Kaistella koreensis gen. nov., sp. nov., a novel member of the *Chryseobacterium*–*Bergeyella*–*Riemerella* branch

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Gram-negative, non-spore-forming, rod-shaped, yellow-pigmented bacteria isolated from a freshwater stream in Korea were investigated to determine their taxonomic position. Complete 16S rRNA gene sequence analysis indicated that the organisms should be placed in the *Chryseobacterium*–*Bergeyella*–*Riemerella* branch in the family *Flavobacteriaceae*. Phylogenetically, the strains were most closely related to *Chryseobacterium balustinum* ATCC 33487^T and *Chryseobacterium scophthalmum* LMG 13028^T (94.3 and 94.1 % 16S rRNA gene sequence similarity, respectively) and they clustered on a separate well-supported branch. The strains contained menaquinone MK-6 as the predominant respiratory quinone and showed higher G + C contents (41.7 mol%) than other species in the *Chryseobacterium*–*Bergeyella*–*Riemerella* branch and i-C_{15:0} as a major fatty acid (47–52 %). The phylogenetic distances from any species with validly published names and their phenotypic properties confirmed that the strains constitute a separate species in a new genus, for which the name *Kaistella koreensis* gen. nov., sp. nov. is proposed (type strain Chj707^T = KCTC 12107^T = IAM 15050^T).

INTRODUCTION

The *Cytophaga*–*Flavobacterium*–*Bacteroides* (CFB) group, rRNA superfamily V, is recognized as a separate line of descent within the domain *Bacteria* (Segers *et al.*, 1993; Woese *et al.*, 1990). The family *Flavobacteriaceae*, emended by Bernardet *et al.* (1996), in the CFB group contains many species in the genera *Chryseobacterium*, *Bergeyella*, *Riemerella*, *Ornithobacterium*, *Empedobacter*, *Weeksella* and *Flavobacterium* (Holmes, 1992; Jooste & Hugo, 1999). The

genera *Bergeyella*, *Chryseobacterium* and *Riemerella* form a separate branch, the so-called *Bergeyella*–*Chryseobacterium*–*Riemerella* branch, on the basis of rRNA cistron similarity (Bernardet *et al.*, 1996; Vandamme *et al.*, 1994) and phenotypic characteristics. The genus *Chryseobacterium* contains eight recognized species, the genus *Bergeyella* accommodates a single species (Holmes *et al.*, 1986; Vandamme *et al.*, 1994) and the genus *Riemerella* comprises two species (Segers *et al.*, 1993; Vancanneyt *et al.*, 1999). Members of these genera are yellow-pigmented rods and have very low G + C contents of 30–38 mol%.

In the present study, three strains, Chj707^T, Ko2 and Ko10, were isolated from a freshwater stream in Korea and we studied their taxonomic parameters, including genotypic, chemotaxonomic and classical phenotypic parameters, in order to establish their phylogenetic affiliation and to describe comprehensively the taxonomic position of these strains.

Lack of flagellation, low G + C content, the presence of

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Abbreviations: CFB, *Cytophaga*–*Flavobacterium*–*Bacteroides*; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Chj707^T, Ko2 and Ko10 are AF344179, AY294611 and AY299974.

menaquinones as the predominant respiratory quinones, the presence of branched-chain fatty acids in high percentages, the absence of carbohydrate fermentation and similar hydrolytic enzyme patterns confirmed that these strains belong to the *Bergeyella*–*Chryseobacterium*–*Riemerella* branch. However, some characteristics such as phylogenetic distance from any type strains of species with validly published names and their phenotypic properties, higher G + C content and specific discriminatory fatty acids confirmed that the strains constitute a separate genus. Below, we show that these isolates constitute a novel genus and species, for which we propose the name *Kaistella koreensis* gen. nov., sp. nov.

METHODS

Bacterial strains and growth conditions. Unless specified otherwise, strains were grown on nutrient agar or broth and incubated at 30 °C. The strains and their sources are listed in Table 1.

Phenotypic characterization. The API 20NE and API ID32 GN microtest systems were used according to the recommendations of the manufacturer (bioMérieux). Cell morphologies were observed under a light microscope (1000× magnification) with cells grown for 3 days. For determination of the Gram reaction, the non-staining method (3% KOH; Buck, 1982) was used. Motility was examined by phase-contrast microscopy. Oxidase activity was tested using Bactident-Oxidase test strips (Merck), catalase activity with 3% H₂O₂. Growth was investigated at different temperatures (15, 25, 30, 37 and 42 °C) and on MacConkey agar. Additional biochemical tests were performed: oxidative or fermentative acid production from 1% glucose (Hugh & Leifson, 1953), H₂S production using peptone iron agar (Difco 289100) and starch hydrolysis using starch agar (Difco 272100).

DNA extraction and 16S rRNA gene sequencing. Chromosomal DNA was extracted and purified using the DNeasy tissue kit

(Qiagen). PCR amplification of extracted 16S rRNA genes was done as described previously by Yoon *et al.* (1997). Primers (Weisburg *et al.*, 1991) annealing at the 5' and 3' ends of the 16S rRNA genes were 9F (5'-GAGTTTGATCCTGGCTCAG-3'; positions 9–27, *Escherichia coli* 16S rRNA numbering) and 1512R (5'-ACGGCT-ACCTTGTTACGACTT-3'; positions 1512–1492). PCR amplification was performed in a final reaction volume of 100 µl and the reaction mixture contained each primer at 1 µM, 100 ng extracted DNA, each dNTP at 0.1 µM, 10× reaction buffer and 2.5 U *Taq* DNA polymerase. PCR was run for 35 cycles and the following thermal profile was used: denaturation at 94 °C for 1 min, primer annealing at 60 °C for 1 min and extension at 72 °C for 2 min. The final cycle included extension for 10 min at 72 °C. Direct sequencing of amplified 16S rRNA genes was done using the following primers (Stackebrandt & Liesack, 1993; Yoon *et al.*, 1998): 9F (5'-GAGTTTGATCCTGGCTCAG-3'; 9–27), 341F (5'-CCTACGGGAGGCAG-CAG-3'; 341–357), 519F (5'-CAGCAGCCGCGGTAATAC-3'; 519–536), 907F (5'-AAACTCAAAGGAATTGACGG-3'; 907–926), 536R (5'-GTATTACCGCGGCTGCTG-3'; 536–519), 1100R (5'-GGGTT-GCGCTCGTTG-3'; 1114–1100) and 1512R (5'-ACGGCTACCTT-GTTACGACTT-3'; 1512–1492).

Phylogenetic analysis. The full 16S rRNA gene sequences were compiled after sequencing using SeqMan software and sequences of the test strains were edited using the BioEdit program (Hall, 1999) and aligned using CLUSTAL X (Thompson *et al.*, 1997). The distance matrix was calculated by the BioEdit program (Hall, 1999) after deleting regions containing ambiguous nucleotides. The phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) using the Mega2 program (Kumar *et al.*, 2001).

DNA base composition. Genomic DNA was extracted and purified using the Qiagen Genomic-tip system 100/G and enzymically degraded into nucleosides as described previously (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). A 10 µl volume of a solution containing 10 µg DNA was heated in a boiling water bath for 5 min and rapidly cooled in an ice-water bath. The denatured DNA solution was mixed with 10 µl nuclease P1 solution (20 U ml⁻¹) and incubated at 37 °C for 1 h. Then, 10 µl alkaline phosphatase

Table 1. Strains studied

KCTC, Korean Collection for Type Cultures, Daejeon, Korea; ATCC, American Type Culture Collection, Manassas, VA, USA; LMG, Culture collection, Laboratorium voor Microbiologie Gent, Universiteit Gent, Belgium.

Strain	Other designation(s)	Received from
Chj707 ^T	KCTC 12107 ^T = IAM 15050 ^T	Own isolate
Ko2	KCTC 12108 = IAM 15051	Own isolate
Ko10	KCTC 12109 = IAM 15052	Own isolate
<i>Chryseobacterium balustinum</i> KCTC 2903 ^T	ATCC 33487 ^T = LMG 8329 ^T	KCTC
<i>Chryseobacterium defluvii</i> B2 ^T	DSM 14219 ^T = CIP 107207 ^T	DSMZ
<i>Chryseobacterium gleum</i> KCTC 2904 ^T	ATCC 35910 ^T = LMG 8334 ^T	KCTC
<i>Chryseobacterium indologenes</i> KCTC 2905 ^T	ATCC 29897 ^T = LMG 8337 ^T	KCTC
<i>Chryseobacterium indoltheticum</i> KCTC 2920 ^T	ATCC 27950 ^T = LMG 4025 ^T	KCTC
<i>Chryseobacterium joostei</i> Ix 5a ^T	LMG 18212 ^T = CCUG 46665 ^T	LMG
<i>Chryseobacterium meningosepticum</i> KCTC 2906 ^T	ATCC 13253 ^T = LMG 12279 ^T	KCTC
<i>Chryseobacterium scophthalmum</i> KCTC 2907 ^T	LMG 13028 ^T	KCTC
<i>Bergeyella zoohelcum</i> KCTC 2910 ^T	ATCC 43767 ^T = LMG 12996 ^T	KCTC
<i>Riemerella anatipestifer</i> KCTC 2911 ^T	ATCC 11845 ^T = LMG 11054 ^T	KCTC
<i>Riemerella columbina</i> LMG 11607 ^T		LMG

(1 U ml⁻¹) was added to the sample and incubated at 37 °C for 3 h. The nucleoside mixture obtained was then separated by HPLC using a Waters Nova-Pak C₁₈ column (3.9 × 300 mm) and eluted with a mixture of 0.2 M NH₄H₂PO₄ and acetonitrile (40:1, v/v) at a flow rate of 0.7 ml min⁻¹ and detected by UV absorbance at 270 nm. DNA of *E. coli* (Sigma) and two other type strains, *Chryseobacterium indologenes* KCTC 2905^T and *Chryseobacterium scophthalmum* KCTC 2907^T, were used as the calibration reference.

Fatty acid methyl ester (FAME) analysis. Strains were grown for 24 h and then two loops of well-grown cells were harvested. FAMES were prepared, separated and identified using the Sherlock Microbial Identification System (MIS) by MIDI, Inc. (Sasser, 1990).

Respiratory isoprenoid quinone analysis. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by TLC on Merck Kieselgel 60 F254 plates (20 × 20 cm, 0.5 cm thick) using petroleum benzene/diethyl ether (85:15, v/v) as the solvent and then analysed by HPLC as described previously (Collins & Jones, 1981; Shin *et al.*, 1996).

RESULTS AND DISCUSSION

Respiratory isoprenoid quinone analyses

Analyses of the respiratory isoprenoid quinones of isolates Chj707^T, Ko2 and Ko10 and other related taxa gave only one characteristic peak, which corresponded to menaquinone

MK-6. As presented in Table 2, it is clear that all species of the genera *Chryseobacterium*, *Bergeyella* and *Riemerella* and the three novel isolates have MK-6 as their common respiratory isoprenoid quinone.

Phylogenetic relationships

Comparative 16S rRNA gene sequence analyses indicated the phylogenetic position of strains Chj707^T, Ko2 and Ko10 as members of the *Chryseobacterium*–*Bergeyella*–*Riemerella* branch of the *Flavobacteriaceae* in rRNA superfamily V, the CFB phylum, which has been studied previously in detail by the DNA–rRNA hybridization technique. The 16S rRNA gene sequences of strains Chj707^T, Ko2 and Ko10, consisting of continuous stretches of 1466 bp (positions 28–1493, according to *E. coli* numbering), were used to search GenBank for related sequences. The sequences of strains Ko2 and Ko10 were identical and differed from the sequence of Chj707^T in only two nucleotide positions. Sequence searches showed that strains Chj707^T, Ko2 and Ko10 were phylogenetically most closely related to representatives of the family *Flavobacteriaceae*. The results of sequence similarity calculations indicated that the nearest relatives of strain Chj707^T are *Chryseobacterium balustinum* (94.3 % sequence similarity over 1430 bp sequence), *C. scophthalmum*

Table 2. Chemotaxonomic characteristics of the novel isolates with species in the *Chryseobacterium*–*Bergeyella*–*Riemerella* branch

Strains/species: 1, *C. balustinum* KCTC 2903^T; 2, *C. defluvii* B2^T; 3, *C. gleum* (n=5); 4, *C. indologenes* (n=45); 5, *C. indoltheticum* KCTC 2920^T; 6, *C. joostei* (n=11); 7, *C. meningosepticum* KCTC 2906^T; 8, *C. scophthalmum* (n=7); 9, *B. zoohelcum* KCTC 2910^T; 10, *R. anatipestifer* (n=16); 11, *R. columbina* (n=13); 12, Chj707^T; 13, Ko2; 14, Ko10. Values are percentages of total fatty acids; only means are given and standard deviations are not given. Fatty acids that account for less than 1 % of the total fatty acids are not shown; the percentages therefore do not add up to 100 %. tr, Trace (<1 %); –, not detected; ECL, equivalent chain-length (i.e. the identity of the fatty acids is unknown). Summed feature 4 contains 15:0 iso 2-OH and/or 16:1 ω 7*clt*. The respiratory quinones of *C. gleum*, *C. indoltheticum*, *C. scophthalmum*, *B. zoohelcum* were analysed in this study (ND, not determined). Data were taken from Bernardet *et al.* (1996), Dees *et al.* (1985), Holmes *et al.* (1984, 1986), Hugo *et al.* (2003), Jooste & Hugo (1999), Kämpfer *et al.* (2003), Mudarris *et al.* (1994), Segers *et al.* (1993), Vancanneyt *et al.* (1999) and Yabuuchi *et al.* (1983).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fatty acids:														
13:0 iso	tr	3	tr	tr	–	tr	1	tr	2	15	10	10	11	10
ECL 13:566	2	tr	1	2	2	1	2	3	2	2	tr	–	–	–
14:0 iso	–	–	–	–	–	–	–	–	–	–	1	2	2	2
15:0 iso	33	59	35	34	29	35	41	35	48	53	45	52	50	47
15:0 iso 3-OH	3	3	3	3	2	3	4	3	4	8	4	3	3	3
15:0 anteiso	tr	3	tr	tr	6	tr	2	tr	–	6	22	11	13	14
16:0	2	1	1	tr	1	tr	tr	1	tr	tr	–	–	–	1
16:0 3-OH	1	tr	1	1	tr	1	2	1	–	–	–	–	–	–
16:0 iso 3-OH	tr	tr	–	–	1	–	tr	tr	–	1	–	2	2	3
ECL 16:580	1	tr	2	2	1	2	2	2	1	tr	–	–	–	–
17:0 iso	1	2	2	tr	tr	tr	tr	1	–	–	–	–	–	–
17:0 iso 3-OH	17	14	22	19	14	20	16	17	14	13	7	9	10	9
17:1 iso ω 9c	27	5	20	24	26	23	7	21	18	–	–	8	5	6
Summed feature 4	9	8	12	11	11	12	18	12	9	–	3	2	3	4
Respiratory quinone	MK6	MK6	MK6	MK6	MK6	ND	MK6	MK6	MK6	MK6	ND	MK6	MK6	MK6

Fig. 1. Phylogenetic tree of strains of Chj707^T, Ko2 and Ko10 and related taxa of the *Chryseobacterium*–*Bergeyella*–*Riemerella* branch of the *Flavobacteriaceae*, drawn with 1348 nt of the 16S rRNA gene by using the complete deletion mode in MEGA2. The tree was constructed using the neighbour-joining method. Bootstrap values indicated at branches were calculated from 1000 trees. Accession numbers are indicated in parentheses.

(94.1% over 1442 bp) and *Chryseobacterium indoltheticum* (93.7% over 1418 bp). Sequence similarity of 92.6–94.3% was observed to all species of the genus *Chryseobacterium*. Lower sequence similarities (87.6–92.9%) were found to species from the genera *Bergeyella* and *Riemerella*. Phylogenetic analyses revealed that strains Chj707^T, Ko2 and Ko10 formed a distinct lineage that belonged to a well-supported cluster containing the genera *Chryseobacterium*, *Bergeyella* and *Riemerella* (Fig. 1). The strains form a distinctly separate cluster separate from all species of the closest genus, *Chryseobacterium*. This distinct branch in the phylogenetic tree provides strong support that these strains belong to a new genus. The isolates have a phylogenetically much more distant position from species of the genera *Bergeyella* and *Riemerella*. Members of *Bergeyella* and *Riemerella* are very different in that they are micro-aerophilic and can not grow on nutrient agar, unlike the novel isolates and all species of *Chryseobacterium*. More than 99.9% 16S rRNA gene sequence similarity between Chj707^T, Ko2 and Ko10 showed the intraspecific relationship among the three strains (Rosselló-Mora & Amann, 2001).

DNA base composition

G+C content analyses strongly support the proposal of a new genus. As shown in Table 3, species of the genus *Chryseobacterium* have G+C contents of 30–38 mol% and strains Chj707^T, Ko2 and Ko10 have higher G+C contents of 41.2–42.3 mol%. It is now generally accepted that bacteria with DNA G+C contents differing by more than 5 mol% should not be assigned to the same species and those differing by more than 10 mol% should not be classified in the same genus (Busse *et al.*, 1996; Rosselló-Mora & Amann, 2001; Schleifer & Stackebrandt, 1983; Stackebrandt & Liesack, 1993; Vandamme *et al.*, 1996). The significantly higher G+C contents of strains Chj707^T, Ko2 and Ko10 compared with species of the genera *Chryseobacterium*, *Bergeyella* and *Riemerella* support the inclusion of these strains in a new genus.

FAME analysis

The long-chain fatty acid contents of strains Chj707^T, Ko2 and Ko10 and related taxa are presented in Table 2. FAME analyses revealed some differences between these strains and their nearest phylogenetic neighbours in the genera *Chryseobacterium*, *Bergeyella* and *Riemerella*. Dominant fatty acids for all strains investigated (mean levels above 1%) are 15:0 iso and 17:0 iso 3-OH. All species of the genus *Chryseobacterium*, including CDC group IIb strains (Hugo *et al.*, 1999), have additional characteristic dominant fatty acids summed feature 4 (15:0 iso 2-OH, 16:1 ω 7c and/or 16:1 ω 7t) and 17:1 iso ω 9c. However, significant differences were observed between the genera *Chryseobacterium*, *Bergeyella* and *Riemerella* and the novel strains. In strains Chj707^T, Ko2 and Ko10, the fatty acid 15:0 iso was dominant, with significant levels of 15:0 anteiso, 17:0 iso 3-OH and 17:1 iso ω 9c. Strains Chj707^T, Ko2 and Ko10 had larger relative amounts of 15:0 iso and smaller amounts of 17:0 iso 3-OH, summed feature 4 and 17:1 iso ω 9c than members of the genus *Chryseobacterium*. The three strains all had distinct fatty acids 13:0 iso and 15:0 anteiso, similar to those of *Bergeyella* and *Riemerella*. Although representatives of the genera *Bergeyella* and *Riemerella* showed similar fatty acid profiles, differentiation on the basis of the amounts of several other fatty acids is possible (15:0 anteiso to *Bergeyella* and 17:1 iso ω 9c to *Riemerella*).

Phenotypic characterization

Phenotypic features that distinguish the novel strains from their nearest relatives are summarized in Table 3. Strains Chj707^T, Ko2 and Ko10 are different from species of the genus *Chryseobacterium* in that they did not produce acid from glucose, did not have β -galactosidase activity, could not hydrolyse starch or assimilate glucose, arabinose or 3-hydroxybutyrate and could not grow on MacConkey agar. They differ from species of the genera *Bergeyella* and *Riemerella* in that they produced indole, had β -glucosidase

Table 3. Differentiating phenotypic characteristics for the novel isolates and other related type species

Strains/species: 1, *C. balustinum*; 2, *C. defluvii*; 3, *C. gleum*; 4, *C. indologenes*; 5, *C. indoltheticum*; 6, *C. joostei*; 7, *C. meningosepticum*; 8, *C. scopthalmum*; 9, *B. zoohelcum*; 10, *R. anatipestifer*; 11, *R. columbina*; 12, Chj707^T; 13, Ko2; 14, Ko10. All strains were Gram-negative, non-motile, rod-shaped and oxidase- and catalase-positive. +, Present in all strains; –, absent in all strains; (+), present in most strains; (–), absent in most strains; V, variable; ND, not determined. See legend to Table 2 for sources of data for reference species.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pigment production	+	+	+	+	+	+	–	+	–	–	–	+	+	+
Growth at:														
5 °C	V	ND	–	–	+	+	–	V	–	ND	ND	–	–	–
37 °C	+	+	+	–	–	–	+	–	+	+	+	+	+	+
42 °C	–	ND	(+)	–	–	–	–	–	(–)	(+)	+	–	+	–
Optimum growth temperature (°C)	ND	25–30	30–37	30	ND	ND	ND	15–25	30–37	37	ND	30	30	30–37
Nitrate reduction	+	–	V	V	–	–	–	–	–	–	–	+	–	–
Indole production	+	+	+	+	+	+	V	–	(+)	V	–	+	+	+
H ₂ S production	–	ND	–	–	+	–	–	–	–	–	ND	–	–	–
Acid production from glucose	+	+	+	+	+	+	+	–	–	V	+	–	–	–
Urease	–	–	(–)	–	–	V	V	+	(+)	V	V	–	+	–
β-Galactosidase	ND	ND	(+)	ND	ND	ND	ND	ND	–	–	ND	–	–	–
β-Glucosidase (aesculin hydrolysis)	V	+	+	+	+	+	(+)	(–)	–	–	+	+	+	+
Protease	+	ND	+	ND	+	+	+	–	–	(+)	ND	+	+	+
Starch hydrolysis	–	ND	+	+	–	+	–	–	–	+	ND	–	–	–
Growth on MacConkey agar	+	–	+	V	+	+	+	–	–	–	–	–	–	–
Growth on nutrient agar	+	+	+	+	+	+	+	+	–	–	ND	+	+	+
DNA G + C content (mol%)	35.0	38.0	37.0	37.7	30.0	36.7	37.0	34.1	35.2	35.0	36.0	42.3	41.2	41.6

activity and grew aerobically on nutrient agar. From the results of analyses of 16S rRNA gene sequence similarity, G + C content, fatty acid profiles and phenotype, it is evident that strains Chj707^T, Ko2 and Ko10 should be assigned to a species in a new genus, for which the name *Kaistella koreensis* gen. nov., sp. nov. is proposed.

Description of *Kaistella* gen. nov.

Kaistella (Ka.is.tel'la. L. dim. suff. -ella; N.L. fem. n. *Kaistella* arbitrary name after KAIST, Korea Advanced Institute of Science and Technology).

Cells are Gram-negative, non-sporulating rods. All strains grow aerobically. Colonies are circular, entire, low-convex, smooth, opaque and yellow. Catalase and oxidase positive. Nitrate is not reduced to N₂. Indole is produced and acid is not produced from glucose. Arginine dihydrolase and β-galactosidase are negative. Aesculin and gelatin are hydrolysed. At present, it is not possible to give a general biochemical profile for the genus as only a single species is known. The DNA base composition ranges from 41.2 to 41.6 mol% G + C. The major isoprenoid quinone is MK-6. The type species is *Kaistella koreensis*.

Description of *Kaistella koreensis* sp. nov.

Kaistella koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* of Korea, from where the novel organisms were isolated).

The description is the same as that of the genus. Mannitol,

maltose, caprate, adipate, malate, citrate, phenylacetate, propionate, 4-hydroxybenzoate, L-proline, rhamnose, suberate, acetate, L-alanine and glycogen are assimilated. Glucose, arabinose, mannose, N-acetylglucosamine, salicin, melibiose, fucose, sorbitol, valerate, histidine, 2-ketogluconate, 3-hydroxybutyrate, D-ribose, inositol, D-sucrose, itaconate, malonate, DL-lactate, 5-ketogluconate, 3-hydroxybenzoate and L-serine are not assimilated. The G + C content of the DNA is 41.2–41.6 mol%.

Strains were isolated from natural mineral water from Daejeon City, Korea. The type strain is Chj707^T (=KCTC 12107^T=IAM 15050^T) and Ko2 (=KCTC 12108=IAM 15051) and Ko10 (=KCTC 12109=IAM 15052) are reference strains.

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