Reclassification of *Leifsonia ginsengi* (Qiu *et al.* 2007) as *Herbiconiux ginsengi* gen. nov., comb. nov. and description of *Herbiconiux solani* sp. nov., an actinobacterium associated with the phyllosphere of *Solanum tuberosum* L.

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In the context of studying the effects of transgenic fructan-producing potatoes on the community structure of phyllosphere bacteria, a group of strains closely related to the species *Leifsonia ginsengi* was isolated. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the new isolates and *L. ginsengi* DSM 19088^T formed a lineage at the genus level and this finding was supported by chemotaxonomic characterization. The peptidoglycan type of the representative isolate, K134/01^T, and *L. ginsengi* DSM 19088^T was B2 γ , with D- and L-diaminobutyric acid as the diagnostic diamino acid and glycine, alanine and *threo*-3-hydroxyglutamic acid. The almost-complete substitution of glutamic acid by *threo*-3-hydroxyglutamic acid supported the differentiation of the new strains from recognized species of the genus *Leifsonia*. Furthermore, the detection of substantial amounts of the fatty acid cyclohexyl-C_{17:0} in the new isolates and *L. ginsengi* DSM 19088^T was a prominent chemotaxonomic feature for a clear demarcation of these strains from all genera of the family *Microbacteriaceae* that display the B2 γ cell-wall type. Comparative phylogenetic and phenotypic analyses of the isolates and *L. ginsengi* DSM 19088^T revealed the separate species status of the isolates. On the basis of these results, it is proposed that *L. ginsengi* should be classified as the type species of a novel genus, *Herbiconiux* gen. nov., with the name *Herbiconiux ginsengi* gen. nov., comb. nov. (type strain wged11^T=CGMCC 4.3491^T=JCM 13908^T=DSM 19088^T=NBRC 104580^T). The phyllosphere isolates are assigned to a novel species, *Herbiconiux solani* sp. nov. (type strain K134/01^T=DSM 19813^T=LMG 24387^T=NBRC 106740^T).

The genus *Leifsonia* is one of the genera of the family *Microbacteriaceae* that are characterized by a B-type peptidoglycan with 2,4-diaminobutyric acid (DAB) as the diagnostic diamino acid. It was established to accommodate six species that were characterized by typical chemotaxonomic features (Suzuki *et al.*, 1999; Evtushenko *et al.*, 2000). As shown by Evtushenko & Takeuchi (2006), these species

formed a monophyletic branch according to 16S rRNA gene sequence analysis and, thus, they could be clearly differentiated from other genera. With the description of novel species of the genus *Leifsonia* on the one hand (Reddy *et al.*, 2003, 2008; Qiu *et al.*, 2007; Dastager *et al.*, 2008, 2009; Pindi *et al.*, 2009) and of related genera on the other (An *et al.*, 2008; Lee *et al.*, 2008; Matsumoto *et al.*, 2008), the genus has become increasingly heterogeneous. As demonstrated in the phylogenetic tree of the Living Tree project by Yarza *et al.* (2008), several *Leifsonia* species cluster outside of the core of the genus, which harbours the type species. Moreover, a clear demarcation of the genus *Leifsonia* from recently described genera by means of chemotaxonomy

Abbreviations: DAB, 2,4-diaminobutyric acid; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K134/01^T is FN432340.

A supplementary figure is available with the online version of this paper.

is complicated as almost all of the typical characteristics are shared (An *et al.*, 2008; Lee *et al.*, 2008; Matsumoto *et al.*, 2008). Thus, the taxonomic affiliation of new strains to taxa falling in the radiation of the genus *Leifsonia* on the basis of phylogenetic and phenotypic analyses has become difficult.

Leifsonia ginsengi is one of the species that represents a separate taxon by 16S rRNA gene sequence analysis and forms a branch far from the original cluster of the genus (Yarza *et al.*, 2008). Furthermore, the fatty acid profile of this species is dominated by ω -cyclohexylundecanoic acid (Qiu *et al.*, 2007), an unusual fatty acid whose occurrence among members of the family *Microbacteriaceae* is only known so far for a single species of the genus *Curto-bacterium* (Suzuki & Komagata, 1983; Aizawa *et al.*, 2007) and the recently described genus *Humibacter* (Vaz-Moreira *et al.*, 2008). In the context of studying the effects of transgenic fructan-producing potatoes on the community structure of phyllosphere bacteria (Becker *et al.*, 2008), a group of bacterial strains was isolated that showed a close relationship to *L. ginsengi* DSM 19088^T by partial 16S rRNA gene sequence comparison. In the course of extensive comparative phylogenetic and phenotypic studies, it could be demonstrated that *L. ginsengi* and the phyllosphere isolates represented a novel genus in the family *Microbacteriaceae*. Furthermore, the separate species position of the phyllosphere isolates became evident.

To study the bacterial community composition, samples from the phyllosphere of a transgenic potato line, which was able to synthesize inulin molecules of different chain lengths, and an isogenic control were processed, as described by Becker *et al.* (2008). Culturable bacteria were investigated on various media after incubation at 21 °C for 7 days. To determine the abundance of bacterial taxa, a representative number of colonies was isolated and grouped on the basis of morphological and physiological tests (Becker *et al.*, 2008). Groups with an uncertain affiliation at the genus level were investigated using partial sequence analysis of the 16S rRNA gene, as described by Ulrich *et al.* (2008). Isolates of a group tentatively affiliated to the genus *Leifsonia* because of their phylogenetic relationship to *L. ginsengi* DSM 19088^T were subjected to further taxonomic investigation. All strains originated from the phyllosphere of the transgenic potato line (Becker *et al.*, 2008). Strains K134/01^T and K133/02 were isolated from 100-fold-diluted nutrient broth (Hattori, 1976) solidified with 15 g agar l⁻¹ and strain K11/01 from nutrient agar (SIFIN).

According to the method of Tóth *et al.* (2008), the phyllosphere isolates and *L. ginsengi* DSM 19088^T were investigated by means of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to study their intra- and interspecific relationships. On the basis of their mass spectra, the isolates formed a tight cluster that was distant from *L. ginsengi* DSM 19088^T (data not shown). This high spectral similarity of the

phyllosphere isolates to each other supported their affiliation to the same separate species. Furthermore, a clear demarcation between the new isolates and *L. ginsengi* DSM 19088^T was demonstrated by this method.

Phylogenetic analysis on the basis of the 16S rRNA gene sequence was performed for the representative isolate, K134/01^T, as described by Behrendt *et al.* (2003). Phylogenetic trees of the type strains of all recognized species of the genus *Leifsonia* and related genera were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms (PHYLP version 3.6; Felsenstein, 1993). As shown in Fig. 1, strain K134/01^T and *L. ginsengi* wged11^T formed a separate branch by both treeing methods, which was supported by a high bootstrap value of 85 % in the neighbour-joining tree. The position of the branch differed in the neighbour-joining and maximum-likelihood trees. Some branches for members of the genus *Leifsonia* clustered frequently with members of other genera and at varying positions according to the clustering algorithm. Thus, the heterogeneity of the genus *Leifsonia* became impressively obvious by both treeing methods. However, despite of the instabilities of the phylogenetic trees, the separate position of *L. ginsengi* wged11^T and the phyllosphere isolates in relation to the type strains of recognized species of the genus *Leifsonia* was demonstrated (Fig. 1).

Pairwise comparisons of the 16S rRNA gene sequences between strain K134/01^T and members of the genus *Leifsonia* showed the lowest 16S rRNA gene sequence similarities with *Leifsonia aurea* CMS 81y^T and the type strain of the type species, *Leifsonia aquatica* JCM 1368^T (94.6 and 94.8 %, respectively) and the highest similarity with *Leifsonia kafniensis* KFC-22^T (96.7 %), which was comparable to results obtained by comparison with the type strains of the type species of the genera *Microterricola*, *Schumanella* and *Chryseoglobus* (96.7–97.0 %). *L. ginsengi* wged11^T showed highest sequence similarity to *Leifsonia poae* VKM Ac-1401^T (97.4 %), *Leifsonia pindariensis* PON10^T (97.2 %) and *Microterricola viridarii* KV-677^T (97.3 %). These values demonstrated that differences in the 16S rRNA gene sequences between strains of species of the genus *Leifsonia* were in the same range as differences between strains of type species of related genera. These results indicated that *L. ginsengi* and the phyllosphere isolates merited the status of a separate genus from the phylogenetic point of view. Comparison of 16S rRNA gene sequences between *L. ginsengi* wged11^T and strain K134/01^T revealed 98.2 % similarity, which indicated that the isolates belonged to a species separate from *L. ginsengi* (Stackebrandt & Ebers, 2006).

To support the result of the 16S rRNA gene sequence analysis, DNA–DNA relatedness between *L. ginsengi* DSM 19088^T and strain K134/01^T was studied. Cells were disrupted by using a French pressure cell (Thermo Spectronic) and DNA in the crude lysate was purified by chromatography on hydroxyapatite, as described by

Fig. 1. Neighbour-joining phylogenetic tree showing the position of strain K134/01^T in the family *Microbacteriaceae*, based on an alignment of 1463 nt (*Escherichia coli* positions 28–1469) of 16S rRNA gene sequences. Bootstrap values (> 70 %) based on 1000 resamplings are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood method. *Arthrobacter globiformis* (GenBank accession no. M23411) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). DNA–DNA hybridization in 2 × SSC plus 10 % formamide at 70 °C revealed a reassociation value of 28.6 % (repetition, 28.0 %).

According to the recommendation of Wayne *et al.* (1987) for species delineation, the separate species status of the phyllosphere isolate was clearly demonstrated at the genomic level.

A comprehensive chemotaxonomic characterization of strain K134/01^T and *L. ginsengi* DSM 19088^T was performed. For elucidation of the cell-wall peptidoglycan structure, cells were prepared on nutrient agar at 27 °C for

Table 1. Differential characteristics of the investigated strains and closely related genera of the family *Microbacteriaceae*

Genera: 1, *Herbiconiux* gen. nov.; 2, *Agreia*; 3, *Agrococcus*; 4, *Chryseoglobus*; 5, *Glaciibacter*; 6, *Leifsonia*; 7, *Microcella*; 8, *Microterricola*; 9, *Phycicola*; 10, *Rhodoglobus*; 11, *Salinibacterium*; 12, *Schumannella*; 13, *Subtercola*; 14, *Yonghaparkia*. Data were taken from this study and Groth *et al.* (1996), Evtushenko *et al.* (2000, 2001), Han *et al.* (2003), Sheridan *et al.* (2003), Schumann *et al.* (2003), Mayilraj *et al.* (2006), Tiago *et al.* (2005, 2006), Yoon *et al.* (2006), An *et al.* (2008), Lee *et al.* (2008), Matsumoto *et al.* (2008), Baik *et al.* (2010) and Katayama *et al.* (2009). C, Cocci; DAB, diaminobutyric acid; DMA, dimethyl acetal; F, filaments; R, rods; +, positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Morphology	R	R	R, C	R	R	R, F	R	R	R, C	R	R	R	R	R
Motility	–	+	–	+	+	+	–	+	+	+	–	–	–	–
Growth (°C)														
Range	4–37	ND	<37	20–40	–5–25	7–37	15–40	10–38	4–30	–2–21	4–37	8–35	–2–28	10–37
Optimum	21–30	24–26	28	30	18–20	24–28	35	15–30	25	18	25–28	ND	15–17	30
Diagnostic diamino acid	DL-DAB	L-DAB, D-Orn	L-DAB	Lys	DAB	DL-DAB	Lys, Orn	DAB	DAB	Orn	Lys, Orn	DAB	DAB	DL-DAB
DNA G+C content (mol%)	66	65–67	74–75	68	65	66–71	67–69	70	70	62	61	59	64–68	71–72
Major fatty acids	i-C _{15:0} , ai-C _{15:0} , Ch-17:0	ai-C _{15:0} , i-C _{16:0} , ai-C _{17:0} , ai-C _{15:0} DMA	ai-C _{15:0} , i-C _{16:0} , i-C _{15:0} , ai-C _{17:0}	i-C _{16:0} , ai-C _{15:0} , i-C _{14:0} , C _{16:0}	ai-C _{15:0} , ai-C _{17:0} , i-C _{16:0} , i-C _{15:0}	ai-C _{15:0} , ai-C _{17:0} , i-C _{16:0}	i-C _{16:0} , ai-C _{15:0} , i-C _{14:0} , i-C _{15:0}	ai-C _{15:0} , ai-C _{17:0}	ai-C _{15:0} , ai-C _{17:0}	ai-C _{15:0} , ai-C _{17:0} , i-C _{16:0}	ai-C _{15:0} , i-C _{16:0} , i-C _{14:0}	ai-C _{15:0} , i-C _{16:0}	ai-C _{15:0} , i-C _{16:0} , ai-C _{17:0} , ai-C _{15:0} DMA, i-C _{16:0} DMA	ai-C _{15:0} , i-C _{16:0} , ai-C _{17:0}
Major menaquinones	10	10, 11	11, 12, 10	12, 13, 14	11, 12, 13	11, 10, 12	12, 13, 14	12	11	11, 12	11, 10	11, 10	9, 10	12, 11

3 days. Preparation of purified peptidoglycan was performed as described by Vaz-Moreira *et al.* (2008). The amino acids and peptides in the peptidoglycan hydrolysates were analysed by two-dimensional TLC on cellulose plates using the solvent system described by Schleifer & Kandler (1972) and amino acid derivatives were analysed by GC (GC 14A; Shimadzu) and GC-MS (320 Singlequad; Varian) according to Schumann *et al.* (1997). Analysis of enantiomeric diamino acid isomers was performed using HPLC (LC-20AB; Shimadzu), as described by Nozawa *et al.* (2007). Strain K134/01^T and *L. ginsengi* DSM 19088^T contained the peptidoglycan amino acids glycine, alanine, *threo*-3-hydroxyglutamic acid and L- and D-isomers of the diagnostic diamino acid DAB. The presence of *threo*-3-hydroxyglutamic acid in the cell wall of *L. ginsengi* DSM 19088^T was confirmed by two-dimensional TLC with the peptidoglycan hydrolysate of *Agreia pratensis* DSM 14246^T, which contains hydroxylated glutamic acid (Behrendt *et al.*, 2002; Schumann *et al.*, 2003), and by the detection of characteristic mass spectrometric fragment ions of the *N*-heptabutyl isobutylester at 253, 279, 296, 323, 341 and 353 *m/z*. However, its presence was contradictory to the results of Qiu *et al.* (2007), which detected glutamic acid. To confirm the results obtained in this study, the investigation of the cell-wall composition was repeated for *L. ginsengi* DSM 19088^T grown in nutrient broth with shaking at 28 °C for 24 h and for *L. ginsengi* NBRC 104580^T grown in NBRC medium 802 [consisting of 1.0 % polypeptone (Nihon Pharmaceutical), 0.2 % yeast extract (Difco) and 0.1 % MgSO₄ · 7H₂O] with shaking at 30 °C for 24 h. Under both of these culture conditions, the substitution of glutamic acid by substantial amounts of *threo*-3-hydroxyglutamic acid was confirmed and only traces of glutamic acid were found. Such substitution can be found also in other genera of the family *Microbacteriaceae*, such as *Subtercola*, *Agreia*, *Microbacterium* and *Curtobacterium* (Behrendt *et al.*, 2002; Schumann *et al.*, 2009). However, *threo*-3-hydroxyglutamic acid has not been reported so far for recognized species of the genus *Leifsonia*. Thus, the presence of this amino acid was a chemotaxonomic feature that supported the separate genus status of *L. ginsengi* and the phyllosphere isolates that was previously indicated by the phylogenetic analysis.

A further chemotaxonomic feature, the isoprenoid quinone composition, was determined as described by Groth *et al.* (1996). The quinone system of strain K134/01^T consisted of the major menaquinone MK-11 and minor amounts of MK-10 (molar ratio 10:3). This result corresponds to the menaquinone composition of *L. ginsengi* (Qiu *et al.*, 2007). The major menaquinone of several species of the genus *Leifsonia* is also MK-11 (Suzuki *et al.*, 1999; Evtushenko & Takeuchi, 2006) and, thus, a demarcation of the isolates and *L. ginsengi* on the basis of the isoprenoid quinone composition was not possible. Chemotaxonomic features that differentiated the investigated strains from phylogenetically related genera of the family *Microbacteriaceae* are summarized in Table 1.

The cellular fatty acid composition of the phyllosphere isolates and *L. ginsengi* DSM 19088^T was determined for cells grown on tryptic soy agar (Becton Dickinson) at 28 °C for 48 h with the Sherlock Microbial Identification system (version 4.5; MIDI) according to the description by Behrendt *et al.* (1999). Cyclohexyl-C_{17:0} was identified by GC-MS because it is misidentified as C_{18:1ω7c} by the MIDI system on the basis of its retention time (Monciardini *et al.*, 2009). The major fatty acids of the phyllosphere isolates were (mean ± SD) anteiso-C_{15:0} (45.10 ± 0.13 %), iso-C_{15:0} 15.61 ± 0.65 %, anteiso-C_{17:0} (13.55 ± 0.69 %), iso-C_{16:0} (9.63 ± 1.10 %) and cyclohexyl-C_{17:0} (7.95 ± 0.45 %). Minor amounts of iso-C_{17:0} (3.28 ± 0.17 %) and iso-C_{14:0} (1.25 ± 0.24 %) were found (Table 2). The fatty acid profiles of the phyllosphere isolates were very similar to each other, which confirmed the results of MALDI-TOF MS, that the isolates are affiliated to one species. Comparisons between the new isolates and *L. ginsengi* DSM 19088^T revealed remarkable differences of the proportions of several fatty acids. Thus, anteiso-C_{15:0} was the main fatty acid of the phyllosphere isolates whereas cyclohexyl-C_{17:0} was the predominant fatty acid of *L. ginsengi* DSM 19088^T, which corresponded to the results reported by Qiu *et al.* (2007). Furthermore, iso-C_{15:0} was one of the major fatty acids in the isolates but was found only in trace amounts in *L. ginsengi* DSM 19088^T. Therefore, the fatty acid profiles contributed to the differentiation of the phyllosphere isolates from *L. ginsengi* DSM 19088^T. Otherwise, the presence of the unusual fatty acid cyclohexyl-C_{17:0} in *L. ginsengi* DSM 19088^T as well as in the phyllosphere isolates emphasized their separate position with respect to other species of the genus *Leifsonia*, as already demonstrated by 16S rRNA gene sequence analysis and cell-wall composition. Species of the genus *Curtobacterium* (Suzuki & Komagata, 1983; Aizawa *et al.*, 2007) and *Humibacter albus* (Vaz-Moreira *et al.*, 2008), which are

Table 2. Cellular fatty acid profiles of the phyllosphere isolates and *Leifsonia ginsengi* DSM 19088^T

Strains: 1, K134/01^T; 2, K133/02; 3, K11/01; 4, *Leifsonia ginsengi* DSM 19088^T. Data are taken from this study. tr, Trace (<1 %); ND, not detected.

Fatty acid (%)	1	2	3	4
iso-C _{14:0}	1.02	1.50	1.24	2.28
iso-C _{15:0}	15.89	14.87	16.07	tr
anteiso-C _{15:0}	45.05	45.00	45.25	24.25
C _{16:0}	tr	tr	tr	tr
iso-C _{16:0}	8.93	10.90	9.06	15.83
C _{16:1ω11c}	tr	tr	tr	tr
iso-C _{17:0}	3.41	3.09	3.33	tr
anteiso-C _{17:0}	14.26	12.89	13.52	12.84
anteiso-C _{17:1}	tr	tr	tr	ND
Cyclohexyl-C _{17:0}	7.43	8.26	8.16	42.24
Unknown	2.59	2.09	2.38	ND

Table 3. Physiological characteristics of the phyllosphere isolates and *Leifsonia ginsengi* DSM 19088^T

Strains: 1, K134/01^T; 2, K133/02; 3, K11/01; 4, *Leifsonia ginsengi* DSM 19088^T. Data are taken from this study. All strains are positive for aesculin hydrolysis, β -galactosidase, oxidase and catalase, growth with 0.05–3 % NaCl, utilization of L-alaninamide, L-arabinose, D-cellobiose, D-fructose, L-fucose, α -D-glucose, glycerol, γ -hydroxybutyric acid, D-maltose, D-mannitol, D-mannose, methylpyruvate, pyruvic acid, D-ribose, salicin, D-sorbitol, sucrose, turanose and D-xylose and acid production from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, maltose, mannitol, D-mannose, rhamnose, ribose, salicin, sucrose, D-turanose and D-xylose. All strains are negative for starch and casein hydrolysis, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, gelatinase, citrate utilization, H₂S production from sodium thiosulfate and indole production, utilization of acetic acid, N-acetyl-D-glucosamine, N-acetyl L-glutamic acid, N-acetyl β -D-mannosamine, adenosine 5'-monophosphate, D- and L-alanine, D-arabitol, 2,3-butanediol, α - and β -cyclodextrin, 2'-deoxyadenosine, glucose 1-phosphate, glycogen, inosine, *myo*-inositol, D-melezitose, α - and β -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketoglutaric acid, α -ketovaleric acid, D-melibiose, methyl α -D-galactoside, methyl β -D-galactoside, methyl α -D-glucoside, methyl β -D-glucoside, lactamide, L-lactic acid, D-lactic acid methyl ester, α -D-lactose, lactulose, monomethyl succinate, D-malic acid, methyl α -D-mannoside, propionic acid, putrescine, L-pyroglutamic acid, sedoheptulosan, succinamic acid, succinic acid, thymidine 5'-monophosphate, uridine 5'-monophosphate and xylitol and acid production from N-acetylglucosamine, D- and L-arabitol, arbutin, D-adonitol, D-arabinose, dulcitol, erythritol, D-fucose, gluconate, glycogen, *myo*-inositol, 2-keto-D-gluconate, 5-keto-D-gluconate, methyl D-glucoside, methyl D-mannoside, D-melezitose, D-melibiose, methyl xyloside, L-sorbitol, sorbose, starch, xylitol and L-xylose.

+, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
Acetoin production (Voges-Proskauer reaction)	–	–	–	+
Reduction of nitrate to nitrite	–	–	–	+
pH range for growth	4–8	4–8	4–8	6–8
Utilization of (GP2 MicroPlate):				
Dextrin	+	w	+	–
Inulin	+	+	+	–
Mannan	w	+	+	–
Tween 40	–	+	+	–
Tween 80	w	+	+	–
Amygdalin	+	+	+	–
Arbutin	+	+	+	–
D-Galactose	+	+	+	–
D-Galacturonic acid	+	w	+	–
Gentiobiose	+	+	+	–
D-Gluconic acid	+	w	+	–
Maltotriose	+	+	+	–
3-Methyl D-glucose	+	+	+	–
Palatinose	+	+	+	–
D-Psicose	+	+	+	–
D-Raffinose	+	+	+	–

Table 3. cont.

Characteristic	1	2	3	4
L-Rhamnose	+	+	+	w
Stachyose	+	+	+	–
D-Tagatose	+	+	+	–
D-Trehalose	+	+	+	–
L-Malic acid	–	–	w	–
L-Alanyl glycine	+	w	+	–
L-Asparagine	+	+	+	–
L-Glutamic acid	+	–	+	–
Glycyl L-glutamic acid	+	+	+	–
L-Serine	–	+	+	–
Adenosine	+	+	+	–
Thymidine	–	+	+	–
Uridine	–	+	+	–
D-Fructose 6-phosphate	+	+	+	–
D-Glucose 6-phosphate	+	+	+	–
DL- α -Glycerol phosphate	+	+	+	–
Acid production from (API 50 CH):				
Amygdalin	+	+	+	–
Lactose	–	–	+	+
D-Trehalose	+	–	+	+
Inulin	+	w	+	–
D-Raffinose	+	–	+	–
Gentiobiose	+	–	–	+
D-Lyxose	–	–	–	+
D-Tagatose	–	–	–	+
L-Fucose	+	+	+	–

also characterized by formation of the fatty acid cyclohexyl-C_{17:0}, differed clearly from the new isolates and *L. ginsengi* by their diagnostic diamino acids in the cell-wall peptidoglycan. Therefore, of the members of the family *Microbacteriaceae* with DAB as the diagnostic diamino acid in the cell wall, only the investigated strains displayed cyclohexyl-C_{17:0}.

The composition of polar lipids of strain K134/01^T was analysed as described by Schumann *et al.* (1997). The polar lipids of strain K134/01^T consisted of diphosphatidylglycerol, phosphatidylglycerol, minor amounts of two unknown phospholipids and two unknown glycolipids (see Supplementary Fig. S1, available in IJSEM Online). The detection of the phospholipids, diphosphatidylglycerol and phosphatidylglycerol almost always in combination with glycolipids is typical of all genera of the family *Microbacteriaceae* displaying the peptidoglycan type B2 γ , as demonstrated by Schumann *et al.* (2009).

The morphological and physiological characterization of the phyllosphere isolates was performed as described by Behrendt *et al.* (1999). Growth at pH 4.0–11.0 was conducted in nutrient broth with the buffer systems described by Lee *et al.* (2002). Additionally, growth at pH 4.0 and 5.0 was tested in nutrient broth containing 100 mM citric acid/200 mM Na₂HPO₄. Growth with varying NaCl concentrations was tested in nutrient broth.

Investigation of oxidative acid production from carbohydrates was carried out using API 50 CH strips (bioMérieux) inoculated with a bacterial suspension from a 24-h-old culture grown at 25 °C on TSA (Merck) in CHB/E medium. Additional physiological and enzymic characteristics were determined using the API 20E and API 20NE strips (bioMérieux). The reading of the results was performed for up to 3 days. An extensive investigation of carbon substrate assimilation was conducted using GP2 MicroPlates (Biolog), according to the manufacturer's instructions. Results were scored visually after 24 and 48 h of incubation at 30 °C. The detailed phenotypic characteristics of the phyllosphere isolates are given in the species description and Table 3. Considering the multitude of phenotypic features tested, the phyllosphere isolates displayed a relatively high level of conformity among each other which supported the conclusions drawn from MALDI-TOF MS and fatty acid analysis that all three isolates represented one species. Nevertheless, some differences were found in the API 50 CH and GP2 MicroPlate results, which demonstrated an intraspecific diversity among the isolates. Comparison between the phyllosphere isolates and *L. ginsengi* DSM 19088^T revealed differences, particularly in the assimilation of a number of substrates tested with GP2 MicroPlates, which enabled them to be clearly differentiated on the basis of their biochemical characteristics (Table 3).

In summary, phylogenetic, chemotaxonomic and phenotypic analyses demonstrated that the isolates from the potato phyllosphere and the species *L. ginsengi* deserve the status of a separate genus. Consequently, it is proposed that *L. ginsengi* should be placed in a novel genus, *Herbiconiux* gen. nov., as the type species, *Herbiconiux ginsengi* gen. nov., comb. nov. Furthermore, the phyllosphere isolates merit a separate species status because a clear demarcation from *L. ginsengi* DSM 19088^T was demonstrated by comparisons of 16S rRNA gene sequences, DNA–DNA relatedness, spectral patterns by MALDI-TOF MS, fatty acid profiles and physiological characteristics. Therefore, the assignment of the phyllosphere isolates to a novel species, *Herbiconiux solani* sp. nov., is proposed.

Description of *Herbiconiux* gen. nov.

Herbiconiux (Her.bi.con'iux. L. n. *herba* plant; L. fem. n. *coniux* wife, female spouse; N.L. fem. n. *Herbiconiux* the associate of a plant, plant spouse).

Cells are Gram-positive, straight or curved, non-motile rods. Endospores are not formed. Mesophilic. Growth is aerobic. The major respiratory quinone is MK-11, while MK-10 is a minor component. The predominant fatty acids are cyclohexyl-C_{17:0}, anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. The cell-wall peptidoglycan contains L- and D-DAB, glycine, alanine and *threo*-3-hydroxyglutamic acid. The DNA G+C content of the type species is approximately 66 mol% (Qiu *et al.*, 2007). Phylogenetically, the genus belongs to the family *Microbacteriaceae*. The type species is *Herbiconiux ginsengi*.

Description of *Herbiconiux ginsengi* (Qiu *et al.* 2007) comb. nov.

Herbiconiux ginsengi (gin.sen'gi. N.L. gen. n. *ginsengi* of ginseng, the source of the type strain of this species).

Basonym: *Leifsonia ginsengi* Qiu *et al.* 2007.

The description is the same as that given for *Leifsonia ginsengi* by Qiu *et al.* (2007) with the additional physiological characteristics as given in Table 3 and the following peptidoglycan data. The cell-wall peptidoglycan contains L- and D-DAB, glycine, alanine and *threo*-3-hydroxyglutamic acid.

The type strain, wged11^T (=CGMCC 4.3491^T=JCM 13908^T=DSM 19088^T=NBRC 104580^T), was isolated from a tissue suspension of a surface-sterilized ginseng root.

Description of *Herbiconiux solani* sp. nov.

Herbiconiux solani (so.la'ni. N.L. gen. neut. n. *solani* of the potato).

Cells are asporogenous, non-motile, straight or curved short rods (0.6–0.75 × 0.8–1.0 µm). Gram-positive and aerobic. Colonies on nutrient agar are yellow, smooth, circular and glistening with regular margins. Optimal growth occurs at 21–30 °C, but not at 4 or 37 °C. Oxidase- and catalase-positive. Does not grow on MacConkey agar. Physiological characteristics are as given in Table 3. Cell-wall peptidoglycan contains L- and D-DAB, glycine, alanine and *threo*-3-hydroxyglutamic acid. The major respiratory quinone is MK-11, while MK-10 is a minor component. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, unidentified glycolipids and minor amounts of unknown phospholipids. The fatty acid profile consists of the predominant components (>5%) anteiso-C_{15:0}, iso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and cyclohexyl-C_{17:0} and the minor components iso-C_{17:0} and iso-C_{14:0}.

The type strain, K134/01^T (=DSM 19813^T=LMG 24387^T=NBRC 106740^T), was isolated from the phyllosphere of transgenic potato plants in Dahnsdorf, Germany.

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