**Globicatella sulfidifaciens** sp. nov., isolated from purulent infections in domestic animals

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DNA–DNA hybridization experiments and comparative 16S rDNA sequence analysis revealed that six isolates from purulent joint and lung infections in calves, from a lung lesion in a sheep, and from a joint infection of a pig represented a novel species belonging to the genus *Globicatella*. Whole-cell protein electrophoresis and biochemical activity testing revealed that the isolates formed a homogeneous group differing from *Globicatella sanguinis*, the only species of this genus described to date. These animal isolates were classified as *Globicatella sulfidifaciens* sp. nov. with LMG 18844T ( = CCUG 44365T), isolated from the lung of a calf, as the type strain. A detailed description of its phenotypic characteristics is presented. Hydrogen sulphide production was found to be a very useful diagnostic feature.

**Keywords:** *Globicatella sulfidifaciens* sp. nov., cattle, sheep, pig

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**INTRODUCTION**

*Globicatella* has been described as a new genus comprising the single species *Globicatella sanguinis* (Collins et al., 1992). This specific epithet was later corrected to ‘sanguinis’ (Trüper & de’ Clari, 1997). The description was based on the study of strains from human clinical sources including blood, urine and spinal fluid (Collins et al., 1992). Phenotypically, the strains showed some similarity to *Streptococcus uberis* and the ‘bovis’ and ‘viridans’ streptococcal groups, as well as the aerococci. Later studies demonstrated that the genus is phylogenetically most closely related to the recently described *Emerococcus* genus, to *Ignavigranum*, *Facklamia* and *Abiotrophia*, and to the well-established genus *Aerococcus* (Collins et al., 1999).

The present paper describes the characterization and classification of relatively slow-growing Gram-variable cocci from purulent infections in animals as members of the genus *Globicatella*. Despite having a certain resemblance to *G. sanguinis*, the animal isolates were distinct and appeared to constitute a new species – *Globicatella sulfidifaciens* sp. nov. – a description of which is given below.

**METHODS**

**Strains.** The present study covers six field isolates which were obtained from severe (usually purulent) lesions in lungs of calves (three strains), from a lung sample from a lamb with acute broncho pneumonia, and from joint fluids of a pig and a calf with polyarthritis (one strain each) (Table 1). All were from necropsies carried out on animals from different farms in Belgium. The infections appeared to be sporadic and of a mixed nature. The bacteria were obtained in large numbers from lesions, but only one strain was found in pure culture in a joint-fluid sample from a calf. *Arcanobacterium pyogenes* was co-isolated in three out of the six cases. *G. sanguinis* reference strains and their sources are listed in Table 1.

**SDS-PAGE of whole-cell proteins.** All strains were grown for 24 h on brain–heart infusion (BHI) agar (Difco) and incubated at 36–37 °C in a microaerobic atmosphere containing approximately 5% O2, 10% CO2 and 85% N2. Preparation of cellular protein extracts, PAGE, densitometric analysis, normalization and interpolation of the protein profiles, and numerical analysis were performed as described by Pot et al. (1994) using the GelCompar version 4.2 software package (Applied Maths). The profiles were recorded and stored on a PC. The similarity between all pairs of traces was expressed by the Pearson product-moment correlation coefficient converted, for convenience, to a percentage value.

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**Abbreviations:** AMC, 7-amino-4-methyl coumarin; MU, methylumbelliferyl.

The GenBank accession number for the 16S rRNA sequence of strain LMG 18844T is AJ297627.
Table 1. *Globicatella* strains investigated

<table>
<thead>
<tr>
<th>Species and LMG no.</th>
<th>Received as</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. sulfidifaciens</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18837</td>
<td>GEM 618</td>
<td>Cattle lung</td>
</tr>
<tr>
<td>18839</td>
<td>T2137-2</td>
<td>Pig joint</td>
</tr>
<tr>
<td>18842</td>
<td>GEM 571</td>
<td>Sheep lung</td>
</tr>
<tr>
<td>18844&lt;sup&gt;T&lt;/sup&gt;</td>
<td>GEM 604&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Cattle lung</td>
</tr>
<tr>
<td>18846</td>
<td>GEM 565</td>
<td>Cattle joint</td>
</tr>
<tr>
<td>18847</td>
<td>GEM 615</td>
<td>Cattle lung</td>
</tr>
<tr>
<td><em>G. sanguinis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14367</td>
<td>CCUG 26778</td>
<td>Human urine</td>
</tr>
<tr>
<td>18987&lt;sup&gt;T&lt;/sup&gt;</td>
<td>CCUG 32999&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Human blood</td>
</tr>
<tr>
<td>18988</td>
<td>CCUG 33000</td>
<td>Human blood</td>
</tr>
<tr>
<td>18996</td>
<td>CCUG 3367</td>
<td>Human blood</td>
</tr>
</tbody>
</table>

Preparation of high-molecular-weight DNA. High-
molecular-weight DNA was prepared as described by
Pitcher *et al.* (1989), modified as outlined below. Prior to
lysis using guanidium thiocyanate, cells were incubated for
1 h at 37 °C in a buffer containing RNase A (200 mg l<sup>-1</sup>; Sigma),
mutanolysin (100 U ml<sup>-1</sup>; Sigma) and lysozyme
(250 mg ml<sup>-1</sup>; Serva). This was followed by treatment with
proteinase K (200 mg l<sup>-1</sup>; Merck) for 15 min at 37 °C.

16S rRNA gene sequence analysis. Part of the rDNA operon,
comprising the nearly complete 16S DNA, was amplified by
using the PCR. The forward primer was 5′-CTGGCTCA-
GGAC/TGAACGCTG-3′, corresponding to positions 19–
38 of the *Escherichia coli* 16S rRNA numbering system. The
reverse primer was 5′-AAGGAGGTGATCCAGCCGC-
GAGGTGATCCAGCCGCA-3′, complementary to positions 1541–1522 of the *E. coli* 16S
rRNA numbering system. PCR-amplified 16S rDNAs were
purified using the QIAquick PCR purification kit (Qiagen).
Sequence analysis was performed using an Applied Bio-
systems 377 DNA sequencer and the protocols of the
manufacturer (Perkin-Elmer/Applied Biosystems), using
the BigDye Terminator Cycle Sequencing Ready Reaction
kit (with AmpliTaq DNA Polymerase, Fs). The sequencing
primers were those described by Coenye *et al.* (1999).
Sequence assembly was performed by using the program
AUTOASSEMBLER (Applied Biosystems). The consensus
sequence of strain LMG 18844<sup>T</sup> and the sequences of strains
belonging to the same phylogenetic group (retrieved from
the EMBL database) were aligned and a phylogenetic tree
was constructed on the basis of the neighbour-joining method
by using the BIONUMERICS software package (Applied
Maths). The nearly complete sequences (1375 bases) of the
16S rRNA genes were used for the calculation of similarity
levels and for tree construction; unknown bases were
excluded from the calculations.

Determination of the DNA base composition. DNA was
enzymically degraded into nucleosides as described by
Mesbah *et al.* (1989). The nucleoside mixture obtained was
then separated by HPLC, using a Waters Symmetry Shield
C8 column with the thermostat set at 37 °C. The solvent was
0.02 M NH<sub>4</sub>H<sub>2</sub>P<sub>O<sub>4</sub></sub> (pH 4.0) with 1.5% (v/v) acetonitrile.
Non-methylated lambda-phage DNA (Sigma) was used as the
calibration reference.

DNA–DNA hybridization. DNA–DNA hybridization experi-
ments were performed with photobiotin-labelled probes in
microplate wells as described by Ezaki *et al.* (1989), using
a HTS7000 Bio Assay Reader (Perkin-Elmer) for the
fluorescence measurements. The hybridization temperature
was 50 °C in 50% (v/v) formamide. Each value given is the
mean of at least two hybridization experiments.

Growth characteristics and biochemical activity. Cultural
characteristics were studied as described previously
(Devriese *et al.*, 1998). API 20 STREP, API 50CH
(bioMérieux) and the BBLCRYSTAL Gram-positive ID
System (Becton Dickinson) were used to test biochemical
activity.

RESULTS AND DISCUSSION

SDS-PAGE of whole-cell protein

The whole-cell-protein profiles of the six *G. sanguinis-
like* isolates were compared with each other and with
those of *G. sanguinis* reference strains that were of
human origin. The result of the numerical analysis is
shown in Fig. 1. The six *G. sanguinis*-like isolates had
virtually identical whole-cell protein profiles that were

![Fig. 1. Dendrogram derived from the unweighted pair group average linkage of correlation coefficients between the whole-
cell-protein patterns of all *Globicatella* strains examined.](image-url)
clearly different from those of G. sanguinis reference strains. In addition, the latter reference isolates had very similar protein profiles.

16S rRNA gene sequence analysis

The 16S rDNA sequence of strain LMG 18844T was examined to determine its phylogenetic position (GenBank no. AJ297627). The level of similarity to the 16S rRNA gene of G. sanguinis LMG 18987T was 99.2%; the level of similarity to the 16S rRNA genes of other organisms was below 94%. Fig. 2 shows the result of neighbour-joining cluster analysis of the 16S rDNA sequences of strain LMG 18844T and related bacteria.

DNA base composition analysis and DNA–DNA hybridization results

The close relationship between both taxa was confirmed by DNA base composition analysis and DNA–DNA hybridization experiments. DNA was prepared from strains LMG 18844T and LMG 18842, and from the G. sanguinis type strain, LMG 18987T. The DNA base ratio of strains LMG 18844T and LMG 18842 was 35.8 mol%; that of LMG 18987T was 35.7%. The DNA–DNA binding value between strains LMG 18844T and LMG 18842 was 90%. The mean value for binding with G. sanguinis LMG 18987T was calculated as 68%.

Morphology, growth characteristics and biochemical activity

The protein electrophoretic differences between these organisms were further supported by salient biochemical differences (Table 2). The cells were predominantly Gram-negative, although Gram-positive cells were always present in the preparations. Most cells tended to occur separately or in pairs, but chains were formed occasionally. Colonies on blood agar were pinpoint-sized after 1 d and became grey-white with a diameter of 0.5–1 mm after 2 d incubation. They appeared crumbly and dry when removed. Corrosion of the agar surface was seen underneath the colonies. Growth occurred at 25 °C but was better at 37 and 42 °C. Supplementation of the incubation atmosphere with 5% CO₂ did not influence growth. The strains showed homogeneous growth after 1 d in BHI broth, but this sedimented after 2 d. Double-zone haemolysis (an inner, opaque, brownish zone and an outer, semi-transparent, greenish ring) was seen on sheep-blood agar.

The strains were unable to grow on bile–aesculin medium but they grew on Edwards agar, and hydrolysed aesculin on this medium. Growth was seen in 6.5% NaCl BHI broth after 2 d incubation. Most characteristically, the strains produced a zone of blackening along the stab inoculation in Kligler’s iron agar, indicating hydrogen sulphide production from sulphur-bearing amino acids. These zones were narrow at the tops of the agar columns (in contact with air) and broadened towards the bottom. The slants and the remaining parts of the agar columns turned yellow because of glucose fermentation. Weak amylase production was evident on starch-containing plates. All strains were resistant to neomycins and to the macrolide antibiotics erythromycin, clindamycin and lincomycin. Other biochemical test results are given in Table 2.

Taxonomic position and identification of the G. sanguinis-like isolates

Together, these data indicate that the human and animal isolates examined are closely related and obviously represent the same genus. Given the clear biochemical differences between both taxa, the differences in whole-cell-protein patterns, and the mean DNA–DNA binding level of 68%, it is appropriate to accommodate the six animal isolates into a novel Globicatella species, for which we propose the name G. sulfidificiens.

The production of hydrogen sulphide, as demonstrated by the deposition of black iron sulphide in Kligler and similar media, is a reaction widely used in the identification of Gram-negative bacteria. The test can be applied to the recognition of Erysipelothrix, but, otherwise, the ability to produce hydrogen in these media is apparently rare in Gram-positive bacteria. The four strains of G. sanguinis available for study...
were negative in this test. This indicates that sulphide production is possibly useful in the search for *G. sulfidifaciens* isolates, and in their identification. However, as only a few strains of the two species were investigated, this characteristic should be used cautiously. Other characteristics differentiating the two *Globicatella* species are listed in Table 2. The differential scheme given by Collins *et al.* (1999) can be useful for differentiating the *Globicatella* strains from other recently described phylogenetically related genera. The reactions listed in this table, which may be of use with respect to *G. sanguinis*, are not, however, entirely applicable to *G. sulfidifaciens*. Notably, the positive hippurate, β-galactosidase, ribose and mannitol reactions cannot be used as genus-specific characteristics, since they are absent in *G. sulfidifaciens*. Acid production from melibiose appears to be a characteristic of the genus *Globicatella* not shared by the related genera as described to date.

*G. sulfidifaciens* was found most often in clinical samples from ruminants, despite the fact that this category of samples is not the most frequently examined in the laboratory in which the strains were examined in the laboratory in which the strains were detected. As most of the strains were not isolated in pure culture, the pathological significance of these bacteria is a matter of conjecture. Certainly, their role in lung infections is secondary (much like the situation with *A. pyogenes*). The natural habitat of *G. sulfidifaciens* remains unknown.

**Description of *Globicatella sulfidifaciens* sp. nov.**


Gram-variable cocci showing the characteristics of Gram-positive cocci, occurring singly, in pairs and in short chains. Colonies are dry and corrodng. Growth takes 2 d. α-Haemolytic. Facultatively anaerobic and catalase-negative. Grows in 6–5% NaCl but not on bile–aesculin agar. Glucose is fermented. Other characteristics are given in Table 2. G+C content of the DNA is 35–36 mol%. Isolated from lung and joint lesions in ruminants and a pig. Type strain is LMG 18844T ( = CCUG 44365T), which was isolated from a bovine lung in Belgium; its G+C content is

### Table 2. Biochemical characteristics and differentiation between *Globicatella sulfidifaciens* and *Globicatella sanguinis*

Differential results are given in body of the table. *Globicatella sulfidifaciens* strains gave positive reactions in the following tests: α-galactosidase, 4-MU β-d-glucoside, 4-MU α-d-glucoside, l-tryptophan–aesculin–agar. Glucose is fermented. Other characteristics differentiating the two *Globicatella* species are listed in Table 2. The differential scheme given by Collins *et al.* (1999) can be used with respect to *G. sanguinis*, are not, however, entirely applicable to *G. sulfidifaciens*. Notably, the positive hippurate, β-galactosidase, ribose and mannitol reactions cannot be used as genus-specific characteristics, since they are absent in *G. sulfidifaciens*. Acid production from melibiose appears to be a characteristic of the genus *Globicatella* not shared by the related genera as described to date.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>G. sulfidifaciens</em></th>
<th><em>G. sanguinis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S production (Kligler’s iron agar)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>l-Phenylalanine AMC hydrolysis</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4-MU N-acetyl β-d-glucosaminide</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4-MU-β-d-glucuronide hydrolysis</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Methyl β-glucoside</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*This table is based on the results of Collins *et al.* (1999) and our results obtained with the four collection strains used in this study for comparative purposes.*
35.8 mol%. Its biochemical characteristics are as described above for the species.

REFERENCES


