Chryseobacterium hispalense sp. nov., a plant-growth-promoting bacterium isolated from a rainwater pond in an olive plant nursery, and emended descriptions of Chryseobacterium defluvii, Chryseobacterium indologenes, Chryseobacterium wanjuense and Chryseobacterium gregarium

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A novel non-motile, Gram-staining-negative, yellow-pigmented bacterium, designated AG13[T], isolated from a rain water pond at a plant nursery in Spain and characterized as a plant-growth-promoting bacterium, was investigated to determine its taxonomic status. The isolate grew best over a temperature range of 15–40 °C, at pH 5.0–8.0 and with 0–4 % (w/v) NaCl. Chemotaxonomic and molecular characteristics of the isolate matched those described for members of the genus *Chryseobacterium*. The DNA G+C content of the novel strain was 37.2 mol%. The strain had a polyamine pattern with *sym*-homospermidine as the major compound and produced flexirubin-type pigments. MK-6 was the dominant menaquinone and the major cellular fatty acids were iso-$C_{15:0}$, $C_{17:1}\omega9c$ and iso-$C_{17:0}$ 3-OH. The main polar lipids were phosphatidylethanolamine, aminolipids and several unidentified lipids. The 16S rRNA gene showed 92.0–97.2 % sequence similarity with those of the members of the genus *Chryseobacterium*. Based on chemotaxonomic and phenotypic traits, and DNA–DNA hybridizations with the type strains of the most closely related species, the isolate is proposed to represent a novel species, *Chryseobacterium hispalense*, type strain AG13[T] (=DSM 25574[T]=CCUG 63019[T]). Emended descriptions of the species *Chryseobacterium defluvii*, *Chryseobacterium indologenes*, *Chryseobacterium wanjuense* and *Chryseobacterium gregarium* are also provided.

The genus *Chryseobacterium* (Vandamme *et al.*, 1994; Bernardet *et al.*, 2011), whose type species is *Chryseobacterium gleum* (Holmes *et al.*, 1984), is included within the family *Flavobacteriaceae* (emended by Bernardet *et al.*, 2002), the main bacterial lineage in the phylum *Bacteroidetes*. At the time of

writing, 65 species with validly published names were considered to be members of the genus *Chryseobacterium* according to the 'List of prokaryotic names with standing in nomenclature' (http://www.bacterio.net/c/chryseobacterium.html). Members of the genus *Chryseobacterium* can be found in a wide variety of environments, including freshwater (Strahan *et al.*, 2011), soil (Li & Zhu, 2012), rhizosphere (Cho *et al.*, 2010), phyllosphere (Behrendt *et al.*, 2007), sludge (Pires *et al.*, 2010), fish (Zamora *et al.*, 2012a, b, c), midgut of insects (Kämpfer *et al.*, 2010a), raw dairy products (Hantsis-Zacharov *et al.*, 2008), raw chicken (de Beer *et al.*, 2005), faeces of millipede (Kämpfer *et al.*, 2010b), industrial plants (Herzog *et al.*, 2008) and clinical samples (Holmes *et al.*, 1984).

Based on previous studies, members of the genus *Chryseo-bacterium* are considered to be an important bacterial group associated with plants (Lee *et al.*, 2006; Brown *et al.*, 2012; Anderson & Habiger, 2012) and currently there is enough evidence to show that strains of plant-associated species of the genus *Chryseobacterium* exhibit plant-growth-promoting activities (Shin *et al.*, 2007; Dardanelli *et al.*, 2010).

During the optimization of alternative systems to produce organically grown olive plants by using plant-growth-promoting bacteria in a commercial nursery located in Burguillos, Seville, Spain (37° 34′ 38.1714″ N 5° 58′ 18.5592″ W), in 2006, it was found that the percentage of rooted cuttings in the negative control (treated with water from a rainwater pond) was similar to the percentage of rooted cuttings in the positive control (treated with indolbutyric acid, IBA) (unpublished data). This observation suggested the existence of some microorganism in the rainwater pond that helped to root olive cuttings. This study, based on a polyphasic approach, describes the taxonomic position of a plant-growth-promoting bacterial strain, representing a novel species within the genus *Chryseobacterium*, isolated from a rainwater pond.

Filtered water samples (>0.55 mm particles) from the rainwater pond were serially diluted in sterile mineral buffer (Vincent, 1970) and inoculated on plate count agar (PCA; Difco) diluted to 50 % and NFb agar (Döbereiner, 1980) supplemented with cycloheximide (100 mg l^{-1}), adjusted to pH 7.0 and incubated at 28 °C for 5 days. Strain AG13^{T} was firstly isolated on the basis of colony morphology, purified by subculturing on trypticase soy agar (TSA; Difco) diluted to 10 % and then selected by its ability to produce indole-3-acetic acid (IAA), a desirable plant-growth-promoting feature, and to promote rooting in model plants and olive cuttings (Montero-Calasanz *et al.*, 2013). The culture was suspended in 0.5 % peptone and 15 % (w/v) glycerol for storage at −80 °C.

The colony morphology and pigmentation of strain AG13^{T} were observed on TSA after 24 and 72 h under a binocular microscope according to the protocol of Pelczar (1957). Exponentially growing bacterial cultures were observed with an optical microscope (AxioScope A1; Zeiss) with a 100-fold magnification and phase-contrast illumination. Micrographs of bacterial cells grown in trypticase soy broth (TSB) for 48 h were taken with a field-emission scanning

electron microscope (FE-SEM Merlin; Zeiss). Gram staining was performed according to the method of Halebian *et al.* (1981) and checked by the KOH test (Gregersen, 1978). Anaerobic growth was investigated by incubation in an anaerobic pouch (Merck) for 7 days at 28 °C on TSA. Activity of oxidase was analysed using filter-paper discs (grade 388; Sartorius) impregnated with 1 % solution of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (Sigma–Aldrich); a positive test was indicated by the development of a blue–purple colour after applying biomass to the filter paper. Catalase activity was tested by the observation of bubbles following the addition of drops of 3 % H$_2$O$_2$. The KOH test to assess whether the pigments produced by strain AG13^{T} were of the flexirubin type was performed according to the method of Bernardet *et al.* (2002). Temperature and pH tolerance of strain AG13^{T} were determined on plates of TSA at temperatures from 5 to 45 °C in steps of 5 °C and at pH 4.0–12.5 (in steps of 0.5 pH units) using the buffer system described by Xu *et al.* (2005). Degradation of specific substrates was examined using agar plates with various basal media, where results were considered to be positive by the appearance of clear zones around the colonies: casein degradation was tested on medium containing skimmed milk (5 %, w/v) and NaCl (0.5 %) solidified with 1 % agarose; tyrosine degradation was investigated as described by Gordon & Smith (1955) on plates containing peptone (0.5 %), beef extract (0.3 %), L-tyrosine (0.5 %) and agarose (1.5 %); the decomposition of xanthine and hypoxanthine was investigated by the same test, replacing L-tyrosine with hypoxanthine or xanthine (0.4 %); starch degradation was tested on plates containing nutrient broth (0.8 %), starch (1 %) and agarose (1.5 %), and developing these plates by flooding with iodine solution (1 %). Enzyme activities of strain AG13^{T} were tested using API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Other biochemical tests, such as cellulase activity (method 15.2.18), methyl red (method 15.2.52) and Voges–Proskauer reactions (method 15.2.82), were performed as described by Tindall *et al.* (2007). The utilization of carbon sources and acid production were determined using API 20NE and API 20E strips (bioMérieux) as well as using GEN III Microplates in an Omnilog device (Biolog). The GEN III Microplates were inoculated with a cell suspension made in a 'gelling' inoculating fluid (IF) at a cell density of 90 % transmittance, yielding a running time of 4 days in phenotype microarray mode at 28 °C. The exported data were further analysed with the opm package for R (Vaas *et al.*, 2012) v.0.9.23, using its functionality for merging subsequent measurements of the same plate, statistically estimating parameters from the respiration curves such as the maximum height and automatically 'discretizing' these values into negative and positive reactions. Comparison of strain AG13^{T} with the reference strains *Chryseobacterium defluvii* DSM 14219^{T}, *C. daecheongense* DSM 15235^{T}, *C. gleum* DSM 16776^{T}, *C. taiwanense* JCM 21767^{T}, *C. gregarium* DSM 19109^{T}, *C. indologenes* DSM 16777^{T}, *C. gambrini*

DSM 18014^T, *C. wanjuense* DSM 17724^T, *C. joostei* DSM 16927^T, *C. vrystaatense* DSM 25206^T, *C. luteum* DSM 18605^T, *C. shigense* DSM 17126^T, *C. jejuense* DSM 19299^T and *C. ureilyticum* DSM 18017^T was performed only in the GEN III Microplates, in two independent determinations. Reactions that gave contradictory results between the two repetitions were regarded as ambiguous.

The extraction and analysis of cellular fatty acids was carried out from biomass grown on TSA plates held at 28 °C for 24 h and harvested always from the same sector (the last quadrant streak) using the above-mentioned reference strains in parallel experiments. 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). Polar lipids were extracted and separated by two-dimensional TLC according to the protocols of Bligh & Dyer (1959) and Tindall *et al.* (2007) and identified according to the method of Minnikin *et al.* (1984) as modified by Kroppenstedt & Goodfellow (2006) using the reference strains *C. defluvii* DSM 14219^T, *C. gregarium* DSM 19109^T, *C. indologenes* DSM 16777^T and *C. wanjuense* DSM 17724^T in parallel for comparison. Respiratory lipoquinones were extracted from freeze-dried cell material with methanol/hexane as described by Tindall (1990a, b), separated into their functional classes by TLC and analysed by reversed-phase HPLC (Tindall, 1990a, b). Polyamines were extracted as reported by Busse & Auling (1988) and analysed according to the protocol of Stolz *et al.* (2007) using biomass cultivated in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract at pH 7.2, 28 °C with shaking at 180 r.p.m.) and harvested at 70 % of the maximum OD. The G+C content of the chromosomal DNA was determined by HPLC according to the method of Mesbah *et al.* (1989). Chromosomal DNA was isolated with a Wizard Genomic DNA Purification kit (Promega). The 16S rRNA gene was amplified by PCR using universal primers 27f and 1492r (Lane, 1991) at an annealing temperature of 55.5 °C. Amplified DNA fragments were cloned using a TOPO TA cloning kit 2.1 (Invitrogen). The sequencing of purified plasmid DNA was performed by using a BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems) as directed by the manufacturer's protocol and an 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 based on an alignment inferred with POA version 2.0 (Lee *et al.*, 2002) and filtered with GBLOCKS (Castresana, 2000). Phylogenetic trees were inferred under maximum-likelihood and maximum-parsimony as optimality criteria using RAxML version 7.2.8 (Stamatakis *et al.*, 2008) and PAUP* 4b10 (Swofford, 2002), respectively. Bootstrap support values were calculated using the bootstopping criterion (Pattengale *et al.*, 2009) as implemented in RAxML and 1000 replicates in the case of PAUP*. Rooting was done using the midpoint method (Hess & De Moraes Russo,

2007) and then checked for its agreement with the classification. Pairwise similarities were calculated from exact pairwise sequence alignments using the Smith–Waterman algorithm as implemented in the EMBOSS suite (Rice *et al.*, 2000). DNA–DNA hybridization experiments were performed in double 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 spectrophotometer (Agilent).

Cells of strain AG13^T were Gram-staining-negative, non-motile, (Fig. S1, available in IJSEM Online), non-spore-forming and strictly aerobic. The colonies were convex, circular and translucent with a shiny, smooth and mucoid surface and entire edges. A non-diffusible yellow–orange pigment of the flexirubin type was produced. Strain AG13^T grew best at 15–40 °C; no growth was observed below 10 °C or above 40 °C. Growth was observed in the presence of 0–4 % NaCl but not 8 % NaCl and at pH 5.0–8.0. The full phenotype microarrays obtained using the OmniLog device in comparison with the type strains of other species of the genus *Chryseobacterium* showed strain AG13^T to be distinct from related species (Fig. S2); a summary of some differential phenotypic characteristics is presented in Table 1.

Strain AG13^T contained primarily menaquinone MK-6 (80.9 %), a feature shared by all members of the family *Flavobacteriaceae* (Bernardet *et al.*, 2011), but also MK-5 (16.5 %). Polyamine analysis revealed *sym*-homospermidine (40.0 μmol) as a major compound, minor amounts of spermidine (4.4 μmol) and spermine (2.2 μmol) and traces of putresceine, cadaverine and 1,3-diaminopropane ($\leqslant$0.1 μmol), which is consistent with the characteristic pattern for species of the genus *Chryseobacterium* (Hamana & Matsuzaki, 1990, 1991; Kämpfer *et al.*, 2003). The polar lipid profile of strain AG13^T consisted of the predominant compounds phosphatidylethanolamine (PE), two unknown lipids (L6 and L8) and two unknown aminolipids (AL1 and AL2) with Rf values documented in Fig. 1. Furthermore, moderate amounts of an unknown lipid L1 and an aminolipid AL3 and minor amounts of three unknown lipids (L3, L4 and L10) and an aminolipid (AL4) were detected. This is in accordance with chromatographic profiles observed for reference strains in this study and those of Kämpfer *et al.* (2003, 2009) and Herzog *et al.* (2008), although it is worthy of note that in the original description of *C. defluvii* an unknown aminophospholipid was detected that had an Rf value similar to that of the lipid labelled as L8 in this study. L8 was present in all strains tested, strongly suggesting that this lipid was not correctly identified in the original work. Hence, the species description of *C. defluvii* should be emended, as well as those of the species *C. indologenes*, *C. wanjuense* and *C. gregarium*, since these results were missing from the original descriptions. The presence of a single phospholipid (phosphatidylethanolamine) and absence of any additional phospholipids is consistent with the reports that phosphosphingolipids are absent, in line with the original genus description (Vandamme *et al.*, 1994). The major fatty acids were the saturated branched-chain acid iso-C$_{15:0}$ (44.3 %), the monounsaturated iso-C$_{17:1}\omega 9c$ (24.3 %) and the hydroxylated iso-C$_{17:0}$ 3-OH

Table 1. Differential physiological and biochemical characteristics of strain AG13[T] and the type strains of closely related species of the genus *Chryseobacterium*

Strains: 1, *Chryseobacterium hispalense* sp. nov. AG13[T]; 2, *C. defluvii* DSM 14219[T]; 3, *C. daecheongense* DSM 15235[T]; 4, *C. gleum* DSM 16776[T]; 5, *C. taiwanense* JCM 21767[T]; 6, *C. gregarium* DSM 19109[T]; 7, *C. indologenes* DSM 16777[T]; 8, *C. gambrini* DSM 18014[T]; 9, *C. wanjuense* DSM 17724[T]; 10, *C. joostei* DSM 16927[T]; 11, *C. vrystaatense* DSM 25206[T]; 12, *C. luteum* DSM 18605[T]; 13, *C. shigense* DSM 17126[T]; 14, *C. jejuense* DSM 19299[T]; 15, *C. ureilyticum* DSM 18017[T]. All physiological data are from this study. +, Positive reaction; −, negative reaction; +/−, ambiguous.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Utilization of (GEN III Microplates):															
Trehalose	−	+	+	+	−	−	+	+	+	+	+	+	+	+	+
Cellobiose	+/−	+	+	+/−	−	+/−	−	+	+	+	+/−	−	−	+/−	−
Sucrose	−	+/−	+	−	−	+	−	+	−	−	+/−	+	+	−	−
Turanose	+	+	−	+/−	−	+	−	+	−	+	+/−	+	−	+/−	+/−
D-Salicin	−	−	−	+	−	+	−	−	−	−	−	+	+	−	−
D-Galactose	+	+/−	−	−	−	+	−	−	+/−	−	+/−	−	−	+/−	+/−
D-Fucose	+	+/−	+/−	−	−	+/−	+	+	+/−	+	+/−	−	−	+/−	+
D-Mannitol	−	−	−	−	−	−	−	+	−	−	+	−	+	−	−
Glycerol	−	+	+	+	−	−	+	+	+	+	+	+	+	+	+
D-Glucose 6-phosphate	+	+/−	+/−	−	−	+	−	−	+	−	−	+	−	−	+/−
D-Fructose 6-phosphate	+	+	+/−	−	−	+	+	+/−	+	+	+	−	+/−	+/−	+
D-Aspartic acid	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
L-Histidine	−	−	−	−	−	+	−	−	−	+	−	−	−	−	+/−
L-Serine	−	+	+	+	+	−	+	−	−	+	+	+	+	+	+
Pectin	+	−	+	+/−	+	+	−	+	+	+	+/−	−	+	−	−
D-Galacturonic acid	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+
Glucuronamide	+	+/−	+/−	−	−	+	+	−	+	+/−	+/−	+	−	+/−	+
Quinic acid	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Methyl pyruvate	−	−	−	−	−	+	−	−	−	−	+	+/−	+	−	+/−
Citric acid	+	+	+	+	−	+	+	−	+/−	+	+	+/−	−	+	−
α-Ketoglutaric acid	+	−	−	−	−	+/−	−	−	+	−	−	+	+	−	−
L-Malic acid	+	−	−	−	−	+	−	−	−	−	+/−	−	−	−	−
Growth with 4 % NaCl	+	+/−	−	+	+	−	+/−	−	−	−	+/−	−	−	−	+/−

(12.1 %) complemented by a fatty acid with an equivalent chain length (ECL) that indicates the presence of iso-$C_{15:0}$ 2-OH despite the fact that it is annotated as part of summed feature 3 (iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$, 8.9 %). Smaller amounts of iso-$C_{15:0}$ 3-OH (2.9 %) and anteiso-$C_{15:0}$ (1.5 %) were also present. This is in agreement with the fatty acid patterns obtained for the other species of the genus *Chryseobacterium* investigated in this study (Table 2). It should be noted that earlier work on the fatty acids of members of the genus *Chryseobacterium* (also previously referred to as CDC group IIb) unambiguously detected the presence of iso-$C_{15:0}$ 2-OH (Yano *et al.*, 1976; Moss & Dees, 1978; Vandamme *et al.*, 1994; Weyant *et al.*, 1995) whereas more recent reports of novel species no longer record the presence of this fatty acid (see for example Nguyen *et al.*, 2013; Sang *et al.*, 2013; Zamora *et al.* 2012a, b, c; Li & Zhu, 2012). This has implications when one refers back to the original genus description (Vandamme *et al.*, 1994) as well as the elucidation of the underlying chemical structures from which these fatty acids are derived. Similarly, some of the more recent evaluations also no longer report the presence of only iso-$C_{17:1}\omega9c$, but now record it as part of

summed feature 9. The annotation of the iso-$C_{17:1}\omega9c$ fatty acid differs in both earlier publications (Moss & Dees, 1978; Weyant *et al.*, 1995) as well as the work of Herzog *et al.* (2008) from that given in other publications. The work of Herzog *et al.* (2008) implies that the correct structure should be 9Z, 15-methyl hexadecenoic acid, while the MIDI annotation implies 7Z, 15-methyl hexadecenoic acid.

The DNA G+C content of strain AG13[T] was 37.2 mol%. This value is consistent with those observed for species of the genus *Chryseobacterium* (Vandamme *et al.*, 1994; Bernardet *et al.*, 2011).

The almost complete (1480 bp) 16S rRNA gene sequence of strain AG13[T] was determined. The 16S rRNA gene sequence showed the highest similarity with those of *C. daecheongense* DSM 15235[T] (97.2 %) and *C. defluvii* DSM 14219[T] (97.1 %). Furthermore, strain AG13[T] and its closest relatives were placed within the same phylogenetic group by both maximum-likelihood and maximum-parsimony estimations (Fig. 2). The 16S rRNA gene sequence analysis thus leaves no doubt that the novel strain belongs to the
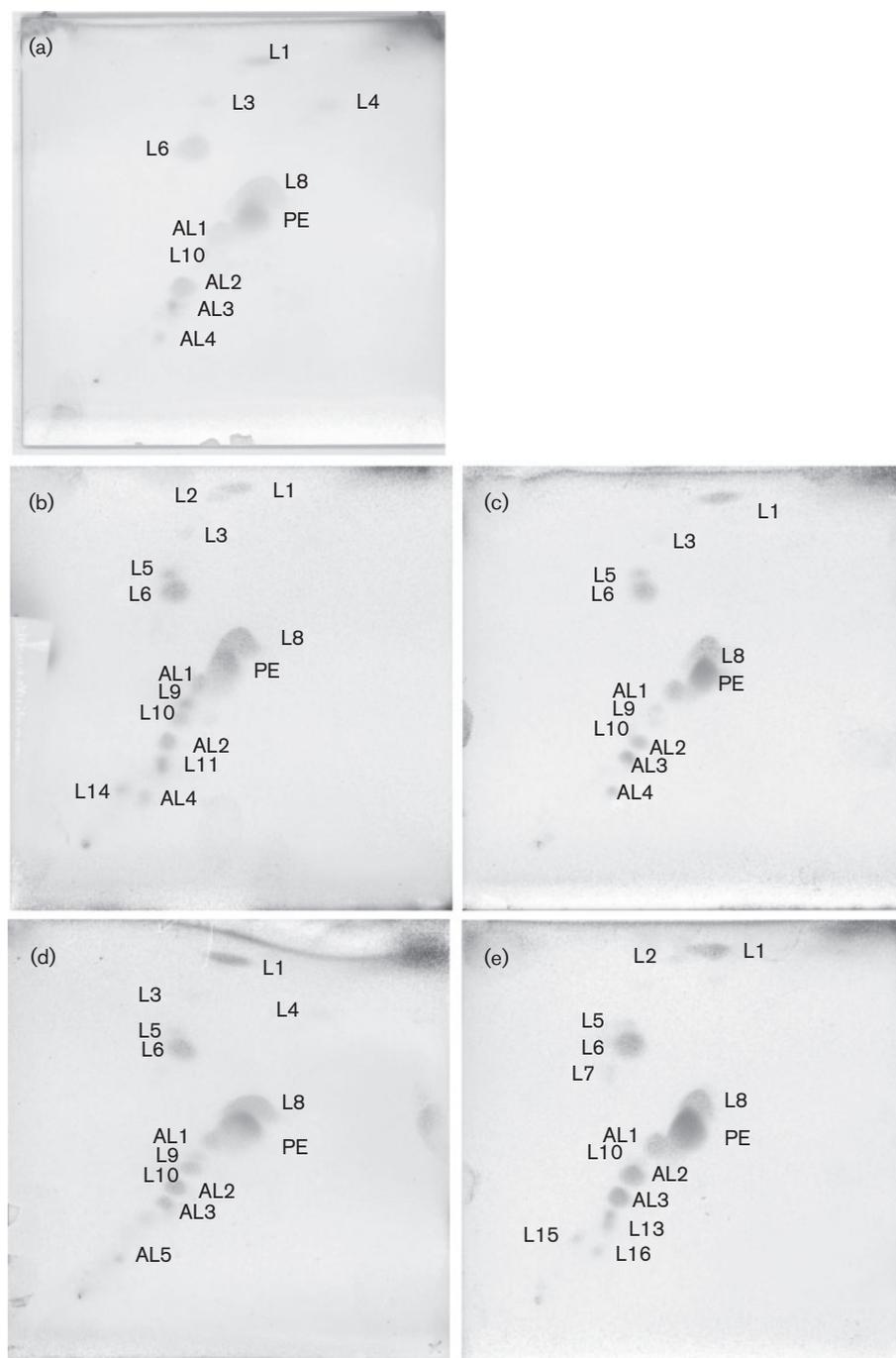
Fig. 1. Polar lipids profiles (labelled by the Rf values) of *Chryseobacterium hispalense* sp. nov. AG13[T] (a), *C. defluvii* DSM 14219[T] (b), *C. indologenes* DSM 16777[T] (c), *C. wanjuense* DSM 17724[T] (d) and *C. gregarium* DSM 19109[T] (e), after separation by two-dimensional TLC using the solvents chloroform/methanol/water (65 : 2 : 4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) in the second dimension. Plates were sprayed with molybdatophosphoric acid (3.5 %; Merck) for detection of the total polar lipids. PE, phosphatidethanolamine; AL1–5, unknown aminolipids; L1–16, unknown lipids. All data are from this study.

genus *Chryseobacterium* and represents a distinct species. However, the degree of 16S rRNA gene sequence difference of strain AG13[T] from the above-mentioned closely related strains indicated the need to prove the genomic distinctness of

the type strain representing the novel species by DNA–DNA hybridization. Strain AG13[T] displayed DNA–DNA relatedness of 16.9 ± 4.5 % with *C. daecheongense* DSM 15235[T] and 24.6 ± 3.2 % with *C. defluvii* DSM 14219[T], both values being

Table 2. Cellular fatty acid profiles (%) of strain AG13[T] and closely related species of the genus *Chryseobacterium*

Strains: 1, *Chryseobacterium hispalense* sp. nov. AG13[T]; 2, *C. defluvii* DSM 14219[T]; 3, *C. daecheongense* DSM 15235[T]; 4, *C. gleum* DSM 16776[T]; 5, *C. taiwanense* JCM 21767[T]; 6, *C. gregarium* DSM 19109[T]; 7, *C. indologenes* DSM 16777[T]; 8, *C. gambrini* DSM 18014[T]; 9, *C. wanjuense* DSM 17724[T]; 10, *C. joostei* DSM 16927[T]; 11, *C. vrystaatense* DSM 25206[T]; 12, *C. luteum* DSM 18605[T]; 13, *C. shigense* DSM 17126[T]; 14, *C. jejuense* DSM 19299[T]; 15, *C. ureilyticum* DSM 18017[T]. TR, Trace (<1 %); ND, not detected; ECL, equivalent chain length (i.e. the identity of the fatty acids is unknown). Fatty acids amounting to <1 % of the total fatty acids in all strains are not shown. All strains were grown on TSA at 28 °C for 24 h. All data are from this study.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
iso-$C_{13:0}$	TR	3.1	1.3	TR	TR	TR	TR	1.6	TR	1.0	TR	1.0	1.6	TR	TR
ECL 13.565	ND	6.4	6.9	2.8	4.2	4.8	5.0	7.8	9.6	ND	ND	7.6	2.9	ND	3.0
iso-$C_{15:0}$	44.3	54.8	48.8	35.7	44.5	34.6	34.50	51.7	43.1	41.4	42.8	37.7	41.7	36.2	32.5
anteiso-$C_{15:0}$	1.5	2.7	2.0	TR	TR	8.2	TR	1.3	TR	TR	1.2	3.8	TR	TR	TR
iso-$C_{16:0}$	ND	TR	TR	TR	ND	TR	TR	TR	ND	ND	TR	TR	TR	TR	ND
$C_{16:00}$	TR	1.3	1.2	1.3	TR	TR	1.1	1.4	1.2	1.1	TR	1.1	1.0	1.4	1.1
iso-$C_{15:0}$ 3-OH	2.9	2.2	2.3	2.8	3.1	1.9	2.3	2.1	2.8	2.7	2.8	2.1	2.4	3.0	2.9
iso-$C_{17:1}$ $\omega9c$*	24.3	5.3	7.9	23.6	16.2	21.0	25.5	8.0	11.4	23.7	22.4	21.2	17.8	23.7	28.2
ECL 16.582	ND	1.3	1.6	1.7	1.6	1.3	7	1.7	1.7	ND	ND	1.6	1.8	ND	1.6
iso-$C_{17:0}$	TR	2.4	2.5	1.5	1.3	TR	1.0	2.2	2.2	1.4	TR	TR	1.0	1.4	TR
$C_{16:0}$ 3-OH	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	1.0	1.2	1.0
iso-$C_{17:0}$ 3-OH	12.1	10.8	13.3	15.1	17.1	8.6	12.7	11.5	13.1	15.6	15.3	10.6	16.3	18.3	14.4
$C_{17:0}$ 2-OH	TR	TR	TR	ND	ND	1.7	TR	ND	ND	TR	TR	TR	TR	TR	TR
Summed features†															
3 (iso-$C_{15:0}$ 2-OH)	8.9	6.8	9.5	11.6	8.4	10.7	11.3	8.3	10.9	9.6	9.2	7.2	9.9	11.2	10.2
4	TR	ND	ND	TR	ND	TR	1.0	ND	TR	TR	1.0	TR	TR	TR	TR

*This fatty acid may be incorrectly identified (see text for details) and is recorded as 9Z, 15-methyl hexadecenoic acid for *C. defluvii*, *C. daecheongense*, *C. taiwanense*, *C. gambrini*, *C. joostei* and *C. ureilyticum* by Herzog *et al.* (2008).

†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. Summed feature 3 is listed as iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ and annotated here as iso-$C_{15:0}$ 2-OH; summed feature 4 is listed as anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I (iI).

far below the threshold value of 70 % recommended by Wayne *et al.* (1987) for a decision on the species status of novel strains.

Several phenotypic characteristics apart from the phylogenetic analysis based on 16S rRNA gene sequences also support the distinctiveness of strain AG13[T] from members of all other species of the genus *Chryseobacterium* (Tables 1 and 2). Based on the phenotypic and genotypic data presented above, we propose that strain AG13[T] represents a novel species within the genus *Chryseobacterium*, with the name *Chryseobacterium hispalense* sp. nov.

Based on a review of the literature and new data obtained in this study, emended descriptions of the species *C. defluvii*, *C. indologenes*, *C. wanjuense* and *C. gregarium* are also provided. Although data are accumulating that indicate the genus description should be further emended to include the polar lipid composition, in the absence of data for all species considered to be members of the genus *Chryseobacterium* this would be premature. However, this does not preclude their inclusion in new species descriptions.

Emended description of *Chryseobacterium defluvii* Kämpfer *et al.* 2003

The properties are as given in the species description by Kämpfer *et al.* (2003) with the following emendation. In

addition to phosphatidylethanolamine, the polar lipid profile contains thirteen unidentified polar lipids (ten lipids and three aminolipids, the Rf values of which are documented in Fig. 1b). In contrast to the fatty acids listed by Kämpfer *et al.* (2003) the cellular fatty acids consist mainly of iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{15:0}$ 2-OH and iso-$C_{17:0}$ 3-OH, with smaller amounts of iso-$C_{15:0}$ 3-OH and anteiso-$C_{15:0}$. The annotation of iso-$C_{17:1}\omega9c$ may be incorrect. Phosphosphingolipids are absent.

Emended description of *Chryseobacterium indologenes* (Yabuuchi *et al.* 1983) Vandamme *et al.* 1994

The properties are as given in the species description for *Flavobacterium indologenes* by Yabuuchi *et al.* (1983) and in the description of the genus *Chryseobacterium* by Vandamme *et al.* (1994) with the following emendation. In addition to phosphatidylethanolamine, the polar lipid profile contains 11 unidentified polar lipids (seven lipids and four aminolipids, the Rf values of which are documented in Fig. 1c). Since there are contradictions in the fatty acid patterns given by Yabuuchi *et al.* (1983) and Vandamme *et al.* (1994), the cellular fatty acids consist mainly of iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{15:0}$ 2-OH and iso-$C_{17:0}$ 3-OH, with smaller amounts of
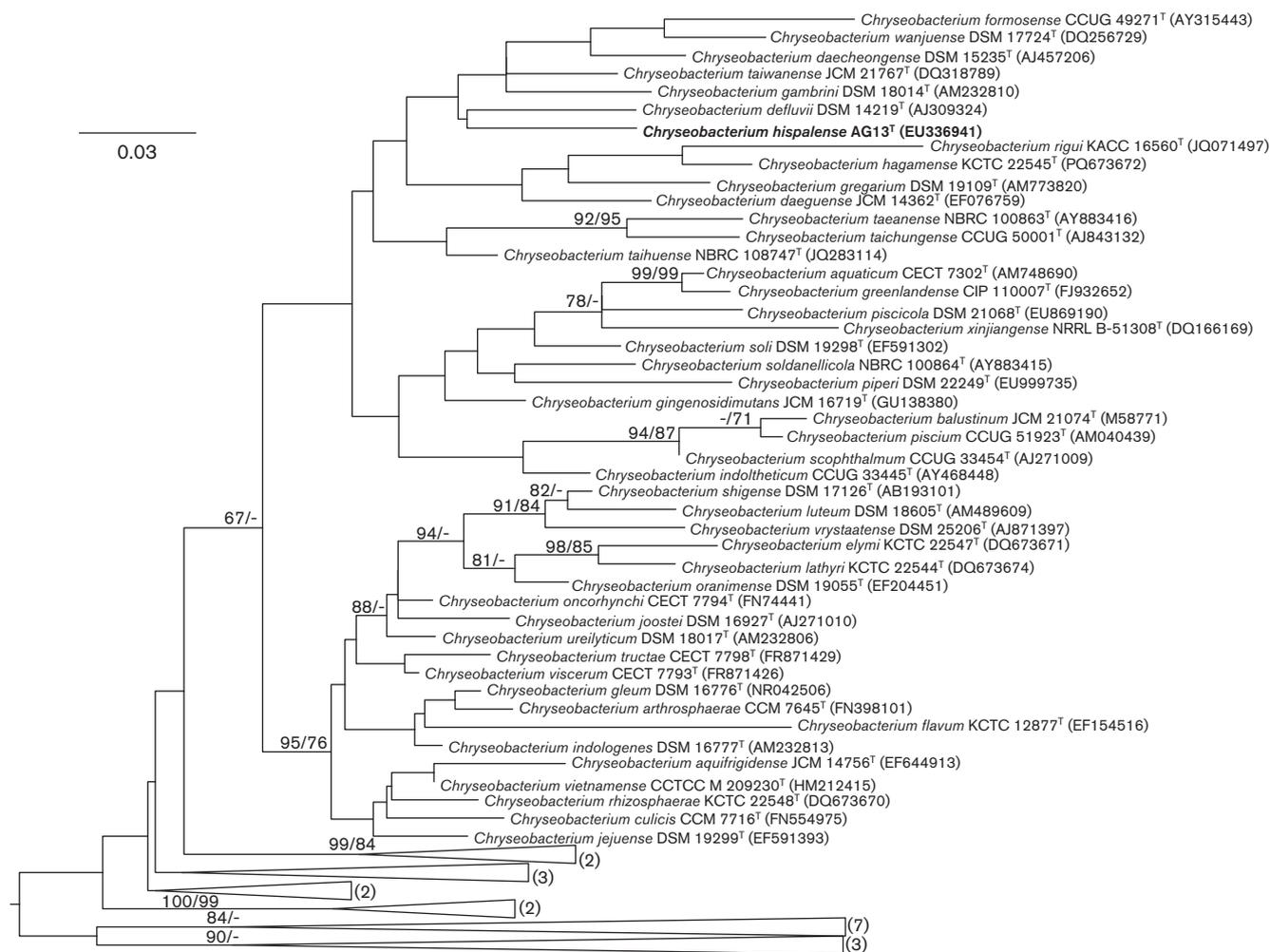
Fig. 2. Maximum-likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain AG13[T] relative to the type strains of species within the genus *Chryseobacterium*. Support values from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping are shown above the branches if equal to or larger than 70 %. Single-digit numbers in parentheses indicate the number of type strains of species of the genus *Chryseobacterium* in each of the collapsed branches. Bar, 0.03 expected substitutions per site.

iso-$C_{15:0}$ 3-OH and anteiso-$C_{15:0}$. The annotation of iso-$C_{17:1}\omega 9c$ may be incorrect. Phosphosphingolipids are absent.

Emended description of *Chryseobacterium wanjuense* Weon *et al.* 2006

The properties are as given in the species description by Weon *et al.* (2006) with the following emendation. In addition to phosphatidylethanolamine, the polar lipid profile contains 12 unidentified polar lipids (eight lipids and four aminolipids, the Rf values of which are documented in Fig. 1d). Cellular fatty acids consist mainly of iso-$C_{15:0}$, iso-$C_{17:1}\omega 9c$, iso-$C_{15:0}$ 2-OH and iso-$C_{17:0}$ 3-OH, with smaller amounts of iso-$C_{15:0}$ 3-OH and anteiso-$C_{15:0}$. The annotation of iso-$C_{17:1}\omega 9c$ may be incorrect. Phosphosphingolipids are absent.

Emended description of *Chryseobacterium gregarium* Behrendt *et al.* 2008

The properties are as given in the species description by Behrendt *et al.* (2008) with the following emendation. In addition to phosphatidylethanolamine, the polar lipid profile contains 13 unidentified polar lipids (10 lipids and three aminolipids, the Rf values of which are documented in Fig. 1e). The annotation of iso-$C_{17:1}\omega 9c$ in the original description may be incorrect. Phosphosphingolipids are absent.

Description of *Chryseobacterium hispalense* sp. nov.

Chryseobacterium hispalense [his.pal.en′se L. neut. adj. *hispalense* of or belonging to the city of *Hispalis*, the

Latin name of Seville (Spain), from where the type strain was isolated].

Colonies are circular with a shiny surface and entire edges and produce a yellow to orange non-diffusible flexirubin-type pigment. Cells are Gram-staining-negative, strictly aerobic, non-spore-forming, non-motile, frequently paired rods with cell dimensions of 0.2–0.6 × 1.1–1.8 μm, catalase- and oxidase-positive, and Voges–Proskauer- and methyl red reaction-negative. In GEN III Microplates and API 20 NE and API 20E strips, dextrin, maltose, β-gentiobiose, D-glucose, D-mannose, D-fructose, D-galactose, D-fucose, L-rhamnose, sodium lactate, D-glucose 6-phosphate, D-fructose 6-phosphate, L-tryptophan, glycyl L-proline, L-arginine, turanose, L-aspartic acid, D-glucuronic acid, mucic acid, quinic acid, L-glutamic acid, pectin, D-galacturonic acid, glucuronamide, citric acid, α-ketoglutaric acid, L-malic acid, potassium tellurite, Tween 40, α-hydroxybutyric acid, acetoacetic acid, acetic acid and butyric acid are utilized as sole carbon source for energy and growth, but sucrose, fusidic acid, glycerol, stachyose, raffinose, trehalose, melibiose, guanidine hydrochloride, α-lactose, methyl β-D-galactoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, 3-*O*-methyl-D-glucose, L-fucose, inosine, D-sorbitol, D-mannitol, *myo*-inositol, D-aspartic acid, DL-serine, L-histidine, L-pyroglutamic acid, L-galactonic acid-γ-lactone, D-saccharic acid, methyl pyruvate, D-malic acid, bromosuccinic acid, γ-amino-*N*-butyric acid, α-ketobutyric acid, propionic acid, sodium formate and sodium bromate are not utilized. Acid is produced from glycyl L-proline, L-arginine, L-aspartic acid and L-glutamic acid (that can be used as sole nitrogen sources), but not from guanidine hydrochloride, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, inosine, D-aspartic acid, DL-serine, L-histidine and L-pyroglutamic acid. Positive for the reduction of nitrate, indole production, and casein, amygdalin, starch, aesculin and gelatin degradation but negative for cellulose, tyrosine, xanthine and hypoxanthine degradation. In API ZYM, API 20E and API 20NE strips, alkaline phosphatase, valine arylamidase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β-glucosaminidase and α-glucosidase activities are present, but esterase lipase (C8), β-glucosidase, esterase (C4), lipase (C14), urease, arginine hydrolase, lysine decarboxylase, ornithine decarboxylase, cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidases, β-glucuronidase, α-mannosidase and α-fucosidase activities are absent. NaCl tolerance ranges from 0 to 4 % (w/v). Growth occurs from 10 to 40 °C (optimal growth at 15–40 °C) and pH 5.0 to 8.0. The predominant menaquinone is MK-6 but a significant amount of MK-5 is also present. The major polyamine is *sym*-homospermidine but minor amounts of spermidine and spermine are also present. The main polar lipids are phosphatidylethanolamine, four unknown aminolipids and six unknown lipids (the Rf values of which are documented in Fig. 1a). Phosphosphingolipids are absent. Cellular fatty acids consist mainly of iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{15:0}$ 2-OH

and iso-$C_{17:0}$ 3-OH, with smaller amounts of iso-$C_{15:0}$ 3-OH and anteiso-$C_{15:0}$. The annotation of iso-$C_{17:1}\omega9c$ may be incorrect.

The type strain, AG13[T] (=DSM 25574[T]=CCUG 63019[T]), was isolated in 2006 from a rainwater pond located in a commercial olive tree nursery in Burguillos (Seville), Spain. The type strain has a genomic DNA G+C content of 37.2 mol%.

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