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Simultaneous isolation of two species, *Brachyspira pilosicoli* and *Brachyspira aalborgi*, from a patient with ulcerative colitis

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ABSTRACT. We succeeded in the simultaneous isolation of *Brachyspira* (*B.*) *aalborgi* and *B. pilosicoli* from a patient with ulcerative colitis. *B. pilosicoli* grew quickly and formed colonies within 7 days, while the growth of *B. aalborgi* was very slow and took over 21 days. Simultaneous isolation of *B. pilosicoli* and *B. aalborgi* from a common specimen is generally recognized to be difficult, mainly due to differences in their growth requirements and the growth rates. However, we succeeded in isolating both species from a patient with ulcerative colitis and this is first evidence. The present results suggest that ulcerative colitis may be caused by simultaneous infection with *B. pilosicoli* and *B. aalborgi*.

KEY WORDS: 16S rRNA, *B. aalborgi*, *B. pilosicoli*, isolation, ulcerative colitis

Brachyspira (*B.*) *pilosicoli* and *B. aalborgi* are candidate causal agents of large intestinal spirochetosis. These spirochetes are also related to adenocarcinoma, polyps, diarrhea containing blood and ulcerative colitis (UC) [2, 4]. However, the pathogenesis mechanisms of these agents are not yet fully understood. We are interested in the relationship between the pathogenesis of ulcerative colitis and intestinal brachyspiral infection. Recently, the presence of brachyspiras was confirmed by histological investigation of patients with intestinal spirochetosis syndrome i.e. adenocarcinoma, UC, polyps, diarrhea with blood and septicemia. In addition, the presence of two different genes of the brachyspiral species has been reported on a patient with large intestinal spirochetosis [10]. We therefore attempted and succeeded in simultaneous isolation of both species, *B. pilosicoli* and *B. aalborgi,* from a patient.

MATERIALS AND METHODS

Strains used

B. aalborgi NCTC11492 and *B. pilosicoli* ATCC51139 were used in this experiment.

Isolation

The biopsy samples from the colon of a patient with ulcerative colitis were mixed with 500 μl of tripticae soy broth (Difco, Sparks, MD, U.S.A.) into a 1.5 m*l* centrifuge tube. Isolation was carried out according to the procedure reported by Songer *et al*. (1976) [11]. Sheep blood agar containing 400 μg/m*l* spectinomycin was prepared and used for isolating brachyspiras under anaerobic conditions using AnaeroPack (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan). For genetic diagnosis, DNAs were extracted from biopsy samples and/or grown colonies by InstaGene matrix (Bio-Rad, Hercules, CA, U.S.A.) and identification of the isolates was performed by PCR (2720 Thermal Cycler, Applied Biosystems, San Mateo, CA, U.S.A.) with specific primers, i.e. Acoli1/R583 for *B. pilosicoli* and aalF161/R596 for *B. aalborgi* [6].

Sequencing

For sequencing, we used universal primers for 16SrDNA, i.e. F3/500R [8] and performed as previously described using BigDye Terminator v3.1. The base alignment was performed as previously described [7].

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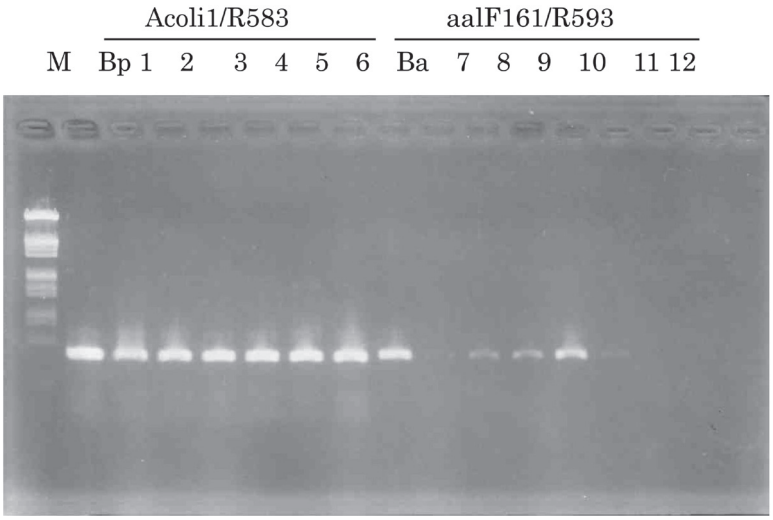

Fig. 1. The specific bands of both species, i.e. *B. pilosicoli* and *B. aalborgi* in colonic biopsy samples from PCR using specific primers, i.e., Acoli1/R583 and aalF161/R596. Biopsy samples are indicated as follows; 1 and 7, cecum; 2 and 8, ascending colon; 3 and 9, transverse colon; 4 and 10, descending colon; 5 and 11, sigmoid colon; 6 and 12, rectum; Bp, *B. pilosicoli* ATCC51139; Ba, *B. aalborgi* NCTC11492; M, Molecular size makers (λDNA/*Eco*RI+*Hind*III). Bp, 1, 2, 3, 4, 5, and 6 were amplified by PCR with Acoli1/R583. Ba, 7, 8, 9, 10, 11 and 12 were amplified by PCR with aalF161/R596.

Table 1. Comparison of biological and genetic characteristics among the isolates

Isolates from	wβ hemolysis	Days spent in colony formation	-TTTTTT- on 16SrDNA	Accession No.
C	+	7	+	LC315389
A	+	7	+	LC315388
T	+	30	−	LC315389
D	+	30	−	LC315390
S	+	7	+	LC315392
R	+	7	+	LC315391
Bp	+	7	+	Reference
Ba	+	30	−	Reference

C, cecum; A, ascending colon; T, transverse colon; D, descending colon; S, sigmoid colon; R, rectum; Bp, *B. pilosicoli* ATCC51139; Ba, *B. aalborgi* NCTC11492; +, presence; −, no presence.

The homology matrix was prepared using a homology % obtained by BLAST techniques using DDBJ.
A phylogenetic tree was constructed by sequencing data and a phylogenetic tree was prepared by the neighbor-joining method [9].

RESULTS

After extraction of DNA from biopsy samples of a female patient (37 years old) with UC, PCR was performed using specific primers and the results are shown in Fig. 1. They demonstrated the presence of both species, i.e., *B. aalborgi* and *B. pilosicoli*. Thereafter, the samples were anaerobically cultured on sheep blood agar containing spectinomycin. Seven days later, the blood agar plates were observed and the growth of *B. pilosicoli* was confirmed by the PCR using specific primers (Acoli1/R583). Thirty days later, many colonies including *B. pilosicoli and B. aalborgi* on the blood agar plates were confirmed (Table 1), selected and subcultured. Then, the anaerobic culture was continued for at least 3 months at 37°C and the colonies were again selected. All colonies were covered with *B. pilosicoli*, but colonies grown independently on the plastic side were found and we selected those colonies. In general, colonies with *B. aalborgi* were covered with *B. pilosicoli* because the growth speed and swarming ability of *B. pilosicoli* was very high. Thereafter, PCR with universal primers for brachyspiras, i.e. F3/500R, was performed and as shown in Table 2, two types of base alignment were confirmed. One was for *B. pilosicoli* with typical base alignment of -TTTTTT- and the other was for *B. aalborgi* without -TTTTTT-. In addition, a phylogenetic tree, which was constructed by base sequence data, indicated the presence of two different lineages. One lineage was *B. pilosicoli*, the other was *B. aalboirgi* (Fig. 2). These results demonstrated that we succeeded in the isolation of both species from one patient in this experiment.

Table 2. Homology matrix of *Brachyspra* isolates from a patient with UC syndrome

Isolates from	Homology % among the isolates used								Typical base alignment (-TTTTTT-)
	C	A	T	D	S	R	Bp	Ba	
C	100.00	100.00	93.63	93.23	99.78	99.56	99.34	93.65	+
A	100.00	100.00	93.63	93.23	99.78	99.56	99.34	93.65	+
T	93.63	93.63	100.00	99.12	93.83	93.65	94.07	99.34	–
D	93.23	93.23	99.12	100.00	93.39	93.17	94.04	99.34	–
S	99.78	99.78	93.83	93.39	100.00	99.79	98.90	93.22	+
R	99.56	99.56	93.65	93.17	99.78	100.00	98.69	93.84	+
Bp	99.34	99.34	94.07	94.04	98.90	98.69	100.00	95.38	+
Ba	93.65	93.65	99.34	99.34	93.22	93.84	95.38	100.00	-

Isolation names are indicated in this Table and C, A, T, D, S and R refer to the cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum, respectively. Bp, *B. pilosicoli* ATCC51139; Ba, *B. aalborgi* NCTC11492; +, presence; –, no presence.

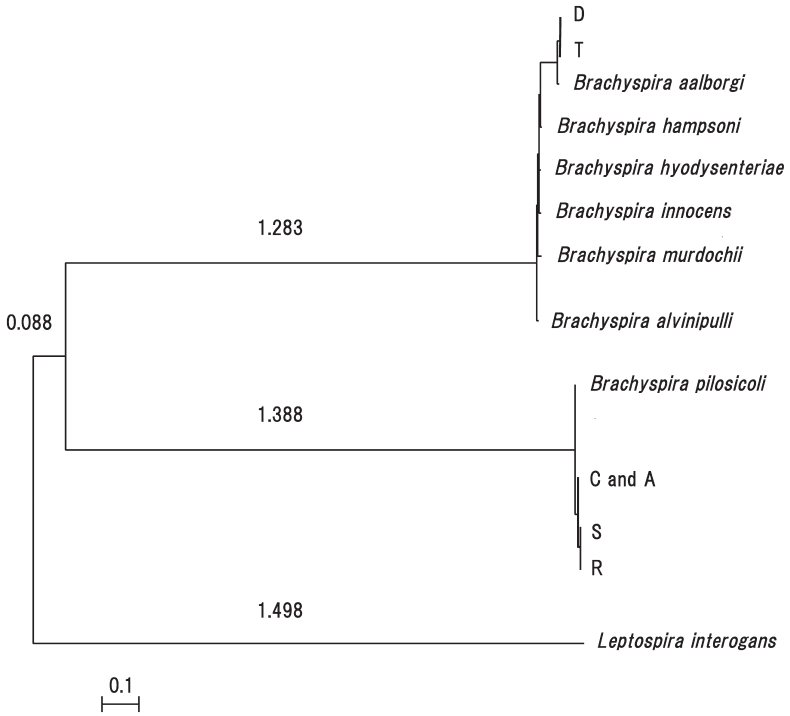

Fig. 2. Phylogenic tree constructed on the basis of the base alignment of the isolates from a patient with UC. The tree was prepared by the neighbor-joining method (Saitou and Nei, 1987). C, A, T, D, S, R and C1 in this tree refer to the isolate names for the cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum, respectively.

DISCUSSION

Since 1982 [3], there has been no report of simultaneously isolating both species, i.e. *B. aalborgi* and *B. pilosicoli*, from one patient with intestinal spirochetosis. We attempted to isolate the two species from one patient and succeeded first.

In general, the number of intestinal spirochete cells was influenced by the antibody to the spirochetes. When the titer was high, the intestinal spirochete cells were decreased in number and vis versa in case of pigs [1, 5]. In the case of a patient with UC, the growth of *B.pilosicoli* could be inhibited by the antibody with high titer to *B.pilosicoli* and the number of cells in the large intestine could be decreased. Meanwhile the growth of *B.aalborgi* having been inhibited, can be started under the low and no antibody titer to *B.aalborg*i. This may be exchange between *B.pilosicoli* and *B.aalborgi*. We could confirmed the presence of two different genes of those species by PCR. Therefore, under those conditions, we may have succeeded in isolating both species from one patient with ulcerative colitis.

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