NOTE ***Bacteriology***

Brachyspira pilosicoli Isolated from Pigs in Japan

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ABSTRACT. Two of four weak β-hemolytic isolates of intestinal spirochetes isolated from pigs in Japan possessed a unique base alignment of TTTTTT on the 16S ribosomal DNA of *Brachyspira pilosicoli* and were identified as *B. pilosicoli*. The other two isolates were not identified by this technique. The identified isolates were 4.2 to 11 μm in length and 0.2 to 0.3 μm in diameter, 4 periplasmic flagella at each end were observed dominantly. The isolates were hippurate positive but indole negative. This is the first report on th e isolation of *B. pilosicoli* from pigs in Japan.

KEY WORDS: *Brachyspira pilosicoli*, Japan, swine.

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Brachyspira pilosicoli was isolated from pig with diarrhea in 1980 [18] and the diarrhea induced by *B. pilosicoli* was named porcine intestinal spirochetosis, differing from swine dysentery. Pure cultures of *B. pilosicoli* ATCC 51139^T were used to inoculate laboratory pigs. Diarrhea, containing clear mucus and in one case, blood occurred in four of the eight animals inoculated [18]. In 1986, Adachi and Minato [1] reported on the experimental infection of young chicks with weak beta-hemolytic intestinal spirochetes (*B. pilosicoli* ATCC 51139^T) differing from *Brachyspira hyodysenteriae*. The colonization of the spirochetes was observed in ceca of all infected one-day-old chicks [1]. The former names, *Anguillina coli* [11] and *Serpulina pilosicoli* [20] were changed to *B. pilosicoli* [13]. Weak β-hemolytic *B. pilosicoli* causes porcine intestinal spirochetosis, whereas other intestinal spirochetes, *B. intermedia*, *B. murdochii* and *B. innocens* are non-pathogenic [9, 16, 20].

Although porcine intestinal spirochetosis has been recorded in all major pig producing countries in the world and the diarrhea is common [3, 5, 7, 8, 12, 17, 19], there is no report on isolation of *B. pilosicoli* from pigs in Japan. In this paper, weak β-hemolytic isolates from pigs were investigated biochemically and genetically by partial sequencing by polymerase chain reaction (PCR) assay.

Four isolates, NK1f, NK2f, TK39 and TK40 isolated from pigs with diarrhea in 1979 and stored at –80°C were grown on trypticase soy agar (TSA, BBL, U.S.A.) containing 5% sheep blood under anaerobic conditions with a GasPak™ System (BBL, U.S.A.). DNA was extracted from the spirochete cells by treatment with InstaGene Matrix (BIO-RAD, U.S.A.) as described previously [13]. The 497 bp of the 16S rDNA were amplified by PCR with the primers M3F (5'-TGTAAAACGACGC CAGTG GCGATCTG TCTTA AGCA-3') and R500 (5'-AAATC-CGAGCA ACGT TTG-3'). Two μl of the spirochetes DNA as the template, 20 pM of each primer, 10 mM dNTP, 5 μl of 10x Ex Taq buffer (Mg^{2+} free) (TaKaRa, Japan), 0.075 mM magnesium chloride (TaKaRa, Japan), 0.5 U of Ex Taq polymerase (TaKaRa, Japan) contained in a total volume of 50 μl of reaction mixture. The amplification of DNA by PCR was carried out as described previously [13]. A thermocycler (ABI, U.S.A.) was used as follows: 94°C for 3 min, 30 cycles at 94°C for 30 sec, at 52°C for 60 sec and at 72°C for 90 sec. The purified PCR products were sequenced by using a BigDye® Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystem, U.S.A.). The base alignment was determined in both directions, 5' and 3' end with a capillary sequencer (ABI PRISM®, 3100 Genetic Analyzer, Applied Biosystems, Hitachi, Japan). The isolates were observed under dark-field and transmission electron microscopes. Negative staining of spirochete cells was prepared as described previously [13]. Hippurate hydrolysis and indole production tests were performed as described previously [20].

NK1f, NK2f, TK39 and TK40 showed weakly β-hemolysis on blood agars. As shown in Table 1, one base alignment of 5'-G--TTTTTTC-3' on the 16S rDNA *B. pilosicoli* was confirmed in two of the isolates, NK1f and NK2f, but base alignment on the 16S rDNA of TK 39 and TK40 was not

Table 1. Comparison of the base alignment of 16S rDNA among *B. pilosicoli* ATCC 51139^T, *B. hyodysenteriae* ATCC 27164^T and four isolates of intestinal spirochetes from pigs

Strains used	The base alignment from 172–181 corresponding to 16S rDNA of *B. hyodysenteriae* ATCC 27164^T
NK1f	G--TTTTTTC
NK2f	G--TTTTTTC
TK39	GGAGCAATCC
TK40	GGAGCAATCC
B. pilosicoli ATCC 51139^T*	G--TTTTTTC
B. hyodysenteriae ATCC 27164^T*	GGAGCAATCC

* Ochiai *et al.*, 1997 [13].

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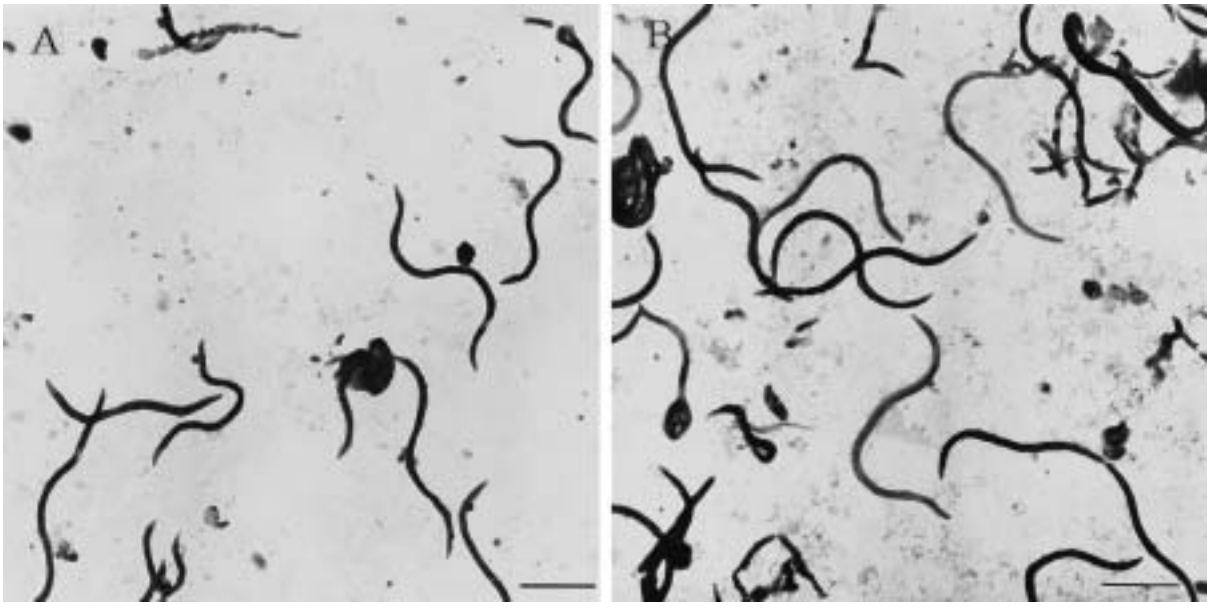

Fig. 1. Japanese isolates of *B. pilosicoli* from pigs, showing cells of a regular coiled type. A, NK1f; B, NK2f. Negatively stained with uranyl acetate. Bars = 1 μm.

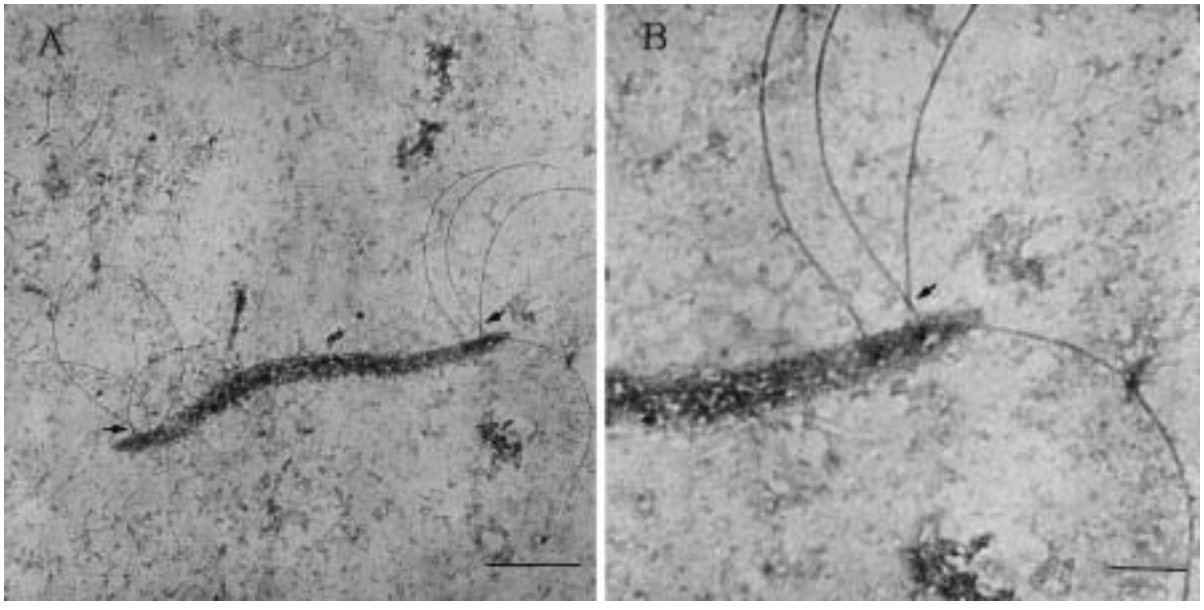

Fig. 2. NK1f. A, cell treated with 1.4 mM SDS, bar = 1 μm; B, magnification of the end of the cell, bar = 250 nm. The arrows show 4 periplasmic flagella at the end of the cell.

confirmed. The observation under the dark-field microscope demonstrated similar morphology among four isolates. Furthermore, the isolates NK1f and NK2f were 4.2 to 11 μm in length and 0.2 to 0.3 μm in diameter (Fig. 1). Four periplasmic flagella at each end of 90% of the cells counted (n=10) in each isolate of *B. pilosicoli* were observed under the electron microscope, while 5 periplasmic flagella at each end of 10% of the cells (n=10) were observed (Fig. 2). Two isolates were hippurate positive but indole negative.

The colonies of *B. pilosicoli* on blood agar were rough. Weak β-hemolysis was observed around the colonies. The intestinal spirochete, *B. pilosicoli* ATCC 51139^T has a specific base alignment of 5'-G--TTTTTTC-3' at positions corresponding to the base positions 172 to 181 of the 16S rDNA sequence of *B. hyodysenteriae*, differing from the other *Brachyspira* species [13]. The base alignment has been used to design it as primer for PCR-based identification of *B. pilosicoli* [2, 10, 14, 15]. Typical *B. pilosicoli* is 4

to 12 μm in length and 0.2 to 0.3 μm in diameter with a pointed end and possesses 8 to 10 periplasmic flagella per cell [20]. Eight periplasmic flagella per cell were dominantly observed in NK1f and NK2f. The isolates hydrolyzed hippurate and did not produce indole. The biological properties are typical characteristics of *B. pilosicoli*, but the sequencing of 16S rDNA indicate that hippurate-positive isolates belonged to the species *B. pilosicoli* [6]. The sequencing of 16S rDNA for analyses of an unique alignment of TTTTTT may be useful for rapid and accurate diagnosis of *B. pilosicoli*. In general, *B. pilosicoli* is pathogenic intestinal spirochetes and has been isolated from dogs, chickens and humans suffering from intestinal spirochetosis [4]. This indicates zoonotic infection, and an epidemiological survey in Japan may be important. This is the first report on the isolation of *B. pilosicoli* from pigs in Japan.

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