NOTES

Sanguibacter inulinus sp. nov.

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Six strains of coryneform bacteria were isolated from blood samples obtained from healthy cows. Phenotypic and molecular genetic studies showed that these isolates represent a new species of the genus *Sanguibacter*, for which the name *Sanguibacter inulinus* is proposed. The type strain of *S. inulinus* is strain ST-50 (= NCFB 3024).

Recently, the new genus *Sanguibacter* was described (2) to accommodate some gram-positive coryneform bacteria that were isolated from blood samples from apparently healthy dairy cows. Currently, two species, *Sanguibacter keddii* and *Sanguibacter suarezii*, are recognized in the genus *Sanguibacter* (2). During the course of the characterization of these bacteria, six isolates which were phenotypically similar to, but nevertheless distinct from, *S. keddii* and *S. suarezii* were recovered. In this paper we describe the cultural and biochemical characteristics of these previously unidentified bovine coryneform isolates and demonstrate that on the basis of chromosomal DNA-DNA pairing and 16S rRNA gene sequencing data they represent a new species of the genus *Sanguibacter*, for which the name *Sanguibacter inulinus* is proposed.

Six strains (ST-19, ST-34, ST-50^T [T = type strain], ST-70, ST-77 and ST-178) of coryneform bacteria were recovered from aseptically drawn venous blood samples obtained from apparently healthy cows. The blood was plated onto listeria selective agar III as described by Dominguez Rodriguez et al. (1) except that the acriflavine concentration was reduced to 6 mg/liter. The strains were tested to determine their Gram reaction and motility, catalase and oxidase production, gelatin and casein hydrolysis, and nitrate reduction tests were performed (5). Production of acetoin from glucose was examined by performing the Voges-Proskauer test. Growth at different temperatures was determined in tryptose soy broth (Difco, East Molesey, United Kingdom) after incubation at 10°C for 10 days and at 25, 30, 37, and 42°C for 4 days. Fermentation of carbohydrates was determined with the API 50CH system (Bio-Merieux, Basingstoke, United Kingdom). Fermentation reactions were recorded after incubation for 2, 5, 10, and 15 days at 30°C. The cell wall murein of the strains was determined by the method of Schleifer and Kandler (8). The DNA used for DNA-DNA relatedness studies was isolated and purified by the method of Marmur (7). DNAs from isolate ST-50^T and *S. suarezii* NCFB 3023^T were labelled with ³H-labelled nucleotides by using a Megaprime kit (Megaprime labelling systems; Amersham International, Amersham, United Kingdom). DNA-DNA hybridization experiments were performed by using an S1 nuclease-trichloroacetic acid procedure (4), and the reaction mixtures were incubated at 70°C for 16 h. ΔT_m values were

determined as described by Grimont et al. (4) (ΔT_m is the difference between the denaturation temperature of a homoduplex and the denaturation temperature of a heteroduplex). The 16S rRNA genes of the isolates were amplified by using PCR, and sequences were determined directly as described by Hutson et al. (6). The 16S rRNA sequences which we determined were aligned with the sequences of other high-G+C-content actinomycetes available from the EMBL Data Library. Levels of sequence similarity and nucleotide substitution rates (K_{nuc}) were calculated and used to produce an unrooted phylogenetic tree by using the algorithm of Fitch and Margoliash (3).

The six bovine bacterial isolates (which were designated the ST-50^T group) were gram-positive, short, irregularly shaped, motile, rod-shaped organisms. On tryptose soy agar plates they formed pale yellow colonies (diameter, 0.5 to 1.0 mm) in 48 h and had an optimum growth temperature of 25 to 30°C. None of the ST-50^T group strains grew at 42°C, and all of these

FIG. 1. Dendrogram showing the phylogenetic relationships of *S. inulinus* ST-50^T and some other high-G+C-content gram-positive bacteria. The tree is based on the results of an analysis of a continuous stretch of approximately 1,372 nucleotides of the 16S rRNA. Bar = 2%.

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TABLE 1. Differential characteristics of *Sanguibacter* species

Species	Nitrate reduction	Fermentation of ^a :							
		Mannitol	α -Methyl-D-mannoside	α -Methyl-D-glucoside	N-Acetylglucosamine	Amygdalin	Inulin	Gluconate	Sorbitol
<i>S. inulinus</i>	V ^b	V	+	+	+	+	+	+	+
<i>S. keddieii</i>	—	—	+	+	+	+	—	V	—
<i>S. suarezii</i>	V	—	—	—	—	+	—	—	—

^a V, variable reaction; +, positive reaction; —, negative reaction.^b The reaction is weak or delayed.

strains were facultatively anaerobic, catalase positive, and oxidase negative. The cell wall murein type of a representative strain (strain ST-50^T) was type A4 α (L-Lys-Ser-D-Glu), like other *Sanguibacter* spp. Although the ST-50^T group was phenotypically similar to *S. keddieii* and *S. suarezii*, it could be readily distinguished from these species by the results of the biochemical tests shown in Table 1.

To establish the genealogical affinity of the ST-50^T group, the almost complete 16S rRNA gene sequence of representative strain ST-50^T was determined by using PCR direct sequencing. The sequence which we determined consisted of a continuous stretch of 1,480 nucleotides representing approximately 97% of the complete 16S rRNA molecule. Partial 16S rRNA gene sequences which included highly variable regions V1 to V3 (approximately positions 40 to 500 [*Escherichia coli* numbering]) of the other blood isolates (ST-19, ST-34, ST-70, ST-77, ST-178) were also determined and were found to be identical to the sequence of strain ST-50^T, which demonstrated

the close genotypic relatedness of the six strains. A comparison of the 16S rRNA gene sequence of strain ST-50^T with the sequences of other high-G+C-content gram-positive bacteria revealed particularly high levels of sequence similarity with *S. suarezii* (98.9%, corresponding to 16 differences in approximately 1,480 positions) and *S. keddieii* (99.1%, corresponding to 14 differences in approximately 1,480 positions). We also observed relatively high levels of sequence relatedness (approximately 94 to 96%) with other members of the *Cellulomonadaceae* (Table 2). Figure 1 is a tree which shows the phylogenetic relationships of strain ST-50^T with members of the family *Cellulomonadaceae* and their relatives. This tree clearly shows that previously unidentified strain ST-50^T is a member of the genus *Sanguibacter*.

In view of the high levels of 16S rRNA sequence relatedness of strain ST-50^T with *S. keddieii* and *S. suarezii*, chromosomal DNA-DNA hybridization experiments were performed to establish whether the ST-50^T group strains form a distinct species of the genus *Sanguibacter*. The results of the hybridization experiments are summarized in Table 3. Three representative strains belonging to the ST-50^T group exhibited more than 72% similarity with strain ST-50^T at 70°C with insignificant divergence (ΔT_m , <2.5°C). In contrast, strain ST-50^T exhibited much lower levels of reassociation (generally <40%) with *S. keddieii* and *S. suarezii* strains. These findings confirm that the ST-50^T group strains are genomically homogeneous and represent a species that is distinct from *S. keddieii* and *S. suarezii*. Thus, on the basis of the molecular findings described above and the phenotypic distinctiveness of the ST-50^T group of strains, we propose that these strains should be classified as members of a new species, *Sanguibacter inulinus*.

Description of *Sanguibacter inulinus* sp. nov. *Sanguibacter inulinus* (i.nu.li'nus. M.L. n. *inulum*, inulin; M.L. adj. *inulinus*, pertaining to inulin) cells are gram-positive, short, irregularly shaped, motile rods. Colonies are circular, convex with entire edges, and pale yellow. Colonies range from 0.5 to 1.0 mm in

TABLE 2. Levels of sequence similarity for a 1,372-nucleotide region of 16S rRNAs of *S. inulinus* ST-50^T and some representatives of the family *Cellulomonadaceae*

Strain ^a	% 16S rRNA similarity												
	<i>Sanguibacter keddieii</i> NCFB 3025 ^T	<i>Sanguibacter suarezii</i> NCFB 3023 ^T	<i>Oerskovia turbata</i> NCIMB 10587	<i>Oerskovia xanthineolytica</i> NCIMB 11025	<i>Cellulomonas biazotea</i> DSM 20112	<i>Cellulomonas cartae</i> DSM 20106	<i>Cellulomonas cellasea</i> DSM 20118	<i>Cellulomonas cellulans</i> DSM 43879	<i>Cellulomonas flavigena</i> NCIMB 8073	<i>Cellulomonas fermentans</i> DSM 3133	<i>Cellulomonas fimi</i> DSM 20113	<i>Cellulomonas gelida</i> NCIMB 8076	<i>Cellulomonas uda</i> NCIMB 8200
<i>Sanguibacter inulinus</i> ST-50 ^T	99.2	99.4	96.0	95.5	95.0	95.6	95.8	95.6	94.2	94.6	94.9	93.6	93.6
<i>Sanguibacter keddieii</i> NCFB 3025 ^T		98.6	96.1	95.7	94.9	95.8	95.7	95.8	94.0	94.7	94.9	93.4	93.4
<i>Sanguibacter suarezii</i> NCFB 3023 ^T			95.8	95.2	94.7	95.3	95.6	95.5	93.7	94.2	94.9	93.4	93.4
<i>Oerskovia turbata</i> NCIMB 10587				95.1	95.8	95.1	96.5	95.1	95.9	94.9	95.7	95.8	95.6
<i>Oerskovia xanthineolytica</i> NCIMB 11025					95.2	99.7	94.8	99.6	93.9	94.3	95.2	94.2	94.9
<i>Cellulomonas biazotea</i> DSM 20112						95.1	97.9	94.9	97.4	95.9	99.6	96.0	95.9
<i>Cellulomonas cartae</i> DSM 20106							94.8	99.7	94.0	94.4	95.1	94.4	95.2
<i>Cellulomonas cellasea</i> DSM 20118									97.0	96.3	98.0	96.7	96.6
<i>Cellulomonas cellulans</i> DSM 43879									93.7	94.1	94.9	94.3	95.1
<i>Cellulomonas flavigena</i> NCIMB 8073										95.6	97.2	97.7	97.1
<i>Cellulomonas fermentans</i> DSM 3133											96.0	94.9	95.2
<i>Cellulomonas fimi</i> DSM 20113												96.1	96.0
<i>Cellulomonas gelida</i> NCIMB 8076													98.9

^a The accession numbers of the 16S rRNA sequences of reference cellulomonads and oerskoviae are as follows: *O. turbata*, X79454; *O. xanthineolytica*, X79453; *C. biazotea*, X79462; *C. cartae*, X79456; *C. cellasea*, X79459; *C. cellulans*, X79455; *C. flavigena*, X79463; *C. fermentans*, X79458; *C. fimi*, X79460; *C. gelida*, X79461; and *C. uda*, X79457.

TABLE 3. Levels of DNA-DNA similarity for *S. inulinus* ST-50^T, *S. suarezii* NCFB 3023^T, and other *Sanguibacter* strains

Source of unlabelled DNA	% Reassociation at 70°C with ³ H-labelled DNA from:	
	<i>S. inulinus</i> ST-50 ^T	<i>S. suarezii</i> NCFB 3023 ^T
<i>S. inulinus</i> strains		
ST-50 ^T (= NCFB 3024 ^T)	100 (0.0) ^a	18.2
ST-70	72.2 (1.0)	14.4
ST-77	99.0 (2.5)	23.0
ST-178	89.9 (1.0)	20.1
<i>S. suarezii</i> strains		
ST-26 ^T (= NCFB 3023 ^T)	16.5	100
ST-30	18.9	95.1
ST-76	14.0	92.1
ST-86	20.7	89.4
ST-162A	22.3	77.6
<i>S. keddii</i> strains		
ST-74 ^T (= NCFB 3025 ^T)	36.1	18.4
ST-167	40.7	20.2

^a The values in parentheses are ΔT_m values (in degrees Celsius).

diameter after 48 h of incubation on tryptose soy agar. Facultatively anaerobic and catalase positive. Cellulose and Tween 80 are not hydrolyzed. Acid is produced from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, rhamnose, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, glycogen, gluconate, β -gentibiose, D-turanose, D-lyxose, α -methyl-D-mannoside, α -methyl-D-glucoside, N-acetylglucosamine, amygdalin, inulin, 5-ketogluconate, and D-raffinose. Acid is not produced from erythritol, D-arabinose, adonitol, sorbitol, L-sorbose, ino-

sitol, melezitose, xylitol, D-tagatose, D-fucose, D-arabitol, L-xylose, dulcitol, and 2-ketogluconate. Some strains produce acid from mannitol and β -methylxyloside. Some strains reduce nitrate. MK-9(H₄) is the major menaquinone. The cell wall murein type is L-Lys-Ser-D-Glu. The G+C content of the DNA of the type strain is 70.5 mol%. Isolated from bovine blood samples. The type strain is strain ST-50 (= NCFB 3024).

Nucleotide accession number. The nucleotide sequence of the 16S rRNA gene of *S. inulinus* ST-50^T (= NCFB 3024^T) has been deposited in the EMBL database under accession number X79451.

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