Isolation of a Human Intestinal Anaerobe, *Bifidobacterium* sp. Strain SEN, Capable of Hydrolyzing Sennosides to Sennidins

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A strictly anaerobic bacterium capable of metabolizing sennosides was isolated from human feces and identified as *Bifidobacterium* sp., named strain SEN. The bacterium hydrolyzed sennosides A and B to sennidins A and B via sennidin A and B 8-monoglucosides, respectively. Among nine species of *Bifidobacterium* having β-glucosidase activity, only *Bifidobacterium dentium* and *B. adolescentis* metabolized sennoside B to sennidin B, suggesting that the sennoside-metabolizing bacteria produce a novel type of β-glucosidase capable of hydrolyzing sennosides to sennidins.

Sennosides A and B, main constituents of senna and rhubarb, are considered to be inactive as laxatives themselves but to be transformed to an active component by intestinal bacteria in the gut (3–5, 7, 11, 14). We have proposed two different pathways for sennoside metabolism by intestinal bacterial flora (1, 8–10), as shown in Fig. 1. One is a pathway by which sennosides A and B are first hydrolyzed to sennidins A and B via sennidin A and B monoglucosides, respectively, by β-D-glucosidase(s); this is followed by reduction to give rhein anthrone, a genuine purgative component. The other pathway is that by which sennosides are first reduced to 8-glucosyl-rhein anthrone and then hydrolyzed by β-D-glucosidase(s) to give rhein anthrone. As human and rat intestinal flora metabolize sennosides A and B to sennidins A and B via the respective monoglucoside intermediate (8, 9), the former pathway is considered to be the main route of metabolism in the intestines. Many strains of intestinal bacteria are able to reduce sennidins to rhein anthrone (8). However, intestinal bacterial strains capable of hydrolyzing sennosides to sennidins have not been isolated hitherto.

In the present paper, we report the isolation from human feces and characterization of a bacterium capable of hydrolyzing sennosides to sennidins.

Media. General anaerobic medium (Nissui Co., Tokyo, Japan) and EG agar (Eiken Chemical Co., Ltd., Tokyo, Japan) were used. Peptone-yeast extract-Fildes solution (PYF), consisting of 10 g of Trypticase, 5 g of yeast extract, 40 ml of Fildes solution (peptic digest of human blood), 40 ml of salts solution (0.2 g of $CaCl_2$, 0.2 g of $MgSO_4$, 1 g of KH_2PO_4, 1 g of K_2HPO_4, 10 g of $NaHCO_3$, and 2 g of NaCl in 1 liter), and 0.5 g of L-cysteine-HCl in 1 liter (12), was used as a basal medium.

Bacterial strains and culture conditions. *Bifidobacterium adolescentis* ATCC 15703, *B. animalis* ATCC 25527, *B. bifidum* DSM 20082, *B. breve* ATCC 15700, *B. dentium* DSM 20221, *B. infantis* JCM 7007, *B. infantis* subsp. *lactentis* JCM 7085, *B. infantis* subsp. *liberorum* JCM 2838, and *B. longum* ATCC 15707 were purchased from the Japan Collection of Microorganisms (The Institute of Physical and Chemical Research, Saitama, Japan). *Peptostreptococcus intermedius* EBF 77/25 was

donated by T. Mitsuoka (The Institute of Physical and Chemical Research).

Each bacterial strain (0.2 ml) precultured overnight in general anaerobic medium broth was inoculated into 10 ml of PYF broth containing 1.2 mM sennoside A or B (purified from crude sennoside powder [Nippon Funmatsu Yakuhin Co. Ltd., Tokyo, Japan]). Cultivation was performed at 37°C in an anaerobic chamber (Forma Scientific). A portion (1 ml) of the culture was taken out at the indicated intervals; one-half was used for quantitative analysis of sennidins, and the half was used for quantitative analysis of the azometin derivative of rhein anthrone as described below.

Analysis and assay. Sennosides and their metabolites were analyzed by thin-layer chromatography as described in a previous paper (10). Rhein anthrone was detected as an azometin derivative (adduct with *N*,*N*-dimethyl-*p*-nitrosoaniline) (8). The fructose-6-phosphate phosphoketolase activity of *Bifidobacterium* sp. strain SEN, a strain capable of hydrolyzing sennosides to sennidins, was measured by using crude extract of cells cultured in general anaerobic medium broth, essentially as described by Racker (13). After cultivation of the isolated strain for 1 week in PYF broth containing 0.5% glucose, volatile fatty acids of the culture medium were analyzed by gas chromatography, as described in a previous report (2). For determination of L-lactic acid content, an aliquot (50 μl) of the culture medium was mixed with NAD^+ (10 μmol) in 3 ml of glycine-hydrazine buffer (pH 9.5). The difference in the A_{340} of the mixture with and without L-lactate dehydrogenase (1 U) was measured (6).

β-D-Glucosidase activity was measured as follows. The assay mixture contained 25 μmol of *p*-nitrophenyl-β-D-glucopyranoside (Nacalai Tesque, Kyoto, Japan), a bacterial suspension, and 100 mM potassium phosphate buffer (pH 6.5) in a final volume of 1.0 ml. The mixture was incubated for 10 to 30 min at 37°C, and then the reaction was stopped by adding 0.25 ml of 1 M NaOH. After centrifugation, the supernatant solution was measured at 405 nm. The reaction rate was calculated by using the calibration line for *p*-nitrophenol.

Isolation of a bacterium capable of hydrolyzing sennosides. A bacterial suspension from fresh feces of a healthy human (33 years old) was repeatedly cultured in PYF broth containing 1.2 mM sennosides (a mixture of A and B) under anaerobic conditions at 37°C. The metabolism of sennosides to rhein anthrone was monitored visually by green coloration resulting

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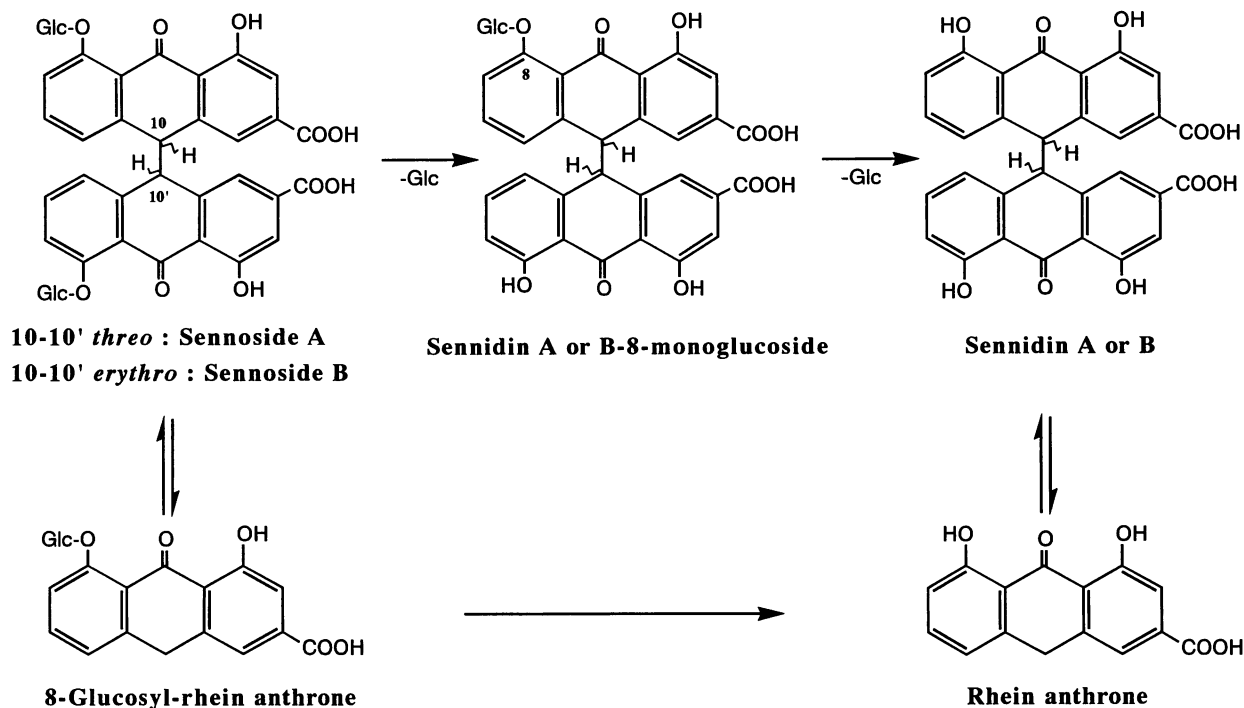
FIG. 1. Pathways of sennoside metabolism by human intestinal bacteria.

from the addition of N,N-dimethyl-p-nitrosoaniline reagent. From culture medium having potent metabolizing activity, a bacterium transforming sennosides to sennidins was isolated as a colony on an EG agar plate. The bacterium was a strictly anaerobic, gram-positive, bud-forming rod and produced acetic acid and L-lactic acid from D-glucose. Because the strain produced fructose-6-phosphate phosphoketolase, it was found to belong to the genus *Bifidobacterium*, named strain SEN, and showed fermentation characteristics similar to those of *B. dentium* (15), in which the only difference between strain SEN and *B. dentium* was in arabinose fermentation.

The isolated strain transformed sennosides A and B to sennidins A and B, respectively. Figure 2 shows the metabolism of sennoside B by the strain in PYF broth. After 10 h of culture, more than 80% of sennoside B was metabolized, and a large amount of sennidin B and a small amount of sennidin A in addition to an appreciable amount of sennidin B 8-monoglucoside were found in spite of a low bacterial growth. During further cultivation, sennidin B 8-monoglucoside disappeared, sennidin B decreased, and sennidin A increased. Throughout 30 h of cultivation, a small amount of rhein anthrone was detected. These results indicate that the strain first hydrolyzes sennoside B to sennidin B via sennidin B 8-monoglucoside and then sennidin B is gradually isomerized to sennidin A via rhein anthrone, similar to the metabolism of sennosides by human intestinal flora (9).

P. intermedius, a human intestinal anaerobe, reduces sennidins to rhein anthrone (8) but does not hydrolyze sennoside B (Table 1). With a mixed culture of strain SEN and *P. intermedius* in PYF broth, all sennoside B was rapidly transformed to rhein anthrone without detection of sennidins, as shown in Fig. 3. Accordingly, in human intestine, sennosides seem to be metabolized stepwise to rhein anthrone by sennosides-hydrolyzing bacteria such as strain SEN and a number of other species of sennidin-reducing bacteria.

As strain SEN was found to belong to the genus *Bifidobacterium*, the sennoside-metabolizing activities of nine species of *Bifidobacterium*, all of which had potent β-D-glucosidase activity for p-nitrophenyl-β-D-glucopyranoside, were examined in PYF broth. Although seven of these species did not metabolize sennoside B even after cultivation for 3 days, *B. dentium* and *B. adolescentis* transformed sennoside B to sennidins A and B. Table 1 shows their metabolic activity
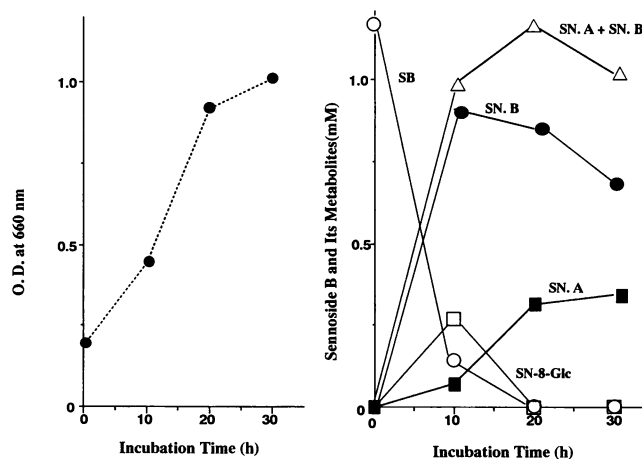

FIG. 2. Time course of sennoside B metabolism in PYF broth by *Bifidobacterium* sp. strain SEN. The presence of sennoside B (SB; ○), sennidin B (SN. B; ●), sennidin B 8-monoglucoside (SN-8-Glc; □), sennidin A (SN. A; ■), and sennidins A and B (SN. A+SN. B; △) was determined as described in Materials and Methods. Bacterial growth (– – –) was monitored by measuring the optical density (O.D.) at 660 nm.

TABLE 1. Sennoside B-metabolizing activities of 10 species of *Bifidobacterium* and of *P. intermedius*[a]

Bacterial species	Recovery of metabolites (%)			
	Sennoside B	Sennidin B monoglu-coside	Sennidin B	Sennidin A
B. bifidum	71.7	ND[b]	ND	ND
B. longum	85.0	ND	ND	ND
B. infantis	71.7	ND	ND	ND
B. infantis ss. liberorum	74.2	ND	ND	ND
B. linfantis ss. lactentis	77.5	ND	ND	ND
B. breve	78.3	ND	ND	ND
B. adolescentis	32.5	24.2	32.5	ND
B. dentium	16.7	45.8	32.5	ND
B. animalis	72.5	ND	ND	ND
Bifidobacterium sp. strain SEN	1.7	4.2	57.5	21.7
P. intermedius	75.8	ND	ND	ND

[a] Each bacterium was cultured for 10 h in PYF medium containing 1.2 mM sennoside B, and then an aliquot of the medium was analyzed for sennoside B and its metabolites.

[b] ND, not detected.

after 10 h of culture, indicating that the activities of *B. dentium* and *B. adolescentis* were lower than that of *Bifidobacterium* sp. strain SEN. β-D-Glucosidase from almonds does not hydrolyze sennosides (16). Moreover, 18 species of human intestinal
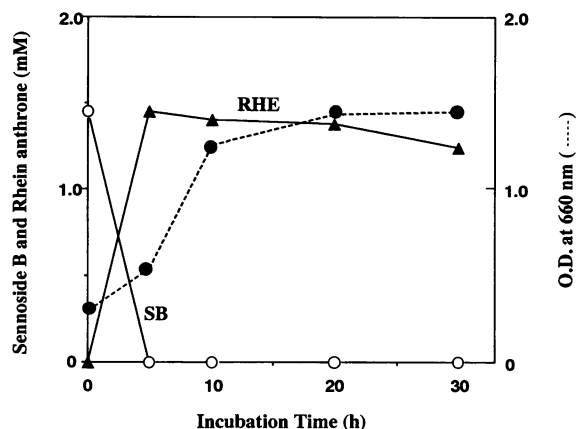

FIG. 3. Metabolism of sennoside B in PYF broth by a mixed culture of *Bifidobacterium* sp. strain SEN and *P. intermedius*. Rhein anthrone (RHE; ▲) was detected as an azometin derivative by adding *N,N*-dimethyl-*p*-nitrosoaniline. O.D., optical density.

bacteria (10) and 7 species of *Bifidobacterium* had β-D-glucosidase activity in spite of their inability to hydrolyze sennosides. Thus, some species of *Bifidobacterium* capable of hydrolyzing sennosides to sennidins via their monoglucosides seem to produce a novel type of β-D-glucosidase hydrolyzing sennosides in addition to a common type of β-D-glucosidase (EC 3.2.1.21).

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