JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 7537–7542 7537
DOI:10.1021/jf900902p

Further Studies on a Human Intestinal Bacterium *Ruminococcus* sp. END-1 for Transformation of Plant Lignans to Mammalian Lignans

JONG-SIK JIN[†,‡] AND MASAO HATTORI*[,†]

[†]Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan, and [‡]Benno Laboratory, Center for Intellectual Property Strategies, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

A human intestinal bacterium *Ruminococcus* (*R.*) sp. END-1 capable of oxidizing (−)-enterodiol to (−)-enterolactone, enantioselectively, was further investigated from the perspective of transformation of plant lignans to mammalian lignans; A cell-free extract of the bacterium transformed (−)-enterodiol to (−)-enterolactone through an intermediate, enterolactol. The bacterium showed not only oxidation but also demethylation and deglucosylation activities for plant lignans. Arctiin and secoisolariciresinol diglucoside were converted to (−)-dihydroxyenterolactone and (+)-dihydroxyenterodiol, respectively. Moreover, by coincubation with *Eggerthella* sp. SDG-2, the bacterium transformed arctiin and secoisolariciresinol diglucoside to (−)-enterolactone and (+)-enterodiol, respectively.

KEYWORDS: Mammalian lignan; human intestinal bacteria; enterolactol; enantioselective oxidation

INTRODUCTION

Enterodiol and enterolactone are metabolites of several plant lignans such as pinoresinol diglucoside, secoisolariciresinol diglucoside, sesamin, and arctiin by gut microflora (*1*−*4*). Since metabolites of these lignans are categorized as phytoestrogens, their biological activities have been extensively studied. They showed estrogenic and antiestrogenic activities (*5*−*7*) as well as antioxidant activity (*8*). Moreover, it is reported that enterolactone inhibits the growth of prostate cancer cell lines in vitro (*9*, *10*) and mammary carcinomas in vivo (*11*), indicating the possible association with epidemiological studies that phytoestrogens have potential health benefits in hormone-dependent diseases such as breast, colon, and prostate cancers (*12*−*14*).

The metabolic processes of precursors to enterodiol and enterolactone by intestinal bacteria include deglucosylation, demethylenation, ring cleavage, demethylation, dehydroxylation, and oxidation of diols to γ-butyrolactones. The bacteria responsible for these processes have been reported. (*1*, *2*, *15*−*18*). However, both compounds occur as enantiomeric, mirror image forms in nature (**Figure 1**). Enterolactone, isolated from incubation of arctiin and pinoresinol diglucoside with intestinal bacteria, was a (−)-form, while that isolated from incubation of secoisolariciresinol diglucoside was a (+)-form. Interconversion between the respective enantiomers did not occur during bacterial metabolism (*19*). Therefore, metabolic specificity must be considered between enantiomers as well as their biological activities.

We recently reported enantioselective dehydroxylation by *Eggerthella* (*Eg.*) sp. SDG-2 (Genbank accession no. EF413638) and strain ARC-1 (EF413639), which were isolated from human feces. *Eg*. sp. SDG-2 transformed (+)-dihydroxyenterodiol and (−)-dihydroxyenterolactone to (+)-enterodiol and (−)-enterolactone, respectively, but left out (−)-dihydroxyenterodiol and (+)-dihydroxyenterolactone. Conversely, strain ARC-1 transformed (−)-dihydroxyenterodiol and (+)-dihydroxyenterolactone to (−)-enterodiol and (+)-enterolactone, respectively, but left out (+)-dihydroxyenterodiol and (−)-dihydroxyenterolactone.

Ruminococcus (*R.*) sp. END-1 (EF451052) and strain END-2 (EF451053) are human intestinal bacteria capable of oxidizing enterodiol to enterolactone (*20*). The former transformed (−)-enterodiol to (−)-enterolactone, selectively. The latter oxidized (+)-enterodiol to (+)-enterolactone.

In this article, we report additional properties of *R*. sp. END-1 in the metabolism of plant lignans, as regards kinetics in transformation, substrate specificity, and coculture with another bacterium, *Eg*. sp. SDG-2.

MATERIALS AND METHODS

General. An anaerobic incubator, model EAN-140 (Tabai Co., Osaka, Japan), was used for the incubation of fecal suspensions and intestinal bacteria. Optical rotations were measured in MeOH solutions with a DIP-360 automatic polarimeter (Jasco Co., Tokyo, Japan). ^{1}H- and ^{13}C NMR spectra were measured with Varian Unity 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) and Varian Gemini 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz). Thin-layer chromatography (TLC) was carried out on silica gel precoated 60 F_{254} plates (0.25 mm, Merck Co., Darmstadt, Germany), and spots were detected under a UV lamp or exposing I_2 vapor. Silica gel BW-820 MH (Fuji Silysia, Aichi, Japan) was used for column chromatography.

Chemicals and Media. General anaerobic medium (GAM) was purchased from Nissui Co. (Tokyo, Japan). (−)-Arctigenin, secoisolariciresinol diglucoside, (+)-secoisolariciresinol, (+)-dihydroxyenterodiol,

*Corresponding author. Tel: +81-76-434-7630. Fax: +81-76-434-5060. E-mail: saibo421@inm.u-toyama.ac.jp.

Published on Web 07/24/2009

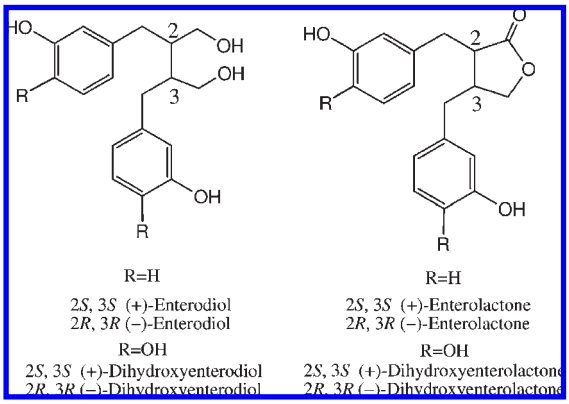

Figure 1. Enantiomers of enterodiol, enterolactone, dihydroxyenterodiol, and dihydroxyenterolactone.

(−)-dihydroxyenterodiol, (+)-dihydroxyenterolactone, (−)-dihydroxyenterolactone, (+)-enterodiol, (−)-enterodiol, (+)-enterolactone, and (−)-enterolactone were prepared by the same methods of Jin et al. (*21*). Enterolactol was prepared by a modified method of Xia et al. (*22*). Enterolactol [(3*R*,4*R*)-3,4-bis(3-hydroxybenzyl)-tetrahydrofuran-2-ol]: amorphous powder. EI-MS *m*/*z*: 300 $[M]^+$. ^{1}H NMR (CD_3OD, 300 MHz): δ 2.10, 2.55 (m, H-3, 4, 7′, 7″), 3.52 (t, J = 8.0 Hz, H_a-5), 3.70 (t, J = 8.5 Hz, H_a-5), 3.83 (t, J = 8.5 Hz, H_b-5), 3.99 (t, J = 8.0 Hz, H_b-5), 5.12 (m, H-2), 6.64, 7.07 (m, H-1′, 2′, 3′, 4′, 5′, 6′, 1″, 2″, 3″, 4″, 5″, 6″). ^{13}C NMR (CD_3OD, 75 MHz): δ 34.9, 39.6, 40.0, 44.1, 47.5, 53.0, 54.8, 72.5, 73.1, 99.8, 104.2, 113.7, 113.8, 113.9, 114.0, 116.3, 116.3, 116.7, 116.8, 120.7, 120.8, 121.1, 121.2, 130.1, 130.2, 142.5, 143.0, 143.2, 143.6, 158.1, 158.2, 158.2, 158.3.

(−)-Secoisolariciresinol was prepared from pinoresinol diglucoside as follows: a bacterial suspension (50 mL) of strain PUE (*23*) and *Eg*. sp. SDG-2 was inoculated to 500 mL of GAM broth containing pinoresinol diglucoside (100 mg) and incubated at 37 °C in an anaerobic incubator for 180 h. The reaction mixture was then extracted three times with 500 mL of ethyl acetate. The organic layer was evaporated under reduced pressure to yield a residue. The residue was applied to a column of silica gel (45 g), which was eluted with a solvent system of $CHCl_3$−MeOH (20:1). Then, fractions including (−)-secoisolariciresinol were concentrated under reduced pressure and applied to a column of Sephadex LH-20, which was eluted with a solvent system of H_2O−MeOH (1:1) to yield (−)-secoisolariciresinol (36.8 mg). The compound was identified by comparing the ^{1}H- and ^{13}C NMR spectra with those published (*1*). (+)-Desdimethylpinoresinol was prepared from pinoresinol diglucoside as follows: a bacterial suspension (2 mL) of *Eubacterium* (*E*.) sp. ARC-2 was inoculated to 200 mL of GAM broth containing 50 mg of pinoresinol diglucoside and incubated at 37 °C in an anaerobic incubator for 132 h. The reaction mixture was then extracted three times with 300 mL of ethyl acetate. The organic layer was evaporated under reduced pressure to yield a residue. The residue was applied to a column of silica gel (62 g), which was eluted with a solvent system of $CHCl_3$−MeOH (15:1). Then, fractions including (+)-desdimethylpinoresinol were concentrated under reduced pressure and applied to a column of Sephadex LH-20, which was eluted with a solvent system of H_2O−MeOH (1:1) to yield (+)-desdimethylpinoresinol (8 mg). (+)-Desdimethylpinoresinol: amorphous powder. $[\alpha]_D^{23}$ 58.0 (c = 0.729, MeOH). ^{1}H NMR (CD_3OD, 300 MHz): δ 3.07 (2H, m, H-8, 8′), 3.78 (2H, dd, J = 3.5, 9.0 Hz, H_a-9, 9′), 4.19 (2H, dd, J = 7.0, 9.0 Hz, H_b-9, 9′), 4.62 (2H, d, J = 4.5 Hz, H-7, 7′), 6.68 (2H, dd, J = 2.5, 8.0 Hz, H-6, 6′), 6.73 (2H, d, J = 8.0 Hz, H-5, 5′), 6.80 (2H, d, J = 2.5 Hz, H-2, 2′). ^{13}C NMR (CD_3OD, 75 MHz): δ 55.2 (C-8, 8′), 72.5 (C-9, 9′), 87.3 (C-7, 7′), 114.3 (C-2, 2′), 116.1 (C-5, 5′), 118.7 (C-6, 6′), 133.6 (C-1, 1′), 145.8 (C-3, 3′), 146.2 (C-4, 4′). EI-MS *m*/*z*: 331 $[M + H]^+$.

LC/MS Analysis. HPLC/MS was performed on an Agilent 1100 system (Agilent Technologies, Waldbronn, Germany) with a photodiode array detector, an Agilent 1100 series binary pump, an Esquire 3000plus mass spectrometer (Bruker Daltonic GmbH, Bremen, Germany) coupled with an ESI interface, and an ion trap mass analyzer, under the following conditions: column, TSK-gel ODS-80Ts (Tosoh Co., Tokyo, Japan, 4.6 mm × 150 mm); mobile phase, 0.1% trifluoroacetic acid (TFA, solvent system A) and CH_3CN (solvent system B) in gradient modes [(−)-enterodiol, (−)-enterolactone, and enterolactol analysis, B from 20 to 50% for 30 min, (−)-hydroxyenterolactone analysis, B from 10 to 35% in 50 min]; flow rate, 1.0 mL/min; detection, UV at 280 nm; temperature, 30 °C. High-purity nitrogen was used as dry gas at a flow rate of 10 L/min, with a temperature of 360 °C. Helium was used as nebulizer at 50 psi. The ESI interface and mass spectrometric parameter were optimized to obtain maximum sensitivity.

Bacterial Growth and Kinetics in Biotransformation with *R*. sp. END-1. GAM broth (4 mL) containing (−)-enterodiol (a final concentration of 0.25 mM) was incubated with a bacterial suspension of *R*. sp. END-1 (400 μL) at 37 °C under anaerobic conditions. A 100 μL aliquot was taken out at 3 h intervals and extracted three times with 200 μL of ethyl acetate. After evaporation of ethyl acetate in vacuo, the residue was dissolved in 0.3 mL of MeOH. The MeOH solution was filtered through a 0.45 μm membrane filter, and a 10 μL portion was injected into a column for HPLC analysis. HPLC was performed on a CCPM-II (Tosoh, Tokyo, Japan) equipped with a Tosoh UV-8020 spectrometer and a Shimadzu C-R8A chromatopac (Shimadzu, Kyoto, Japan) under the following conditions: column, TSK-gel ODS-80Ts (Tosoh Co., Tokyo, Japan, 4.6 mm × 150 mm); mobile phase, 0.1% TFA (solvent system A) and CH_3CN (solvent system B) in a gradient mode (B from 30 to 50% in 20 min); flow rate, 1.0 mL/min; detection, UV 280 nm; temperature, room temperature. Concentrations of (−)-enterodiol and (−)-enterolactone were calculated from calibration curves of the respective authentic samples. Optical density at 540 nm was measured with a UV-2200 UV−vis recording spectrophotometer (Shimadzu Co., Kyoto, Japan).

Preparation of a Cell-Free Extract. *R*. sp. END-1 was cultured under anaerobic conditions at 37 °C for 12 h in GAM broth containing 0.2 mM (−)-enterodiol (inducer), harvested, and then suspended in 100 mM phosphate buffer (pH 7.3). The cell suspensions were sonicated by 10 sonic bursts of 30 s each (Branson Sonifier 250, Branson Ultrasonics Corporation, Danbury, CT, USA) on ice and then centrifuged at 100,000*g* for 60 min (Ultracentrifuge Beckman Optima XL-70, Beckman Instruments, Fullerton, CA, USA) at 4 °C. The supernatants were filtered with a 0.45 μm microfilter and then used as cell-free extracts.

Incubation of *R*. sp. END-1 with Substrates. Each substrate (0.1 mM) was anaerobically incubated with *R*. sp. END-1 for 120 h at 37 °C, and then a 100 μL aliquot was taken out and extracted three times with 200 μL of ethyl acetate. After evaporation of ethyl acetate in vacuo, the residue was dissolved in 0.3 mL of MeOH. The MeOH solution was filtered through a 0.45 μm membrane filter, and a 10 μL portion was injected onto a column for LC/MS analysis. The conversion of substrates was confirmed by TLC.

Coincubation of *R*. sp. END-1 and *Eg*. sp. SDG-2. Each substrate (0.1 mM) was anaerobically incubated with *R*. sp. END-1 and *Eg*. sp. SDG-2 for 120 h at 37 °C, and then a 100 μL aliquot was taken out and treated as described above.

RESULTS

Bacterial Growth and Biotransformation of (−)-enterodiol to (−)-enterolactone by *R*. sp. END-1. Figure 2 shows bacterial growth and time course of (−)-enterolactone formation by *R*. sp. END-1 in GAM broth containing 0.25 mM (−)-enterodiol. The bacterium grew maximal within 18 h under anaerobic conditions monitored by turbidity at 540 nm. The metabolic activity of oxidizing (−)-enterodiol to (−)-enterolactone was observed accompanied by bacterial growth. (−)-Enterodiol was decreased in amount from 9 h, while (−)-enterolactone was gradually increased. The maximal concentration of (−)-enterolactone was obtained at 30 h. A metabolic intermediate was detected during the cultivation but not measured quantitatively because of a small amount (data not shown).

Identification of an Intermediate in the Transformation of (−)-enterodiol by *R*. sp. END-1. Since a little amount of the metabolic intermediate was detected, we obtained a relatively large amount of it for structural elucidation by incubation of (−)-enterodiol with a cell-free extract of *R*. sp. END-1. The same

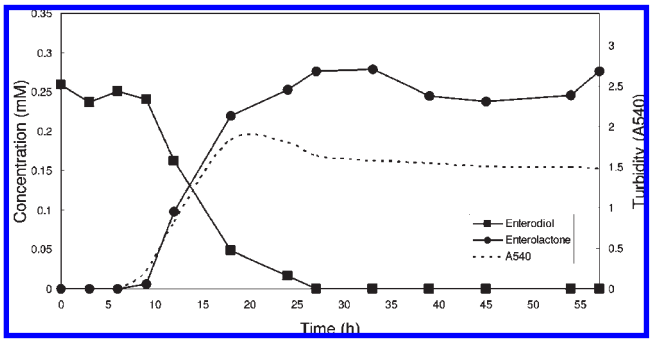

Figure 2. Transformation kinetics of (−)-enterodiol to (−)-enterolactone by *R.* sp. END-1.

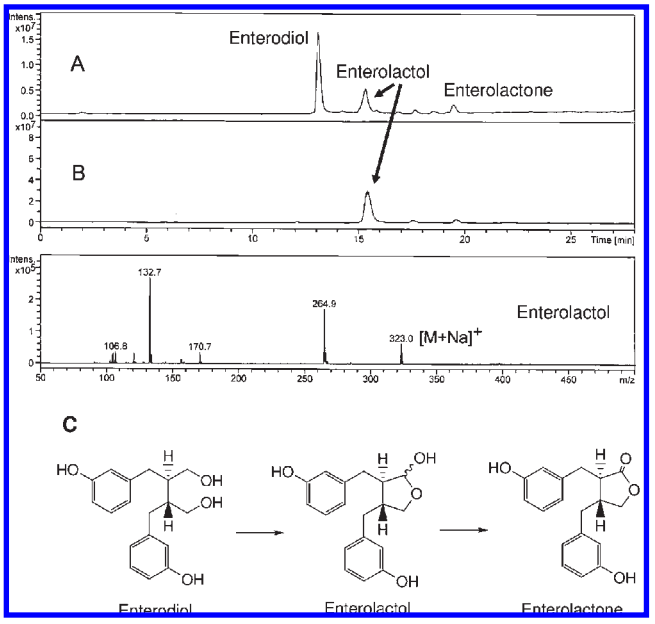

Figure 3. Transformation of (−)-enterodiol by cell-free extract of *R.* sp. END-1 and ESI-MS spectra of enterolactol. (**A**) LC/MS total ion chromatogram profile of the transformation of (−)-enterodiol to (−)-enterolactone by cell-free extract of *R.* sp. END-1. (**B**) LC/MS total ion chromatogram profile of synthesized enterolactol. (**C**) Possible metabolic pathway of (−)-enterodiol by *R.* sp. END-1.

metabolite was detected by LC/MS analysis (**Figure 3A**). The metabolite had a quasi-molecular ion peak at m/z 323 $[M + Na]^+$ in the MS spectrum, indicating 2 mass units higher than that of enterolactone and 2 mass units lower than that of enterodiol, suggesting a reduction product of enterolactone. We synthesized it from (−)-enterolactone by reduction with $LiEt_3BH$ and analyzed it under the same conditions. The synthesized compound showed the retention time and mass fragment patterns (**Figure 3B**) same as those of an isolated metabolite in bacterial transformation.

Substrate Specificity of *R.* sp. END-1 in Oxidation Activity. (−)-Dihydroxyenterodiol was transformed to (−)-dihydroxyenterolactone through oxidative lactonization by anaerobic incubation with *R.* sp. END-1 (data not shown). However, (+)-dihydroxyenterodiol, an enantiomer of (−)-dihydroxyenterodiol, was not transformed by this bacterium, indicating the strain has enantioselectivity to a substrate in oxidation. For studying selectivity in oxidation of other dibenzylbutanediols, *R.* sp. END-1 was incubated with (−)-hydroxyenterodiol (0.1 mM), and we obtained two isomers of 4′- and 4″- hydroxyenterolactone with retention times of 36.9 and 36.5 min, respectively (**Figure 4**). The former was identical with that obtained by incubation of (−)-dihydroxyenterolactone with *Eg.* sp. SDG-2.

Demethylation Activity of *R.* sp. END-1. The demethylation activity of *R.* sp. END-1 was demonstrated by incubation of either (−)-arctigenin or (−)-secoisolariciresinol, which resulted in the formation of (−)-dihydroxyenterolactone. Moreover, (+)-secoisolariciresinol was transformed to (+)-dihydroxyenterodiol by the bacterium (**Figure 5**).

***β*-Glucosidase Activity of *R.* sp. END-1.** *R.* sp. END-1 showed β-glucosidase activity for some plant lignans. Pinoresinol diglucoside and secoisolariciresinol diglucoside were converted to (+)-desdimethylpinoresinol and (+)-dihydroxyenterodiol, respectively, by elimination of a glucose moiety, in which the latter product was further demethylated (**Figure 5**).

Transformation of Plant Lignans by Coincubation of *R.* sp. END-1 and *Eg.* sp. SDG-2. When pinoresinol diglucoside was anaerobically incubated with both *R.* sp. END-1 and *Eg.* sp. SDG-2 at 37 °C, (−)-enterolactone was detected in an ethyl acetate extract from the bacterial suspension by analytical LC/MS with a chiral column. In the conversion of pinoresinol diglucoside to (−)-enterolactone, *R.* sp. END-1 participated in deglucosylation, demethylation, and oxidation, while *Eg.* sp. SDG-2 participated in ring cleavage and dehydroxylation (**Figure 6**). In the same way, *R.* sp. END-1 was responsible for deglucosylation and demethylation of secoisolariciresinol diglucoside and *Eg.* sp. SDG-2 for dehydroxylation to produce (+)-enterodiol. Moreover, arctiin was also converted to (−)-enterolactone by co-operation of both bacteria.

DISCUSSION

The oxidative lactonization of dibenzylbutane-1,4-diols is essential in the formation of enterolactone from plant lignans such as pinoresinol diglucoside, secoisolariciresinol diglucoside, and sesamin by intestinal flora. Some bacteria capable of oxidizing enterodiol to enterolactone have been reported; Clavel et al. isolated strain ED-Mt61/PYG-s6, which was classified into *Lactonifactor longoviformis* as an enterolactone-producing bacterium from human feces (*16*, *18*). We also isolated two bacterial species, *R.* sp. END-1 and strain END-2, capable of enantioselectively oxidizing enterodiol to enterolactone from human feces.

R. sp. END-1 enantioselectively converted diols such as (−)-dihydroxyenterodiol, (−)-hydroxyenterodiol, and (−)-enterodiol to the corresponding γ-lactones, indicating that the oxidation proceeds regardless of the presence or absence of a para hydroxyl group in the phenyl rings. 4′- Hydroxyenterolactone and 4″- hydroxyenterolactone were detected during the transformation of pinoresinol diglucoside (*1*), indicating that oxidation takes place both sides at C-1 and C-4 in the butane-1,4-diol moiety.

When (−)-enterodiol was incubated with *R.* sp. END-1, enterolactol was detected as an intermediate; however, we could not analyze it quantitatively because of a faint amount of formation during the incubation. Although this compound was newly identified in the present experiment as an intermediate of the transformation of precursor lignans to enterolactone, a similar compound, (−)-lactol, was already reported in plant metabolic processes. Secoisolariciresinol dehydrogenase from *Forsythia intermedia* converted (−)-secoisolariciresinol to (−)-matairesinol via (−)-lactol (*22*). The secoisolariciresinol dehydrogenase was enantioselective in the oxidation of secoisolariciresinol. A similar dehydrogenase has not been isolated from *R.* sp. END-1 yet. However, because cell-free extract of the strain converted to (−)-enterodiol to enterolactol and (−)-enterolactone, further experiments will make clear the enzymatic transformation.

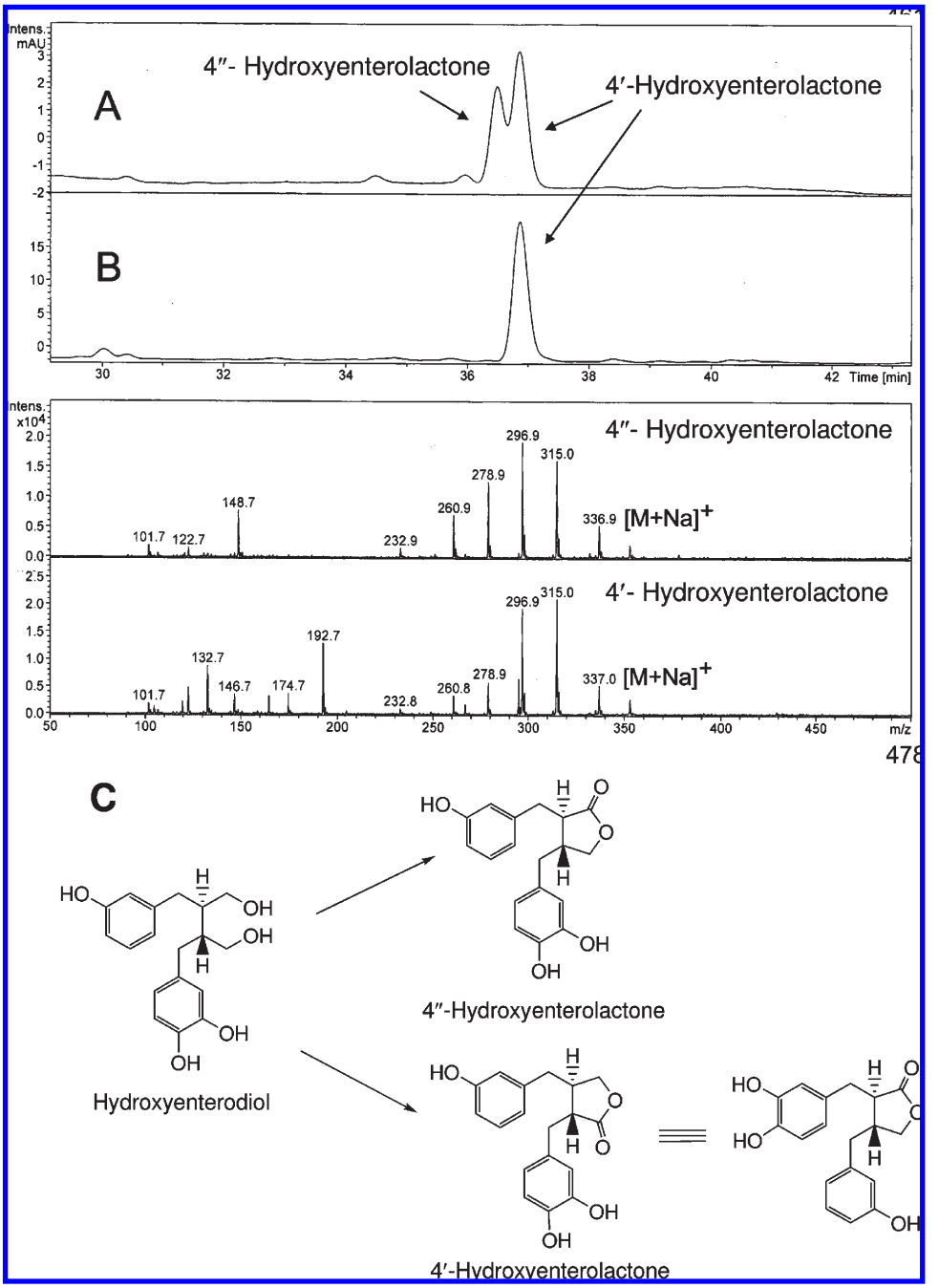

Figure 4. Transformation of (−)-hydroxyenterodiol by *R.* sp. END-1 and ESI-MS spectra of two metabolites. (**A**) HPLC elution profile of the transformation of (−)-hydroxyenterodiol to two types of hydroxyenterolactone. (**B**) HPLC elution profile of 4′- hydroxyenterolactone isolated from incubation of *Eg.* sp. SDG-2 with (−)-dihydroxyenterolactone. (**C**) Possible metabolic pathway of (−)-hydroxyenterodiol by *R.* sp. END-1.

R. sp. END-1 takes part in not only oxidation but also demethylation and deglucosylation of plant lignans, in which methoxy groups of (−)-arctigenin and both (+)- and (−)-secoisolariciresinols were converted to free hydroxyl groups without any enantioselectivity. Since demethylation and deglucosylation are essential metabolic processes in the transformation of precursor lignans to enterodiol and enterolactone, several secoisolariciresinol diglucoside-deglycosylating and secoisolariciresinol-demethylating bacteria were reported (*2*, *16*, *24*). Wang et al. reported the *Peptostreptococcus* (*P.*) sp. strain SDG-,1 which was isolated from a human fecal suspension as a bacterium capable of demethylating. *P.* sp. SDG-1 demethylated (+)-secoisolariciresinol to (+)-dihydroxyenterodiol. However, this strain could not convert dimethylsecoisolariciresinol, i.e., only methoxy groups having a vicinal hydroxyl group could be eliminated by *P.* sp. SDG-1. *R. s*p. END-1 showed low substrate specificity compared with that of *P.* sp. SDG-1. These bacteria having demethylation activity may contribute to a variety of metabolic processes converting some lignans to phytoestrogens in the gastrointestinal tract. Moreover, the bacteria with diverse metabolic activities such as *R.* sp. END-1 bring complicated processes into metabolism. The metabolic intermediates or final metabolites can be absorbed and show several biological activities.

Eg. sp. SDG-2 had ring cleavage activity as well as dehydroxylation like *Eg. lenta* (AF292375) and showed 99% similarity with the latter in the 16S rRNA gene sequencing analysis (*21*, *25*). Hence, pinoresinol diglucoside was converted to (−)-enterolactone by coincubation of only two bacteria, *R.* sp. END-1 and

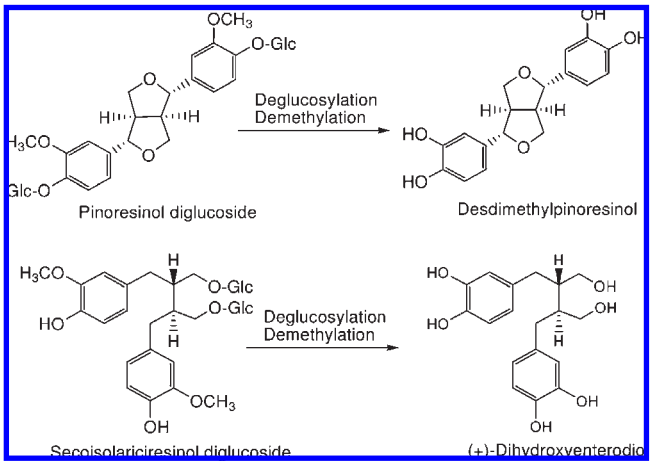

Figure 5. Demethylation and deglucosylation activity of *R.* sp. END-1 in the transformation of pinoresinol diglucoside and secoisolariciresinol diglucoside.

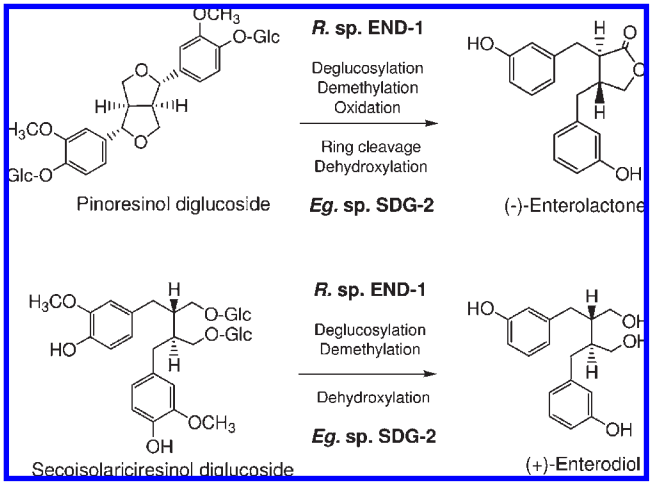

Figure 6. Transformation of plant lignans by coincubation of *R.* sp. END-1 and *Eg.* sp. SDG-2.

Eg. sp. SDG-2. In order to produce enterolactone from pinoresinol diglucoside, five metabolic processes are concerned; *R.* sp. END-1 participates in deglucosylation, demethylation, and oxidation, while *Eg.* sp. SDG-2 participates in ring cleavage and dehydroxylation activity. The bacterial cooperation leads to the formation of enterolactone from pinoresinol diglucoside. Because cooperation and/or competition between many bacteria occur in the gastrointestinal tract, bacterial transformation may be influenced by several factors such as individual diversity of bacterial composition and numbers of the responsible bacteria. Investigation of metabolic activity by defined bacteria promotes a better understanding on the in vivo metabolic processes of lignans to phytoestrogens.

LITERATURE CITED

(1) Xie, L. H.; Akao, T.; Hamasaki, K.; Deyama, T.; Hattori, M. Biotransformation of pinoresinol diglucoside to mammalian lignans by human intestinal microflora, and isolation of *Enterococcus faecalis* strain PDG-1 responsible for the transformation of (+)-pinoresinol to (+)-lariciresinol. *Chem. Pharm. Bull.* **2003**, *51*, 508–515.

(2) Wang, L. Q.; Meselhy, M. R.; Li, Y.; Qin, G. W.; Hattori, M. Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone. *Chem. Pharm. Bull.* **2000**, *48*, 1606–1610.

(3) Penalvo, J. L.; Heinonen, S. M.; Aura, A. M.; Adlercreutz, H. Dietary sesamin is converted to enterolactone in humans. *J. Nutr.* **2005**, *135*, 1056–1062.

(4) Xie, L. H.; Ahn, E. M.; Akao, T.; Abdel-Hafez, A. A.; Nakamura, N.; Hattori, M. Transformation of arctiin to estrogenic and antiestrogenic substances by human intestinal bacteria. *Chem. Pharm. Bull.* **2003**, *51*, 378–384.

(5) Adlercreutz, H.; Mousavi, Y.; Clark, J.; Hockerstedt, K.; Hamalainen, E.; Wahala, K.; Makela, T.; Hase, T. Dietary phytoestrogens and cancer: in vitro and in vivo studies. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 331–337.

(6) Mueller, S. O.; Simon, S.; Chae, K.; Metzler, M.; Korach, K. S. Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ERalpha) and ERbeta in human cells. *Toxicol. Sci.* **2004**, *80*, 14–25.

(7) Schottner, M.; Gansser, D.; Spiteller, G. Lignans from the roots of *Urtica dioica* and their metabolites bind to human sex hormone binding globulin (SHBG). *Planta Med.* **1997**, *63*, 529–532.

(8) Prasad, K. Antioxidant activity of secoisolariciresinol diglucoside-derived metabolites, secoisolariciresinol, enterodiol, and enterolactone. *Int. J. Angiol.* **2000**, *9*, 220–225.

(9) Lin, X.; Switzer, B. R.; Demark-Wahnefried, W. Effect of mammalian lignans on the growth of prostate cancer cell lines. *Anticancer Res.* **2001**, *21*, 3995–3999.

(10) Chen, L. H.; Fang, J.; Li, H.; Demark-Wahnefried, W.; Lin, X. Enterolactone induces apoptosis in human prostate carcinoma LNCaP cells via a mitochondrial-mediated, caspase-dependent pathway. *Mol. Cancer Ther.* **2007**, *6*, 2581–2590.

(11) Saarinen, N. M.; Huovinen, R.; Wärri, A.; Mäkelä, S. I.; Valentín-Blasini, L.; Sjöholm, R.; Ammälä, J.; Lehtilä, R.; Eckerman, C.; Collan, Y. U.; Santti, R. S. Enterolactone inhibits the growth of 7,12-dimethylbenz(a)anthracene-induced mammary carcinomas in the rat. *Mol. Cancer. Ther.* **2002**, *1*, 869–876.

(12) Rowland, I.; Faughnan, M.; Hoey, L.; Wahala, K.; Williamson, G.; Cassidy, A. Bioavailability of phyto-oestrogens. *Br. J. Nutr.* **2003**, *89*, S45–58.

(13) Pietinen, P.; Stumpf, K.; Mannisto, S.; Kataja, V.; Uusitupa, M.; Adlercreutz, H. Serum enterolactone and risk of breast cancer: a case-control study in eastern Finland. *Cancer Epidemiol. Biomarkers Prev.* **2001**, *10*, 339–344.

(14) Morton, M. S.; Chan, P. S.; Cheng, C.; Blacklock, N.; Matos-Ferreira, A.; Abranches-Monteiro, L.; Correia, R.; Lloyd, S.; Griffiths, K. Lignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom. *Prostate* **1997**, *32*, 122–128.

(15) Clavel, T.; Henderson, G.; Alpert, C. A.; Philippe, C.; Rigottier-Gois, L.; Doré, J.; Blaut, M. Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. *Appl. Environ. Microbiol.* **2005**, *71*, 6077–6085.

(16) Clavel, T.; Henderson, G.; Engst, W.; Doré, J.; Blaut, M. Phylogeny of human intestinal bacteria that activate the dietary lignan secoisolariciresinol diglucoside. *FEMS Microbiol. Ecol.* **2006**, *55*, 471–478.

(17) Clavel, T.; Borrmann, D.; Braune, A.; Doré, J.; Blaut, M. Occurrence and activity of human intestinal bacteria involved in the conversion of dietary lignans. *Anaerobe* **2006**, *12*, 140–147.

(18) Clavel, T.; Lippman, R.; Gavini, F.; Doré, J.; Blaut, M. *Clostridium saccharogumia* sp. nov. and *Lactonifactor longoviformis* gen. nov., sp. nov., two novel human faecal bacteria involved in the conversion of the dietary phytoestrogen secoisolariciresinol diglucoside. *Syst. Appl. Microbiol.* **2007**, *30*, 16–26.

(19) Saarinen, N. M.; Smeds, A.; Mäkelä, S. I.; Ammälä, J.; Hakala, K.; Pihlava, J. M.; Ryhänen, E. L.; Sjöholm, R.; Santti, R. Structural determinants of plant lignans for the formation of enterolactone in vivo. *J. Chromatogr., B* **2002**, *777*, 311–319.

(20) Jin, J. S.; Kakiuchi, N.; Hattori, M. Enantioselective oxidation of enterodiol to enterolactone by human intestinal bacteria. *Biol. Pharm. Bull.* **2007**, *30*, 2204–2206.

(21) Jin, J. S.; Zhao, Y. F.; Nakamura, N.; Akao, T.; Kakiuchi, N.; Min, B. S.; Hattori, M. Enantioselective dehydroxylation of enterodiol

and enterolactone precursors by human intestinal bacteria. *Biol. Pharm. Bull.* **2007**, *30*, 2113–2119.

(22) Xia, Z. Q.; Costa, M. A.; Pelissier, H. C.; Davin, L. B.; Lewis, N. G. Secoisolariciresinol dehydrogenase purification, cloning, and functional expression. Implications for human health protection. *J. Biol. Chem.* **2001**, *276*, 12614–12623.

(23) Jin, J. S.; Nishihata, T.; Kakiuchi, N.; Hattori, M. Biotransformation of *C*-glucosylisoflavone puerarin to estrogenic (3*S*)-equol by co-culture of two human intestinal bacteria. *Biol. Pharm. Bull.* **2008**, *31*, 1621–1625.

(24) Jin, J. S.; Zhao, Y. F.; Nakamura, N.; Akao, T.; Kakiuchi, N.; Hattori, M. Isolation and characterization of a human intestinal bacterium, *Eubacterium* sp. ARC-2, capable of demethylating arctigenin, in the essential metabolic process to enterolactone. *Biol. Pharm. Bull.* **2007**, *30*, 904–911.

(25) Wang, L. Q.; Meselhy, M. R.; Li, Y.; Nakamura, N.; Min, B. S.; Qin, G. W.; Hattori, M. The heterocyclic ring fission and dehydroxylation of catechins and related compounds by *Eubacterium* sp. strain SDG-2, a human intestinal bacterium. *Chem. Pharm. Bull.* **2001**, *49*, 1640–1643.

Received March 17, 2009. Revised manuscript received June 18, 2009. Accepted July 02, 2009.