***Ornithinicoccus hortensis* gen. nov., sp. nov., a soil actinomycete which contains L-ornithine**

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Two Gram-positive coccoid, non-motile bacteria with L-ornithine as diagnostic diamino acid of the peptidoglycan and an interpeptide bridge of L-Orn ← Gly_(1,2) ← D-Glu were isolated from a sample of garden soil. The major menaquinone is MK-8(H₄). 13-methyl and 12-methyl tetradecanoic acids are the predominant fatty acids. The polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine and two unknown phospholipids. Mycolic acids are absent. The DNA base composition is 72 mol% G+C. Recent comparative 16S rDNA studies revealed that strains HKI 0125^T and HKI 0131 represent a novel lineage adjacent to the family *Intrasporangiaceae* of the order *Actinomycetales* but distinct from the previously described genera of this family. On the basis of the genotypic, chemotaxonomic, morphological and physiological characteristics of these two isolates it is proposed to classify HKI 0125^T and HKI 0131 in a new genus and species for which the name *Ornithinicoccus hortensis* gen. nov., sp. nov. is proposed. The type strain is HKI 0125^T (= DSM 12335^T).

Keywords: *Ornithinicoccus hortensis* gen. nov., sp. nov., L-ornithine-containing actinomycete

INTRODUCTION

A few actinomycete genera contain ornithine as diagnostic diamino acid in their peptidoglycans. Those exhibiting peptidoglycans with cross-linkage A (Schleifer & Kandler, 1972) are the genera *Cellulomonas* (Bergey *et al.*, 1923) and *Rarobacter* (Yamamoto *et al.*, 1988) belonging to the family *Cellulomonadaceae*. Genera having peptidoglycans with cross-linkage B are members of the family *Microbacteriaceae*, i.e. *Curtobacterium* (Yamada & Komagata, 1972b) and *Microbacterium* (Orla-Jensen, 1919; Takeuchi & Hatano, 1998), the latter of which now comprises members of the former genus *Aureobacterium* (Collins *et al.*, 1983a, b; Yokota *et al.*, 1993). The two families belong to the suborder *Micrococccineae* (Stackebrandt *et al.*, 1997).

Of about 25 actinomycete isolates originating from a soil sample collected in a garden near Giessen (Germany), four strains were found having in common ornithine in the peptidoglycan and the respiratory

menaquinones of the type MK-8(H₄). This combination of significant diagnostic chemotaxonomic features has not been found before in actinomycetes as the ornithine containing genera are characterized by isoprenoid quinones MK-9 (*Curtobacterium*, *Rarobacter*), MK-10, MK-11, MK-12, MK-13, MK-14 (*Microbacterium*) and MK-9(H₄) (*Cellulomonas*). By studying the morphological, physiological and chemotaxonomic characteristics of these four isolates we found that two of these strains should be placed in the same taxon because of the high similarity of their tested features. However, they differed remarkably from the other two isolates of the same origin (I. Groth, unpublished).

On the basis of our results presented in this study we conclude that strain HKI 0125 and HKI 0131 are phylogenetically identical and members of a new genus and a new species for which the name *Ornithinicoccus hortensis* is proposed. The type strain is HKI 0125^T (= DSM 12335^T).

METHODS

Bacterial strains and cultural conditions. Strains HKI 0125^T and HKI 0131 were isolated from a soil sample collected in

The EMBL accession numbers for the 16S rDNA sequences of *Ornithinicoccus hortensis* HKI 0125^T and *Rarobacter faecitabidus* DSM 4813^T are Y17869 and Y17870, respectively.

Fig. 1. 16S rDNA sequence-based phylogenetic dendrogram constructed from evolutionary distances (De Soete, 1983) showing the phylogenetic position of strain HKI 0125^T next to members of the family Intrasporangiaceae and other families of the suborder Micrococccineae (Stackebrandt *et al.*, 1997). Numbers at branching points refer to bootstrap values (500 trees resampled). Scale bar, two inferred nucleotide substitutions per 100 nucleotides.

a garden near Giessen, Germany. One gram of soil was suspended in 10 ml sterile phosphate buffer (pH 7.2) and thoroughly mixed by shaking. Soil particles were allowed to sediment, the liquid phase was serially diluted and spread onto the surface of peptone/yeast extract/brain-heart infusion agar plates [PY-BHI medium (Yokota *et al.*, 1993)] which contains per litre: 10 g peptone, 2 g yeast extract, 2 g brain-heart infusion, 2 g NaCl and 2 g glucose (pH 7.2). Biomass for biochemical analyses was prepared by growing the strains in liquid rich (R) medium (Yamada & Komagata, 1972a) and Bacto tryptic soy broth (Difco) and harvesting the cells by centrifugation. Solidified R medium was used for general laboratory cultivation and morphological and physiological studies unless otherwise indicated.

Morphological and physiological characteristics. Cell morphology was examined by phase-contrast microscopy (Olympus BH-2 microscope). Motility was studied by the hanging drop technique. Cell dimensions were measured microscopically [Olympus BX 50, combined with the image analysing software VIS 5504 H (Jentech)]. Gram staining and acid-fast staining were performed as described previously (Groth *et al.*, 1997). For scanning electron microscopy a 24-h-old culture grown on a R agar plate was suspended in phosphate buffer (pH 7.2). Preparation of cells was as described by Groth *et al.* (1997). Cell morphology was studied with a field emission scanning electron microscope (LED 1530) with a working distance of 3 mm. Colony morphology was studied with a stereo microscope (Olympus SZ 11). Growth dependence on different incubation temperatures, increasing concentrations of NaCl in the culture medium and on the supply with oxygen, as well as the utilization of various organic compounds and selected enzyme activities, were studied as described by Groth *et al.* (1997). The susceptibility to antibiotics was tested by

placement of antibiotic discs (Oxoid) on R agar plates seeded with suspensions of HKI 0125^T and HKI 0131, respectively.

Cell wall analysis. Purified cell wall preparations were obtained by the method of Schleifer & Kandler (1972). The amino acids and peptides in cell wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates using the solvent systems of Schleifer & Kandler (1972). The amino terminal amino acid of the interpeptide bridge was determined by dinitrophenylation as described by Schleifer (1985). Whole-cell sugars were determined as alditol acetates by GC as described by Groth *et al.* (1996). The molar ratios of amino acids were determined by GC and GC-MS of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987). Analysis of enantiomers of cell wall amino acids was performed by GC of *N*-pentafluoropropionyl amino acid isopropyl esters (Frank *et al.*, 1980) on a L-Chirasil-Val column (25 m × 0.25 mm i.d.; Macherey-Nagel) using helium as carrier gas at a linear velocity of 200 mm s⁻¹ and at a temperature programmed from 80 to 190 °C at a rate of 2 °C min⁻¹. Instruments used for GC and GC-MS were as specified previously (Groth *et al.*, 1996). The glycolate content of bacterial cell walls was determined by the colorimetric method of Uchida & Aida (1984).

Lipid analysis. Cellular fatty acid methyl esters obtained from cells grown in Bacto tryptic soy broth at 28 °C by the method of Stead *et al.* (1992) were separated by GC (Groth *et al.*, 1996) and identified as described previously (Schumann *et al.*, 1997). Menaquinones were extracted as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC and spraying with specific reagents

(Collins & Jones, 1980). Absence of mycolic acids was demonstrated by TLC (Minnikin *et al.*, 1975).

DNA base composition. DNA was isolated and its G+C content determined by HPLC of deoxyribonucleosides as described by Groth *et al.* (1996).

16S rDNA sequence determination and phylogenetic analysis. Preparation of genomic DNA from strains HKI 0125^T and HKI 0131 was done as described previously (Rainey *et al.*, 1996). Sequences were aligned manually with sequences published previously. These were stored in the DSMZ database consisting of several thousand 16S rDNA sequence entries, including those from the Ribosomal Database Project (Maidak *et al.*, 1997) and EMBL. Similarity values were transformed into phylogenetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The algorithm of De Soete (Maidak *et al.*, 1997) and the neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993) were used in the construction of phylogenetic dendrograms. Bootstrap values were determined according to Felsenstein (1993). All analyses were done on a SUN SparcII workstation.

Nucleotide accession numbers. The accession numbers of the reference strains used in the phylogenetic analysis are shown in Fig. 1.

RESULTS

Morphological and physiological characteristics

The strains HKI 0125^T and HKI 0131 were very similar in colony and cell morphology. They mainly formed cream-coloured, circular, glistening, opaque colonies with an entire margin on R medium. However, a few colonies were observed having a depressed centre surrounded by a uniform or radial structured wall and

Fig. 2. Micrograph of strain HKI 0125^T grown on R agar for 6 d at 28 °C. Bar, 10 µm.

Fig. 3. Scanning electron micrograph of cells from a 24-h-old culture of strain HKI 0125^T grown on R agar. Bar, 1 µm.

an irregular wrinkly marginal zone. The colony sizes were in the range 0.6–4.3 mm. Cells were irregularly spherical, occurring singly, in pairs, short chains and in clusters (Figs 2 and 3). The cell diameters varied from 0.8 to 1.3 µm. Spore formation and motility of the cells were not observed.

Both strains grew well on complex organic media under aerobic and microaerophilic conditions. No growth occurred in an anaerobic atmosphere. Optimal temperatures for growth were 28 to 37 °C. At a temperature of 42 °C the strains did not grow. NaCl in combination with R medium was tolerated up to a concentration of 4% (w/v). Addition of 6% (w/v) of NaCl to the culture medium resulted in a strong decrease in growth. At concentrations of 8% (w/v) and 10% (w/v) of NaCl only traces of growth were observed and at a concentration of 12% (w/v) of NaCl growth was completely inhibited. Liquid cultures of both strains were maintained at –80 °C and in the vapour phase of liquid nitrogen. The latter is recommended for long-term storage.

The cells of strains HKI 0125^T and HKI 0131 were Gram-positive and not acid-fast.

The physiological properties are listed in Table 1. Both isolates HKI 0125^T and HKI 0131 were very similar in most of the tested characteristics. The acid production from carbohydrates was in the case of positive reactions (Table 1) generally low and indicated an oxidative metabolism. On aesculin-iron agar both strains produced a brown pigment after a prolonged incubation time of more than 7 d. This pigment was different from the black staining of the agar in the case of positive aesculin hydrolysis. It stained the colonies and penetrated into the agar. Sodium malate was readily used as sole carbon source while disodium succinate, sodium formate and sodium aconitate were utilized after a prolonged incubation time (about 7 d). HKI 0125^T and HKI 0131 were susceptible to all

Table 1. Physiological properties of strains HKI 0125^T and HKI 0131

—, Negative; +, positive; w, weakly positive.

Test	HKI 0125 ^T	HKI 0131	Test	HKI 0125 ^T	HKI 0131	Test	HKI 0125 ^T	HKI 0131
Decomposition of:			Utilization of:			Growth in the presence of:		
Adenine	—	—	Acetate	—	w	2% NaCl	+	+
Casein	+	+	Aconitate	+	+	4% NaCl	+	+
Aesculin	—	—	Benzoate	—	—	6% NaCl	w	w
Gelatin	+	+	Citrate	—	+	8% NaCl	w	w
Hippurate	w	+	Formate	+	w	10% NaCl	w	w
Hypoxanthine	+	+	Malate	+	+	12% NaCl	—	—
Potato starch	—	—	Succinate	+	+	Antibiotic susceptibility:		
Tween 80	+	+	DL-Tartrate	—	—	Ampicillin, 10 µg	+	+
Tyrosine	—	—	Enzyme assay (API ZYM)			Chloramphenicol, 30 µg	+	+
Urea	—	w	Phosphatase alkaline	+	+	Ciprofloxacin, 5 µg	+	+
Xanthine	+	+	Esterase (C 4)	+	+	Erythromycin, 15 µg	+	+
Acid produced from:			Esterase lipase (C 8)	+	+	Gentamicin, 10 µg	+	+
L-Arabinose	—	—	Lipase (C 14)	w	+	Kanamycin, 30 µg	+	+
Lactose	—	—	Leucine arylamidase	+	+	Lincomycin, 2 µg	+	w
D-Cellobiose	—	—	Valine arylamidase	w	+	Neomycin, 30 µg	+	+
Dextrin	+	w	Cystine arylamidase	+	+	Nitrofurantoin, 300 µg	+	+
D-Fructose	+	—	Trypsin	—	—	Oxacillin, 5 µg	—	w
D-Galactose	—	—	Chymotrypsin	+	+	Oxytetracycline, 30 µg	+	+
D-Glucitol	w	w	Phosphatase acid	w	w	Penicillin G, 2 IU	+	+
D-Glucose	+	w	Naphthol-AS-BI-phosphohydrolase	+	w	Polymyxin B, 300 IU	+	+
Glycerol	w	—	α-Galactosidase	—	—	Rifampin, 2 µg	+	+
Inulin	—	—	β-Galactosidase	—	—	Streptomycin, 10 µg	+	+
Maltose	+	w	β-Glucuronidase	—	—	Sulfonamide, 300 µg	—	—
D-Mannitol	w	w	α-Glucosidase	+	+	Nitrate reduction	+	+
D-Mannose	w	—	β-Glucosidase	—	—	Production of H ₂ S	+	+
D-Raffinose	—	—	N-Acetyl-β-glucosamidase	—	—	Catalase reaction	+	+
L-Rhamnose	—	—	α-Mannosidase	—	—	Methyl red test	—	—
D-Ribose	—	—	α-Fucosidase	—	—	Voges-Proskauer test	—	—
Salicin	—	—	Growth at:			Oxidase test	—	—
Sucrose	w	w	28 °C	+	+	Indole test	—	—
Potato starch	+	+	37 °C	+	+			
Trehalose	w	w	42 °C	—	—			
D-Xylose	—	—						

antibiotics tested except sulfonamide. They were strongly inhibited by penicillin G, chloramphenicol, erythromycin and rifampin.

Cell wall analysis

The peptidoglycan of strains HKI 0125^T and HKI 0131 contained Orn, Ala, Glu and Gly in a molar ratio of 0.8:1.5:2.0:1.6 (HKI 0131, 1.0:1.6:2.0:1.7), respectively. Dinitrophenylated Glu and traces of dinitrophenylated Gly were detected in hydrolysates of cell wall preparations treated with 1-fluoro-2,4-dinitrobenzene. Enantiomeric analysis of cell wall amino acids resulted in detection of L-Orn, D-Ala, L-Ala, D-Glu and L-Glu (traces). From these results and from

two-dimensional thin-layer chromatographic peptide patterns of partial hydrolysates of cell walls (data not shown), it was concluded that strains HKI 0125^T and HKI 0131 contain a peptidoglycan of type A4β (Schleifer & Kandler, 1972) with an L-Orn ← Gly_(1,2) ← D-Glu interpeptide bridge. The acyl type was acetyl. Glucose was found as only whole-cell sugar.

Lipid analysis

The polar lipid patterns consisted of phosphatidyl-inositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine and two unknown phospholipids. The cellular fatty acid profiles (Table 2) were of the iso and anteiso branched type and were

Table 2. Cellular fatty acid composition (%) of strains HKI 0125^T and HKI 0131

The abbreviations for fatty acids are illustrated by the following examples: C_{16:0}, hexadecanoic acid; C_{18:1}, octadecenoic acid; i-C_{15:0}, 13-methyl tetradecanoic acid; ai-C_{17:0}, 14-methyl hexadecanoic acid.

Strain	i-C _{13:0}	ai-C _{13:0}	i-C _{14:0}	C _{14:0}	i-C _{15:0}	i-C _{15:1}	ai-C ₁₅	C _{15:0}	C _{15:1}	i-C _{16:0}	i-C _{16:1}	C _{16:0}	C _{16:1}	i-C _{17:0}	ai-C _{17:0}	i-C _{17:1}	C _{17:0}	C _{17:1}	C _{18:0}	C _{18:1}
HKI 0125 ^T	0.7	0.4	0.9	3.1	36.6	3.6	19.4	8.5		3.5	0.4	4.7	1.0?	1.3	3.0	5.5				0.8
HKI 0131	0.5	0.2	0.3	0.6	46.6	4.5	16.8	4.1	0.2	2.2	0.2	1.4	0.5	2.6	3.3	10.1	0.2	0.2	0.1	0.8

distinctly dominated by the occurrence of 13-methyl and 12-methyl tetradecanoic acids.

The major menaquinone was MK-8(H₄). MK-8(H₂) occurred in minor amounts. Strain HKI 0125^T showed MK-6(H₄) and strain HKI 0131 showed MK-9(H₂) and MK-8 as additional minor components. Mycolic acids were not present.

DNA base composition

Strains HKI 0125^T and HKI 0131 had a DNA G + C content of 72 mol %.

16S rDNA analyses

Phylogenetic information on *Rarobacter faecitabidus* DSM 4813^T had already been incorporated into the analysis of the class *Actinobacteria* (Stackebrandt *et al.*, 1997), without, however, having deposited the 16S rDNA sequence. The EMBL number is Y17870.

The almost complete 16S rDNA sequence was determined for strain HKI 0125^T. The 5' terminal 500 nucleotides of strain HKI 0131 were identical with those of strain HKI 0125^T. Comparison of the sequence of strain HKI 0125^T with those of the class *Actinobacteria* revealed that this strain is a phylogenetic member of the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997), with which strain HKI 0125^T shared between 90 and 95% similarity. The highest similarity values, ranging between 94.5 and 95.2% were found with members of the genera *Janibacter*, *Intrasporangium* and *Terrabacter* while those obtained with *Terracoccus luteus* and some members of the genera *Cellulomonas*, *Sanguibacter* and *Kytococcus* were slightly lower (92.1–94.0% similarity).

Assessment of the phylogenetic position of strain HKI 0125^T within the radiation of a varying selection of reference organisms, using the algorithms of De Soete (Maidak *et al.*, 1997) and the neighbour-joining method (Felsenstein, 1993), consistently showed this strain to branch adjacent to *Janibacter* and certain genera of the family *Intrasporangiaceae*, i.e. *Intrasporangium*, *Terrabacter* and *Terracoccus* (Fig. 1). The position of neighbouring genera, however, changed with the algorithms applied. While according to the neighbour-joining algorithm the neighbouring cluster contains members of the family *Dermatophilaceae* (not shown), the distance matrix analysis of De Soete indicates that the phylogenetic neighbours are

members of *Sanguibacter* and *Cellulomonadaceae* (Fig. 1). In none of the trees the genus *Sanguibacter*, described to be a member of the family *Intrasporangiaceae*, and sharing the same set of signature nucleotides, clustered with the other members of the family. As noted before (Stackebrandt *et al.*, 1997) almost none of the branching points among actinobacterial families is supported by high bootstrap values; this phenomenon extends even to some intra-family relationships when the radiation of lineages occurs within a short evolutionary time span.

DISCUSSION

Both isolates under study showed 100% homology in their 16S rDNA sequences. Therefore strain HKI 0131 was considered to be a member of the same species as HKI 0125^T. This affiliation was confirmed by the high similarity in morphological, physiological and chemotaxonomic characteristics. The minor differences we stated however in various properties between the two isolates are an expression of phenotypic variation within the species and indicate that the strains despite of the same origin are not identical.

The results of the 16S rDNA sequence comparison show clearly that strain HKI 0125^T forms a distinct clade neighbouring the evolutionary radiation of the family *Intrasporangiaceae* and *Janibacter limosus*. The question whether strain HKI 0125^T should be considered a member of the family *Intrasporangiaceae* could be decided upon the presence of family signature nucleotides in the sequence of HKI 0125^T. Of the 17 signatures described for the genera *Sanguibacter*, *Terrabacter* and *Intrasporangium*, only 12 signatures are present in the sequence of HKI 0125^T. *Janibacter limosus*, a taxon that branches intermediate to strain HKI 0125^T and members of the family *Intrasporangiaceae*, possessed only 13 of the 17 family-specific signatures. These significant differences may indicate that neither strain HKI 0125^T nor *Janibacter limosus* should be considered as members of the family *Intrasporangiaceae*. At this point a decision about higher taxon affiliation should await analysis of additional strains of both taxa.

The phylogenetic separateness of *Janibacter* and strain HKI 0125^T from *Intrasporangiaceae* is extended to the epigenetic level. Members of this family contain LL-A₂pm in their peptidoglycans, the structure of which is unique within members of the suborder *Micrococcineae* (Prauser *et al.*, 1997). On the other hand, the

Table 3. Differential characteristics of strain HKI 0125^T and related taxa

Abbreviations: D-Orn, D-ornithine; L-Orn, L-ornithine; meso-A₂pm, meso-diaminopimelic acid; LL-A₂pm, LL-diaminopimelic acid; DPG, diphosphatidylglycerol; GL, unknown glycolipid(s); PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; PSer, phosphatidylserine; PLs, unknown phospholipids; A, anteiso methyl-branched; I, iso methyl-branched; S, straight-chain saturated; U, monounsaturated; ND, not determined.

Taxon	Cell morphology	Wall diamino acid	G + C content (mol %)	Murein type	Major menaquinone(s)	Polar lipid	Fatty acid type
HKI 0125 ^T	Cocci, non-motile	L-Orn	72	A4β	MK-8(H ₄)	PI, PG, DPG, PSer, PLs	S, A, I
<i>Cellulomonas</i> *	Irregular rods, motile or non-motile	L-Orn	71–76	A4β	MK-9(H ₄)	DPG, PI, PGL	S, A, I
<i>Rarobacter</i> †, ‡	Rods	L-Orn	65–66	A4β	MK-9	ND	S, A, I
<i>Curtobacterium</i> †	Irregular rods, motile	D-Orn	68–75	B2β	MK-9	DPG, PG, GLs	S, A, I
<i>Microbacterium</i> ‡, §	Irregular rods	D-Orn, L-Lys	67–70	B2β, B2α	MK-11, MK-12, MK-13, MK-14	DPG, PG, GL	S, A, I
<i>Intrasporangium</i>	Hyphae	LL-A ₂ pm	68	A3γ	MK-8	PI, PIM, PG, DPG	S, A, I
<i>Terrabacter</i> ¶	Rod/coccus cycle	LL-A ₂ pm	70–73	A3γ	MK-8(H ₄)	DPG, PI, PE, PL	S, A, I
<i>Terracoccus</i> #	Cocci in packets, non-motile	LL-A ₂ pm	73	A3γ	MK-8(H ₄)	PE, PI, PG, DPG	S, A, I
<i>Janibacter</i> ¶	Coccoid to rod-shaped, non-motile	meso-A ₂ pm	70	A1γ	MK-8(H ₄)	DPG, PG, PI	S, I, U

* Data from Stackebrandt *et al.* (1995).

† Data from Yokota *et al.* (1993).

‡ Data from Goto-Yamamoto *et al.* (1993).

§ Data from Takeuchi & Hatano (1998).

|| Data from Schumann *et al.* (1997).

¶ Data from Martin *et al.* (1997).

Data from Prauser *et al.* (1997).

peptidoglycans of strains HKI 0125^T and HKI 0131 contain L-ornithine while *Janibacter limosus* possesses meso-A₂pm as the diagnostic diamino acid (Martin *et al.*, 1997). Other differences in taxonomically important diagnostic features are shown in Table 3.

Strains HKI 0125^T and HKI 0131 are assumed to be a novel taxon among members of the peptidoglycan A type-containing *Actinobacteria* because of the unusual combination of L-ornithine as diagnostic diamino acid in the peptidoglycan and the presence of tetrahydrogenated menaquinones with eight isoprene units as predominant isoprenologue. In contrast to the two ornithine-containing peptidoglycan A type genera *Rarobacter* and *Cellulomonas*, strains HKI 0125^T and HKI 0131 possess peptidoglycan variation A4β (Schleifer & Kandler, 1972) defined by an L-Orn ← Gly_(1,2) ← D-Glu interpeptide bridge. This type has not yet been described before in *Actinobacteria*. This feature and additional chemotaxonomic, morphological and physiological characteristics (Table 3) justify a separation of our two isolates on the genus level from the above-mentioned genera. The moderate degree of phylogenetic relatedness to the two members of the family *Cellulomonadaceae* confirms our results (Fig. 1). While peptidoglycan A-type organisms are common in actinomycetes and encompass several separate phylogenetic lineages it was shown that B-type organisms are more closely related among each other, comprising the family *Microbacteriaceae* (Park *et al.*, 1993; Rainey *et al.*, 1994; Takeuchi & Yokota, 1994; Stackebrandt *et al.*, 1997). It is pointed out in the dendrogram that strain HKI 0125^T is unrelated to

the ornithine-containing genera *Curtobacterium* and *Microbacterium*. This is supported by chemotaxonomic features as well as morphological and physiological characteristics. On the basis of the distinct phylogenetic position at the basis of the family *Intrasporangiaceae* and the pronounced differences in morphological, physiological and chemotaxonomic characteristics we propose that the genus *Ornithinicoccus* should be established for the strains HKI 0125^T and HKI 0131. The type species of the genus *Ornithinicoccus* is *Ornithinicoccus hortensis* gen. nov., sp. nov., which is represented by type strain HKI 0125^T and strain HKI 0131 which are deposited in the DSMZ as DSM 12335^T and DSM 12336, respectively.

Description of *Ornithinicoccus* gen. nov.

Ornithinicoccus (or'ni.thi.ne. Gr. n. *ornithos* bird, ornithine an amino acid named after birds; Gr. n. *coccus* a grain; M.L. masc. n. *Ornithinicoccus* a coccus with ornithine).

Cells are irregularly spherical occurring singly, in pairs, short chains and clusters. Cell diameters vary from 0.8 to 1.3 µm. Gram-positive, not acid-fast, non-motile, no formation of spores. Aerobic to microaerophilic. Oxidase-negative, catalase-positive. The peptidoglycan type is A4β with L-Orn ← Gly_(1,2) ← D-Glu as interpeptide bridge. The acyl type is acetyl. The major menaquinone is MK-8(H₄). The major fatty acids are 13-methyl and 12-methyl tetradecanoic acids. The polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine

and two unknown phospholipids. The G + C content of the DNA is 72 mol %. Phylogenetically this genus is a member of the suborder *Micrococcineae* in which it is closely related to members of the family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997). The type species is *Ornithinicoccus hortensis*.

Description of *Ornithinicoccus hortensis* sp. nov.

Ornithinicoccus hortensis (hor.ten'sis. L. masc. n. hortus garden; M.L. adj. hortensis referring to the place where the organism was isolated).

Cells are irregularly spherical occurring singly, in pairs, short chains and clusters. Cell diameters vary from 0.8 to 1.3 µm. Gram-positive, not acid-fast, non-motile, no formation of spores. Aerobic to microaerophilic. Oxidase-negative, catalase-positive. Colonies are 0.6–4.3 mm in diameter, cream coloured, circular, convex with an entire or structured surface and marginal zone. Acids produced in a low amount from D-glucose, maltose, D-mannitol, D-glucitol, trehalose, starch, dextrin, sucrose. No acid production from L-arabinose, lactose, D-cellobiose, D-galactose, inulin, D-raffinose, L-rhamnose, D-ribose, salicin and D-xylose. Acid production is variable from D-fructose, glycerol and D-mannose. Aconitate, formate, malate and succinate are used as sole carbon sources. Utilization of acetate and citrate is variable. Benzoate and DL-tartrate are not utilized. Nitrate is reduced to nitrite, H₂S and catalase are produced, indole and oxidase are not produced. Methyl red and Voges–Proskauer reactions are negative. Casein, gelatin, hippurate, hypoxanthine, xanthin and Tween 80 are decomposed; aesculin and tyrosin are not decomposed. Urease activity is variable. NaCl in combination with R medium is well tolerated up to a concentration of 4%. Good growth occurs at 28 and 37 °C; no growth at 42 °C. Cells are susceptible to ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg), penicillin G (2 IU), polymyxin B (300 IU), rifampin (2 µg) and streptomycin (10 µg). Susceptibility to oxacillin (5 µg) is unclear, no susceptibility to sulfonamide. Tested by the API ZYM enzyme assay HKI 0125^T and HKI 0131 are positive for phosphatase alkaline, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, phosphatase acid, naphthol-AS-BI-phosphohydrolase, α-glucosidase and negative for trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. The peptidoglycan is of the A4β-type with an L-Orn ← Gly_(1,2) ← D-Glu as interpeptide bridge. The acyl type is acetyl. The fatty acid pattern is of the iso and anteiso branched type. The major menaquinone MK-8(H₄). The polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine and two unknown phospholipids. Glucose is the only whole-cell sugar. The G + C content of the DNA is

72 mol %. Mycolic acids are absent. The habitat is soil. The type strain is HKI 0125^T which has been deposited in the DSMZ as DSM 12335^T.

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