suaedae* was also described as a novel species within the genus by Bibi *et al*. (2014).

The strain Cs25^T was isolated in 2013 in Lebrija, Seville, Spain (36º 64' 29" N - 6º 12' 92" W) during a study on plant growth promoting bacteria from the rhizosphere of the halophyte *Arthrocnemum macrostachyum,* a C3 shrup typically found in ecosystems characterized by pouring annual rainfall and changes in temperature and wind direction (El-Morsy, 2010) at the coasts of the southwestern Iberian Peninsula, the Mediterranean region, and the Middle East and Asia (Redondo-Gómez *et al*., 2010) known as salt marshes. This study, based on a polyphasic approach, refers the taxonomic position of Cs25^T, a halophilic strain representing a novel species within the genus *Labrenzia* isolated from a rhizosphere soil sample of the plant *A. macrostachyum*.

Rhizosphere soil samples were suspended and homogenised in sterile mineral buffer (0.5 g PO_4HK_2, 0.2 g SO_4Mg .$7H_2O$, 0.1 g ClNa and 0.05 g Cl_2Ca in 1 l distilled water; Vincent, 1970). Serial dilutions were plated on Nutrient Agar (NA, Scharlau^{TM} : 1 g meat extract, 2 g yeast extract, 5 g peptone, 5 g NaCl in 1 l distilled water, plus 16 g/l agar) supplemented with 17,5 g/l NaCl (NA-salt) and cycloheximide (100 mg/l), adjusted to pH 7.4. The inoculated plates were incubated at 28 ºC for 5 days. Strain Cs25^T was firstly isolated based on colony morphology and purified by sub-culturing on NA-salt plates. The culture was suspended in 0.5 % peptone and 15 % (w/v) glycerol for preservation at – 80 ºC.

The colony morphology and pigmentation of strain Cs25^T were observed on NA-salt after 5 days under a binocular microscope according to Pelczar (1957). Exponentially growing bacterial cultures were observed with an optical microscope (Zeiss AxioScope A1) with a 100-fold magnification and phase-contrast illumination. Micrographs of bacterial cells grown on Marine Broth (MB) for 5 days were taken with a field-emission scanning electron microscope (FE-SEM Merlin, Zeiss, Germany). Motility was detected by stabbing a wire loop on tubes containing NA-salt medium with 0.3 % agar and was considered positive when a hazy zone was observed around the stab line. Gram staining was performed according to Halebian *et al.* (1981) and checked by the KOH test (Gregersen, 1978). Activity of oxidase was analysed using filter-paper disks (Sartorius grade 388) impregnated with 1 % solution of N,N,N',N'-tetramethyl-*p*-phenylenediamine (Sigma-Aldrich); a positive test was indicated by the immediate development of a blue-purple colour after applying biomass on the filter paper. Catalase activity was tested by the observation of bubbles following the addition of drops of 3

% H_2O_2. Anaerobic growth was assessed on NA-salt and NA-salt supplemented with potassium nitrate (0.2 %) by incubation in an anaerobic pouch at 28 ºC for 20 days. Growth rates were determined on NA-salt plates for temperatures from 0 to 50 °C in steps of 5 °C and in NB-salt tubes adjusted to pH 4.0-11.0 (in steps of 1.0 pH units) using the organic buffers homopipes, MOPS and Tris at concentration of 20 mM each, for pH 4-5, 6-7 and 8-9, respectively, and 0.05 M Na_2HPO_4 / 0.1 M NaOH buffer for pH 10-11. To determine the requirements for Na^+, Mg^{2+} and Ca^{2+}, a peptone-yeast extract-glucose medium was used as a base containing 5 g bacto peptone (Difco), 1 g bacto yeast extract (Difco), 10 g glucose, 0.1 g ferric citrate, 5.9 g $MgCl_2.7H_2O$ and 1.8 g $CaCl_2.2H_2O$ in 1 l distilled water at pH 7.6. Growth, if any, was observed with or without either 2 % (w/v) NaCl or equimolar KCl to examine Na^+ requirement and with 2 % (w/v) NaCl added with $MgCl_2$, $CaCl_2$ or both omitted to examine requirements for Ca^{2+} and/or Mg^{2+}. Tolerance to sodium chloride was tested on NA (0.5 % NaCl) supplemented with 0.5-11.5 % (w/v) NaCl for a final NaCl concentration of 1-12 %. Degradation of specific substrates was examined using agar plates with various basal media, where results were considered as positive with the appearance of clear zones around the colonies: casein degradation was tested on medium containing skimmed milk (5 %) and NaCl (2 %) solidified with 1 % agarose; tyrosine degradation was investigated on plates containing peptone (0.5 %), beef extract (0.3 %), L-tyrosine (0.5 %), NaCl (2%) and agarose (1.5 %); the decomposition of xanthine and hypoxanthine was investigated by the same test, replacing L-tyrosine by hypoxanthine or xanthine (0.4 %), respectively; starch degradation was tested on plates containing nutrient broth (0.8 %), starch (1 %), NaCl (2 %) and agarose (1.5 %), developing these plates by flooding with iodine solution (1 %) after incubation for 5 days. Enzyme activities of strain Cs25^T were tested using API ZYM galleries according to the instructions of the manufacturer (bioMérieux) preparing the cell suspensions in API NaCl 0.85 % Medium. The oxidation of carbon compounds and resistance to inhibitory chemicals of strain Cs25^T were determined using GEN III Microplates incubated in an Omnilog device (BIOLOG Inc., Hayward, CA, USA) in comparison with the reference strains *L. alba* DSM 18320^T, *L. aggregata* DSM 13394^T and *L. marina* DSM 17023^T in parallel assays. The GEN III Microplates were inoculated with a cell suspension made in the viscous inoculating fluid (IF) A at a cell density of 96 % transmittance (T), adding NaCl to a final concentration of 1 %, for *L. alba* DSM 18320^T, and yielding a running time of 5 days in Phenotype Microarray mode at 28 °C. The exported data were further analysed with the `opm` package for R (Vaas *et al.*, 2012, 2013) v.0.9.23., statistically estimating parameters from the respiration curves such as the maximum height, and automatically “discretizing” these values into negative and

positive reactions. All the tests were run in two independent determinations. Contradictory results between the two repetitions were regarded as ambiguous.

The extraction of cellular fatty acids was carried out from biomass grown on MA plates held at 28 °C for 3 days and harvested always from the same sector (the second and third quadrant streak). Analysis was conducted using the Microbial Identification System (MIDI) Sherlock Version 6.1 (results evaluated against the TSBA40 peak naming table database) as described by Sasser (1990). Biomass used to extract polar lipids and quinones was grown on MB medium. Polar lipids were extracted and separated by two-dimensional thin-layer chromatography (TLC) according to Bligh and Dyer (1959) and Tindall *et al.* (2007) and identified by Minnikin *et al.* (1984) as modified by Kroppenstedt & Goodfellow (2006). Respiratory lipoquinones were extracted from freeze-dried cell material with methanol:hexane as described by Tindall (1990 a, b), separated into their functional classes by TLC and analysed by reverse phase HPLC (Tindall, 1990 a, b). Analysis of cellular fatty acids, polar lipids and respiratory lipoquinones were carried out using the reference strains listed above in parallel experiments.

The G + C content of the chromosomal DNA was determined by HPLC according to Mesbah *et al.* (1989). Chromosomal DNA was isolated with Wizard Genomic DNA Purification Kit (Promega). The 16S rRNA coding gene was amplified by PCR using universal primers 8f and 1522r (Giovannoni, 1991) at an annealing temperature of 55.0 °C. The sequencing of purified DNA was performed by BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems) as directed by the manufacturer´s protocol and ABI PRISM 3730 DNA analyser (Applied Biosystems). Sequence data were edited and assembled manually using BIOEDIT (Sequence Alignment Editor) v.7.0.5 (Hall, 1999). Phylogenetic analysis was carried out according to Montero-Calasanz *et al.* (2012). Pairwise similarities were calculated as recommended by Meier-Kolthoff *et al.* (2013). DNA–DNA hybridization tests were performed in double reciprocal analysis as described by De Ley *et al.* (1970) with the modifications suggested by Huss *et al.* (1983) using a Cary 100 Bio UV/VIS.

The presence of the CO dehydrogenase (*coxL)* gene of strain Cs25^T was amplified using the forwards primers OMPf and BSMf, and the reverse OMPr, as described by King (2003). Amplification of the *pufLM* genes was carried out following the protocol of Kim *et al* (2006) to detect the presence of genes related to the synthesis of BChl a.

Cells of strain Cs25^T were Gram-staining-negative motile rods of 1-2.5 x 0.5-1 µm which tended to form aggregates as previously described for the species *L. alexandrii* (Biebl *et al*., 2007), *L. alba* (Pujalte *et al*., 2005) and *L. marina* (Kim *et al*., 2006) (Supplementary Fig 1). Strain Cs25^T grew best at 15-40 °C on NA-salt and MA, not growing below 15 °C and above 40 °C. On NA-salt and MA, at 28 ºC, colonies were light ivory coloured, convex and with entire margin. Colonies are 1.0-2.0 mm diameter (NA-salt and MA) after 3 days incubation at 28 ºC. Growth was observed at pH 7.0-10.0 and in the presence of 1-11 % NaCl. No growth is observed in 0.5 % of NaCl. Strain Cs25^T required Na^+, which could not be replaced by K^+. The isolate grew properly in peptone-yeast extract glucose medium containing 2.0 % NaCl in the presence of either Mg^{2+} or Ca^{2+}, but its growth was delayed in the absence of both. According to the BIOLOG System: it oxidises D-maltose, D-trehalose, D-cellobiose, sucrose, turanose, D-salicin, N-acetyl-D-glucosamine, D-glucose, D-mannose, D-fructose, D-fucose, L-fucose, L-rhamnose, sodium lactate, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, glycyl-L-proline, L-glutamic acid, L-pyroglutamic acid, D-gluconic acid, glucuronamide, mucic acid, D-saccharic acid, p-hydroxy-phenylacetic acid, L-lactic acid, citric acid, α-keto-glutaric acid, L-malic acid, β-hydroxy-butyric acid, acetoacetic acid, propionic acid and acetic acid, but not β-gentiobiose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl-neuraminic acid, D-galactose, 3-O-methyl-D-glucose, inosine, fusidic acid, D-aspartic acid, D and L-serine, gelatin, L-alanine, L-arginine, L-aspartic acid, L-histidine, guanidine hydrochloride, pectin, L-galactonic acid-ϒ-lactone, D-glucuronic acid, quinic acid, methyl pyruvate, D-lactic acid methyl ester, D-malic acid, bromo-succinic acid, tween 40, ϒ-amino-n-butyric acid, α-hydroxy-butyric acid, α-keto-butyric acid, sodium formate and butyric acid. The full phenotype microarrays obtained using the OmniLog device in comparison to the type strains of other *Labrenzia* species showed the distinction of strain Cs25^T from related species (Supplementary Fig. S2); a summary of some differential phenotypic characteristics is presented in Table 1.

The predominant respiratory lipoquinone found in strain Cs25^T was an ubiquinone with ten isoprene units (Q-10) at a peak area ratio of 77.7 %. This ubiquinona is characteristic in the most of the member of the *Alphaproteobacteria* and has been described in all validly named species of *Labrenzia* (Biebl *et al.,* 2007; Bibi *et al.,* 2014). Strain Cs25^T possesses another unindentified ubiquinona (retention time: 17,123 min) with a peak area ratio of 12 %. The polar lipids profile of strain Cs25^T consisted of the predominant compounds phosphatidylmonomethylethanolamine (PMME), phosphatidylcholine (PC),

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sulphoquinovosyldiacylglyceride (SQDG), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE). Similar patterns were observed for the reference strains investigated in this study (Figure 1), being in accordance with those revealed by Biebl *et al.* (2007) for the same reference strains. The unambiguous absence of PE observed in *L. alba* DSM 18320^T (Fig 1.c) and *L. marina* DSM 17023^T (Fig 1.d) could be hypothesised, as already commented by Biebl *et al.* (2007), as result of variations in the growth conditions or growth phase that may affect the relative composition of PE and PMME. Similar results regarding a latter modification of PE (hydroxylation) have shown to be related to the microbial growth phase in other phyla (unpublished data). According to all members of the *Alphaproteobacteria*, the unsaturated fatty acid $C_{18:1}\omega7c$ was the most abundant (55 %). A significant amount of 11-methyl $C_{18:1}\omega7c$ (19 %) was revealed in agreement with the polar lipids pattern but also $C_{20:1}\omega7c$ (11 %) and $C_{18:0}$ (7.5 %) (Table 2). Nevertheless, considering data obtained by GC/MS reported by Biebl *et al.* (2007), the annotation of 11-methyl $C_{18:1}\omega7c$ may be erroneous, being the correct configuration of the double bonds 11-methyl $C_{18:1}\omega6t$. The presence of 3-OH fatty acids was limited to $C_{14:0}$ 3-OH (2.3 ± 0.2 %) annotated as part of summed feature 2 ($C_{14:0}$ 3-OH/iso-$C_{16:1}$ I) but unambiguously detected by Biebl *et al.* (2007) in all the studied species. Similarly according to Montero-Calasanz *et al.* (2014) and the ECL (15.816) of summed feature 3 ($C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH), the detection of $C_{16:1}\omega7c$ should be unambiguously considered in the fatty acids profile of *L. marina*. The presence of this component was already described in such species by Biebl *et al.* (2007) and as a part of the summed feature 3 ($C_{16:1}\omega7c$ /$C_{16:1}\omega6c$) by Bibi *et al.* (2014).

The almost complete (1360 bp) 16S rRNA gene sequence of strain Cs25^T was determined and showed the highest similarity with the type strains of the species *L. alba* DSM18320^T (99.19 %), *L. aggregata* DSM13394^T (98.6 %) and *L. marina* DSM17023^T (98.59 %). Furthermore, the strain Cs25^T and the closest relatives were placed within the same phylogenetic group with maximum support by both maximum-likelihood and maximum-parsimony methods (Fig. 2). DNA-DNA hybridization between strain Cs25^T and its relative *S. alba* displayed a DNA-DNA relatedness of 18.4 ± 0.4 %. Additional DNA-DNA hybridizations were omitted based on the observations reported by Meier-Kolthoff *et al.* (2013), who statistically established a 16S rRNA threshold at 98.7 % with a maximum probability of error of 1.00 % to get DNA-DNA hybridization values above the 70 % threshold recommended by Wayne *et al.* (1987) to confirm the species status of novel strains.

Amplification of the *coxL* gene of strain Cs25^T yielded a fragment of, approximately, 1300 bp which corresponds with the form II *coxL* gene. This result suggests that strain Cs25^T may oxidize CO, as well as other members of the genus *Labrenzia* and the related genera *Stappia* (Biebl *et al*., 2007; Bibi *et al*., 2014) and *Roseibium* (Suzuki *et al*., 2000; Zhong *et al*., 2014). Conversely, no amplification was obtained for the *pufLM* genes, indicating that strain Cs25^T did not possess the photosynthetic reaction centre described for *L. alexandrii*, *L. marina* and *L. suaedae* (Biebl *et al*., 2007; Bibi *et al*., 2014) and members of the related genus *Roseibium* (Suzuki *et al*., 2000; Zhong *et al*., 2014). The genomic DNA G+C content of strain Cs25^T was 62.4 mol %.

Several phenotypic characteristics apart from the phylogenetic analysis based on 16S rRNA gene sequence support the distinctiveness of strain Cs25^T from other *Labrenzia* species. Based on the phenotypic and genotypic data presented above, we suggest that strain Cs25^T represents a novel species within the genus *Labrenzia*, for which the name *Labrenzia salina* sp. nov. is proposed.

Description of *Labrenzia salina* sp. nov.

Labrenzia salina (sa.li'na. L. fem. adj. *salina*, salty). Cells are Gram-staining-negative, strictly aerobic, non-spore-forming, motile, catalase negative and oxidase positive and require sodium ions for growth. Colonies are light ivory coloured on NA-salt and on MA. Sea salt-based medium is required to growth (1-11 % w/v). Growth occurs from 15 to 40 °C and pH 7.0 to 10.0. The oxidation of carbon compounds and resistance to inhibitory chemicals are shown in Table 1 and Supplementary Fig. 2. Positive for hypoxanthine degradation but negative for starch, tyrosine, xanthine and casein. In API ZYM test, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, ß-glucosidase and N-acetyl-ß-glucosamidase activities are positive but, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α- and ß-galactosidase, ß- glucuronidase, α-glucosidase, α-mannosidase and α-fucosidase activities are absent. The predominant ubiquinone is Q-10. The main polar lipids are phosphatidylmonomethylethanolamine, phosphatidylcholine, sulphoquinovosyldiacylglyceride, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Cellular fatty acids consist mainly of $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{20:1}\omega7c$ and $C_{18:0}$. The type strain has a genomic DNA G + C content of 62.4 mol %.

The type strain, Cs25^T (= DSM 28920^T = CECT8799^T), was isolated in 2013 from the rhizosphere of the halophyte *A. macrostachyum* in the Lebrija marshes (Seville), Spain. The INSDC accession number for the 16S rRNA gene sequence of strain Cs25^T is LN794846.

Acknowledgements

This research was supported by INIA Project RTA-2012-00006 co-funded by FEDER. Maria Camacho was the recipient of a Research Stay grant (Ref. Code A/13/09442) from the DAAD at the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany).

Appendix A. Supplementary data.

Supplementary data associated with this article can be found, in the online version, at <*to be added by the publisher*>.

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Figure 1. Polar lipids profile (labelled by the chromatographic mobility) of *Labrenzia rhizosphaerae* sp. nov. Cs25^T (a), *L. alba* DSM18320^T (b), *L. aggregata* DSM13394^T (c) and

L. marina DSM17023^T (d) after separation by two-dimensional TLC using the solvents chloroform:methanol:water (65:25:4, by vol.) in the first dimension and chloroform:methanol:acetic acid:water (80:12:15:4, by vol.) in the second one. Plates were sprayed with molybdatophosphoric acid (3.5 %; Merck^{TM}) for detection of the total polar lipids. PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PMME, phosphatidylmonomethylethanolamine; GL SQDG, glycolipid sulphoquinovosyldiacylglyceride; AL, unidentified aminolipid; L, unidentified lipid. All data are from this study.

Figure 2. Maximum likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain Cs25^T relative to the type strains within the genus *Labrenzia*. The branches are scaled in terms of the expected number of substitutions per site. Support values from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping are shown above the branches if equal to or larger than 60 %.

Table 1. Differential physiological and biochemical characteristics of strain Cs25^T and the type strains of closely related species of the genus *Labrenzia.* Strains: 1, *Labrenzia rhizosphaerae* Cs25^T sp. nov.; 2, *L. alba* DSM18320^T; 3, *L. aggregata* DSM13394^T; 4, *L. marina* DSM17023^T. All data are from this study, unless indicated. +, positive reaction; –, negative reaction, ND, not determined.

Characteristics	**1**	**2**	**3**	**4**
Isolation source	Halophyte	Oyster*[a]	Marine-sediment[b]	Seawater[d]
Colour of colonies	Sand yellow	Opaque[a]	White-cream[c]	Cream[d]
Cell morphology	Rods	Large Rods[a]	Large Rods, joined [c]	Club-shaped[d]
Growth at:				
0 % NaCl	-	-[a]	+[e]	-[d]
10 % NaCl	+	-[a]	+[e]	-[d]
37 ºC	+	-[a]	+[e]	+[e]
Presence of *pufLM* genes	-	ND	-[f]	+[f]
Activities				
Oxidase	+	+[a]	ND	+[d]

Catalase	-	+ [a]	ND	+[d]
Oxidation of (Gen III Microplates):				
β-Gentiobiose	-	+	+	+
D-Melibiose	-	+	+	-
N-Acetyl-D-Galactosamine	-	+	-	-
D-Galactose	-	+	+	+
D-Fucose	+	-	+	-
D-Arabitol	+	-	-	+
Glycerol	+	-	-	+
D-Glucose-6-Phosphate	+	-	+	-
glycyl-L-proline	+	-	-	-
L-Glutamic Acid	+	-	+	+
D-Gluconic Acid	+	-	-	-
Mucic Acid	+	-	-	-
D-Saccharic Acid	+	-	-	-
Methyl Pyruvate	-	-	+	+
DNA G+C content (mol %)	62.4	ND	59[c]	60[d]

*Data from : a, Pujalte *et al*. (2005); b, Ahrens, R. (1968); c, Uchino *et al*. (1998); d, Kim *et al*. (2006); e, Bibi *et al.* (2014); f, Biebl *et al*. (2007)

Table 2. Cellular fatty acids profiles (%) of strain Cs25^T and closely related species of the genus *Labrenzia*. Strains: 1, *Labrenzia rhizosphaerae* Cs25^T sp. nov.; 2, *L. alba* DSM18320^T; 3, *L. aggregata* DSM13394^T; 4, *L. marina* DSM17023^T. Fatty acids that represented <1.0 % in all strains are not shown. tr = Trace (< 1 %); -, not detected. All strains were grown on MA plates at 28 °C for 3 days. All data (Mean ± SD of two determinations) are from this study

Fatty acids (%)	**1**	**2**	**3**	**4**
Straight-chain:				
$C_{16:0}$	tr	tr	tr	1.3 ± 0.0
$C_{18:0}$	7.6 ± 0.1	6.8 ± 0.1	4.0 ± 0.1	8.8 ± 0.2
$C_{19:0}$ cyclo ω8c	2.8 ± 0.1	-	-	5.9 ± 0.3
Unsaturated:				

$C_{18:1}$ ω7c	55.0 ± 0.1	72.8 ± 0.0	75.6 ± 0.2	52.0 ± 0.1
$C_{20:1}$ ω7c	11.2 ± 0.3	9.3 ± 0.4	8.1 ± 0.3	6.1 ± 0.1
11-Methyl $C_{18:1}$ ω7c#	19.0 ± 0.0	7.1 ± 0.0	8.0 ± 0.1	13.7 ± 0.3
Hydroxy:				
$C_{18:0}$ 3-OH	tr	tr	tr	1.1 ± 0.0
Summed features*				
2 ($C_{14:0}$ 3-OH)	2.3 ± 0.2	2.2 ± 0.1	2.1 ± 0.0	1.9 ± 0.0
3 ($C_{16:1}$ω7c)	-	-	tr	7.7 ± 0.1

*Summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately (Montero-Calasanz *et al.*, 2013). Summed feature 2 listed as $C_{14:0}$ 3-OH / $C_{16:1}$ iso I and annotated here as $C_{14:0}$ 3-OH; Summed feature 3 listed as $C_{16:1}$ω7c/iso-$C_{15:0}$ 2-OH and annotated here as $C_{16:1}$ω7c. #This fatty acid may be incorrectly identified, see text for details and is recorded as 11-methyl $C_{18:1}$ω6t for *L. aggregata, L. alba*, *L. alexandrii* and *L. marina* by Biebl *et al.* (2007)

Supplementary Fig. S1. Scanning electron micrograph showing cells of strain Cs25^T grown on MB for 5 d at 28 °C.

Supplementary Fig. S2. The parameter "Maximum Height" estimated from the respiration curves as measured with the OmniLog phenotyping device and visualized as heatmap using the opm package v.8.0. Plates and substrates are rearranged according to their overall similarity (as depicted using the row and column dendrograms).