Isolation of *Streptobacillus moniliformis* from a Pet Rat

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(Received 22 August 2007/Accepted 26 December 2007)

ABSTRACT. We isolated *Streptobacillus moniliformis*, the causative agent of rat-bite fever in humans, from the salivary gland of a pet rat postmortem. The isolate was a Gram-negative pleomorphic coccobacillus, which produced acid from glucose and showed enzymatic activities for eight items in the API ZYM system. The results were consistent with those of the reference strain, ATCC 14647^T, except for acid production from dextrin. Partial sequencing of 16S rRNA (1,440 bp) and *gyrB* genes (514 bp) of the isolate revealed similarities of 100% and 99.8%, respectively, to those of *S. moniliformis* in GenBank. Therefore, the isolate was identified as *S. moniliformis*. These results suggested the potential risk of rat-bite fever arising from pet rats in Japan.

KEY WORDS: isolation, pet rat, *Streptobacillus moniliformis*.

J. Vet. Med. Sci. 70(5): 493–495, 2008

Streptobacillus moniliformis is a microaerophilic, pleomorphic, Gram-negative rod bacterium, which is known to be the causative agent of rat-bite fever (RBF) and Haverhill fever in humans. This bacterium is considered part of the normal nasopharyngeal flora in rats, but there have been few reports of the isolation of *S. moniliformis* from rats. Reports in the literature have described isolation of this bacterium only from wild and laboratory rats [7, 8, 10]. Detection of *S. moniliformis* in pet rats by molecular techniques, such as PCR, has been reported [2, 11], but there have been no previous reports of its actual isolation from these animals. Here, we describe the isolation of *S. moniliformis* from a postmortem pet rat.

The rat (*Rattus norvegicus*), an albino female of unknown age, was obtained from a pet store, and had been reared as a pet for 6 months. The rat died of unknown causes, and was presented to our center for necropsy. The rat was transported to our center with a refrigerant, and necropsy was performed approximately 40 min after death.

The examination for *S. moniliformis* was performed at necropsy by culture methods using ATCC medium 488 supplemented with nalidixic acid (Wako Pure Chemical Industries, Osaka, Japan) at 30 mg/l (488NA) for elimination of *Enterobacteriaceae* [12]. The composition of ATCC medium 488, the medium recommended by ATCC (American Type Culture Collection, Manassas, VA, U.S.A.) for culture of *Streptobacillus moniliformis* ATCC 49940, was as follows: 25.0 g of Heart Infusion Broth (Becton Dickinson Japan, Tokyo, Japan), 10.0 g of Bacto peptone (Becton Dickinson Japan), 15.0 g of Bacto agar (Becton Dickinson Japan), 0.5 g of glucose (Wako Pure Chemical Industries), 200.0 ml of horse serum, and 900.0 ml of distilled water. The salivary gland of the rat was removed and homogenized with 500 μ l of sterilized PBS, and aliquots of 50 μ l were

inoculated onto 488NA. Tracheal and oral swabs were also inoculated onto 488NA. The inoculated media were incubated at 37°C in an atmosphere of 5% CO₂ in air for 72 hr.

To examine the phenotypic traits of the isolate, Gram staining, and oxidase and catalase assays were performed, and acid production from carbohydrates was tested for arabinose, dextrin, glucose, lactose, mannose, raffinose, sorbitol, sucrose, trehalose, and xylose. The acid production assays were performed according to the methods reported previously [6] with some modifications. Phenol-red Broth (Merck, Darmstadt, Germany) was used in this study instead of Nutrient Broth. The results were determined after incubation for 5 days. The isolate was also tested using the API ZYM chromogenic assay system (bioMérieux Japan, Tokyo, Japan) for examination of enzymatic activities in accordance with the manufacturer's instructions. ATCC 14647, the type strain of *S. moniliformis*, was used as a reference strain for all tests of phenotypic traits.

The 16S rRNA gene of the isolate was amplified by PCR using the previously reported universal primers A and H [3, 4], and directly sequenced using a Taq Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) and automated DNA sequencer (model 310; Applied Biosystems). In addition, the *gyrB* gene of the isolate was amplified by PCR using *S. moniliformis*-specific primers (MZK-F: 5'-AAG ATA GGG TAA TGC TTA CAG AAG GAG-3' and MZK-R: 5'-AAT CTA CCT TGT TTT GCA GAT CCA C-3') and directly sequenced as described above. The primers designed for this study were based on common sequences of *S. moniliformis* (MZK-F: position 453–479 for AB353277, 476–502 for AB353278, MZK-R: 1068–1044 for AB353277, 1091–1066 for AB353278) and confirmed as species-specific by comparison with all data in the GenBank/EMBL/DBJ databases.

No gross lesions were observed in the rat at necropsy. Small (1–2 mm in diameter), clear, smooth, and butyrous colonies were recovered from the ATCC488NA medium inoculated with the salivary gland homogenate. No bacte-

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Table 1. Acid production from carbohydrates and enzymatic activities of isolate

Acid production from carbohydrates	
Arabinose	–
Dextrin	–
Glucose	+
Lactose	–
Mannose	–
Raffinose	–
Sorbitol	–
Sucrose	–
Trehalose	–
Xylose	–
Enzymatic activity	
Alkaline phosphatase	4*
Butyrate esterase	4
Caprylate esterase	4
Myristate lipase	1
Leucine arylamidase	5
Valine arylamidase	0
Cystine arylamidase	0
Trypsin	0
Chymotrypsin	5
Acid phosphatase	3
Naphthol-AS-BI-phosphohydrolase	0
α -galactosidase	0
β -galactosidase	0
β -glucuronidase	1
α -glucosidase	0
β -glucosidase	0
N-acetyl- β -glucosaminidase	0
α -mannosidase	0
α -fucosidase	0

* 0=Negative, 5=strongly positive, 1–4=intermediate reaction; all results were read by comparison with the manufacture's color charts.

rial colonies were detected from the tracheal swab, and a large number of bacterial colonies that were mostly Gram-positive cocci and bacilli were detected from the oral swab.

Gram-staining of these colonies identified Gram-negative, pleomorphic coccobacilli (Fig. 1). The isolate showed negative results on the oxidase and catalase assay, but was positive for acid production from glucose, alkaline phosphatase, butyrate esterase, caprylate esterase, myristate lipase, leucine arylamidase, chymotrypsin, acid phosphatase, and β -glucuronidase activities. The results are shown in Table 1. The results of acid production assays agreed with those of the ATCC 14647 strain with the exception of dextrin, which was positive in ATCC 14647. The results of enzymatic activity assays also agreed with those of strain ATCC 14647. Partial sequencing of the 16S rRNA gene (1,440 bp) and *gyrB* gene (514 bp) of the isolate revealed high degrees of similarity with the 16S rRNA (DQ325537: 100.0%) and *gyrB* genes (AB353278: 99.8%) in the GenBank/EMBL/DDBJ databases. Based on these results, the isolate was identified as *S. moniliformis*.

Although RBF is a rare disease, it has a fatality rate of 7–10% among untreated patients [5], and is therefore regarded as an important zoonosis. Rats, which are carriers, show no

Fig. 1. Gram-stain of isolate. Cultivation on ATCC 488 medium supplemented with nalidixic acid at 37°C, 5 % CO₂ for 72 hr.

symptoms but can effectively transmit the infection by a bite or through infected excreta. The isolation of *S. moniliformis* from patients is the best method of diagnosis of RBF, and this agent is often isolated from the affected part of the skin or body fluids of patients [1, 2, 9]. However, *S. moniliformis* has rarely been isolated from rats that were suspected as the source of infection in cases of RBF, and there have been only a few reports of cases detected using molecular techniques [2, 11]. In the previous reports, *S. moniliformis* was detected from saliva of the rat by PCR [11], but *S. moniliformis* was not detected from the oral cavity because of the overgrowth of Gram-positive bacteria on the media in this study. Molecular detection methods such as PCR may be necessary for more sensitive detection of the agent, and improvement of selective media will be necessary for more specific detection of the agent by culture methods.

Here, we reported the isolation of *S. moniliformis* from a pet rat at necropsy. Although rat-bite fever has rarely been reported in Japan, our results indicated the potential of rat-bite fever arising from pet rats in Japan.

We are grateful to Dr. Koichi Imaoka and Mr. Masanobu Kimura (National Institute of Infectious Diseases) for helpful discussions.

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