Facklamia sourekiiの泌乳牛からの同定

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NOTE *Bacteriology*

Identification of *Facklamia sourekii* from a Lactating Cow

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ABSTRACT. A gram-positive, catalase-negative, facultatively anaerobic coccus was isolated from a lactating cow with hematuria and urodynia in Japan. The isolate was identified by 16S rRNA gene sequence analysis as *Facklamia sourekii*. The biochemical and culture characteristics of the isolate were well consistent with those of *F. sourekii* type strain. Since all *F. sourekii* strains reported so far were isolated from human clinical specimens, this is the first reported case of *F. sourekii* isolated from veterinary clinical specimen.
KEY WORDS: *Facklamia sourekii*, hematuria, lactating cow.

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F. sourekii is a recently described gram-positive, catalase-negative, facultatively anaerobic cocci [2]. In the genus *Facklamia*, five more species, *Facklamia hominis*, *Facklamia ignava, Facklamia languida, Facklamia tabacinasalis*, and *Facklamia miroungae*, have been described to date [1, 3, 4, 7, 10]. Although *F. tabacinasalis* and *F. miroungae* strains were isolated from powdered tobacco and a nasal swab of a juvenile elephant, respectively [3, 7], all other *Facklamia* strains were isolated from human clinical specimens [1, 2, 4, 5, 10]. In this report, we describe a case of *F. sourekii* isolated from an animal source.

Late in April 2005, a 5-year- and 5-month-old lactating cow (Holstein), which was kept in a farm in Ishikawa prefecture, Japan, presented with hematuria and urodynia. On the first examination in May 2005, the cow had normal body temperature and a good appetite. Arched back was not observed. After drying off, the cow was treated intramuscularly with 4 g of oxytetracycline. Since, in addition to the hematuria, the cow showed arched back and poor appetite 6 days after the first examination, the cow was additionally treated intramuscularly with 2 g of enrofloxacin on that day, 2 g of enrofloxacin and 2.5 g of tranexamic acid 2 days later, and 2 g of enrofloxacin 5 days after the second examination. Based on persistent hematuria, urinary tract infection was presumed, and urine sample was obtained for further investigation. Microscopic examination of the urine showed numerous red blood cells, white blood cells, epithelial cells, *Streptococcus*-like bacteria, but no urinary casts were observed. Further urinalysis demonstrated normal urobilinogen, positive protein, and a pH of 8–9, with negative results for glucose, ketones, bilirubin, and ascorbic acid.

The urine sample was inoculated onto Trypticase soy agar plates and Columbia agar plates containing 5% sheep blood or 5% horse blood, and incubated at 37°C in air plus 5% CO_2. After 24–48 hr of incubation, small gray to colorless colonies (maximal diameter of 1.0 mm) with little hemolytic activity on sheep blood cells were observed in pure culture. The isolate (NIAH 13370) was gram positive and ovoid in shape and formed single cells, pairs, or short chains. The strain grew in 6.5% NaCl at 37°C, but not at all at 10 or 45°C. The strain was non-spore-forming, catalase-negative, oxidase-negative, leucine aminopeptidase-positive, pyrrolidonylarylamidase-positive facultative anaerobe (Table 1). Biochemical profile (00230111500; Table 1) generated by the API ID32 STREP system (bioMérieux) identified the isolate as *Aerococcus viridans* and *Streptococcus acidominimus* with only 65.7% and 34.2% probabilities, respectively. Considering the insufficiency of this identification, the 16S rRNA gene sequence was determined as described previously [12], and comparison of the sequence (1,502 bp; DDBJ/EMBL/GenBank accession no. AB248259) with all bacterial sequences available from DDBJ/EMBL/GenBank databases was performed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). The isolate was identified as *Facklamia sourekii* on the base of a 99.87% identity of the 16S rRNA gene sequence with that of *F. sourekii* type strain CCUG 28783A^T (GenBank accession number Y17312). Of note, the biochemical and culture characteristics of NIAH 13370 were well consistent with those of *F. sourekii* CCUG 28783A^T, and only two differences were noted (sorbitol and lactose fermentation; Table 1). These results further support the above identification.

Since the milk cow, from which *F. sourekii* NIAH 13370 was isolated, was treated with oxytetracycline and enrofloxacin, the MICs of strain NIAH 13370 and the type strain CCUG 28783A^T for these two antibiotics were determined using the methods described by the National Committee for Clinical Laboratory Standards (NCCLS) [11]. As shown in Table 2, reduced susceptibility to oxytetracycline was observed in strain NIAH 13370, compared with strain CCUG 28783A^T. However, the enrofloxacin MIC for strain NIAH13370 was fourfold lower than that for strain CCUG 28783A^T. In this case, the cow's urinary symptoms eventually resolved. However, because the cow's condition did

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Table 1. Biochemical characteristics of *F. sourekii* NIAH 13370 isolated from hematuria of a milk cow and *F. sourekii* CCUG 28783A^T (the type strain)

Characteristics or tests[b]	Results[a] for:	
	F. sourekii NIAH 13370	*F. sourekii* CCUG 28783A^T[c]
Growth		
In NaCl-BHI[d]	+	+
At 10°C in BHI	–	–
At 45°C in BHI	–	–
Production of		
Leucine aminopeptidase	+	+
Pyrrolidonylarylamidase	+	+
Oxidase	–	–
Catalase	–	–
API ID32 STREP results[e]		
Hydrolysis of:		
Hippurate (HIP)	+	+
Arginine (ADH)	–	–
Acid production from:		
Ribose (RIB)	–	–
Mannitol (MAN)	+	+
Sorbitol (SOR)	**–**	**+**
Lactose (LAC)	**+**	**–**
Trehalose (TRE)	+	+
Raffinose (RAF)	–	–
Sucrose (SAC)	+	+
L–Arabinose (LARA)	–	–
D–Arabitol (DARL)	+	+
Cyclodextrin (CDEX)	–	–
Glycogen (GLYG)	–	–
Pullulan (PUL)	–	–
Maltose (MAL)	+	+
Melibiose (MEL)	–	–
Melezitose (MLZ)	–	–
Methyl-β-D-Glucopyranoside (MβDG)	–	–
Tagatose (TAG)	–	–
Voges-Proskauer reaction (VP)	–	–
Enzyme activity		
β-Glucosidase (βGLU)	–	–
β-Galactosidase (βGAR)	–	–
β-Glucuronidase (βGUR)	–	–
α-Galactosidase (αGAL)	–	–
Alkaline phosphatase (PAL)	–	–
Alanine-phenylalanine-proline arylamidase (APPA)	–	–
β-Galactosidase (βGAL)	–	–
Pyroglutamic acid arylamidase (PryA)	+	+
N-Acetylglucosaminidase (βNAG)	–	–
Glycyl-tryptophan arylamidase (GTA)	–	–
β–Mannosidase (βMAN)	–	–
Urease (URE)	–	–

a) +, positive; –, negative.
b) Different biochemical characteristics between strains NIAH 13370 and CCUG 28783A^T are indicated by bold type.
c) The type strain CCUG 28783A^T was obtained from American Type Culture Collection as ATCC 700629. No clinical information was available on the strain.
d) NaCl-BHI, brain heart infusion plus 6.5% NaCl at 37°C.
e) Abbreviations in parentheses are those used in the API ID32 STREP system.

not drastically improve even after a series of the oxytetracycline and enrofloxacin treatments, it is unclear whether the recuperation could be attributed to the antibiotic treatments or the cow's inherent immunity.

Because of the limited clinical information, the natural habitat and pathogenesis of *F. sourekii* strains remain unknown at present. Since most of the *Facklamia* strains from human clinical specimens were isolated from female

Table 2. MICs for *F. sourekii* NIAH 13370 and CCUG 28783A^T

Antibiotics	MIC (mg/liter)	
	F. sourekii NIAH 13370	*F. sourekii* CCUG 28783A^T
Enrofloxacin	0.125	0.5
Oxytetracycline	2	0.25

patients [9], and three of the six *F. hominis* strains in the original description of the species were isolated from vaginal swabs [1], it has been postulated that the natural habitat of the *Facklamia* species is the female genitourinary tract [9]. Isolation of *F. hominis* from urine of a girl [1] and *F. languida* from the blood of a woman with a urinary tract infection [10], as well as a recent case of chorioamnionitis caused by *F. hominis* [6], may support this assumption. Consistent with this assumption, in the case presented here, *F. sourekii* NIAH 13370 was isolated from the urine of a female cow. In addition, the cow presented with hematuria and urodynia. These facts suggest that the habitat of *F. sourekii* in milk cows is also the female genitourinary tract, and that the species may cause opportunistic genitourinary infections in compromised hosts. For investigation of these possibilities and pathogenesis of the organism, extended epidemiological and molecular biological studies of the *Facklamia* species will be necessary.

Using the bioMérieux API ID32 STREP system, the *F. sourekii* isolate was not identified correctly. Instead, the obtained profile misidentified the isolate as *A. viridans* or *S. acidominimus*. Similar misidentification of *F. sourekii* strains by this rapid identification system has been reported previously [8]. In the report, the first of three *F. sourekii* strains tested was identified as "unaccepted ID", the second was misidentified as *S. acidominimus* (%ID=99.9), and the last was misidentified as *A. viridans* (%ID=94.5, doubtful ID). Similarly, using two other rapid identification systems (the BBL Crystal rapid gram-positive identification and the Remel IDS RapID STR), the three strains could not be identified as *F. sourekii* [8]. Therefore, when gram-positive cocci-like isolates are identified as *S. acidominimus* or *A. viridans* by the API ID32 STREP system, further confirmation using molecular techniques should be performed to obtain an accurate identification.

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REFERENCES

1. Collins, M. D., Falsen, E., Lemozy, J., Åkervall, E., Sjödén, B. and Lawson, P.A. 1997. *Int. J. Syst. Bacteriol.* **47**: 880–882.
2. Collins, M. D., Hutson, R. A., Falsen, E. and Sjödén, B. 1999. *Int. J. Syst. Bacteriol.* **49**: 635–638.
3. Collins, M. D., Hutson, R. A., Falsen, E. and Sjödén, B. 1999. *Int. J. Syst. Bacteriol.* **49**: 1247–1250.
4. Collins, M. D., Lawson, P. A., Monasterio, R., Falsen, E., Sjödén, B. and Facklam, R. R. 1998. *J. Clin. Microbiol.* **36**: 2146–2148.
5. Facklam, R. 2002. *Clin. Microbiol. Rev.* **15**: 613–630.
6. Healy, B., Beukenholt, R. W., Tuthill, D. and Ribeiro, C. D. 2005. *J. Infect.* **50**: 353–355.
7. Hoyles, L., Foster, G., Falsen, E., Thomson, L. F. and Collins, M. D. 2001. *Int. J. Syst. Evol. Microbiol.* **51**: 1401–1403.
8. LaClaire, L. L. and Facklam, R. R. 2000. *J. Clin. Microbiol.* **38**: 2037–2042.
9. LaClaire, L. and Facklam, R. 2000. *Antimicrob. Agents Chemother.* **44**: 2130–2132.
10. Lawson P. A., Collins, M. D., Falsen, E., Sjödén, B. and Facklam, R. R. 1999. *J. Clin. Microbiol.* **37**: 1161–1164.
11. National Committee for Clinical Laboratory Standards. 2002. Performance Standards for Antimicrobial Susceptibility Testing, vol. 22. M31-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. Takamatsu, D., Osaki, M. and Sekizaki, T. 2002. *J. Bacteriol.* **184**: 2050–2057.