**INTRODUCTION**

One of the first systematic descriptions of the pigmentation of bacteria and of the taxonomic ranks of pigmented micro-organisms was published by the German microbiologist J. Schroeter, based on his culture experiments in the years 1868–70 at the Institute of Physiology of Plants in Breslau (now Wroclaw, Poland). He proposed ‘Bacteridium luteum’ (Schroeter, 1875), which was included by Cohn (1875) as Micrococcus luteus (Schroeter) Cohn 1872 in the characterization of the genus Micrococcus. Since this first description of ‘micrococi’ [in the present study, the authors used the term ‘micrococi’ in quotes to indicate members of the genus Micrococcus Cohn 1872 as understood before the emendation by Stackebrandt et al. (1995)], the taxon has been revised several times and various novel species, including Micrococcus sedentarius (ZoBell & Upham, 1944), have been added (Kocur, 1986). In 1995, the genus Micrococcus Cohn 1872 was taxonomically dissected into five genera, which have been reclassified into the new hierarchic classification system of Actinobacteria classis nov. (Stackebrandt et al., 1995, 1997). In this context, the genus Kytococcus was established with the type species Kytococcus sedentarius and had been classified as a member of the family Dermatophilaceae. Both the Micrococcaceae and the Dermatophilaceae are included in the suborder Micrococcosinae (Stackebrandt et al., 1997). Presently, Kytococcus sedentarius (ZoBell & Upham 1944) Stackebrandt et al. 1995 is the only recognized species of this genus, which is characterized by Gram-positive, coccoid, non-encapsulated, non-motile, aerobic and catalase-positive cells. This species differs from ‘micrococi’ by being resistant to penicillin and methicillin and exhibiting arginine dihydrolase activity. Colonies of Kytococcus sedentarius are deep buttercup yellow or cream-white and they grow more slowly than those of other ‘micrococi’ (Kloos & Bannerman, 1999). Kytococcus sedentarius is usually regarded as part of the human skin flora (Kloos & Musselwhite, 1975). Nevertheless, the bacterium has been documented to be a causative organism in various infections (Greene et al., 1980; Nordstrom et al., 1987; Musselwhite, 1975). It has been isolated from various clinical sources, including skin lesions (Kloos & Bannerman, 1999), abscesses (Greene et al., 1980) and from human blood (Bannerman, 1999). The name Kytococcus sedentarius is a combination of the Greek word kyto- meaning cell and the Latin sedens meaning sitting. The species is characterized by its deep buttercup yellow colonies, which are macroscopically evident on agar plates and are easily identifiable. The bacterium is further characterized by its catalase-positive phenotype and its ability to hydrolyze Tween 80 and the lack of ɑ-glucosidase activity. The predominant menaquinones are MK-8 and MK-7. The major cellular fatty acids are iso-C17:0 3OH, iso-C17:0 2OH, iso-C15:0 3OH and anteiso-C17:0. The strain contains catalase and does not produce acid from carbohydrates. The ability to hydrolyse Tween 80 and the lack of ɑ-glucosidase activity are the most characteristic features. The results of comparative 16S rDNA analysis revealed that the strain represents a novel species within the genus Kytococcus, for which the name Kytococcus Schroeteri sp. nov. is proposed. The type strain is strain Muenster 2000T (= DSM 13884T = CCM 4918T).

**Keywords:** Kytococcus Schroeteri, Micrococcineae, taxonomy, phylogeny, 16S rDNA
Old & McNeill, 1979). Here, we report the characterization of a novel muddy yellow-pigmented, Gram-positive coccus that was isolated from four different blood cultures of a patient with endocarditis. Based on the results of phylogenetic and phenotypic studies, we propose a second species of the genus Kytococcus, Kytococcus schroeteri sp. nov.

**METHODS**

**Bacterial strain.** In 1999, three isolates designated K8356, K8473 and K8645 were cultivated in the diagnostic laboratory of the Institute of Medical Microbiology, University of Münster, Germany, and an isolate designated 28997 was cultivated in the Labor Centrum Nordhorn, Germany. They were isolated in pure culture from four different blood specimens (Bectec 9240 System, Becton Dickinson) obtained from a 34-year-old woman with endocarditis. After genotyping, the isolates were recognized as a single strain, named Muenster 2000.

**Morphological and phenotypic analyses.** The morphological and phenotypic analyses described in this paper were performed on the four unidentified isolates. Isolates were grown at 37 °C on Columbia agar (Becton Dickinson) supplemented and 5% sheep blood. Nutrient broth (Oxoid) was used as liquid or semi-solid medium. Biomass for menaquinone and cell wall analyses was obtained by cultivation in Trypticase soy/yeast extract medium no. 92 (DSMZ, 2001) and biomass for analysis of cellular fatty acids was obtained by cultivation on Trypticase soy broth (BBL Microbiology Systems) with agar at 28 °C for 48 h. Pigmentation and morphology were observed after 24-48 h growth. All physiological tests were performed at 37 °C except for studies concerning the temperature range (27, 30, 37, 43, 50 °C). Catalase production was demonstrated on slides by the formation of bubbles after mixing a suspension of culture material with a drop of 3% (v/v) hydrogen peroxide solution. Oxidase activity was tested by oxidase reagent (Biotest) based on tetramethyl p-phenylenediamine (Tarrand & Groschel, 1982). Lysostaphin and lysozyme lysis susceptibility were assessed as described previously (Geary & Stevens, 1986; Kloos et al., 1974). Urease activity, adenosine hydrolysis and hydrolysis of Tween 80 were tested according to published methods (Christensen, 1946; Cowan & Steel, 1974). Hydrolysis of starch was determined on inorganic salts media. Hydrolysis of amylase and gelatin hydrolysis was determined on inorganic salts medium and by using the substrate panels of the API ID 32 STAPH, API CORYNE and API ZYM test strips according to the manufacturer’s instructions (bioMérieux).

**Chemotaxonomic characterization.** Cell wall analysis was performed as described previously (Groth et al., 1999). For GC analysis of cellular fatty acids, 40 mg cells was saponified, methylated, extracted and analysed using the Microbial Identification System described by Miller (1982). Menaquinones were extracted as described by Collins et al. (1977) and were analysed by HPLC as described previously in detail (Stackebrandt et al., 1995).

**PCR fingerprinting.** The clonality of the clinical isolates was examined in arbitrarily primed (AP)-PCR with prolonged ramp times, applying random primer AP 90-I (Becker et al., 2000; Ellinghaus et al., 1999). The resulting banding patterns were displayed on a 2.0 % agarose gel, visualized by ethidium bromide staining.

**DNA–DNA hybridization, determination of 16S rDNA sequences and phylogenetic analyses.** DNA–DNA hybridization analysis was carried out as described previously (Martin et al., 1997). For 16S rDNA sequence determination, genomic DNA extracts were prepared from an 18-h brain/heart infusion broth culture by using the QIAamp tissue kit (Qiagen) following the manufacturer’s recommendations. The 16S rDNA was amplified using universal prokaryotic 16S rDNA primers 27f, 357f and 907r (Lane, 1991) and primer 16S-4 (5'–GGACTACMGGTTACGTAATCC–3'). The PCR products were purified using Centri-Sep columns (Princeton Separations) and sequenced directly by using the Taq Dye Deoxy terminator cycle sequencing kit (Applied Biosystems) and the ABI PRISM 310 Genetic Analyzer automated sequencing system (Applied Biosystems). The sequence determined was aligned with the 16S rDNA sequences of reference organisms of the genera Arthrobacter, Dermacoccus, Dermatophilus, Janibacter, Kocuria, Kytococcus, Micrococcus, Nesterenkonia and Terrabacter obtained from the EMBL database by using omiga software (Oxford Molecular) and the program CLUSTAL W (Thompson et al., 1994). The resulting multiple sequence alignment was corrected manually. Levels of sequence similarity for approximately 1400 nucleotides were determined with the program package DAMBE (Xia, 2000). A phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987), the stability of the groupings was estimated by bootstrap analysis (500 replications) and branch lengths were evaluated by using a program based on the method of Fitch & Margoliash (1967).

**Nucleotide accession numbers.** The accession numbers of the reference strains used in the phylogenetic analysis are shown in Fig. 2.

**RESULTS AND DISCUSSION**

**Bacterial isolates.** The isolates were obtained within a period of 5 days from four different blood specimens of a patient with endocarditis, suggesting a possible disease association. They grew in pure culture after 24 h incubation. A direct stain of blood smears was positive for Gram-positive cocci. All isolates were clonally related, representing one strain (named Muenster 2000), as tested by AP-PCR fingerprinting (Fig. 1).

**Morphological characteristics and phenotypic analysis.** The unidentified isolates consisted of Gram-positive or Gram-variable cells. In accordance with the genus description of Kytococcus (Stackebrandt et al., 1995), the cells were non-motile and non-encapsulated. Endo-
Kytococcus Schroeteri sp. nov.

Fig. 1. Agarose gel electrophoresis patterns of AP-PCR-amplified products of Kytococcus strains. Lanes: M, DNA molecular size marker (combined 100 bp/1 kb ladder; New England Biolabs); 1, amplicon of Kytococcus sedentarius DSM 20547T; 2–4, amplicons of Kytococcus Schroeteri sp. nov. isolates 28997, K8356T (DSM 13884T), K8473 and K8645 of strain Muenster 2000T.

Table 1. Characteristics that differentiate yellowish-pigmented 'micrococci'

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>M. luteus</th>
<th>Kocuria varians</th>
<th>Kytococcus sedentarius DSM 13884T</th>
<th>Kytococcus Schroeteri sp. nov. DSM 13884T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigmentation of colonies</td>
<td>Yellow or yellowish green</td>
<td>Dark yellow</td>
<td>Cream white/deep buttermilk yellow</td>
<td>Muddy yellow</td>
</tr>
<tr>
<td>Growth on Simmons citrate agar</td>
<td>–</td>
<td>+ &gt; ±, –</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+ &gt; ±</td>
<td>&gt; ±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aerobic acid production from: d-Glucose</td>
<td>– &gt; ±</td>
<td>+ &gt; ±</td>
<td>– &gt; ±</td>
<td>–</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>–</td>
<td>+ &gt; ±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>–</td>
<td>+, –</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Activity of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>α-d-Glucosidase</td>
<td>+†</td>
<td>–†</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>+, –, –</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+, –</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>–‡</td>
<td>+, –</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tween 80</td>
<td>–§</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Production of acetoin (VP test)</td>
<td>–</td>
<td>±, –</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Resistance to:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methicillin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Most strains are negative in the oxidase test (Stackebrandt et al., 1995).
† Data from this study (M. luteus DSM 20030T and Kocuria varians DSM 20022T tested).
‡ Most strains negative, few strains weakly positive (Kloos et al., 1974).
§ Strains of biotype II show variable hydrolysis of Tween 80 (Wieser et al., 2002).

After 24 h incubation on Columbia agar supplemented with 5% sheep blood under aerobic conditions, colonies were tiny, increasing in size to about 1.5–2.5 mm in diameter after 48 h. The colonies were circular, entire, convex, smooth and muddy-yellow without a haemolytic reaction. The temperature range for growth on Columbia blood agar was between 27 and 43°C. The optimal growth temperature was 37°C. Growth did not occur at 50°C. The isolates grew well in up to 12% NaCl and sparsely at 15% NaCl, but no growth was detected above 15% NaCl. Growth did not occur in Simmons citrate agar and growth was not observed under anaerobic conditions. The bacterium was oxidase-negative, as are most Kytococcus sedentarius strains. The isolates were resistant to lysostaphin lysis, whereas they were susceptible to lysozyme, indicating an affiliation to the 'micrococci' but not to the staphylococci (Jeffries, 1968; Klesius & Schulhardt, 1988; Kloos et al., 1974; Schleifer et al., 1981; Stackebrandt et al., 1995).
Table 2. Cellular fatty acid compositions of species of 
*Kytococcus*

Values are percentages of total fatty acids; values less than 1% are not shown for *Kytococcus sedentarius* (data taken from Stackebrandt et al., 1995).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Kytococcus sedentarius</th>
<th>Kytococcus Schroeteri sp. nov. DSM 13884T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{15:0})</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>C(_{16:0})</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>C(_{17:0})</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>C(_{17:1})</td>
<td>3.1</td>
<td>1.1</td>
</tr>
<tr>
<td>iso-C(_{15:0})</td>
<td>27.3</td>
<td>17.9</td>
</tr>
<tr>
<td>iso-C(_{15:1})</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>iso-C(_{16:0})</td>
<td>20</td>
<td>3.2</td>
</tr>
<tr>
<td>iso-C(_{17:0})</td>
<td>8.1</td>
<td>21.1</td>
</tr>
<tr>
<td>iso-C(_{17:1})</td>
<td>10.6</td>
<td>32.5</td>
</tr>
<tr>
<td>anteiso-C(_{15:0})</td>
<td>1.2</td>
<td>3.5</td>
</tr>
<tr>
<td>anteiso-C(_{17:0})</td>
<td>29.8</td>
<td>15.3</td>
</tr>
<tr>
<td>anteiso-C(_{17:1})</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>Total</td>
<td>95.7</td>
<td>100.0</td>
</tr>
</tbody>
</table>

1968; Schleifer & Kloos, 1975). Nitrate reduction did not occur. They shared the following physiological properties with the type species of the genus *Kytococcus*, *Kytococcus sedentarius*, as investigated in comparison to the type strain, DSM 20547\(^T\): growth under aerobic conditions; alkaline phosphatase, arginine dihydrolase, pyrazinamidase and catalase were positive; lecithinase, \(\beta\)-galactosidase and urease were negative; acid production from \(\alpha\)-arabinose, \(\beta\)-galactose, \(\beta\)-glucose, maltose, mannose, melibiose, raffinose, \(\alpha\)-rhamnose, \(\alpha\)-ribose, sucrose, \(\beta\)-xylose, adonitol, glycerol and \(\beta\)-mannitol was negative; utilization of D-fructose, D-glucose, lactose, D-mannose, D-galactose, L-rhamnose, sucrose, D-xylose, glycerol, D-mannitol, D-pantothenate, DL-arginine, \(\beta\)-leucine, L-lysine and L-valine was negative; hydrolysis of gelatin was positive; hydrolysis of aesculin and starch was negative; acetoin was not produced. The isolates differed from *Kytococcus sedentarius* in hydrolysis of Tween 80 and lack of \(\alpha\)-glucosidase activity (Table 1).

Tests of susceptibility to antibiotics showed the following results: all four unknown isolates were susceptible to chloramphenicol, ciprofloxacin, gentamicin, tetracycline, vancomycin and teicoplanin; all isolates were resistant to penicillin, oxacillin and erythromycin. In addition, the isolates were susceptible to bacitracin and resistant to nitrofurantoin, as typically described for ‘micrococci’, in contrast to staphylococci, which are noted for the opposite pattern of susceptibility (Baker, 1984; Baker et al., 1986).

Chemo-taxonomy

The peptidoglycan type was \(\alpha\)-Lys–Glu\(_{\alpha}\), variation A4\(_2\) (A11.43), as described for the genus *Kytococcus* (Stackebrandt et al., 1995). The isoprenoid quinones of the unknown bacterium were represented by MK-8, MK-7 and MK-9 at a peak area ratio of 43:36:1 whereas, in *Kytococcus sedentarius*, menaquinones MK-8, MK-9 and MK-10 predominate. The fatty acid profile was dominated by iso-C\(_{15:0}\) with smaller amounts of iso-C\(_{15:0}\) and anteiso-C\(_{15:0}\) (Table 2). In contrast to these findings, the type species of the genus, *Kytococcus sedentarius*, shows, in addition to iso-C\(_{17:0}\) and anteiso-C\(_{17:0}\), predominantly the straight-chain saturated acids C\(_{15:0}\) and C\(_{17:0}\), but only minor amounts of iso-C\(_{15:0}\).

Determination of 16S rDNA sequence, genomic DNA–DNA hybridization and phylogenetic analyses

In order to ascertain the phylogenetic position of the unknown bacterium, its almost complete 16S rDNA sequence was determined and subjected to compara-

![Fig. 2. 16S rDNA-based phylogenetic dendrogram showing the phylogenetic position of *Kytococcus Schroeteri* sp. nov. Numbers at the nodes are bootstrap values. Bar, 1% sequence divergence.](image-url)
Acetoin is not produced. Metabolically inert for acid 
dase. Negative for lecithinase, 
muddy-yellow. Grows well in the presence of NaCl 
smooth and develop rather slowly. Colonies are 
(data not shown).

The level of DNA–DNA relatedness between the 
unknown bacterium (strain DSM 13884³) and the 
type strain of *Kytococcus sedentarius*, DSM 20547⁷, 
was 45-5%, which is clearly below the threshold value for 
deﬁnition of genospecies (Wayne et al., 1987).

A tree constructed by the neighbour-joining method, 
showing the phylogenetic position of the unknown 
human bacterium with respect to some related Gram-
positive taxa, is shown in Fig. 2. The treeing analysis 
clearly demonstrated that the unknown micro-
organism represents a novel subline within the *Kytococcus* 
clade. Bootstrap resampling showed that the relationship 
to *Kytococcus sedentarius* was statistically signiﬁ-

The morphological and biochemical properties of the 
unknown Gram-positive cocci were found to 
be consistent with its assignment to the genus *Kytococcus*, although it did not correspond to the 
hitherto-established species of this genus, *Kytococcus 
sedentarius*. Furthermore, the results of DNA–DNA 
hybridization experiments showed that the yellow-
pigmented bacterium merits classiﬁcation as a distinct 
species. Thus, based on phylogenetic and phenotypic 
evidence, we consider that the unidentified micro-
organism from human blood should be assigned to the 
genus *Kytococcus as Kytococcus Schroeteri* sp. nov.

**Description of Kytococcus Schroeteri** sp. nov.

*Kytococcus Schroeteri* (schro' ter.i. N.L. gen. n. *schroet-
eri* of Schroeter, honouring Joseph Schroeter, a 
German microbiologist).

The description of the physiological and morpho-
logical characteristics is based on results of studies of 
one strain. In addition to the properties given in the 
genus description (Stackebrandt et al., 1995), the 
species has the following properties. Cells are spherical 
(diameter 1–1.5 μm) and occur predominantly in 
pairs or tetrads. Colonies are up to 2-5 mm in diameter 
(after 48 h incubation), circular, entire, convex and 
smooth and develop rather slowly. Colonies are 
muddy-yellow. Grows well in the presence of NaCl 
concentrations up to 12%. No growth above 15% 
NaCl. Growth does not occur in Simmons citrate agar. 
Negative in the oxidase test. Positive for alkaline 
phosphatase, arginine dihydrolase and pyrazinami-
dase. Negative for lecitihase, β-galactosidase and 
urease. Nitrate is not reduced. Hydrolyses gelatin and 
TWEEN 80, but does not hydrolyse aesculin or starch. 
Acetoin is not produced. Metabolically inert for acid 
production from carbohydrates. No acid is pro-
duced from L-arabinose, D-galactose, D-glucose, mal-
tose, D-mannose, D-melibiose, raffinose, L-rhamnose, 
D-ribose, sucrose, D-xylene, adonitol, glycerol or D-
mannitol. The optimum growth temperature is 37 °C. 
Non-haemolytic. Resistant to penicillin G, oxacillin 
and erythromycin and susceptible to ampicillin, chlor-
amphenicol, ciprofloxacin, gentamicin, tetracycline, 
vancocycin and teicoplanin. Menaquinones with eight 
and seven completely unsaturated isoprene units (MK-
8, MK-7) predominate. The major cellular fatty acids 
are iso-C_{17:0}, iso-C_{16:0} iso-C_{15:0} and anteiso-C_{17:0}. 
The type strain is strain Muenster 20000³ (= DSM 13884³ = CCM 4918³).

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