Phylogenetic Evidence that the Gram-Negative Nonsporulating Bacterium *Tissierella* (*Bacteroides*) *praeacuta* Is a Member of the *Clostridium* Subphylum of the Gram-Positive Bacteria and Description of *Tissierella creatinini* sp. nov.

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The 16S rRNA gene sequence of the type strain of *Tissierella praeacuta* (formerly *Bacteroides praeacutus*) was determined by PCR direct sequencing. A comparative sequence analysis showed that *T. praeacuta* is a member of the *Clostridium* subphylum of the gram-positive bacteria and has a close phylogenetic affinity with the species that form *Clostridium* cluster XII (M. D. Collins, P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow, Int. J. Syst. Bacteriol. 44:812–826, 1994). Although *T. praeacuta* is gram negative and does not produce endospores, 16S rRNA sequence data showed that it is closely related genealogically (level of sequence similarity, 99.9%) to *Clostridium hastiforme*. On the basis of our results and the results of previous studies, a second species of *Tissierella*, *Tissierella creatinini* sp. nov., is described.

Tissierella praeacuta (formerly *Bacteroides praeacutus*) consists of obligately anaerobic, gram-negative, nonsporulating, rod-shaped organisms and was originally isolated from infant feces and described by Tissier in 1908 (25). In addition to occurring in human feces, *T. praeacuta* has been isolated, although rarely, from a variety of clinical sources, including lung abscesses, gangrenous lesions, and blood (1, 2, 11). Although well characterized, *T. praeacuta* has had an unsettled taxonomic history; at various times it has been assigned to the genera *Coccobacillus* (25), *Zuberella* (21), *Fusobacterium* (9), and *Bacteroides* (19). Shah and Collins (23), in a chemotaxonomic survey of the family *Bacteroidaceae*, showed that there were major differences between *B. praeacutus* and the group of species that belong to the genus *Bacteroides* sensu stricto (the “*Bacillus fragilis* group”). For example, *B. praeacutus* differs from true *Bacteroides* species by lacking menaquinones, by lacking several key metabolic enzymes, and by having a significantly lower DNA G+C content. Accordingly, Collins and Shah (4) transferred *B. praeacutus* to a new monospecific genus, the genus *Tissierella*. Although the genus *Tissierella* clearly differs from the genus *Bacteroides* and its relatives phenotypically, the higher phylogenetic associations of this taxon remain unknown.

There has been considerable progress in recent years in elucidating the phylogenetic relationships of the genus *Bacteroides* and other obligately anaerobic gram-negative bacteria by using 16S rRNA sequence analysis (20). Therefore, in this study we determined the 16S rRNA gene sequence of the type strain of *T. praeacuta* in order to elucidate the higher phylogenetic affiliations of this organism. A comparative sequence analysis demonstrated that *T. praeacuta* is a member of the *Clostridium* subphylum of the gram-positive bacteria and is closely related genealogically to the type strain of *Clostridium hastiforme*. On the basis of the results of this study and previous studies a new creatinine-degrading species of the genus *Tissierella*, *Tissierella creatinini*, is described.

MATERIALS AND METHODS

Bacterial strains. The type strains of *T. praeacuta* (ATCC 25539, NCTC 11158) and *C. hastiforme* (ATCC 33268, DSM 5675, NCTC 11832) were obtained from the American Type Culture Collection, Rockville, Md., the National Collection of Type Cultures, London, United Kingdom, and the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, and were cultured as recommended in the culture collection catalogs.

Isolation of creatinine-degrading strain BN11^T. Strain BN11^T (T = type strain) was isolated from anaerobic enrichment cultures that had been inoculated with sediment from a wastewater pool at a sugar refinery in Nörten-Hardenberg, Germany. Enrichment medium AM0.1 contained (per liter) 1.17 g of NaCl, 0.4 g of MgCl₂ · 6H₂O, 0.3 g of KCl, 0.15 g of CaCl₂ · 2H₂O, 0.27 g of NH₄Cl, 0.2 g of KH₂PO₄, 2.84 g of Na₂SO₄, 1 ml of trace element solution SL-10 (26), 1 ml of 10^{−4} M NaHSeO₃, 0.5 g of yeast extract, 7.5 g of creatine · H₂O, 0.001 g of resazurin, 10 ml of a vitamin solution (28), 4.5 g of NaHCO₃, and 0.5 g of L-cysteine hydrochloride; the pH of this medium was 7.5. The medium was prepared anaerobically by the Hungate technique under an 80% N₂–20% CO₂ gas atmosphere. During autoclaving, 75% of the creatine was converted to creatinine (18). Enrichment cultures in completely filled 50-ml screw-cap bottles were incubated at 30°C. After two transfers, a sample was streaked onto bicarbonate-free creatinine-containing AM0.1 agar plates and incubated in an anaerobic jar. Single colonies which produced a strong alkaline reaction when they were smeared on phenol red indicator paper were picked and purified by repeated streaking on agar plates of the same composition. Several isolates were obtained, and one strain, strain BN11^T, was selected for further study.

Physiological and biochemical tests. Tests to determine production of lipase, lecithinase, urease, and indole, hemolysis, hydrolysis of gelatin, and digestion of milk and meat were performed as described by Holdeman et al. (10) and Krieg (14). Utilization of sugars and some other substances was determined in AM0.1 medium and in peptone-yeast extract medium at pH 6.8 and 8.6. Utilization of creatinine and structurally related compounds was determined by using AM0.1 medium containing 0.5% yeast extract at pH 8.3. Cultures were checked for spore production after growth in different liquid media or on agar media by phase-contrast microscopy and by performing heating tests. The pH and temperature optima and ranges for growth were determined in AM0.1 medium containing 1% yeast extract and 50 mM N-methylhydantoin as the substrate. Different pH values were obtained by using only 1 g of sodium bicarbonate per liter and buffering the preparations with 0.02 M potassium phosphate or Tris-HCl buffer at pH values ranging from 5.8 to 9.5. A model TN3 temperature gradient incubator (Tokyo Kagaku Sangyo, Ltd., Japan) was used to incubate cultures at temperatures ranging from 18 to 50°C.

Analytical techniques. Acetate contents were determined by gas chromatography; creatinine and N-methylhydantoin contents were determined by high-performance liquid chromatography; sarcosine contents were determined enzymatically with sarcosine oxidase and peroxidase; and N-carbamoylsarcosine contents were determined with N-carbamoylsarcosine hydrolase in a coupled reaction with sarcosine oxidase as described previously (7). Ammonia levels were determined colorimetrically by the Berthelot reaction by using Merckognost urea (E. Merck, Darmstadt, Germany).

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DNA base composition. The G+C content of strain BN11^T was determined by the thermal denaturation method with a Gilford model 2600 spectrophotometer equipped with a model 2527 thermoprogrammer and was calculated by using the equation of Marmur and Doty (17) as modified by De Ley (5). *Escherichia coli* K-12 strain DSM 485 (G+C content, 51.7 mol%) was used as the control.

16S rRNA gene sequence determination. Genomic DNA was extracted from mid-logarithmic-phase cells and was purified as described by Lawson et al. (15). 16S rRNA gene fragments were generated by a PCR, purified, and sequenced as described previously (12).

Analysis of sequence data. The sequence of *T. praeacuta* NCTC 11158^T was aligned with 235 previously determined clostridial (3, 16) and reference sequences obtained from the EMBL and Ribosomal Database Project databases and was analyzed by using the DNADIST program of the PHYLIP package (6). The distance matrix which we obtained was corrected for substitution rates by using Kimura's parameters (13). A phylogenetic tree was constructed by the neighbor-joining method of Saitou and Nei (22) with a VAX computer. The stability of relationships was assessed by using the programs SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE of the PHYLIP package. A total of 1,000 bootstrap trees were generated for the data set.

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S rRNAs of *T. praeacuta* NCTC 11158^T and ATCC 25539^T and *C. hastiforme* DSM 5675^T have been deposited in the EMBL data library under accession numbers X80832, X80833, and X80841, respectively.

RESULTS

The 16S rRNA gene of *T. praeacuta* NCTC 11158^T was amplified by a PCR, and its nucleotide sequence was determined directly. The sequence which we determined consisted of 1,475 nucleotides representing approximately 96% of the complete 16S rRNA primary structure. This 16S rRNA sequence was compared with the sequences of other 16S rRNAs available from the EMBL Data Library and the Ribosomal Database Project. Very low levels of sequence relatedness (generally <77%) were found with members of the genus *Bacteroides* and related gram-negative anaerobes. Much higher levels of sequence similarity were observed with members of the low-G+C-content subphylum of the gram-positive bacteria. Surprisingly, the 16S rRNA sequence of *T. praeacuta* NCTC 11158^T was found to be almost identical (level of similarity, 99.9%, corresponding to 1 base difference in a comparison of 1,475 positions) to the sequence of the gram-positive spore-forming organism *C. hastiforme* DSM 5675^T. To eliminate the possibility of incorrect collection strains or culture contamination, cultures of the type strains of *T. praeacuta* (ATCC 25539) and *C. hastiforme* (ATCC 33268 and NCTC 11832) held in other collections were sequenced, and the original data were confirmed.

To determine the phylogenetic position of *T. praeacuta* within the *Clostridium* subphylum, the sequence of this organism was compared with previously published sequences of more than 200 clostridial species and non-spore-forming relatives of these organisms. A matrix of representative similarity values based on an approximately 1,330-nucleotide region of the 16S rRNA is shown in Table 1. In addition to its close genealogical affinity with *C. hastiforme*, *T. praeacuta* exhibited high levels of sequence relatedness with species belonging to *Clostridium* cluster XII (groups of Collins et al. [3]), which includes *Clostridium acidurici*, *Clostridium purinolyticum*, "*Clostridium filamentosum*," *Eubacterium angustum*, and the previously unnamed non-spore-forming organism strain BN11^T. A phylogenetic tree constructed by using the neighbor-joining method is shown in Fig. 1, and this tree confirms the placement of *T. praeacuta* within this cluster.

DISCUSSION

The recovery of *T. praeacuta* within the *Clostridium* subphylum of gram-positive bacteria was somewhat unexpected as *T. praeacuta* stains gram negative and does not produce spores (4). However, there is a growing recognition that Gram stain-

ing and spore formation are not necessarily good indicators of relatedness. For example, several genera that traditionally have been considered to be gram negative have now been shown to be related to gram-positive bacteria (e.g., members of the genera *Pectinatus*, *Megasphaera*, *Selenomonas*, and *Sporomusa*), while the phylogenetic intermixing of spore-forming and non-spore-forming taxa is now well established (3, 24, 27). To our knowledge, however, this combination of differences in Gram staining and sporulation characteristics has not been reported previously in such phylogenetically closely related organisms. Although it is now recognized that the occurrence of identical (or nearly identical) 16S rRNA sequences does not necessarily imply that species are identical, the data demonstrate that *T. praeacuta* and *C. hastiforme* are either the same species or very closely related species. In the past *C. hastiforme* has been phenotypically associated with the group IV organisms *Clostridium botulinum* and *Clostridium subterminale*. Chromosomal DNA-DNA pairing studies are clearly necessary to determine whether *T. praeacuta* and *C. hastiforme* represent different species and the latter organism should be reclassified. The previously unnamed creatinine-degrading organism strain BN11^T (7, 8), which was isolated from a wastewater pool at a sugar refinery, exhibited >95% sequence relatedness to *T. praeacuta*. Interestingly, strain BN11^T differs from *T. praeacuta* by being gram positive. Furthermore, unlike *T. praeacuta*, which has a wall based on *meso*-diaminopimelic acid, the wall of strain BN11^T contains D-ornithine as the dibasic amino acid. There are, however, now numerous examples of organisms which are genealogically closely related or members of the same genus but which differ in wall composition. Some examples within the *Clostridium* subphylum include the genus *Clostridium* sensu stricto (cluster I [3]), which contains species with *meso*-diaminopimelic acid and species with LL-diaminopimelic acid; and *Clostridium paradoxum* and *Clostridium thermoautotrophicum*, which exhibit approximately 2% 16S rRNA sequence divergence and have walls based on *meso*-diaminopimelic acid and ornithine, respectively. Despite differences in staining and wall composition, *T. praeacuta* and strain BN11^T resemble each other in many ways, including the fact that they are both rod shaped and nonfermentative (carbohydrates are not utilized), the fact that they do not have endospores and respiratory menaquinones, and the fact that they have low DNA base compositions (28 to 32 mol% G+C). Thus, these similarities, together with the observed level of 16S rRNA sequence divergence (<5%), indicate that strain BN11^T should be included in the genus *Tissierella*. It is worth noting that grouping of strain BN11^T with *T. praeacuta* occurred in all 1,000 bootstrapped trees; these data support the close phylogenetic relationship of these organisms. Of the remaining species in *Clostridium* cluster XII, "*C. filamentosum*" exhibited approximately 10% sequence divergence with *T. praeacuta* and therefore in terms of evolutionary distance probably is peripheral to, or lies outside the boundaries of, the genus *Tissierella*. A relationship between "*C. filamentosum*" and the genus *Tissierella* was observed in only 83% of the bootstrapped trees. As pointed out previously (3), "*C. filamentosum*" could conceivably represent a distinct subspecies worthy of separate generic status. To avoid an undesirable proliferation of new genera, the genus *Tissierella* could be considered a possible genus of convenience for "*C. filamentosum*," although phenotypic information supporting this association would be required. *C. acidurici*, *C. purinolyticum*, and *E. angustum* form a distinct group within cluster XII, exhibiting levels of sequence relatedness of >93% with each other. Significantly lower levels of sequence similarity (<90%) were observed with *T. praeacuta* and its relatives. The levels of sequence divergence and tree branching

TABLE 1. Levels of sequence similarity for an approximately 1,330-nucleotide region of the 16S rRNAs of *T. praeacuta* and some representatives of the *Clostridium* subphylum of gram-positive bacteria^a

	% Similarity to:																																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	
1. <i>T. praeacuta</i>		99.9	95.2	90.6	90.3	90.6	90.5	88.5	87.7	86.9	86.3	86.5	86.0	87.8	84.4	83.6	82.3	84.3	85.9	85.2	86.7	85.2	85.6	86.2	86.6	86.2	86.9	85.3	86.4	86.5	84.7	88.3	86.2	90.1	87.2	86.3	80.7	83.5	86.4	87.1	84.6	
2. <i>C. hastiforme</i>			95.1	90.1	90.1	90.4	90.2	88.3	87.6	86.8	86.1	86.4	85.9	87.6	84.4	83.6	81.9	84.0	85.5	84.9	86.5	84.9	85.3	86.0	86.4	85.9	86.5	85.0	86.0	86.2	84.4	87.1	85.9	89.7	87.0	86.0	80.6	83.4	86.4	87.0	84.3	
3. <i>T. creatinini</i>				89.0	90.6	91.1	90.2	89.2	88.7	86.6	86.7	87.3	86.8	88.6	84.8	84.1	83.5	84.9	85.1	86.0	85.6	85.4	85.3	85.7	86.2	85.7	86.4	85.7	86.0	85.9	84.4	87.6	86.1	89.6	86.7	86.1	82.1	84.6	85.3	86.7	85.7	
4. <i>"C. filamentosum"</i>					89.5	86.9	87.3	87.2	87.2	84.3	82.4	82.2	82.3	83.2	80.7	79.5	79.4	84.1	84.3	84.3	85.6	84.2	82.6	85.2	86.1	85.7	86.4	84.7	86.0	86.1	84.0	86.5	84.5	87.1	86.5	85.5	79.6	82.1	83.6	84.1	84.0	
5. <i>C. purinolyticum</i>						94.0	93.8	91.3	90.1	85.1	85.6	86.7	85.6	86.7	83.7	83.7	82.1	86.5	86.5	88.0	88.7	87.8	88.3	87.7	88.0	87.2	87.8	86.8	88.1	87.8	85.8	88.6	87.2	90.1	89.4	87.2	80.6	84.4	87.4	87.6	86.6	
6. <i>C. aciduriaci</i>							92.8	90.4	88.8	84.8	85.6	86.2	86.0	86.1	83.7	83.4	82.6	86.4	86.5	87.2	87.6	87.3	87.4	87.2	87.0	86.7	87.3	85.7	87.8	87.2	85.4	88.7	87.2	89.1	88.8	86.8	81.3	84.1	87.4	88.1	85.4	
7. <i>E. angustum</i>								89.3	88.3	85.4	85.8	86.3	85.6	87.4	83.9	84.1	82.3	86.5	86.4	87.8	87.7	87.9	87.7	87.9	87.4	87.0	87.6	85.7	87.6	87.7	86.0	87.4	87.0	88.7	88.5	86.3	80.7	84.1	86.1	86.6	84.7	
8. <i>"E. thermomarinans"</i>									87.8	81.8	83.2	84.6	84.8	85.9	82.5	82.3	81.6	85.9	86.2	86.1	87.2	85.5	85.8	85.9	87.3	86.7	87.2	86.0	88.2	87.6	84.4	86.8	86.4	89.9	88.3	86.1	79.7	83.7	86.0	86.4	84.7	
9. <i>C. hydroxybenzoicum</i>										84.5	85.6	87.8	85.0	86.4	83.2	83.1	82.3	84.6	84.6	84.5	84.5	84.9	85.4	84.4	85.4	84.1	85.6	85.2	85.3	85.3	84.8	85.6	85.6	89.0	88.0	86.7	82.0	83.9	85.5	85.9	84.4	
10. <i>H. kunzii</i>											85.1	84.5	82.6	83.6	83.7	82.9	82.3	80.7	80.9	81.5	82.1	80.5	81.8	82.2	81.7	81.1	81.8	81.8	81.6	81.6	81.8	82.4	81.2	86.1	81.6	82.0	79.3	81.0	81.1	82.5	81.6	
11. <i>P. magnus</i>												88.4	85.0	87.7	84.6	86.0	84.6	80.9	82.3	81.8	82.4	81.9	81.0	81.2	82.2	81.4	82.0	82.5	82.7	82.3	83.0	82.8	83.5	86.9	84.5	84.4	80.9	81.8	82.4	83.7	82.0	
12. <i>P. micros</i>													85.0	87.4	84.8	84.4	84.2	82.6	84.1	83.5	83.6	83.5	83.0	83.3	84.3	83.0	84.1	83.0	83.8	83.7	83.9	83.6	84.6	87.4	86.4	84.4	82.3	84.0	81.9	82.3	83.6	
13. <i>P. lacrimalis</i>														88.4	84.8	84.2	86.5	81.0	82.6	82.0	82.7	82.0	81.6	82.1	82.7	83.0	82.0	82.7	81.7	83.1	82.7	82.6	83.9	83.3	85.6	85.0	82.9	80.8	81.9	84.0	84.0	81.3
14. <i>P. asaccharolyticus</i>															86.7	85.9	84.9	83.5	84.5	84.6	84.2	84.5	84.5	83.7	85.2	83.2	83.2	85.1	83.5	84.8	85.5	84.2	85.9	84.8	87.2	86.3	84.2	81.6	83.3	83.3	84.4	82.2
15. <i>P. prevotii</i>																94.0	93.0	80.8	81.6	81.1	81.0	81.5	81.2	80.9	81.5	81.3	81.5	81.2	82.4	82.2	81.7	82.3	82.2	85.5	83.7	82.1	80.1	80.8	80.7	81.4	81.2	
16. <i>P. tetradiis</i>																	93.1	80.1	80.6	80.6	81.4	80.8	80.5	80.1	80.8	80.1	80.5	80.5	80.8	82.1	81.5	81.6	81.3	81.4	85.4	82.5	82.5	78.9	80.5	80.8	81.6	80.0
17. <i>P. hydrogenalis</i>																		79.7	79.8	80.0	79.7	79.3	78.6	79.5	78.6	79.3	80.5	80.4	79.4	79.9	80.3	81.8	84.6	82.2	81.2	78.2	79.4	79.4	79.7	79.6		
18. <i>C. difficile</i>																			95.0	95.2	95.4	94.8	94.3	94.6	96.1	95.5	96.0	92.2	91.8	91.4	86.8	89.4	87.5	90.2	87.1	86.1	82.3	86.6	82.7	83.5	84.2	
19. <i>C. mangelotii</i>																				94.3	94.1	93.7	93.7	94.2	94.7	94.0	94.7	92.3	91.0	90.2	88.3	89.6	86.9	89.7	87.5	85.1	82.3	86.5	84.3	83.8	85.2	
20. <i>C. lituseburens</i>																					97.2	96.0	95.7	96.4	96.5	95.4	95.9	92.5	91.2	91.2	88.0	91.6	88.0	90.8	87.8	86.7	82.7	86.8	83.9	84.0	84.9	
21. <i>C. irregularis</i>																						96.7	94.3	96.0	94.8	95.8	92.3	91.8	91.6	89.8	91.0	87.8	91.2	88.1	87.4	83.1	86.4	85.1	83.9	85.1		
22. <i>C. glycolicum</i>																							98.8	94.7	95.3	94.6	95.0	92.1	91.1	91.0	88.9	91.1	87.0	90.3	87.2	87.6	82.7	86.4	84.2	83.5	84.7	
23. <i>C. mayombe</i>																								94.3	95.1	94.2	94.7	91.7	91.1	91.0	88.8	90.5	86.8	89.1	87.3	87.5	81.8	85.2	84.3	83.5	85.3	
24. <i>C. bifementans</i>																									97.9	97.2	97.4	90.9	91.8	91.4	89.2	91.5	86.6	88.7	87.5	87.4	81.8	86.2	84.4	84.0	84.8	
25. <i>C. sordellii</i>																										98.1	99.2	92.5	93.1	93.0	89.9	91.6	87.5	90.2	88.4	87.9	82.3	87.0	84.3	84.2	84.7	
26. <i>C. ghonii</i>																										98.9	91.8	92.4	91.7	89.2	91.6	87.3	90.1	88.0	87.3	81.4	85.8	83.3	83.9	83.5		
27. <i>E. tenue</i>																											92.6	93.4	93.1	90.1	91.5	87.6	90.3	86.7	87.9	82.5	86.8	84.1	83.7	84.4		
28. <i>P. anaerobius</i>																												91.0	90.9	89.2	88.7	87.3	90.6	87.1	87.4	82.1	85.5	83.3	84.1	84.7		
29. <i>C. thermoalcaliphilum</i>																													98.2	89.9	92.2	88.5	90.9	88.8	86.0	81.4	85.4	84.1	84.8	84.1		
30. <i>C. paradoxum</i>																														89.5	91.4	88.2	90.9	88.5	85.9	81.6	85.1	83.7	84.2	84.1		
31. <i>C. stricklandii</i>																															90.0	86.2	90.2	86.1	87.5	83.8	86.8	82.6	83.1	83.4		
32. <i>C. litorale</i>																																90.3	92.5	89.4	87.5	82.3	84.7	83.9	85.4	86.0		
33. <i>C. formicoacetatum</i>																																	96.8	89.5	87.5	82.0	85.7	82.4	83.5	83.7		
34. <i>C. felsineum</i>																																		91.8	89.3	86.1	89.0	85.3	85.9	87.6		
35. <i>C. halophilum</i>																																			88.4	81.8	85.1	83.4	83.7	83.9		
36. <i>C. aminobutyricum</i>																																				82.3	85.0	83.7	83.8	83.8		
37. <i>E. yurii</i>																																					85.4	78.5	79.1	80.0		
38. <i>C. villosum</i>																																						81.1	81.2	82.4		
39. <i>C. barkeri</i>																																							95.3	82.8		
40. <i>E. limosum</i>																																							83.1			
41. <i>C. butyricum</i>																																										

^a Abbreviations: *T.*, *Tissierella*; *C.*, *Clostridium*; *E.*, *Eubacterium*; *H.*, *Helcococcus*; *P.*, *Peptostreptococcus*.

FIG. 1. Dendrogram showing the relationship of *T. praeacuta* to other members of the *Clostridium* subphylum of gram-positive bacteria. The tree was constructed by using the neighbor-joining method, and bootstrap values were calculated from 1,000 trees. Numbers are the percentages of bootstrap replicates.

considerations support the view that *C. acidurici*, *C. purinolyticum*, and *E. angustum* represent a new genus that is related to, but nevertheless is separate from, the genus *Tissierella*.

Taxonomic proposals. (i) **Emendation of the genus *Tissierella* (Collins and Shah).** The description of the genus *Tissierella* given by Collins and Shah (4) should be emended as follows. Gram negative or gram positive: *meso*-Diaminopimelic acid or D-ornithine is present in the cell wall peptidoglycan. The cellular fatty acids are primarily straight-chain saturated and iso-methyl branched-chain or unsaturated fatty acids. Strains preferentially growing on creatinine typically produce acetate, sarcosine, *N*-carbamoylsarcosine, ammonia, and CO₂. The G+C content of the DNA is 28 to 32 mol%.

(ii) **Description of *Tissierella creatinini* sp. nov.** *Tissierella creatinini* (cre.a.ti.ni' ni. N. L. gen. n. *creatinini*, of creatinine). Cells are rod shaped (1.0 by 3.5 µm) and occur mostly singly or in pairs and rarely in chains of four to six cells (Fig. 2). Cells are nonmotile, and spores are not observed. Cells are gram positive and are surrounded by a slime capsule that is easily detectable in India ink preparations. Grows anaerobically in AM0.1 medium containing 0.05% yeast extract, 0.05% Trypticase, and creatinine or *N*-methylhydantoin. Growth is stimulated fivefold by the addition of formate (40 mM), twofold by the addition of serine (2 g/liter) or threonine (2 g/liter), and threefold by the addition of arginine (2 g/liter) and serine (2 g/liter), but not by the addition of arginine alone. In the absence of creatinine or *N*-methylhydantoin, formate and amino

acids do not support growth. No stimulation of growth is produced by lactate, malate, succinate, glycerol, or H₂. Yeast extract (optimal concentration, 0.5 to 1.0%) is required for growth and cannot be replaced by Casamino Acids or pep-

FIG. 2. Phase-contrast micrograph of strain BN11^T. (Inset) India ink preparation showing slime capsules of cells. Bar = 10 µm.

TABLE 2. Substrates utilized by *T. creatinini* BN11^{Ta}

Substrate	Maximum optical density at 578 nm	Doubling time (h)
None	0.170	ND ^b
Creatinine (50 mM)	1.740	4.2
<i>N</i> -methylhydantoin (50 mM)	1.840	4.2
<i>N</i> -carbamoylsarcosine (50 mM)	0.510	11.4
Sarcosine (50 mM)	1.530	9.3
Glycine (50 mM)	0.700	19.5
Hydantoin (50 mM)	0.970	17.0
Hydantoic acid (50 mM)	0.490	18.6

^a The medium used was AM0.1 medium containing 0.5% yeast extract. The optical densities of cultures grown in Hungate tubes were determined directly with a spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). Doubling times were calculated from increases in optical density between 0.1 and 0.3. The following substrates were tested but were not utilized: arabinose, cellobiose, fructose, glucose, inositol, lactose, maltose, mannitol, mannose, melibiose, raffinose, ribose, sucrose, salicin, sorbitol, trehalose, xylose, betaine, choline, methylamine, trimethylamine, lactate, pyruvate, glycerol, methanol, ethanol, formate, formamide, arginine, glutamate, serine, threonine, creatine, cytosine, and uracil.

^b ND, not determined.

tones. Utilizes a limited range of mainly nitrogen-containing compounds, such as creatinine and structurally related substances, for growth. Not able to utilize creatine, carbohydrates, or various other substances (Table 2). Very weak growth occurs in peptone-yeast extract media (10), and biochemical tests for gelatin hydrolysis, production of lipase, lecithinase, indole, and urease, digestion of meat and milk, reduction of nitrate, and hemolysis are negative. Grows at 20 and 39°C, and optimum growth occurs at 37°C. The pH range for growth is 6.7 to 9.1, and the optimum pH is pH 8.3 (determined with *N*-methylhydantoin as the substrate). Sodium bicarbonate and sodium chloride are required for good growth; the optimum concentrations of these compounds are 27 and 60 mM, respectively, in AM0.1 medium containing 0.5% yeast extract and 50 mM *N*-methylhydantoin as the substrate. Creatinine is metabolized via *N*-methylhydantoin, *N*-carbamoylsarcosine, and sarcosine to acetate, ammonia, and CO₂. In a typical growth experiment performed with AM0.1 medium containing 0.5% yeast extract, 2 g of serine per liter, 2 g of threonine per liter, and 46 mM creatinine, the fermentation products formed after 60 h are 22 mmol of acetate per liter, 78 mmol of ammonia per liter, 24 mmol of sarcosine per liter, and 7 mmol of *N*-carbamoylsarcosine per liter. Respiratory menaquinones are not produced. The G+C content of the DNA is 32 mol%. The cell wall murein is based on L-ornithine with D-glutamic acid as the interpeptide bridge (type A4β). Contains predominantly unsaturated fatty acids; C_{18:1} c11/12 (61%) is the major component. Minor amounts of straight-chain saturated, methyl-branched, and 3-hydroxylated long-chain fatty acids are also produced. Isolated from a sugar refinery wastewater pond. The type strain is DSM 9508 (= BN11).

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REFERENCES

- Beerens, H., and M. Demonchy. 1948. Sur une variété non gazogène de *Zuberella praeacuta*. C. R. Seances Soc. Biol. 142:1107-1108.
- Cato, E. P., L. V. Holdeman, and W. E. C. Moore. 1979. Proposal of neotype strains for seven non-saccharolytic *Bacteroides* species. Int. J. Syst. Bacteriol. 29:427-434.
- Collins, M. D., P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int. J. Syst. Bacteriol. 44:812-826.
- Collins, M. D., and H. N. Shah. 1986. Reclassification of *Bacteroides praeacutus* Tissier (Moore and Holdeman) in a new genus, *Tissierella*, as *Tissierella praeacuta* comb. nov. Int. J. Syst. Bacteriol. 36:461-463.
- De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of nucleic acids. J. Bacteriol. 101:738-754.
- Felsenstein, J. 1989. PHYLIP—phylogeny inference package (version 3.2). Cladistics 5:164-166.
- Gaugglitz, U. 1988. Anaerobe mikrobieller Abbau von Kreatin, Kreatinin und *N*-Methylhydantoin. Reihe Biologie, Bd. 1. Uniflex Verlag, Göttingen, Germany.
- Gottschalk, E. M., H. Hippe, and F. Patzke. 1991. Creatinine deiminase (EC 3.5.4.21) from bacterium BN11: purification, properties and applicability in serum/urine creatinine assay. Clin. Chim. Acta 204:223-238.
- Hoffman, H. 1957. Genus II. *Fusobacterium* Knorr, p. 436-440. In R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), *Bergey's manual of determinative bacteriology*, 7th ed. The Williams & Wilkins Co., Baltimore.
- Holdemann, L. V., E. P. Cato, and W. E. C. Moore (ed.). 1977. *Anaerobe laboratory manual*, 4th ed. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg.
- Holdeman, L. V., I. J. Good, and W. E. C. Moore. 1976. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl. Environ. Microbiol. 31:359-375.
- Hutson, R. A., D. E. Thompson, and M. D. Collins. 1993. Genetic interrelationships of saccharolytic *Clostridium botulinum* types B, E and F and related clostridia as revealed by small subunit rRNA sequences. FEMS Microbiol. Lett. 108:103-110.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.
- Krieg, N. R. 1981. Systematics, p. 407-449. In P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), *Manual of methods for general bacteriology*. American Society for Microbiology, Washington, D.C.
- Lawson, P. A., S. E. Gharbia, H. N. Shah, and D. R. Clark. 1989. Recognition of *Fusobacterium nucleatum* subgroups Fn-1, Fn-2 and Fn-3 by ribosomal RNA gene restriction patterns. FEMS Microbiol. Lett. 65:41-46.
- Lawson, P. A., P. L. Perez, R. A. Hutson, H. Hippe, and M. D. Collins. 1993. Towards a phylogeny of the clostridia based on 16S rRNA sequences. FEMS Microbiol. Lett. 113:87-92.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of DNA from its thermal denaturation temperature. J. Mol. Biol. 5:109-118.
- Möller, B., H. Hippe, and G. Gottschalk. 1986. Degradation of various amine compounds by mesophilic clostridia. Arch. Microbiol. 145:85-90.
- Moore, W. E. C., and L. V. Holdeman. 1973. New names and combinations in the genera *Bacteroides* Castellani and Chalmers, *Fusobacterium* Knorr, *Eubacterium* Prevot, *Propionibacterium* Orla-Jensen, and *Lactobacillus* Beijerinck. Int. J. Syst. Bacteriol. 23:69-74. (Author's correction, 24:311, 1974.)
- Paster, B. J., F. E. Dewhirst, I. Olsen, and G. J. Fraser. 1994. Phylogeny of *Bacteroides*, *Prevotella*, and *Porphyromonas* spp. and related bacteria. J. Bacteriol. 176:725-732.
- Prevot, A. R. 1938. Etudes de systematique bacterienne. Ann. Inst. Pasteur (Paris) 60:285-307.
- Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.
- Shah, H. N., and M. D. Collins. 1983. Genus *Bacteroides*: a chemotaxonomical perspective. J. Appl. Bacteriol. 48:75-87.
- Stackebrandt, E. 1992. Unifying phylogeny and phenotypic diversity, p. 19-47. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The prokaryotes*, vol. 1. Springer Verlag, New York.
- Tissier, H. 1908. Recherche sur la flore intestinale normale des enfants ages d'un à cinq ans. Ann. Inst. Pasteur (Paris) 22:189-208.
- Widdel, F., G. W. Kohring, and F. Mayer. 1983. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. sp. nov. and *Desulfonema magnum* sp. nov. Arch. Microbiol. 134:286-294.
- Woese, C. R. 1987. Bacterial evolution. Microbiol. Rev. 51:221-271.
- Wolin, E. A., R. S. Wolfe, and M. J. Wolin. 1964. Viologen dye inhibition of methane formation by *Methanobacillus omelianskii*. J. Bacteriol. 87:993-998.