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NOTE

Microbial metabolism of soy isoflavones by human intestinal bacterial strains

Ryota Tsuchihashi · Seiichi Sakamoto · Mitsuru Kodera · Toshihiro Nohara · Junei Kinjo

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Abstract Intestinal bacteria play an important role for the metabolism of soy isoflavonoids. When soy foods are consumed, the soy isoflavone glucosides are metabolized into their aglycones and the related isoflavonoids by intestinal bacteria. We designed an in vitro microbial metabolic system using 29 commercially available human intestinal bacterial strains and elucidated the metabolism of soy isoflavone glucosides. The strains were classified into three categories, which were 14 facultative anaerobes, 13 obligate anaerobes, and 2 aerobes. Almost all facultative anaerobe strains metabolized soy isoflavone glucosides to their aglycones. The ratio of metabolism from glucoside to aglycone was different in each strain. Contrary to the facultative anaerobes, some of the obligate anaerobes did not metabolize soy isoflavone glucosides at all. Both the aerobic bacteria hardly metabolized soy isoflavone glucosides. The bacterial growth speed might show good correlation to the metabolizing speed of both glucosides. Therefore, the speed of metabolism would be different in each bacterial strain, too.

Keywords Soy isoflavonoid · Genistin · Daidzin · Metabolism · Human intestinal bacterial strain

R. Tsuchihashi · S. Sakamoto · M. Kodera · J. Kinjo (✉)
Faculty of Pharmaceutical Sciences, Fukuoka University,
8-19-1 Nanakuma, Fukuoka 814-0180, Japan
e-mail: kinjojun@fukuoka-u.ac.jp

T. Nohara
Faculty of Pharmaceutical Sciences, Kumamoto University,
5-1 Oehonmachi, Kumamoto 862-0973, Japan

Introduction

Some epidemiological studies have demonstrated that the intake of soy foods containing isoflavonoid phytoestrogens may reduce the risk of some hormone-dependent diseases, such as not only postmenopausal symptoms, but also certain cancers (breast, prostate, and colon) and cardiovascular diseases [1–4]. With the publicity given to the benefit of soy isoflavonoids for human health, the consumption of dietary supplements derived from soybeans has increased significantly.

When soy foods are consumed, the soy isoflavonoids (isoflavone glucosides) are metabolized into their aglycones by intestinal bacteria at first (Fig. 1). Then the metabolites are absorbed through the gastrointestinal membrane and are brought to the liver. Finally, they are further metabolized in the liver to yield various conjugated forms [5–7]. We have already reported about the structures of soy isoflavonoid metabolites excreted in human urine together with their estrogenic activities [8, 9]. In the first step, intestinal bacteria play an important role for the metabolism of soy isoflavone glucosides. They are greatly responsible for catabolism and scission of the isoflavonoids [10]. The reductive products of soy isoflavonoids, for example, equol and *O*-desmethylangolensin (DMA), showed stronger estrogenic activity than soy isoflavone aglycone (daidzein) itself [9, 11]. Therefore, it seems to be very important for elucidation of the soy isoflavonoid metabolism by intestinal bacteria.

Herein, we report the microbial transformation of soy isoflavone glucosides using 29 bacterial strains (14 strains of facultative anaerobe, 13 strains of obligate anaerobe, and 2 strains of aerobe) isolated from the human gastrointestinal tract.

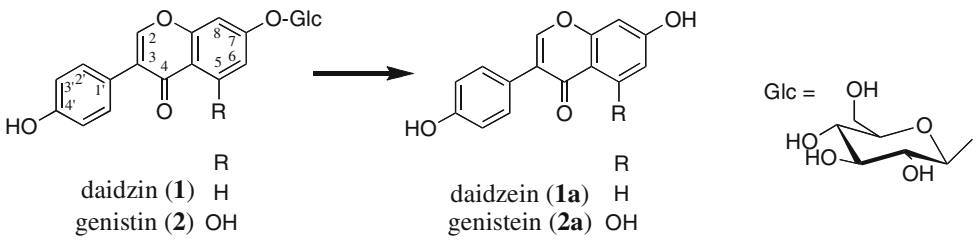

Fig. 1 Metabolic pathway of soy isoflavonoids by intestinal bacteria

Materials and methods

Bacterial strains

Bifidobacterium adolescentis JCM1275, *Bifidobacterium bifidum* JCM1254, *Bifidobacterium longum* JCM1217, *Bifidobacterium breve* JCM7016, *Bifidobacterium pseudocatenulatum* JCM1200, *Bifidobacterium pseudolongum* subsp. *pseudolongum* JCM1205, *Lactobacillus acidophilus* JCM1132, *Lactobacillus gasseri* JCM1131, *Lactobacillus reuteri* JCM1112, *Lactobacillus brevis* JCM1059, *Lactobacillus paracasei* subsp. *Paracasei* JCM1109, *Lactobacillus ermentum* JCM1560, *Streptococcus bovis* JCM7891, *Enterococcus faecalis* JCM5803, *Bacteroides vulgatus* JCM5826, *Bacteroides distasonis* JCM5825, *Bacteroides thetaiotaomicron* JCM5827, *Bacteroides ovatus* JCM5824, *Bacteroides fragilis* JCM11019, *Mitsuokella multacida* JCM2054, *Clostridium celatum* JCM1394, *Clostridium ramosum* JCM1298, *Clostridium spiroforme* JCM1432, *Peptostreptococcus productus* JCM1471, *Peptostreptococcus hydrogenalis* JCM7635, *Fusobacterium nucleatum* JCM6328, *Prevotella veroralis* JCM6290, *Klebsiella pneumoniae* JCM1662, and *Escherichia coli* JCM5491 were purchased from the Japan Collection of Microorganisms (RIKEN BioResource Center, Ibaraki, Japan).

Culture conditions

Each bacterial strain was maintained in general anaerobic medium (GAM) semisolid. It was inoculated into GAM broth (Nissui Co., Tokyo, Japan), then was continuously cultured at 37°C in the anaerobic chamber. The air was replaced by mixed gas (N_2 80%, CO_2 10%, H_2 10%) together with AnaeroPack Kenki (Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan) for anaerobic conditions. Each bacterial strain (1 ml) precultured for 24 h in GAM broth was inoculated into 10 ml of GAM broth containing 2 mg soy isoflavones (genistin and daidzin). The culture medium was incubated at 37°C for 24 h. The cultured medium was extracted three times with ethyl acetate, then was evaporated to dryness under reduced pressure. The residue was analyzed by HPLC.

HPLC analysis of metabolism

The HPLC system consisted of CCPM-II pump, UV-8020 UV detector (Tosoh, Tokyo, Japan). HPLC conditions were as follows: column: C_{18} reversed-phase column (Cadenza CD-C_{18} 3 μm, 150 mm × 4.6 mm i.d., Imtakt Co. Inc., Kyoto, Japan), solvent A: H_2O with 0.05% trifluoroacetic acid (TFA), solvent B: H_2O:CH_3CN (4:6) with 0.05% TFA, column temperature: 40°C, flow rate: 1 ml/min. Elution was done by the following process: solvent A: solvent B (7:3, 0 min) → solvent A: solvent B (35:65, 20 min). All of the samples were detected at 254 nm.

Time course experiments of metabolism

Some bacterial strains (0.1 ml) precultured for 24 h in GAM broth were inoculated into 1.1 ml of GAM broth containing 0.1 mg soy isoflavonoid (daidzin, genistin). The culture medium was incubated under anaerobic conditions in a similar manner as above for designated times (0, 3, 6, 12, and 24 h). The growth of bacteria was evaluated by turbidity (absorbance at 595 nm). Each cultured medium was purified over Oasis HLB cartridges (Waters, Milford, MA); the H_2O–MeOH (19:1) washing step was followed by 100% MeOH. The MeOH fr. was evaporated (under N_2 stream at 40°C), and the residue was taken up by 500 μl of H_2O–MeOH (1:9). The sample solution was analyzed by HPLC. HPLC conditions were as follows: column: C_{18} reversed-phase column (Cadenza CD-C_{18} 3 μm, 150 mm × 3.0 mm i.d., Imtakt), flow rate: 0.75 ml/min. The elution processes for daidzin was [solvent A: solvent B (75:25, 0 min) → solvent A: solvent B (7:93, 10 min)], for genistin was [solvent A: solvent B (70:30, 0 min) → solvent A: solvent B (0:100, 10 min) → solvent A: solvent B (0:100, 11 min)]. All other HPLC conditions were the same as above.

Results

Six strains of *Bifidobacterium* genus and *Lactobacillus* genus along with *Enterococcus faecalis* and *Streptococcus bovis* were used as a typical facultative anaerobe, which

was called lactic acid bacteria (Table 1). Almost all strains metabolized soy isoflavone glucosides to their aglycones, i.e., from genistin and daidzin to genistein and daidzein, respectively. However, one *Bifidobacterium* (*B. bifidum*) and three *Lactobacillus* (*L. brevis*, *L. ermentum* and *L. reuteri*) strains could not appreciably convert isoflavone glucosides to their aglycones. The ratio of metabolism from glucoside to aglycone was different in each strain of facultative anaerobe. Especially *Lactobacillus gasseri* showed 100% productive rate from genistin to genistein, although that from daidzin to daidzein was 60%. This strain seems to recognize the structural difference between genistin and daidzin.

Table 1 Microbial metabolism of soy isoflavone glucosides by intestinal bacterial strains

	Yield (%) of metabolites	
	Daidzein (**1a**)	Genistein (**2a**)
Facultative anaerobe		
Bifidobacterium adolescentis	100	100
B. bifidum	13	6
B. breve	100	100
B. longum	55	45
B. pseudocatenulatum	98	100
B. pseudolongum subsp. *pseudolongum*	19	23
Enterococcus faecalis	100	100
Lactobacillus acidophilus	100	100
L. brevis	12	13
L. ermentum	0	7
L. gasseri	60	100
L. paracasei subsp. *paracasei*	100	100
L. reuteri	1	2
Streptococcus bovis	100	100
Obligate anaerobe		
Bacteroides distasonis	100	100
B. vulgatus	0	0
B. fragilis	100	87
B. ovatus	100	97
B. thetaiotaomicron	80	35
Clostridium celatum	35	14
C. ramosum	96	100
C. spiroforme	0	0
Fusobacterium nucleatum	0	0
Mitsuokella multacida	7	7
Peptostreptococcus hydrogenalis	0	0
P. productus	96	100
Prevotella veroralis	14	6
Aerobe		
Escherichia coli	0	0
Klebsiella pneumoniae	1	0

Five strains of *Bacteroides* genus, three strains of *Clostridium* genus, and two strains of *Peptostreptococcus* genus along with *Fusobacterium nucleatum, Mitsuokella multacida* and *Prevotella veroralis* were used as a typical obligate anaerobes (Table 1). Contrary to the facultative anaerobe, some of obligate anaerobes (*B. vulgatus, C. spiroforme, F. nucleatum*, and *Peptostreptococcus hydrogenalis*) did not metabolize soy isoflavone glucosides at all. In addition, *M. multacida* and *Prevotella veroralis* could not appreciably convert into aglycones. Contrary to *Lactbacillus gasseri* of the facultative anaerobes, *Bacteroides thetaiotaomicron* showed a higher productive rate to daidzein than to genistein. Although the total yield of aglycones was not good, *Clostridium celatum* produced more daidzein than genistein.

Klebsiella pneumoniae and *Escherichia coli* were used as typical aerobes. Both the aerobic bacteria hardly metabolized soy isoflavone glucosides.

Bifidobacteriumadolescentis,Lactobacillus acidophilus, *Bacteroides distasonis*, *Clostridium ramosum*, and *Peptostreptococcusproductus* were used for the time course experiments (Fig. 2) of soy isoflavone metabolism since these strains converted to aglycones in good yield. The order of growth speed was *Bifidobacteriumadolescentis* > *Clostridium ramosum* >> *Peptostreptococcusproductus* >> *Bacteroides distasonis* > *Lactobacillus acidophilus* (Fig. 2c), although the saturated time for growth of each bacterial strain was different. In the hydrolysis, *Bifidobacteriumadolescentis* and *Clostridium ramosum* metabolized both glucosides within 6 h (Figs. 2a, b). On the other hand, *Lactobacillus acidophilus* and *Bacteroides distasonis* metabolized very slowly (within 24 h). *Peptostreptococcusproductus* showed moderate activity. Therefore, it seemed that the bacterial growth speed showed good correlation to the metabolizing speed of both glucosides.

Discussion

These results indicated that the metabolism from soy isoflavone glucosides to their aglycones was performed by various bacterial strains except for aerobes (Table 1). However, Hur et al. [12] reported that *Escherichia coli* of aerobes metabolized daidzin and genistin into their aglycones. It might be caused by the difference between strain levels, even if the bacteria had the same scientific name. The difference between facultative anaerobes and obligate anaerobes was not largely observed. Many microbial enzymes were found to possess broad substrate specificity, yet they catalyze reactions with a high degree of regio- and stereospecificity [13]. Some of the bacterial strains

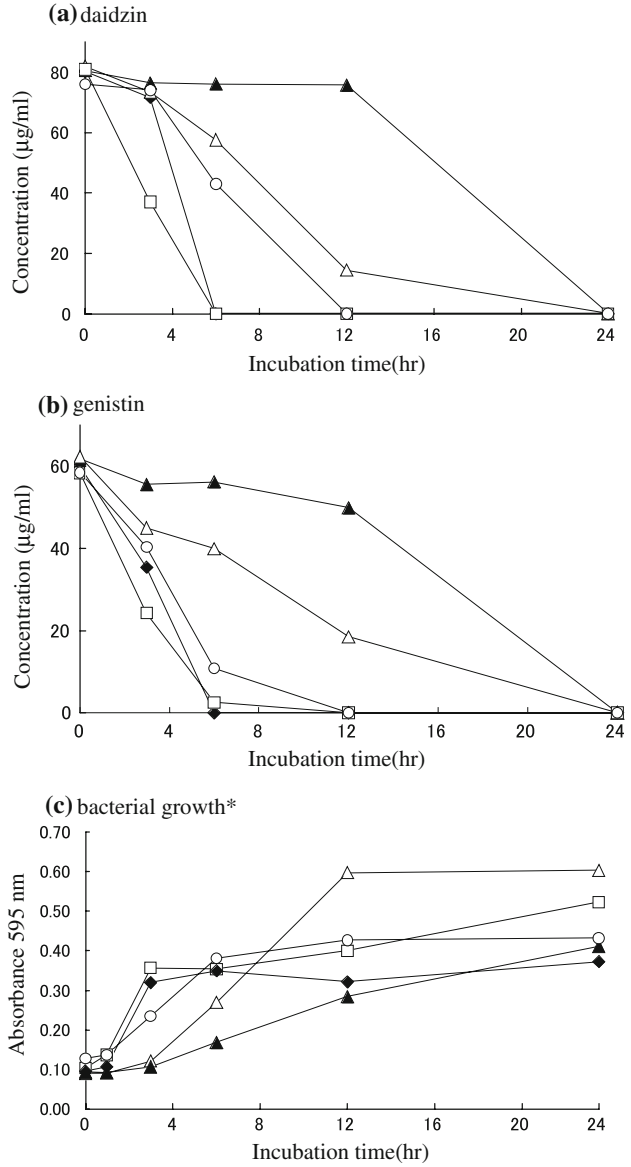

Fig. 2 Time course experiments of soy isoflavone metabolism by several bacterial strains. (**a**) Daidzin, (**b**) genistin, (**c**) bacterial growth,* *Bifidobacterium adolescentis* (*open square*); *Lactobacillus acidophilus* (*filled triangle*); *Bacteroides distasonis* (*open triangle*); *Clostridium ramosum* (*filled diamond*); *Peptostreptococcus productus* (*open circle*). *The growth of bacteria was evaluated by turbidity (absorbance at 595 nm)

(*Lactbacillus gasseri*, *Bacteroides thetaiotaomicron*, and *Clostridium celatum*) showed specific hydrolysis for the glucosidic unit of daidzin rather than that of genistin (Table 1). However, no other reaction, such as reduction or scission, was observed in this experiment. Namely, dihydrodaidzein, equol, *O*-DMA, dihydrogenistein, 5-hydroxyequol, and 6-hydroxy-*O*-DMA were not detected in the metabolic studies. Therefore, the metabolic enzyme in these bacterial strains might have the nature of glucosidase only. In the time course experiments of soy isoflavone metabolism (Fig. 2), *Bifidobacteriumadolescentis* of facultative anaerobes and *Clostridium ramosum* of obligate anaerobes metabolized more rapidly than the other strains, whereas the metabolizing speed of *Lactobacillus acidophilus* of facultative anaerobes and *Bacteroides distasonis* of obligate anaerobes was very slow. The order of metabolizing speed would be parallel to that of the growth speed of each bacterial strain(Fig. 2c).

It was known that the *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* genus colonized in the upper gastrointestinal tract, for example, the oral cavity, stomach, and duodenum. Therefore, soy isoflavone glucosides would be metabolized in the early stage after administration. The greater part of soy isoflavone aglycones could be absorbed before further reductive reaction, since the reductive product appeared later than isoflavone aglycone in plasma [14, 15]. Consequently, long-term incubation in consideration of enterohepatic circulation may be needed to obtain the reductive products of soy isoflavone aglycones [16, 17]. Moreover, since the flora constitution of intestinal bacteria was different in each individual [18, 19], combination culture using some bacterial strains [20] might be usuful to obtain the reductive products.

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