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, phosphatidylerine 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 0125T 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 0125T 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 0125T (= DSM 12335T).

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 0125T (= DSM 12335T).

METHODS

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

...Rarobacter faecitabidus DSM 4813\(^T\) (Y17709)
Promicromonospora enterophila DSM 43852\(^T\) (X83607)
Cellulomonas flavigena DSM 20109\(^T\) (X83760)
Sanguibacter suarezi DSM 10543\(^T\) (X74452)
Sanguibacter keddiei DSM 10542\(^T\) (X77450)
Janibacter limosus DSM 11140\(^T\) (Y08539)
Intrasporangium calvum DSM 43043\(^T\) (Z76214)
Terrabacter tumescens DSM 20308\(^T\) (X83112)
Terracoccus luteus DSM 44267\(^T\) (Y19382)
strain HKI 0125\(^T\) (Y17700)
Bogoriella caselijtica DSM 11294\(^T\) (Y09911)
Dermacoccus nishinomiyaensis DSM 20448\(^T\) (X87757)
Kytoococcus sedentarius DSM 20547\(^T\) (X87755)
Dermatophilus congolensis DSM 43037\(^T\) (L00015)
Demetria terragena DSM 11295\(^T\) (Y14152)
Arthrobacter globiformis DSM 20124\(^T\) (M024411)
Nesterenkonia halobia DSM 20541\(^T\) (X82747)
Kocuria rosea DSM 20447\(^T\) (X87756)
Stomalococcus mucilaginosus DSM 20746\(^T\) (X87758)
Jonesia denitrificans DSM 20603\(^T\) (X83811)
Dermabacter hominis NCIC 2769\(^T\) (A01034)
Brachybacterium faecium NCIC 8860\(^T\) (X83810)
Curtobacterium citreum DSM 20528\(^T\) (X77406)
Microbacterium liquefaciens DSM 20638\(^T\) (X77444)

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 Micrococcineae (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 et al., 1979) and on the supply with oxygen, as well as the enzyme activities, were studied as described by Groth et al. (1997). The susceptibility to antibodies 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-heptfluorobutyryl amino acid isobutyl esters (MacKenzie, 1987). Analysis of enantiomers of cell wall amino acids was performed by GC of N-pentafluorobutyric acid isopropyl esters (Frank et al., 1987). 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...
Ornithinicoccus lzortensis gen. nov., sp. nov. (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 0125T 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 0125T 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 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 0125T and HKI 0131 were Gram-positive and not acid-fast.

The physiological properties are listed in Table 1. Both isolates HKI 0125T 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 0125T and HKI 0131 were susceptible to all
Table 1. Physiological properties of strains HKI 0125\textsuperscript{T} and HKI 0131

<table>
<thead>
<tr>
<th>Decomposition of:</th>
<th>Utilization of:</th>
<th>Growth in the presence of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>Acetate</td>
<td>2% NaCl</td>
</tr>
<tr>
<td>Casein</td>
<td>Aconitate</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin</td>
<td>Benzoate</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Citrate</td>
<td>+</td>
</tr>
<tr>
<td>Hippurate</td>
<td>Formate</td>
<td>+ w</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>Malate</td>
<td>+</td>
</tr>
<tr>
<td>Potato starch</td>
<td>Succinate</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>DL-Tartrate</td>
<td>-</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>w</td>
<td></td>
</tr>
<tr>
<td>Xanthine</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Acid produced from:

| L-Arabinose       | Lipase (C 14)   | +                           |
| Lactose          | Leucine arylamidase | +             |
| D-Cellobiose     | Valine arylamidase | w               |
| D-Fructose       | Cystine arylamidase | +               |
| D-Galactose      | Chymotrypsin    | +                           |
| D-Glucitol       | Phosphatase acid | w                           |
| D-Glucose        | Naphthol-AS-BI- | w                           |
| Glycerol         | Phosphohydrolase| -                           |
| Inulin           | 2-Galactosidase | -                           |
| Maltose          | 2-Galactosidase | -                           |
| D-Mannitol       | 2-Gluconidase   | -                           |
| D-Mannose        | 2-Glucosidase   | +                           |
| D-Raffinose      | 2-Glucosidase   | -                           |
| L-Rhamnose       | N-Acetyl-2-glucosaminase | -           |
| D-Ribose         | 2-Mannosidase   | -                           |
| Salicin          | 2-Fucosidase    | -                           |
| Sucrose          |                  |                             |
| Potato starch    | 28 °C           | +                           |
| Trehalose        | 37 °C           | +                           |
| D-Xylose         | 42 °C           | -                           |

Antibiotic susceptibility:

- Ampicillin, 10 μg
- Chloramphenicol, 30 μg
- Ciprofloxacin, 5 μg
- Erythromycin, 15 μg
- Gentamicin, 10 μg
- Kanamycin, 30 μg
- Lincomycin, 2 μg
- Neomycin, 30 μg
- Nitrofurin, 300 μg
- Oxacillin, 5 μg
- Oxytetracycline, 30 μg
- Penicillin G, 2 IU
- Polymyxin B, 300 IU
- Rifampin, 2 μg
- Streptomycin, 10 μg
- Sulfonamide, 300 μg

Growth at:

- 28 °C
- 37 °C
- 42 °C

Cell wall analysis

The peptidoglycan of strains HKI 0125\textsuperscript{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 l-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\textsuperscript{T} and HKI 0131 contain a peptidoglycan of type A4\textsubscript{2} (Schleifer & Kandler, 1972) with an L-Orn + Gly(l,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 phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine and two unknown phospholipids. The cellular fatty acid profiles (Table 2) were of the iso and anteiso branched type and were
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.

<table>
<thead>
<tr>
<th>Strain</th>
<th>( i-C_{15:0} )</th>
<th>( ai-C_{15:0} )</th>
<th>( C_{16:0} )</th>
<th>( C_{17:0} )</th>
<th>( C_{18:0} )</th>
<th>( C_{18:1} )</th>
<th>( C_{19:0} )</th>
<th>( C_{20:0} )</th>
<th>( C_{20:1} )</th>
<th>( C_{22:0} )</th>
<th>( C_{22:1} )</th>
<th>( C_{24:0} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HKI 0125T</td>
<td>07</td>
<td>04</td>
<td>09</td>
<td>31</td>
<td>36</td>
<td>36</td>
<td>194</td>
<td>85</td>
<td>35</td>
<td>04</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td>HKI 0131</td>
<td>05</td>
<td>02</td>
<td>03</td>
<td>06</td>
<td>46</td>
<td>45</td>
<td>169</td>
<td>41</td>
<td>02</td>
<td>14</td>
<td>05</td>
<td>26</td>
</tr>
</tbody>
</table>

**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 0125T. 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 0125T forms a distinct clade neighbouring the evolutionary radiation of the family *Intrasporangiaceae* and *Janibacter limosus*. The question whether strain HKI 0125T should be considered a member of the family *Intrasporangiaceae* could be decided upon the presence of family signature nucleotides in the sequence of HKI 0125T. Of the 17 signatures described for the genera *Sanguibacter*, *Terrabacter* and *Intrasporangium*, only 12 signatures are present in the sequence of HKI 0125T. *Janibacter limosus*, a taxon that branches intermediate to strain HKI 0125T and members of the family *Intrasporangiaceae*, possessed only 13 of the 17 family-specific signatures. These significant differences may indicate that neither strain HKI 0125T 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 0125T from *Intrasporangiaceae* is extended to the epigenetic level. Members of this family contain LL-Amp in their peptidoglycans, the structure of which is unique within members of the suborder *Micrococccineae* (Prauser et al., 1997). On the other hand, the
Table 3. Differential characteristics of strain HKI 0125 T and related taxa

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Cell morphology</th>
<th>Wall diamino acid</th>
<th>G + C content (mol%)</th>
<th>Murein type</th>
<th>Major menaquinone(s)</th>
<th>Polar lipid</th>
<th>Fatty acid type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HKI 0125 T</td>
<td>Cocci, non-motile</td>
<td>L-Orn</td>
<td>72</td>
<td>Aαβ</td>
<td>MK-8(H4)</td>
<td>PI, PG, DPG, PSer, PLs</td>
<td>S, A, I</td>
</tr>
<tr>
<td>Cellulomonas*</td>
<td>Irregular rods, motile or non-motile</td>
<td>L-Orn</td>
<td>71-76</td>
<td>Aαβ</td>
<td>MK-9(H4)</td>
<td>DPG, PI, PGL</td>
<td>S, A, I</td>
</tr>
<tr>
<td>Rarobacter†</td>
<td>Rods</td>
<td>L-Orn</td>
<td>65-66</td>
<td>Aαβ</td>
<td>MK-9</td>
<td>ND</td>
<td>S, A, I</td>
</tr>
<tr>
<td>Curtobacterium‡</td>
<td>Irregular rods, motile</td>
<td>α-Orn</td>
<td>68-75</td>
<td>Bβ2</td>
<td>MK-9</td>
<td>DPG, PG, Glu</td>
<td>S, A, I</td>
</tr>
<tr>
<td>Microbacterium§</td>
<td>Irregular rods</td>
<td>β-Orn, β-Lys</td>
<td>67-70</td>
<td>Bβ2, Bβ2a</td>
<td>MK-11, MK-12, MK-13, MK-14</td>
<td>DPG, PG, Glu</td>
<td>S, A, I</td>
</tr>
<tr>
<td>Intrasporangiaceae</td>
<td>Hyphae</td>
<td>L-α,pm</td>
<td>68</td>
<td>A3γ</td>
<td>MK-8</td>
<td>PI, PI, PM, PG, DPG</td>
<td>S, A, I</td>
</tr>
<tr>
<td>Terracoccus#</td>
<td>Rod/coccus cycle</td>
<td>L-α,pm</td>
<td>70-73</td>
<td>A3γ</td>
<td>MK-8(H4)</td>
<td>DPG, PI, PE, PL</td>
<td>S, A, I</td>
</tr>
<tr>
<td>Janibacter*</td>
<td>Coccos in packets, non-motile</td>
<td>L-α,pm</td>
<td>73</td>
<td>A3γ</td>
<td>MK-8(H4)</td>
<td>PE, PI, PG, DPG</td>
<td>S, A, I</td>
</tr>
<tr>
<td></td>
<td>Coccos to rod-shaped, non-motile</td>
<td>meso-α,pm</td>
<td>70</td>
<td>A1γ</td>
<td>MK-8(H4)</td>
<td>DPG, PG, PI</td>
<td>S, I, U</td>
</tr>
</tbody>
</table>

* 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).

The peptidoglycans of strains HKI 0125 T and HKI 0131 contain L-ornithine while Janibacter limosus possesses meso-A-pm 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 tetra-hydrogenated menaquinones with eight isoprene units as predominant isoprenologine. 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 intermediate bridge. This type has yet not 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 Cellulomonomadaceae 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. ornthos 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 intermediate 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 phosphatidylglycerol, phosphatidylglycerol, phosphatidylserine...
and two unknown phospholipids. The G+C content of the DNA is 72 mol%. Phylogenetically this genus is a member of the suborder Micrococccaeae 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 fructose, glyceral and D-mannose. Aconitate, formate, malate and succinate xanthin and Tween 80 are decomposed; aesculin and with an entire or structured surface and marginal zone.

Produced. Methyl red and Voges-Proskauer reactions are not utilized. Nitrate is reduced to nitrite, and citrate is variable. Benzoate and DL-tartrate are 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), nitrofurazone (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 0125T and HKI 0131 are positive for phosphatase activity 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 peptido- glycan 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, phosphatidylglucose, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinerine 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 0125T which has been deposited in the DSMZ as DSM 12335T.

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REFERENCES


I. Groth and others


