Human Intestinal Bacteria Capable of Transforming Secoisolariciresinol Diglucoside to Mammalian Lignans, Enterodiol and Enterolactone

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Received March 1, 2000; accepted July 18, 2000

Seven metabolites were isolated after anaerobic incubation of secoisolariciresinol diglucoside (1) with a human fecal suspension. They were identified as (−)-secoisolariciresinol (2), 3-demethyl-(−)-secoisolariciresinol (3), 2-(3-hydroxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)butane-1,4-diol (4), didemethylsecoisolariciresinol (5), 2-(3-hydroxybenzyl)-3-(3,4-dihydroxybenzyl)butane-1,4-diol (6), enterodiol (7) and enterolactone (8).

Furthermore, two bacterial strains, *Peptostreptococcus* sp. SDG-1 and *Eubacterium* sp. SDG-2, responsible for the transformation of 1 to a mammalian lignan 7, were isolated from a human fecal suspension. The former transformed 2 to 3 and 5, as well as 4 to 6, and the latter transformed 5 to 6 and 7.

Key words biotransformation; enterodiol; enterolactone; *Eubacterium* sp.; *Peptostreptococcus* sp.; secoisolariciresinol diglucoside

Fiber-rich foods, such as flaxseed, are consumed in varying quantities in many parts of the world. Such widely used materials are of great concern with regard to their potential biological benefits. Flaxseed is the most significant dietary source of the plant lignan secoisolariciresinol diglucoside (SDG, **1**).[1)] Compound **1** was found to be a precursor of phytoestrogenic lignans, enterodiol (**7**) and enterolactone (**8**), which have been detected in the serum, urine and bile of humans and several animals ingesting flaxseed.[2)] Adlercreutz *et al*. reported that vegetarians excreted large amounts of **7** and **8**, and noted a significant correlation between the excretion of these compounds and the consumption of fiber-rich foods such as flaxseed, grains and legumes.[3,4)] Compounds **7** and **8** were found to have estrogenic activity and to play an important role in the prevention of breast cancer incidence in vegetarians.[5—8)] Jacobs *et al*. reported that hepatic microsomes from rats and humans were able to oxidize **7** and **8**,[9)] and that similar derivatives were excreted in the urine of humans ingesting flaxseed.[10)] However, these derivatives accounted for less than 5% of the total urinary metabolites. Generally, before being absorbed to the body fluids, orally ingested dietary lignans are expected to undergo metabolism by intestinal bacteria. Borriello *et al.*[11)] reported that human intestinal bacteria were able to transform **1** to **7** and **8** *in vitro*. However, it should be pointed out that the pathway to the formation of these metabolites was not clearly explained, and the intestinal bacteria capable of transforming **1** to **7** and **8** have not been isolated from human feces so far. In order to clarify the transformation of **1** by intestinal bacteria, the present study was designed to a) isolate intestinal bacterial metabolites of **1** after anaerobic incubation with a human fecal suspension, and b) to isolate bacterial strains capable of transforming **1** to **7** and **8** from human feces.

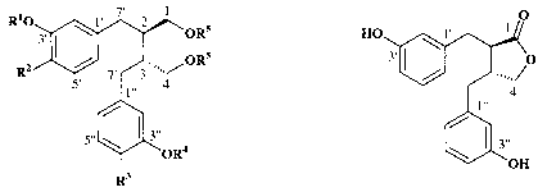
Results

Transformation of Secoisolariciresinol Diglucoside (SDG, 1) by a Human Fecal Suspension After anaerobic incubation of SDG (**1**) with a human fecal suspension, the culture was extracted with BuOH and then purified by silica gel, Sephadex LH-20 and RP-18 column chromatography to afford seven metabolites (**2**—**8**) (Chart 1). The structures of these metabolites were determined by NMR and MS spectroscopy.

Compound **2** (EI-MS *m/z* 362 $[M]^+$) was obtained as one of the major metabolites after incubation for 12 h. The ^{1}H- (see Experimental) and ^{13}C-NMR (Table 1) spectral data of **2** were in good agreement with those reported for secoisolariciresinol, an aglycone of **1**.[12)]

Compound **3** showed a molecular ion peak at *m/z* 348 $[M]^+$ in its EI-MS spectrum, 14 mass units less than that of **2**, suggesting that **3** was a demethylation product of **2**. This was confirmed by the presence of a singlet signal for *O*-methyl protons at δ 3.76 and signals for 6 non-equivalent aromatic protons at δ 6.43, 6.56, 6.57, 6.63, 6.63 and 6.66 in the ^{1}H-NMR spectrum. Therefore, the structure of **3** was identified as 3-demethyl-(−)-secoisolariciresinol previously isolated from the needles of the Himalayan yew, *Taxus baccata*.[13)] Compound **3** has been previously reported as a possible precursor of **7**.[14)]

Most of the signals in the ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of **3** (see Experimental and Table 1) except for additional signals for an aromatic methine at δ 6.60 (δ_C 113.7). The spin-coupling patterns in the aromatic region of the ^{1}H-NMR spectrum showed the presence of 1,3,4-trisubstituted and 1,3-disubstituted phenyl groups. Furthermore, the EI-MS spectrum of **4** showed a molecular ion peak at *m/z* 332 $[M]^+$, 16 mass units less than that of **3**, suggesting



1 R^1=R^4=Me, R^2=R^3=OH, R^5=Glc
2 R^1=R^4=Me, R^2=R^3=OH, R^5=H
3 R^1=R^5=H, R^2=R^3=OH, R^4=Me
4 R^1=R^3=R^5=H, R^2=OH, R^4=Me
5 R^1=R^4=R^5=H, R^2=R^3=OH
6 R^1=R^2=R^4=R^5=H, R^3=OH
7 R^1=R^2=R^3=R^4=R^5=H

8

Chart 1. Structures of **1** and Its Metabolites (**2**—**8**)

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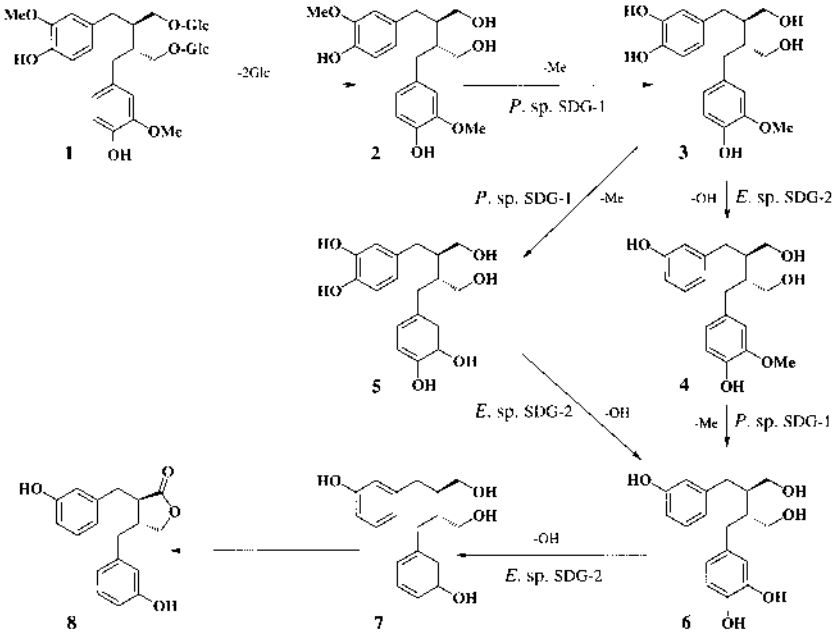

Chart 2. Possible Pathway for the Transformation of **1** by *P.* sp. Strain SDG-1 and *E.* sp. Strain SDG-2, Human Intestinal Bacteria

that **4** was produced by *p*-dehydroxylation of **3**. The structure of **4** was determined to be 2-(3-hydroxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)butane-1,4-diol.

The molecular ion peak of **5** (*m/z* 334 $[M]^+$) in its EI-MS spectrum showed 28 mass units less than that of **2**. The ^{1}H-NMR spectrum of **5** showed close resemblance to that of **2**, but signals for two methoxy groups in **2** were not seen in the spectrum of **5**. These findings indicated that **5** was formed by complete demethylation of two methoxy groups in **2**. The structure of **5** was consequently identified as didemethylsecoisolariciresinol, previously reported as a precursor of **8**.[14]

Compound **6** was a metabolite considered to be formed by *p*-dehydroxylation of **5**, as confirmed by EI-MS (*m/z* 318 $[M]^+$, 16 mass unit less than that of **5**) and NMR, and its structure was established as 2-(3-hydroxybenzyl)-3-(3,4-dihydroxybenzyl)butane-1,4-diol, previously identified by GC/MS as a metabolite of **7** in the urine of humans ingesting flaxseed.[10] Furthermore, this compound was claimed as a metabolic intermediate in the bacterial transformation of **1** to **7**.[10]

Compounds **7** and **8** were identified as enterodiol and enterolactone, respectively, by direct comparison of their spectral data with those of authentic samples.[15,16] After prolonged incubation of **1** for 3 d with a human fecal suspension, **8** was obtained in a yield of 90%, as determined by HPLC.

Isolation of Bacterial Strains Capable of Transforming 2 and 5 from a Human Fecal Suspension In order to clarify the transformation of **1** by human intestinal bacteria, trials were made to isolate bacterial strains capable of transforming **1** from a human fecal suspension. After repeated culture of the human fecal suspension in GAM broth containing **2** or **5** (1 mM) under anaerobic conditions, two colonies (strains SDG-1 and SDG-2) responsible for demethylation and dehydroxylation, respectively, were isolated. The biochemical characteristics of strains SDG-1 and SDG-2 are shown in Table 2. Strain SDG-1 was strictly anaerobic, Gram-positive, and elliptical cocci. Strain SDG-1 hydrolyzed esculin and starch, but was not able to produce indole, H_2S or nitrite. The strain produced acetic acid and lactic acid from D-glucose. Strain SDG-1 grew well in GAM broth (A_{max} value of 0.43 at 540 nm). The cells cultured on an EG agar plate were mainly in chains, while those on a BL agar plate were singly or in pairs. Accordingly, strain SDG-1 was considered to belong to the genus *Peptostreptococcus*. Strain SDG-1 fermented amygdalin, salicin, esculin, and most carbohydrates tested, but did not ferment inulin or inositol. Therefore, strain SDG-1 was concluded to be a *Peptostreptococcus* sp. with characteristics similar to those reported for *P. productus* isolated from human fecal samples (Table 3),[17] and was tentatively named *P.* sp. strain SDG-1. However, *P.* sp. strain SDG-1 did

Table 1. ^{13}C-NMR (100 MHz) Spectral Data of Compounds **2**—**6**

C	**2**[a)]	**3**[b)]	**4**[b)]	**5**[b)]	**6**[b)]
1	60.3	61.9[c)]	61.7[c)]	61.8	61.8[c)]
2	43.7	44.2[d)]	44.2	44.6	44.4[d)]
3	43.7	44.5[d)]	44.2	44.6	44.7[d)]
4	60.3	62.0[c)]	61.8[c)]	61.8	61.9[c)]
1′	132.4	133.9[e)]	143.8	133.9	144.0
2′	111.8	117.2	116.9	117.2	117.0
3′	146.7	146.0	158.1	146.0	158.2
4′	143.7	144.2	113.7[d)]	144.2	113.8
5′	114.3	116.1	130.1	116.1	130.2
6′	121.5	121.4	121.4	121.3	121.4
7′	35.8	35.8[f)]	36.3	35.7	36.3
1″	132.4	133.8[e)]	133.7	133.9	133.9
2″	111.8	113.4	113.4[d)]	117.2	117.2
3″	146.7	148.8	148.7	146.0	146.1
4″	143.7	145.4	145.4	144.2	144.3
5″	114.3	115.8	115.8	116.1	116.1
6″	121.5	122.6	122.5	121.3	121.4
7″	35.8	36.0[f)]	35.9	35.7	35.7
–OMe	55.8	56.3	56.3		

a, *b*) Chemical shifts in δ ppm, measured in: *a*) $CDCl_3$, *b*) MeOH-d_4. *c*—*f*) Assignments with the same superscript may be interchangeable within the same column.

Table 2. Biochemical Characteristics of Strains SDG-1 and SDG-2

Characteristic	Strain SDG-1	Strain SDG-2
Aerobic growth	−	−
Gram stain	+	+
Major product from PYFG	Acetic acid, lactic acid	ND
Growth in 20% bile	−	−
Esculin hydrolysis	+	−
Starch hydrolysis	+	+
Indole production	−	−
H_2S production	−	−
Nitrate reduction	−	+
Genus	*Peptostreptococcus*	*Eubacterium*

Symbols: +: positive; −: negative; ND, not detected.

Table 3. Comparative Fermentation Reactions of Strains SDG-1 and SDG-2

Compound	Strain SDG-1	*Peptostreptococcus productus*[17]	Strain SDG-2	*Eubacterium lentum*[18]
Amygdalin	+	+	−	−
Arabinose	+	+	−	−
Cellobiose	+	+	−	−
Dextrin	−	+	−	−
Esculin	+	±	−	−
Fructose	+	+	−	−
Galactose	+	+	−	−
Glucose	+	+	−	−
Glycerol	−	−	−	−
Glycogen	−	−	−	−
Inositol	−	−	−	−
Inulin	−	−	−	−
Lactose	+	+	−	−
Maltose	+	+	−	−
Mannitol	−	+	−	−
Mannose	−	+	−	−
Melibiose	+	+	−	−
Melezitose	+	+	−	−
Rhamnose	−	−	−	−
Ribose	−	v	−	−
Raffinose	+	+	−	−
Starch	−	v	−	−
Salicin	+	+	−	−
Sorbitol	−	+	−	−
Sucrose	+	+	−	−
Trehalose	+	+	−	−
Xylose	+	+	−	−

Symbols: +, positive reaction (below pH 5.5); −, negative reaction (pH 6.0 or above); v, sometimes positive, sometimes negative; ±, almost positive (negative) but may exhibit a weak reaction.

not utilize dextrin, mannitol, mannose or sorbitol, in contrast with *P. productus*.

In a similar procedure, strain SDG-2 capable of transforming **5** to **6** and **7** was also isolated from the human fecal suspension. This strain was strictly anaerobic, Gram-positive, and non-sporeforming rods (Table 2). The strain reduced nitrate and hydrolyzed starch, but did not ferment carbohydrates, nor produce acids from media containing D-glucose. Indole and H_2S were not produced by this strain. Similar characteristics were reported for *Eubacterium lentum*.[18] The growth of strain SDG-2 in PYFG and GAM broth was very poor (A_{max}: 0.04 at 540 nm), but the growth was significantly enhanced by the addition of arginine to GAM broth (A_{max}: 0.11 at 540 nm in the presence of 1% arginine), similar to the

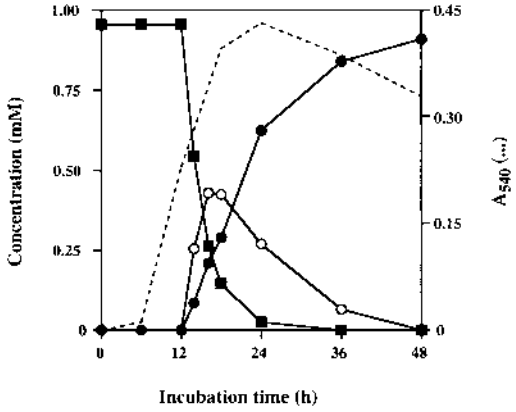

Fig. 1. Time Course of Transformation of **2** (■) to **3** (○) and **5** (●) by *P.* sp. Strain SDG-1

Bacterial growth (···) was monitored by measuring turbidity at 540 nm.

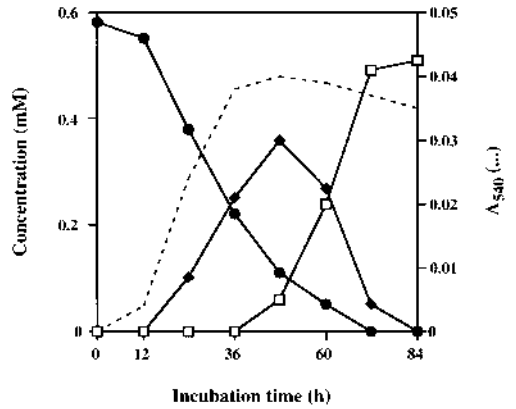

Fig. 2. Time Course of Transformation of **5** (●) to **6** (◆) and **7** (□) by *E.* sp. Strain SDG-2

Bacterial growth (···) was monitored by measuring turbidity at 540 nm.

case of *E. lentum*.[19] Consequently, the isolated strain SDG-2 was concluded to be a *Eubacterium*. sp. and was named *E.* sp. strain SDG-2.

Transformation of (−)-Secoisolariciresinol (2) by *P.* sp. Strain SDG-1 This strain showed high ability to transform **2** to **3** and **5** after incubation for 16 h (Fig. 1). The maximal production of **3** was observed 16 h after the start of incubation, then the amount was gradually decreased. This was accompanied by a gradual increase in the amount of **5** after 12 h. Further transformation of **5** to another metabolite was not demonstrated by this strain. This observation suggested that **5** was produced in a manner of sequential demethylation *via* **3**.

Similarly, *P.* sp. strain SDG-1 was capable of transforming **4** to **6** (data not shown). However, tetra-*O*-methylsecoisolariciresinol was not converted, even after a long period of incubation (data not shown), suggesting that the presence of a free hydroxy group vicinal to the methoxy group (as in **2**—**4**) is necessary for demethylation of these compounds.

Transformation of Didemethylsecoisolariciresinol (5) by *E.* sp. Strain SDG-2 Compound **5** was slowly transformed to **6** and **7** by anaerobic incubation with *E.* sp. strain SDG-2 in GAM broth (Fig. 2). Transformation of **5** to **6** was started after 12 h of incubation, and the amount was slowly increased to reach a maximum after 48 h, then gradually de-

creased. A further metabolite (**7**) was detected more than 60 h after incubation.

In addition, the transformation of **3** to **4** was also demonstrated by this strain (data not shown).

Discussion

The present study provided evidence that human intestinal bacteria is capable of producing phytoestrogenic lignans, **7** and **8**, and other metabolic intermediates from one of the major dietary lignans, **1** after anaerobic incubation *in vitro*. The diphenolic structures of **7** and **8** share similarities with several estrogen-like substances, such as dienoestrol, diethylstilboestrol and its dihydroderivative hexoestrol, which accounts for the hormone-like effects of these compounds.[20] Epidemiological studies showed the low urinary excretion of **7** and **8** in patients with breast cancer, suggesting that the consumption of some lignans may be related with the incidence of such cancer.[3,4] Furthermore, the United States National Cancer Institute considers these compounds to be potential anti-cancer agents.[3]

Compounds **7** and **8** were excreted in the urine of normal rats, but not in the urine of germ-free rats or humans who were administered antibiotics.[2] However, in our *in vitro* experiments, the transformation of **7** to **8** was not observed by incubation with a rat fecal suspension, even after prolonged incubation for one week. In contrast, **8** was the end product of **1** and **7** by incubation with a human fecal suspension. This may be due to the difference in bacterial flora between both fecal suspensions used.

Transformation of **1**, **2** or **7** was effectively achieved by the human fecal suspension under anaerobic conditions, while similar results were not obtained under aerobic conditions (compound **2** was a sole metabolic product from **1**) or when a sterilized fecal suspension was used. These findings suggest that the conversion of **1** to **7** and **8** is not the result of spontaneous chemical reactions, but due to the metabolic reaction of viable intestinal bacteria under anaerobic conditions.

Compound **8** was also reported to be detected after incubation of a plant lignan, matairesinol, with a human fecal suspension.

To date, the metabolic pathway from **1** to **7** and **8** by intestinal bacteria had not been determined, and intestinal bacteria responsible for this transformation had not yet been isolated from human feces. However, Setchell and co-workers proposed a possible metabolic pathway for the formation of **7** and **8** from **1** by incubation with a human faecal flora, and claimed that dehydroxylation was the first step, followed by demethylation.[11] From the present experiment, it seems reasonable that demethylation occurs prior to dehydroxylation since demethylated metabolites **2**, **3** and **5** were formed from **1** after incubation for a short period of time (20—30 h), whereas dehydroxylated ones **4**, **6**—**8** were obtained later than 48 h. In addition, no transformation of **2** or **4** was observed by a newly isolated strain of *E.* sp. SDG-2 having dehydroxylation ability.

Several of these metabolites, such as **6**—**8**, may find their way to the general circulation of humans who ingest flaxseed or dietary lignans, as recently reported by Jacobs *et al.*[10] All the possible precursors in the transformation from **1** to **7** (**3** and **5**) suggested by Kurzer *et al.*[14] were isolated as intermediates in this experiments, and these may be further metabolized by tissue enzymes to be excreted into the urine.

In conclusion, several intestinal bacterial metabolites that can be considered intermediates in the pathway from **1** to **7** and **8** were isolated, in which demethylation was suggested to occur prior to dehydroxylation. Two bacterial strains, *P.* sp. SDG-1 and *E.* sp. SDG-2, responsible for the demethylation and dehydroxylation of the metabolic intermediates were isolated from human feces.

The present study emphasizes the role of human intestinal bacteria in the activation of dietary lignans, and suggests that, following the oral consumption of fiber-rich foods, lignan glycosides may undergo metabolism by intestinal bacteria to give mammalian lignans. Some of the metabolic intermediates may be excreted into the bile as conjugates which will eventually be exposed to intestinal bacteria again in the large intestine, which harbours numerous bacteria capable of metabolizing these compounds to mammalian lignans.

Trials to isolate bacterial strains from human feces capable of transforming **7** to **8** are currently underway in our laboratories.

Experimental

General Optical rotations were measured in MeOH solutions using a JASCO DIP-360 automatic polarimeter at 25 °C. UV spectra were taken on a Shimadzu UV-2200 UV-VIS spectrophotometer (Kyoto, Japan) in MeOH solution. ^{1}H- and ^{13}C-NMR spectra were taken on a JOEL JNM GX-400 with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were measured with a JEOL JMS DX-300 mass spectrometer at an ionization voltage of 70 eV. For anaerobic cultivation, an anaerobic incubator EAN-140 (Tabai Co., Osaka, Japan) was used. A gas chromatograph (Shimadzu Co., Kyoto, Japan) fitted with a DB-1 column [0.25 (i.d.) ×30 m] (J & W Scientific, U.S.A.) (column temperature: 140 °C, carrier gas: He) coupled to an Automass System II Mass spectrometer (JEOL, Japan) was used for determination of fatty acids. HPLC was performed on a CCPM-II (Tosoh, Tokyo, Japan) equipped with a Tosoh UV-8020 spectrometer and a Shimadzu C-R 6A chromatopac (Shimadzu, Kyoto, Japan). Column, TSKgel ODS-80T$_M$ (Tosoh Co., Tokyo, Japan, 250 mm×4.6 mm i.d.); mobile phase, CH_3OH (solvent system A) and H_2O containing 0.1% TFA (solvent system B) in a gradient mode (A from 33 to 50% in 60 min, and then isocratic for 10 min); flow rate, 1.0 ml/min; detection, UV 280 nm. Kieselgel 400 (Wako Pure Chemical Co., Osaka, Japan) and Sephadex LH-20 (Pharmacia, Sweden) were used for column chromatography. Merck Kieselgel 60 F_{254} (layer thickness 0.25 mm) were used for TLC, with solvent system A, $CHCl_3$–MeOH (10 : 1). Spots were detected under a UV lamp and after spraying with anisaldehyde/H_2SO_4 reagent, followed by heating.

Chemicals and Media Secoisolariciresinol diglucoside (**1**) was provided by Suntory Co. and purified in our laboratory. Methylation of **2** with Me_2SO_4 was carried out by the usual method.[21] General anaerobic medium (GAM) was purchased from Nissui Co. (Tokyo, Japan). Peptone yeast extract fildes (PYF) broth was prepared according to the procedure of Mitsuoka.[22]

Preparation of a Human Fecal Suspension A fresh fecal sample (5 g), obtained from a healthy subject, was homogenized with K-phosphate buffer (50 ml, pH 7.3), and the sediments were removed by filtration through gauze. The suspension was made up to 100 ml with the same buffer and was used as a human fecal suspension in this experiment.

Preparation of a Rat Fecal Suspension A fresh fecal sample (5 g), obtained from male Wistar strain rats (250 g; Sankyo Lab. Service, Tokyo, Japan), was homogenized in K-phosphate buffer (50 ml, pH 7.3). After filtration through gauze, the suspension was made up to 100 ml with the same buffer and used in this experiment.

Incubation of Secoisolariciresinol Diglucoside (1) with a Human Fecal Suspension Secoisolariciresinol diglucoside (**1**, 300 mg) dissolved in PYF broth (250 ml) was incubated with a human fecal suspension (50 ml) at 37 °C in an anaerobic incubator for 15 h. The mixture was then extracted with BuOH, saturated with H_2O (300 ml×3), and the BuOH layer was evaporated *in vacuo* to give a residue (950 mg). The residue was applied to a column of silica gel, and elution was started with $CHCl_3$, then with $CHCl_3$ with increasing percentages of methanol. Similar fractions were pooled to give fr. A and B. Purification of fr. A and B through Sephadex LH-20 (eluting with

95% aq. MeOH) and then RP-18 (eluting with 50% aq. MeOH) gave **2** (68 mg) and **4** (10 mg), respectively.

Compound **1** (500 mg) in GAM broth (500 ml) was anaerobically incubated with a human fecal suspension (50 ml) under anaerobic conditions for 30 h. The mixture was then extracted and purified as mentioned above to afford **3** (6 mg), **5** (9 mg), **6** (4 mg) and **7** (70 mg). On the other hand, anaerobic incubation of **1** (50 mg) with a human fecal suspension in GAM broth (50 ml) for 3 d yielded **8** (7 mg) after repeated column chromatography.

Incubation of 2—7 with a Human Fecal Suspension A human fecal suspension (2 ml) containing a test compound (0.8 mM) was anaerobically incubated at 37 °C for 48 h. The mixture (200 μl) was then extracted with BuOH saturated with H_2O (200 μl). Ten μl of the BuOH layer was applied on TLC, which was then developed with solvent system A. Identification was made by direct comparison of *Rf* values with those of authentic samples.

Compound 2 Amorphous powder. EI-MS *m/z*: 362 $[M]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.84 (2H, m, H-2, 3), 2.66 (H, m, H-7′, 7″), 3.46 (4H, m, H-1, 4), 3.80 (6H, s, OCH_3), 6.60 (4H, overlapped, H-2′, 2″, 5′, 5″), 6.75 (2H, dd, J=8.4, 3.0 Hz, H-6′, 6″).

Compound 3 Amorphous powder. EI-MS *m/z*: 348 $[M]^+$. ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 1.91 (2H, m, H-2, 3), 2.60 (4H, m, H-7′, 7″), 3.53 (2H, dd, J=11.1, 5.1 Hz, H_a-1, H_a-4), 3.59 (2H, dd, J=11.1, 4.8 Hz, H_b-1, H_b-4), 3.76 (3H, s, –OC$\underline{H}_3$), 6.43 (1H, dd, J=8.0, 1.9 Hz, H-6′), 6.56 (1H, dd, J=8.0, 1.9 Hz, H-6″), 6.57 (1H, d, J=1.9 Hz, H-2′); 6.63 (1H, d, J=1.9 Hz, H-2″), 6.63 (1H, d, J=8.0 Hz, H-5″), 6.66 (1H, d, J=8.0 Hz, H-5′).

Compound 4 Amorphous powder. $[\alpha]_D$ 24.4° (c=0.16, MeOH). UV (MeOH) nm: 227, 280. EI-MS *m/z*: 332 $[M]^+$. ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 1.95 (2H, m, H-2, 3), 2.63 (4H, m, H-7′, 7″), 3.55 (2H, ddd, J=11.1, 5.7, 2.7 Hz, H_a-1, H_a-4), 3.62 (2H, ddd, J=11.1, 6.8, 2.4 Hz, H_b-1, H_b-4), 3.78 (3H, s, –OC$\underline{H}_3$) 6.57 (1H, dd, J=8.0, 1.9 Hz, H-6″), 6.60 (3H, overlapped, H-2′, 4′, 6′), 6.65 (1H, d, J=1.9 Hz, H-2″), 6.68 (1H, d, J=8.0 Hz, H-5″), 7.04 (1H, t, J=8.0 Hz, H-5′).

Compound 5 Amorphous powder. EI-MS *m/z*: 334 $[M]^+$. ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 1.86 (2H, m, H-2, 3), 2.51 (4H, m, H-7′, 7″), 3.45 (2H, dd, J=11.1, 5.3 Hz, H_a-1, H_a-4), 3.56 (2H, dd, J=11.1, 3.8 Hz, H_b-1, H_b-4), 6.41 (2H, dd, J=8.0, 2.2 Hz, H-6′, 6″), 6.55 (2H, dd, J=2.2 Hz, H-2′, 2″), 6.60 (2H, d, J=8.0 Hz, H-5′, 5″).

Compound 6 Amorphous powder. EI-MS *m/z*: 318 $[M]^+$. ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 1.94 (2H, m, H-2, 3), 2.56 (2H, m, H-7′ or H-7″), 2.62 (2H, m, H-7″ or H-7′), 3.50 (2H, ddd, J=11.1, 6.0, 3.6 Hz, H_a-1, H_a-4), 3.61 (2H, ddd, J=11.1, 7.0, 4.1 Hz, H_b-1, H_b-4), 6.45 (1H, dd, J=8.0, 2.1 Hz, H-4′), 6.58 (1H, ddd, J=8.0, 2.1, 2.1 Hz, H-6′), 6.59 (1H, s, H-2″), 6.59 (1H, t, J=2.1 Hz, H-2′), 6.61 (1H, d, J=8.0 Hz, H-6″), 6.64 (1H, d, J=8.0 Hz, H-5″), 7.03 (1H, t, J=8.0 Hz, H-5′).

Compound 7 Amorphous powder. EI-MS *m/z*: 302 $[M]^+$. ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 1.98 (2H, m, H-2, 3), 2.64 (4H, m, H-7′, 7″), 3.53 (2H, dd, J=11.1, 5.3 Hz, H_a-1, H_a-4), 3.63 (2H, dd, J=11.1, 3.8 Hz, H_b-1, H_b-4), 6.60 (2H, ddd, J=7.8, 2.2, 1.2 Hz, H-4′, 4″), 6.62 (4H, overlapped, H-2′, 2″, 6′, 6″), 7.05 (2H, t, J=7.8 Hz, H-5′, 5″).

Compound 8 Amorphous powder. $[\alpha]_D$ 34.8° (*c*=0.12, MeOH). EI-MS *m/z*: 298 $[M]^+$. ^{1}H-NMR (400 MHz, MeOH-d_4) δ: 2.50 (2H, m, H_a-7″, 3), 2.59 (1H, m, H-2), 2.61 (1H, m, H_b-7″), 2.91 (1H, m, H_a-7′), 3.00 (1H, m, H_b-7′), 3.87 (1H, dd, J=9.1, 7.6 Hz, H_a-4), 4.14 (1H, dd, J=9.1, 7.0 Hz, H_b-4), 6.47 (1H, t, J=1.6 Hz, H-2″), 6.59 (1H, t, J=1.6 Hz, H-2′), 6.60 (1H, ddd, J=7.9, 1.6, 1.6 Hz, H-6″), 6.70 (1H, m, H-4′), 6.73 (1H, dt, J=7.9, 1.6, 1.6 Hz, H-6′), 6.74 (1H, m, H-4″), 7.15 (1H, t, J=7.9 Hz, H-5″), 7.18 (1H, t, J=7.9 Hz, H-5′).

Isolation of Bacterial strains Capable of Transforming 2 and 5 from Human Fecal Suspension A bacterial suspension from fresh feces of a healthy man was repeatedly cultured in GAM broth containing **2** or **5** (1 mM) and incubated at 37 °C in an anaerobic incubator. Five μl of the culture was seeded on BL agar plates. Colonies were picked up and screened for the ability to transform **2** and **5**, respectively.

Time Course of Transformation of 2 by *P.* sp. SDG-1 GAM broth (4 ml) containing **2** (a final concentration of 1 mM) was incubated at 37 °C with a bacterial suspension of *P.* sp. SDG-1 (100 μl) in an anaerobic incubator. A 150 μl portion was taken out at intervals and extracted with BuOH saturated with H_2O (150 μl). The BuOH extract was evaporated *in vacuo* to give a residue. The residue was dissolved in MeOH (150 μl) and the MeOH extract was analyzed by HPLC. Bacterial growth in GAM broth was monitored by measuring turbidity at 540 nm.

Time Course of Transformation of 5 by *E.* sp. SDG-2 GAM broth (6 ml) containing **5** (a final concentration of 0.8 mM) was incubated at 37 °C with a bacterial suspension of *E.* sp. SDG-2 (100 μl) under anaerobic conditions. Samples were taken at intervals and analyzed by HPLC, as mentioned above.

Fermentation Test Carbohydrate fermentation tests were carried out according to the method of Sneath *et al.*[23] Fatty acids obtained in PYF broth containing D-glucose were determined using gas chromatography.

Acknowledgement Part of this study was financially supported by Suntory, Ltd., Shimamotocho, Mishimagun, Osaka, and a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan.

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