Fastidiosipila sanguinis gen. nov., sp. nov., a new Gram-positive, coccus-shaped organism from human blood

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Phenotypic and phylogenetic studies were performed on two strains of an unidentified Gram-positive, fastidious, non-spore-forming, coccus-shaped bacterium recovered from human blood. The organism was catalase-negative and grew under strictly anaerobic conditions and in the presence of 2 and 6% O₂. Comparative 16S rRNA gene sequencing demonstrated that the unidentified bacterium was, phylogenetically, far removed from peptostreptococci and related Gram-positive coccus-shaped organisms, but exhibited a phylogenetic association with *Clostridium* rRNA cluster III [as defined by Collins *et al.*, *Int J Syst Bacteriol* **44** (1994), 812–826]. Sequence divergence values of 15% or more were observed between the unidentified bacterium and all other recognized species within this and related rRNA clostridial clusters. Treeing analysis showed that the unknown bacterium formed a deep line branching at the periphery of rRNA cluster III and represents a hitherto unknown genus within this supra-generic grouping. On the basis of both phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium from blood be classified in a new genus, *Fastidiosipila* gen. nov., as *Fastidiosipila sanguinis* sp. nov. The type strain of *Fastidiosipila sanguinis* is CCUG 47711^T (= CIP 108292^T).

The anaerobic, Gram-positive cocci represent a taxonomically heterogeneous group of organisms. These organisms were at one time classified in a single family, 'Peptostreptococcaceae' (Rogosa, 1974), which included the genera *Ruminococcus*, *Coprococcus*, *Sarcina*, *Peptococcus* and *Peptostreptococcus*. During the past decade or so, knowledge of the phylogenetic inter-relationships of these organisms has been greatly clarified through the use of 16S rRNA gene sequencing, and it is now known that the various genera of the anaerobic Gram-positive cocci are scattered throughout the *Clostridium* subphylum (Collins *et al.*, 1994; Li *et al.*, 1994; Murdoch *et al.*, 1997). Phylogenetically, *Peptostreptococcus anaerobius*, the type species of the genus *Peptostreptococcus*, is a member of *Clostridium* rRNA group XI (see Collins *et al.*, 1994, for group designations), whereas other peptostreptococci are found in *Clostridium* rRNA group XIII and now have been assigned to distinct genera (*Anaerococcus*, *Finegoldia*, *Gallicola*, *Peptoniphilus*;

Murdoch & Shah, 1999; Ezaki *et al.*, 2001). *Sarcina ventriculi* is a member of *Clostridium* rRNA group I, coprococci are related to the members of *Clostridium* rRNA group XVI, whereas ruminococci are located in two different rRNA lines (Collins *et al.*, 1994; Rainey & Janssen, 1995). In addition to these changes, a plethora of novel species of strictly anaerobic, Gram-positive cocci has been described in recent years (e.g. Murdoch *et al.*, 1997; Ezaki *et al.*, 1990; Li *et al.*, 1992) and there are strong indications that much new diversity remains to be discovered, especially from human sources. During the course of a study of taxonomically problematic anaerobic organisms associated with human clinical specimens, we have characterized two isolates that represent a hitherto unknown genus of Gram-positive cocci within *Clostridium* rRNA group III. On the basis of the phenotypic and phylogenetic findings presented here, we describe a new genus and novel species, *Fastidiosipila sanguinis*.

Strain CCUG 47711^T was recovered from a blood specimen taken from an 84-year-old man in Karlstad, Sweden, whereas strain CCUG 47367 was isolated from the blood sample of an 87-year-old man from Göteborg, Sweden. The strains were cultivated on chocolate agar (Difco) and

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anaerobic blood agar (Oxoid) and incubated anaerobically at 37 °C under a gas phase comprising N₂/H₂/CO₂ (86:7:7, by vol.). Aerotolerance was determined by incubating the organism on *Brucella* agar plates (Difco) with and without 5% (v/v) laked sheep blood in an atmosphere containing either 2% or 6% O₂, as described by Wexler *et al.* (1996). The strains were characterized biochemically by using a combination of conventional tests as described previously in the VPI *Anaerobe Laboratory Manual* (Holdeman *et al.*, 1977) and the API Rapid ID 32S, API Rapid ID 32A and API ZYM systems according to the instructions of the manufacturer (bioMérieux). Fermentation tests were performed using pre-reduced, anaerobically sterilized peptone/yeast/glucose broth (Anaerobe Systems) tubes. All biochemical tests were performed in duplicate. The G+C content (mol%) of the DNA was determined by HPLC according to Mesbah *et al.* (1989) except that the methanol content of the chromatographic buffer was decreased to 8% (v/v) and the temperature was increased to 37 °C. The 16S rRNA genes of the isolates were amplified by PCR using universal primers pA (positions 8–28, *Escherichia coli* numbering) and pH* (positions 1542–1522). The amplified products were purified by using a QIAquick PCR purification kit (Applied Biosystems) and directly sequenced using primers directed towards conserved positions of the rRNA gene, and using the dRhodamine terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (Applied Biosystems). The closest known relatives of the novel isolates were determined by performing database searches using the program FASTA (Pearson & Lipman, 1985). These sequences and those of other known related strains were retrieved from GenBank and aligned with the newly determined sequence by using the program SEQtools (Rasmussen, 2002). The resulting multiple sequence alignment was corrected manually using the program GeneDoc (Nicholas *et al.*, 1997). A phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987) with the programs SEQtools and TreeView (Page, 1996) and the stability of the groupings was estimated by bootstrap analysis (1000 replications) using the same programs.

The isolates originating from human blood specimens grew under strictly anaerobic conditions and also in the presence of 2 and 6% O₂. The organism formed small, coccus-shaped cells that stained Gram-positive. Spores were not observed. Colonies on chocolate agar and anaerobic blood agar were small, pinpoint and grey in colour. The organisms were non-haemolytic. Growth was poor in Fastidious Anaerobe Broth (Oxoid). The acid end-products formed in Fastidious Anaerobe Broth with meat granules were found to be acetic and butyric acids, in small amounts. Conventional biochemical methods revealed that the strains were catalase-, lecithinase- and urease-negative. The strains did not reduce nitrate and were indole-negative (both in the spot-indole test and in pre-reduced, anaerobically sterilized indole-nitrate broth). Both strains failed to grow in peptone/yeast broth with fructose, glucose, inositol, lactose, maltose,

mannose, mannitol, melibiose, raffinose, rhamnose, ribose, starch, sucrose or xylose. The unidentified isolates were relatively unreactive in the API Rapid ID 32S tests. They hydrolysed hippurate and displayed α -galactosidase, β -galactosidase, alanine phenylalanine proline arylamidase and β -mannosidase (weak reaction) activities. One isolate (CCUG 47711^T) was β -glucuronidase-positive. All other tests in the API Rapid ID 32S system gave negative results. The organisms were more reactive in the API Rapid ID 32A tests, displaying activity for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, proline arylamidase and α -fucosidase; reactions for serine arylamidase, histidine arylamidase, glycine arylamidase and tyrosine arylamidase were either weakly positive or negative. In the API ZYM tests, the strains gave positive reactions for *N*-acetyl- β -glucosaminidase, α -galactosidase, α -glucosidase and β -glucosidase, whereas reactions for acid phosphatase, ester lipase C8, phosphatase and phosphoamidase were either weakly positive or negative. Reactions for β -glucuronidase and esterase C4 were found to be variable. All other tests in the API ZYM gallery produced negative results. On the basis of their cellular morphology and overall biochemical reactions, the unidentified isolates did not appear to correspond to any currently described species of anaerobic cocci. The G+C content of the DNA of strain CCUG 47711^T was determined and found to be 32.9 mol%, which was consistent with the assignment of the isolates to the low-G+C branch of the Gram-positive bacteria. To elucidate the phylogenetic position of the unidentified isolates, their almost-complete 16S rRNA genes were amplified by PCR and then sequenced. The 16S rRNA gene sequences were found to be identical to each other, and sequence database searches showed that the unidentified organisms displayed the highest sequence relatedness to members of the *Clostridium* subphylum (data not shown), consistent with their low G+C content. On the basis of sequence similarities, the closest named relatives of the unidentified bacterium corresponded to species within *Clostridium* rRNA clusters III and IV (see Collins *et al.*, 1994, for rRNA group designations). Treeing analysis showed that the unidentified bacterium clustered at the periphery of *Clostridium* rRNA cluster III, which includes *Acetivibrio cellulolyticus*, *Bacteroides cellulosolvens* and a variety of misclassified clostridial species (Fig. 1). Values for sequence divergence between the unidentified isolates and species of rRNA cluster III were invariably greater than 15% (data not shown).

From the findings presented, it is evident that the unidentified Gram-positive-staining, asporogenous, coccus-shaped organism recovered from human blood represents a hitherto unknown line within the *Clostridium* subphylum of the Gram-positive bacteria (Collins *et al.*, 1994). Phylogenetically, the unknown bacterium displays an affinity with rRNA cluster III, and is far removed from all other recognized genera of Gram-positive anaerobic cocci (*viz.* *Anaerococcus*, *Coprococcus*, *Finegoldia*, *Gallicola*, *Peptoniphilus*, *Peptostreptococcus* and *Sarcina*) which are found

Fig. 1. Unrooted tree, based on 16S rRNA gene sequences, showing the association between *Fastidiosipila sanguinis* gen. nov., sp. nov. and *Clostridium* rRNA cluster III and some other taxa. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1330 nt. Bootstrap values, expressed as percentages of 1000 replications, are given at the branching points. Bar, 1% sequence divergence.

interspersed throughout a variety of phylogenetic groups (Collins *et al.*, 1994; Murdoch & Shah, 1999; Ezaki *et al.*, 2001; Rainey & Janssen, 1995). The association of the coccus-shaped organism with members of *Clostridium* rRNA clade III, however, was only loose, with the unknown bacterium branching deeply at the periphery of the cluster. Treeing analysis showed that the unknown bacterium did not have a close relationship with any individual member of *Clostridium* rRNA group III, although the branching at the base of the grouping was supported by a high bootstrap value. The distinctiveness of the unknown bacterium was also evident from divergence values of greater than 15% with members of rRNA cluster III and greater than 20% with other recognized Gram-positive, anaerobic cocci. *Clostridium* rRNA cluster III currently represents a small supra-generic grouping, embracing a diverse range of organisms including *Acetivibrio cellulolyticus*, the misclassified *B. cellulosolvens* and a variety of so-called clostridial species (viz. *Clostridium cellobioparum*, *Clostridium cellulolyticum*, *Clostridium josui*, *Clostridium hungatei*, *Clostridium*

papyrosolvens, *Clostridium stercorarium*, *Clostridium termitidis*, *Clostridium thermocellum* and *Clostridium thermosuccinogenes*). In addition to high sequence divergence values and tree topology considerations, support for the separateness of the unknown bacterium from other organisms within *Clostridium* rRNA cluster III also comes from phenotypic considerations. In particular, the unidentified bacterium can be readily distinguished from all other taxa within this cluster by its small, coccoid cells and fastidious nature. Furthermore, the novel bacterium can be distinguished from *Acetivibrio cellulolyticus* and *B. cellulosolvens* by its Gram-positive staining reaction and the absence of cellulolytic activity (Murray *et al.*, 1984; Patel *et al.*, 1980). Similarly, the unknown organism differs from the misclassified clostridia within rRNA cluster III by not producing endospores and in terms of other phenotypic criteria (Table 1). Hence, given the phenotypic distinctiveness of the unknown coccus, together with its long rRNA line branching at the base of *Clostridium* rRNA cluster III, we consider that it merits inclusion in a new genus. We

Table 1. Characteristics that are useful in differentiating *Fastidiosipila sanguinis* from other taxa in rRNA cluster III and some other related organisms

Taxa: 1, *Fastidiosipila sanguinis*; 2, *Acetivibrio cellulolyticus*; 3, *Anaerofilum*; 4, *B. cellulosolvens*; 5, *Clostridium aldrichii*; 6, *C. cellobioparum*; 7, *C. cellulolyticum*; 8, *C. hungatei*; 9, *C. papyrosolvens*; 10, *C. stercorarium*; 11, *C. thermocellum*; 12, *C. thermosuccinogenes*; 13, *Faecalibacterium prausnitzii*; 14, *Papillibacter cinnaminivorans*. Characteristics are scored as follows: +, positive; -, negative; D, strain-dependent; ND, not determined. Data for *Fastidiosipila sanguinis* are from the present study and data for the other taxa are from Cato *et al.* (1986), Defnouv *et al.* (2000), Duncan *et al.* (2002), Madden *et al.* (1982), Monserrate *et al.* (2001), Murray *et al.* (1984), Patel *et al.* (1980), Petitdemange *et al.* (1984), Yang *et al.* (1990) and Zellner *et al.* (1996).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cellular morphology	Cocci	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Spores	-	-	-	-	+	+	+	+	+	D	+	+	-	-
Gram stain	+	-	+	-	+	-	+	-	-	-	-	-	-	-
Motility	-	+	D	-	+	+	+	+	+	D	-	+	-	-
Flagella*	-	M	P	-	PB	P	P	SP	P	P/SL	-	P	-	-
End-products of metabolism†	A, B	^a A, B, P	A, E, F, L, 2,3-butanediol	^a A, E, L	A, B, P, IB, IV, L, S	^b A, E, L, F	A, E, F, L	^b A, E, L, F	^b A, E, L	A, L, E	^b A, E, L	A, E, F, L, S	B, L, F, S	ND
Cellulose degradation	-	+	-	+	+	+	+	+	+	+	+	-	-	-
Growth at 60 °C	-	-	-	-	-	-	-	-	-	+	+	+	-	-
DNA G+C content (mol%)	33	38	54-55	43	40	28	41	40	30	39-43	38-39	36	47-57	56

*Abbreviations: M, monotrichous; P, peritrichous; PB, polar bundle; SL, single lateral; SP, subpolar.

†End-products: A, acetate; B, butyrate; E, ethanol; F, formate; IB, isobutyrate; IV, isovalerate; L, lactate; P, propionate; S, succinate (^acellulose fermentation, ^bcellulose fermentation).

therefore formally propose that the bacterium recovered from human blood be classified in a new genus and species, *Fastidiosipila sanguinis* gen. nov., sp. nov. Tests that are useful in distinguishing *Fastidiosipila sanguinis* from its nearest phylogenetic relatives and morphologically similar organisms are shown in Table 1 and Table 2, respectively.

Description of *Fastidiosipila* gen. nov.

Fastidiosipila (Fas.ti.di.o.si.pi'la. L. adj. *fastidiosus* fastidious; L. fem. n. *pila* ball; N.L. fem. n. *Fastidiosipila* a fastidious ball, because the organisms are difficult to grow).

Cells consist of Gram-positive-staining, non-spore-forming, small cocci. Growth occurs under strictly anaerobic conditions and also in the presence of 2 and 6% O₂. Catalase-negative. Carbohydrates are not fermented. Small amounts of acetic and butyric acids are detected in Fastidious Anaerobe Broth with meat granules. Lipase-, lecithinase- and urease-negative. Non-cellulolytic. Nitrate is not reduced. Indole-negative. The G+C content of the DNA is 32.9 mol%. *Fastidiosipila* is a member of the *Clostridium* subphylum of the Gram-positive bacteria and is phylogenetically loosely associated with *Clostridium* rRNA group III.

The type species is *Fastidiosipila sanguinis*.

Description of *Fastidiosipila sanguinis* sp. nov.

Fastidiosipila sanguinis (san'gui.nis. L. gen. n. *sanguinis* of blood, referring to the source of the organism).

Cells consist of small cocci (approx. 0.5 µm in diameter) that stain Gram-positive. After 48 h anaerobic incubation at 37 °C, colonies are small, pinpoint and grey in colour. Grows under anaerobic conditions and also in 2 and 6% O₂. Catalase-negative. Grows poorly on Fastidious Anaerobe Agar (Oxoid) and Fastidious Anaerobe Broth with meat granules. In the latter, small amounts of acetic and butyric acids are detected. Lecithinase, lipase and urease are not produced. Gelatin is not hydrolysed. In the API Rapid ID 32S system, activity is detected for α-galactosidase, β-galactosidase, β-glucosidase, alanine phenylalanine proline arylamidase and β-mannosidase. No activity is detected for alkaline phosphatase, arginine dihydrolase, glycyl tryptophan arylamidase, pyroglutamic acid arylamidase or urease; β-glucuronidase activity may or may not be detected. Hippurate is hydrolysed and acetoin is not produced. In the API Rapid ID 32A system, positive reactions are obtained for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, alanine arylamidase (weak), arginine arylamidase, proline arylamidase, leucine arylamidase (weak) and α-fucosidase; reactions for serine arylamidase, histidine arylamidase, glycine arylamidase and tyrosine arylamidase are either weakly positive or negative. Activity is not detected for alkaline phosphatase, α-arabinosidase, arginine dihydrolase, β-galactosidase-6-phosphate, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, β-glucuronidase, leucyl glycine arylamidase, phenyl alanine arylamidase, pyroglutamic acid arylamidase or urease. Indole is not produced and nitrate is not reduced. Sensitive to kanamycin

Table 2. Characteristics that are useful in differentiating *Fastidiosipila sanguinis* from some other Gram-positive, coccus-shaped taxa

Taxa: 1, *Fastidiosipila sanguinis*; 2, *Anaerococcus prevotii*; 3, *Anaerococcus tetradius*; 4, *Anaerococcus lactolyticus*; 5, *Anaerococcus hydrogenalis*; 6, *Anaerococcus octavius*; 7, *Anaerococcus vaginalis*; 8, *Peptoniphilus asaccharolyticus*; 9, *Peptoniphilus lacrimalis*; 10, *Peptoniphilus harei*; 11, *Peptoniphilus ivorii*; 12, *Peptoniphilus indolicus*; 13, *Gallicola barnesae*; 14, *Finegoldia magna*; 15, *Micromonas micros*; 16, *Peptostreptococcus anaerobius*; 17, *Peptococcus niger*. Characteristics are scored as follows: +, positive; –, negative; D, strain-dependent; W, weak. Data for *Fastidiosipila sanguinis* are from the present study; other data are from Ezaki *et al.* (2001).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Major terminal volatile fatty acid(s)*	A, B	B	B	B	B	B, C	B	B	B	B	B	B	A, B	A	A	IC, IV	C
Production of:																	
Indole	–	–	–	–	+	–	D	D	–	D	–	+	W	–	–	–	–
Urease	–	D	+	+	D	–	–	–	–	–	–	–	–	–	–	–	–
Alkaline phosphatase	–	–	–	–	D	–	D	–	–	–	–	+	–	D	+	–	–
Fermentation of:																	
Glucose	–	D	D	+	+	+	+	–	–	–	–	–	–	–	–	+	–
Lactose	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Raffinose	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Mannose	–	+	+	+	+	+	D	–	–	–	–	–	–	–	–	+	–
Activity for:																	
α -Galactosidase	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
β -Galactosidase	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
α -Glucosidase	+	+	+	–	D	–	–	–	–	–	–	–	–	–	–	W	–
β -Glucosidase	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Arginine arylamidase	D	+	+	+	–	–	+	+	+	+	–	+	–	+	+	–	–
Proline arylamidase	+	–	–	–	–	+	–	–	–	–	+	–	–	–	+	+	–
Phenylalanine arylamidase	–	–	W	–	–	–	–	–	+	–	–	+	–	–	–	–	–
Leucine arylamidase	D	–	+	–	–	–	+	D	+	D	–	+	–	+	+	–	–
Pyroglutamic acid arylamidase	–	+	W	–	–	W	–	–	–	–	–	–	–	+	+	–	–
Histidine arylamidase	D	+	W	–	–	–	+	W	+	+	–	+	–	D	+	–	–
DNA G + C content (mol%)	33	29–33	30–32	30–34	30–34	26–31	30–34	31–32	30–34	25	29	32–35	32–34	32–34	28–30	34–36	50–51

*A, acetate; B, butyrate; C, caproate; IC, isocaproate; IV, isovalerate.

(1000 µg) and vancomycin (5 µg), but resistant to colistin sulfate (10 µg) and metronidazole (5 µg) identification discs. The G + C content of the DNA is 32.9 mol%. Isolated from human blood. Habitat is not known.

The type strain is CCUG 47711^T (= CIP 108292^T).

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