Administration of Equol-Producing Bacteria Alters the Equol Production Status in the Simulator of the Gastrointestinal Microbial Ecosystem (SHIME)

Karel Decroos, Ellen Eeckhaut, Sam Possemiers, and Willy Verstraete

Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

ABSTRACT  The intestinal microbial transformation of daidzein, one of the principal isoflavones from soy, into the isoflavon equol is subjected to a high interindividual variability. The latter compound is considered to have a higher biological activity than its precursor; hence, there is interest in dietary applications that modulate this important biotransformation. In 2 separate experiments, we administered a mixed microbial culture (EPC4), which we had isolated previously and which efficiently transforms daidzein into equol, to the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). The SHIME was fed soy germ powder and inoculated with fecal samples from two nonequol-producing individuals. Equol production was induced in the distal colon compartments in both experiments, 5–6 d after the start of the treatment; 2 wk after interrupting the addition of EPC4, equol was still produced in high amounts. There are large interregional differences in daidzein metabolism in the simulated colon. Furthermore, no major shifts in the composition and activity of the microbial communities were caused by the supplementation with the microbial consortium. Although further confirmation in vivo is required, these results validate the concept that administering EPC4 could constitute a novel means for converting a nonequol-producer into a producer. J. Nutr. 136: 946–952, 2006.

KEY WORDS: • equol • daidzein • gut bacteria • SHIME • soy germ

Equol has received much attention recently because its biological activities differ from those of its precursor: it has higher estrogenicity (10), is a stronger antioxidant (11), and demonstrates antiandrogenic properties (12). Additionally, equol is easily absorbed through the colon wall, has a slower plasma clearance rate than daidzein, and is metabolically inert (13). However, only 30–50% of the Western population excrete significant amounts of equol (14,15). This fact was suggested as an explanation for the sometimes conflicting results obtained in the past from dietary intervention studies with isoflavones (13). Indeed, some reports suggested a lower disease risk for equol-producers than nonproducers (16–18). However, this is not fully proven and has been contradicted (19,20); more experimental data are necessary to explore the relation between equol production and health effects (21). Because equol was found to be formed exclusively by intestinal bacteria (22), its production in humans may depend on the presence of certain bacterial strains. A number of strains involved in daidzein metabolism were identified (23–25) and recently, we isolated a microbial consortium that catalyzes the conversion from daidzein into equol (26).

The composition and the activity of the gut microbiota are greatly predisposed by diet composition (27). Hence, it can be expected that dietary habits will influence equol production. However, previous reports have given conflicting results at times and have not yet led to the establishment of generic interpretations, if these exist (14,28–31). There is a growing interest in dietary applications that modulate equol production.
in humans. It was suggested that the use of functional foods containing certain bacteria could influence the equol production status of an individual (13). However, in 2 studies it was that reported the administration of probiotic lactic acid bacteria did not affect equol excretion in humans (32, 33). Until the present, no food applications converting a nonequol-producer into a producer have been reported.

The research goal of this study was to investigate the influence of the supplementation of equol-producing bacteria on daidzein metabolism, the general composition and activity of a nonequol-producing intestinal microbial community. For this, we supplemented a microbial consortium (EPC4) efficiently transforming daidzein into equol, which we had isolated previously (26), to the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) inoculated with fecal samples originating from nonequol-producing individuals.

MATERIALS AND METHODS

Simulator of the Human Intestinal Microbial Ecosystem. The reactor set-up was adapted from the SHIME, representing the gastrointestinal tract of an adult human, as described by Molly et al. (34). The SHIME consists of a succession of 5 reactors representing the different parts of the human gastrointestinal tract, i.e., the stomach, small intestine, ascending colon compartment (proximal colon compartment), and transverse and descending colon compartment (distal colon compartments). The first 2 reactors work on the fill-and-draw principle, simulating different steps in food uptake and digestion, with peristaltic pumps adding a defined amount of SHIME feed (3 × 10^8 mL/d) and pancreatic and bile liquid (3 × 10^6 mL/d) and emptying the respective reactors after specified intervals. The last 3 compartments are continuously stirred reactors with a constant volume and pH control. A schematic representation of the SHIME was presented by De Boever et al. (35). Retention time, pH, and temperature settings were described previously by Possemiers et al. (26).

Microbial strains and culture conditions. The bacterial strains used in this study were previously isolated from a human fecal sample at our laboratory (26). This stable mixed culture, which efficiently transforms daidzein into equol, consists of 4 dominant bacterial strains: Enterococcus faecium EPI1, Lactobacillus mucosae EPI2, Finegoldiella magna EPI13, and a Veillonella sp.-related strain EP. The culture was obtained this way was denoted Fecal Inoculum (FI)-1 and 2. Part of the freshly voided samples was processed and assayed for daidzein metabolism as described by Boon et al. (35) before the start of the experiment. The feed remained unchanged throughout the entire SHIME-run and contained ~175 mg/L isoflavones, of which 75 mg/L (175 μmol/L) had daidzein as aglycone. In this way, 41.3 μmol of daidzein equivalents were delivered daily to the SHIME. First, there was a 2-wk baseline period in which only the feed was dosed to the SHIME. This was followed by a 2-wk EPC4 inoculation period in which EPC4 was supplemented daily to the ascending colon compartment. Therefore, 10 mL of a 48-h-old culture of EPC4, whose OD at 590 nm was adapted to 0.5 with sterile brain heart infusion, corresponding to a total of 10^8 bacterial cells, was injected into reactor 3 of the SHIME. Finally, there was a postinoculation period in which no bacteria were supplemented and the SHIME was simply fed with standard SHIME feed, supplemented with 7 g/L soy germ powder. During the different phases, samples from the different colon reactors were taken at different time intervals and analyzed for daidzein and daidzein metabolites, short-chain fatty acids (SCFA) and DNA extraction. The total sampling volume that was retrieved from each compartment amounted to a maximum of 8 mL/d, which is <1.5% of the volume of each compartment. This did not affect the composition or activity of the SHIME.

SCFA determination. Liquid samples (2 mL) from each colon reactor were collected and frozen at −20°C for subsequent analysis. The SCFA were extracted from the samples with diethyl ether and determined with GC as described by Nollet et al. (38).

DNA extraction and RT-PCR. Total DNA extractions of 1 mL liquid SHIME sample were performed following the method described by Boon et al. (39). Using an ABI Prism SDS 7000 instrument (Applied Biosystems), general bacterial DNA as well as specific DNA from Lactobacillus spp., Bifidobacterium spp., and Enterococcus faecium EPC4, whose OD at 590 nm was adapted to 0.5 with sterile brain heart infusion, were analyzed for daidzein and daidzein metabolites, short-chain fatty acids (SCFA) and DNA extraction. The total sampling volume that was retrieved from each compartment amounted to a maximum of 8 mL/d, which is <1.5% of the volume of each compartment. This did not affect the composition or activity of the SHIME.

HPLC analysis. Two different HPLC methods were used in this study. For the extraction and analysis of daidzein, dihydrodaidzein, O-DMA, and equol the protocol described by Decroos et al. (26) was used. For the extraction of 6-O-malonyl-daidzein, 6-O-acetyl-daidzein, and

Experimental set-up. Two identical SHIME-runs were performed with 2 different inocula: IF-1 and IF-2. A schematic representation of the experimental set-up is given in Figure 1. After reactor start-up, the system was allowed to stabilize for 3 wk with standard SHIME feed (37) supplemented with 7 g/L soy germ powder [Soylife™micro25, Acatris Holding BV]; the composition is described in De Boever et al. (35) before the start of the experiment. The feed remained unchanged throughout the entire SHIME-run and contained ~175 mg/L isoflavones, of which 75 mg/L (175 μmol/L) had daidzein as aglycone. In this way, 41.3 μmol of daidzein equivalents were delivered daily to the SHIME. First, there was a 2-wk baseline period in which only the feed was dosed to the SHIME. This was followed by a 2-wk EPC4 inoculation period in which EPC4 was supplemented daily to the ascending colon compartment. Therefore, 10 mL of a 48-h-old culture of EPC4, whose OD at 590 nm was adapted to 0.5 with sterile brain heart infusion, corresponding to a total of 10^8 bacterial cells, was injected into reactor 3 of the SHIME. Finally, there was a postinoculation period in which no bacteria were supplemented and the SHIME was simply fed with standard SHIME feed, supplemented with 7 g/L soy germ powder. During the different phases, samples from the different colon reactors were taken at different time intervals and analyzed for daidzein and daidzein metabolites, short-chain fatty acids (SCFA) and DNA extraction. The total sampling volume that was retrieved from each compartment amounted to a maximum of 8 mL/d, which is <1.5% of the volume of each compartment. This did not affect the composition or activity of the SHIME.

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and daidzin, 1 mL of sample was diluted with ethanol to 70% (v:v) ethanol and then centrifuged for 5 min at 10000 × g. The supernatant was collected and evaporated completely under a flow of N₂ at 37°C. The dried extract was dissolved in 1 mL ethanol:dimethyl sulfoxide (1:1), and samples were analyzed with the protocol described in De Boever et al. (41). An analytical standard for daidzin was kindly provided by Acatris Holding BV. The molar extinction coefficient that was determined for daidzin was used for quantification of malonyl- and acetyl-daidzin because these are very similar (42).

Statistical analysis. A comparison of the mean equol concentration at specific time points between 2 colon compartments within 1 SHIME-run was carried out using Student’s t test. Data for daidzin metabolites, SCFA concentration, and microbial community structure were grouped per period and evaluated by 2-way ANOVA using period and colon compartment as main effects with testing for interactions (period × colon compartment). When significant effects or interactions were observed, means for different periods within 1 compartment and for different compartments within 1 period were compared using 1-way ANOVA and Duncan’s post hoc test. For the daidzin metabolite concentrations, the criterion of homoscedasticy was not fulfilled. Therefore, data were evaluated using Weighted Least Square ANOVA, with a weight equal to the inverse of the variance assigned to each variable. When data were below the detection limit, we assigned a random value between 0 and the detection limit, which was 0.85, 0.96, 1.2, and 0.74 µmol/L for daidzin, DHD, equol, and O-DMA, respectively. Differences were considered significant at P < 0.05. Values in the text are means ± SD. All statistical analyses were performed using SPSS 12.0 for Windows software.

RESULTS

Resistance to acid and bile stress. After incubation under simulated gastric or small-intestinal conditions, EPC4 could not produce equol, although a fraction of the bacteria was still viable as determined by plate counts (data not shown). Therefore, we decided to add the bacteria immediately to the ascending colon compartment of the SHIME during further experiments.

Daidzin metabolism of the fecal inocula in batch tests. Before the start-up of the SHIME, the capacity of the 2 fecal samples (further used to inoculate the SHIME) to metabolize daidzin was tested in batch (Table 1). FI-1 produced DHD and O-DMA, whereas FI-2 produced only DHD. FI-2 degraded more daidzin than FI-1, i.e., 33 ± 3 and 45 ± 5% of the originally added amount of daidzin was transformed for FI-1 and FI-2, respectively.

Microbial metabolism of daidzin in the SHIME. The concentrations of daidzin, DHD, O-DMA, and equol in the different colon compartments were monitored during the SHIME-runs with FI-1 and FI-2 (Fig. 2). In the ascending colon compartment (Fig. 2A and C), no equol was detected during the 2 experiments. Equol was not detected in either the transverse or descending colon compartments (Fig. 2B, C, E and F) during the baseline period in both the runs with FI-1 and FI-2. At d 20 (6 d after start of the EPC4 inoculation), equol appeared rapidly in the transverse colon compartment (Fig. 2B and E) to reach a more or less constant concentration at d 23, varying between 124 ± 12 and 158 ± 8 µmol/L (FI-1) and 146 ± 6 and 167 ± 14 µmol/L (FI-2) until the end of the experiment. In the descending colon compartment (Fig. 2C and F), equol appeared as well, but as a consequence of the sequential reactor set-up, the increase in equol concentration was delayed ~1 d compared with the transverse colon compartment. When the equol concentrations at each time point were compared between the transverse and descending colon compartment within the same SHIME-run, equol concentrations were significantly lower (P ≤ 0.035) in the descending colon compartment from d 20–23 for both SHIME-runs. At every other time point, equol concentrations did not differ (P ≥ 0.087). Coinciding with the appearance of equol, both daidzin and DHD concentrations decreased and remained negligible until d 42 for both experiments.

When the equol concentrations were grouped per period of the SHIME-runs (Table 2), equol was produced only in the distal regions of the colon, and only during and after the inoculation with EPC4. O-DMA also was produced exclusively in the transverse and descending colon, except for a small amount that was detected in the ascending colon during the postinoculation period with FI-1. During the SHIME-run with FI-1, the O-DMA concentration was not significantly affected by EPC4 inoculation in the transverse (P = 0.061) and descending colon (P = 0.351), whereas during the postinoculation period, it was reduced significantly in both compartments, compared with the baseline and EPC4 inoculation period. During the run with FI-2, O-DMA was detected only during the baseline period; during and after the EPC4 inoculation period, O-DMA was completely repressed. During both SHIME-runs, the concentrations of the different daidzin metabolites were not significantly different in the transverse and descending colon compartments (P > 0.078), except for O-DMA in the baseline period of the run with FI-1 (P = 0.009).

In the ascending colon compartment, only daidzin was present during the baseline period of both SHIME-runs (Table 2). Daidzin concentrations had decreased significantly in this compartment in the postinoculation period of the run with FI-1 and already in the EPC4 inoculation period of the run with FI-2, in favor of the DHD concentration. Daidzin was present in the transverse and descending colon compartments only during the baseline period. Inoculation with EPC4 caused complete conversion of daidzin into equol, and this effect lasted until the end of the experiment.

Daidzin metabolism over the entire gastrointestinal tract. The sum of all of the daidzin-related compounds did not differ over the whole simulated gastrointestinal tract for both the runs with FI-1 (P = 0.06, 158 ± 17 µmol/L) and FI-2 (P = 0.19, 152 ± 12 µmol/L). In both SHIME-runs, a shift occurred from the malonyl- and acetyl-glycosylated and glycosylated forms in the feed toward the aglycone in the duodenum and ascending colon compartments and further to O-DMA and equol in the distal colon parts. In the descending colon, equol accounted for 79 ± 4% and O-DMA for 19 ± 4% (on a molar basis) of the daidzin equivalents administered to the SHIME through the feed in the run with FI-1. In the run with FI-2, 99 ± 6% of the administered daidzin was found back as equol in the descending colon.

Production of SCFA. The concentrations of SCFA were measured during the different periods of the SHIME-runs in the different colon compartments of the SHIME (Table 3). The
most important feature that was noted was the significant increase in butyrate production in both SHIME-runs in all of the colon vessels during the EPC4 inoculation period, which lasted into the postinoculation period. In the run with FI-2, this was associated with a decrease in propionate concentrations in all colon compartments.

Microbial community structure. The stability of the microbial community in each colon compartment was assessed by quantifying the abundances of different important microbial groups by RT-PCR in the middle and at the end of each period of the SHIME-run. The abundances of *Bifidobacterium* spp., *Atopobium* spp., and the *Clostridium coccoides-Eubacterium*}

### TABLE 2

Concentrations of daidzein, DHD, O-DMA, and equol in the different colonic compartments of the SHIME, inoculated with FI-1 and FI-2 during baseline, EPC4 inoculation, and postinoculation.\(^1\,2\)

<table>
<thead>
<tr>
<th></th>
<th>FI-1 Ascending</th>
<th>FI-1 Transverse</th>
<th>FI-1 Descending</th>
<th>FI-2 Ascending</th>
<th>FI-2 Transverse</th>
<th>FI-2 Descending</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daidzein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>158 ± 12(^a)</td>
<td>22 ± 6(^b)</td>
<td>26 ± 6(^b)</td>
<td>136 ± 5(^d)</td>
<td>107 ± 3(^c)</td>
<td>105 ± 2°</td>
</tr>
<tr>
<td>EPC4 inoculation</td>
<td>149 ± 4(^d)</td>
<td>2 ± 1(^a)</td>
<td>4 ± 3(^a)</td>
<td>81 ± 27(^b)</td>
<td>3 ± 4(^a)</td>
<td>3 ± 7(^a)</td>
</tr>
<tr>
<td>Postinoculation</td>
<td>91 ± 3(^c)</td>
<td>5 ± 6(^a)</td>
<td>2 ± 3(^a)</td>
<td>58 ± 3(^b)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
</tr>
<tr>
<td><strong>DHD</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>ND(^a)</td>
<td>69 ± 7(^b)</td>
<td>65 ± 4(^c)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>5 ± 5(^a)</td>
</tr>
<tr>
<td>EPC4 inoculation</td>
<td>ND(^a)</td>
<td>1 ± 3(^a)</td>
<td>6 ± 9(^a)</td>
<td>62 ± 28(^b)</td>
<td>ND(^a)</td>
<td>1 ± 3(^a)</td>
</tr>
<tr>
<td>Postinoculation</td>
<td>46 ± 27(^b)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>92 ± 6(^c)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
</tr>
<tr>
<td><strong>Equol</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
</tr>
<tr>
<td>EPC4 inoculation</td>
<td>131 ± 11(^b)</td>
<td>122 ± 18(^b)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>143 ± 24(^b)</td>
<td>127 ± 33(^b)</td>
</tr>
<tr>
<td>Postinoculation</td>
<td>ND(^a)</td>
<td>140 ± 9(^c)</td>
<td>146 ± 10(^c)</td>
<td>ND(^a)</td>
<td>157 ± 7(^b)</td>
<td>156 ± 7(^b)</td>
</tr>
<tr>
<td><strong>O-DMA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0 ± 1(^b)</td>
<td>34 ± 6(^a)</td>
<td>40 ± 13(^d)</td>
<td>ND(^a)</td>
<td>15 ± 4(^d)</td>
<td>9 ± 2(^c)</td>
</tr>
<tr>
<td>EPC4 inoculation</td>
<td>1 ± 1(^a)</td>
<td>27 ± 10(^c)</td>
<td>35 ± 7(^d)</td>
<td>ND(^a)</td>
<td>4 ± 6(^ab)</td>
<td>5 ± 5(^a)</td>
</tr>
<tr>
<td>Postinoculation</td>
<td>9 ± 3(^b)</td>
<td>17 ± 3(^b)</td>
<td>16 ± 4(^d)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
<td>ND(^a)</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD, n = 5, for the last week of each period (wk 2 for the baseline, wk 4 for the EPC4 inoculation and wk 6 for the post-inoculation period). For each variable within a SHIME-run, means with superscripts without a common letter differ, *P* < 0.05.

2 For all variables, period, compartment, and period × compartment were significant (*P* < 0.001), except for compartment on DHD in FI-1 (*P* = 0.147).

3 ND, not detected.
TABLE 3
SCFA concentrations in the different colonic compartments of the SHIME, inoculated with FI-1 and FI-2 at baseline, EPC4 inoculation and postinoculation1,2

<table>
<thead>
<tr>
<th></th>
<th>Acetic acid</th>
<th>Propionic acid</th>
<th>Butyric acid</th>
<th>Other acids</th>
<th>Total SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
</tr>
<tr>
<td></td>
<td>Ascending colon</td>
<td>Transverse colon</td>
<td>Descending colon</td>
<td>P</td>
<td>Ascending colon</td>
</tr>
<tr>
<td>Acetic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>26.7 ± 2.1a</td>
<td>45.9 ± 3.8b</td>
<td>54.4 ± 2.0c</td>
<td>0.109d</td>
<td>40.3 ± 2.4a</td>
</tr>
<tr>
<td>EPC4 inoculation</td>
<td>28.8 ± 2.4a</td>
<td>49.4 ± 3.8b</td>
<td>55.6 ± 3.5c</td>
<td>&lt;0.001e</td>
<td>36.8 ± 3.4a</td>
</tr>
<tr>
<td>Postinoculation</td>
<td>28.9 ± 1.9a</td>
<td>48.3 ± 2.3b</td>
<td>54.7 ± 2.6c</td>
<td>0.898f</td>
<td>38.5 ± 3.6a</td>
</tr>
<tr>
<td>Propionic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9.7 ± 0.8a</td>
<td>18.0 ± 1.8b</td>
<td>18.4 ± 1.0d</td>
<td>0.173d</td>
<td>7.8 ± 1.5a</td>
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<tr>
<td>EPC4 inoculation</td>
<td>9.9 ± 0.8a</td>
<td>19.2 ± 1.8b</td>
<td>18.2 ± 1.3c</td>
<td>&lt;0.001f</td>
<td>6.2 ± 1.5a</td>
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<tr>
<td>Postinoculation</td>
<td>10.6 ± 1.0a</td>
<td>19.8 ± 1.0b</td>
<td>18.7 ± 0.6b</td>
<td>0.728e</td>
<td>6.3 ± 0.9a</td>
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<tr>
<td>Butyric acid</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.6 ± 0.5a</td>
<td>8.3 ± 1.7b</td>
<td>9.1 ± 1.4b</td>
<td>&lt;0.001d</td>
<td>6.3 ± 0.7a</td>
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<tr>
<td>EPC4 inoculation</td>
<td>8.0 ± 0.6a</td>
<td>11.5 ± 2.7c</td>
<td>12.0 ± 1.4e</td>
<td>&lt;0.001b</td>
<td>10.1 ± 1.0b</td>
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<tr>
<td>Postinoculation</td>
<td>9.1 ± 1.1c</td>
<td>14.8 ± 0.8a</td>
<td>13.9 ± 0.9d</td>
<td>0.430d</td>
<td>10.2 ± 0.7b</td>
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<tr>
<td>Other acids</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>0.9 ± 0.1a</td>
<td>2.8 ± 0.3c</td>
<td>2.0 ± 0.5b</td>
<td>&lt;0.001e</td>
<td>0.3 ± 0.1a</td>
</tr>
<tr>
<td>EPC4 inoculation</td>
<td>0.9 ± 0.1a</td>
<td>3.0 ± 0.3b</td>
<td>3.0 ± 0.2e</td>
<td>&lt;0.001f</td>
<td>0.2 ± 0.1a</td>
</tr>
<tr>
<td>Postinoculation</td>
<td>1.0 ± 0.2a</td>
<td>3.0 ± 0.2b</td>
<td>3.0 ± 0.2e</td>
<td>&lt;0.001f</td>
<td>0.1 ± 0.1a</td>
</tr>
<tr>
<td>Total SCFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>42.9 ± 3.0a</td>
<td>74.9 ± 4.7c</td>
<td>83.8 ± 2.9abc</td>
<td>&lt;0.001f</td>
<td>54.7 ± 2.1a</td>
</tr>
<tr>
<td>EPC4 inoculation</td>
<td>47.6 ± 2.9ab</td>
<td>83.2 ± 8.1a</td>
<td>88.9 ± 5.1abc</td>
<td>&lt;0.001c</td>
<td>53.2 ± 3.9a</td>
</tr>
<tr>
<td>Postinoculation</td>
<td>49.6 ± 3.2ab</td>
<td>85.8 ± 3.5de</td>
<td>90.3 ± 3.3a</td>
<td>0.831f</td>
<td>55.1 ± 3.2a</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD, n = 5, for the last week of each period (wk 2 for the baseline, wk 4 for the EPC4 inoculation and wk 6 for the post-inoculation period). For each variable within a SHIME-run, means with superscripts without a common letter differ, P < 0.05.
2 P-values for the effects of period (‘a’), compartment (‘b’) and period × compartment (‘c’).
rectal group did not shift significantly during the 2 SHIME-runs. The number of Lactobacillus spp. targets increased significantly in both runs by 0.7 to 1.1 log-units during EPC4 inoculation. In the postinoculation period, this decreased non-significantly by 0.3 to 0.5 log-units (P = 0.072) compared to the EPC4 inoculation.

DISCUSSION

In the present study, we investigated the influence of the administration of equol-producing bacteria on daidzein metabolism and the general composition and activity of the intestinal microbial community under simulated gastrointestinal conditions. To achieve this, we administered daily a previously isolated microbial consortium (EPC4) efficiently transforming daidzein into equol (26), to the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) inoculated with fecal sample from 2 nonequol-producing individuals in 2 separate experiments. We found that supplementation with EPC4 effectively induced equol production in both cases.

The results presented here are a first “proof of principle” that supplementation with equol-producing bacteria can constitute a novel way to induce or enhance equol production in situ. For a number of reasons, it is was suggested that the presence of equol is an important factor in the positive clinical effects of a diet rich in soy (13). Although this is not yet fully supported by experimental data, and the health benefits of equol production remain subject to further investigation (21), there is much interest in food applications that modulate the intestinal production of equol. Here, we showed that supplementation with EPC4, an equol-producing bacterial consortium we isolated from a human fecal sample, can convert a nonequol-producing into an equol-producing intestinal microbial community in vitro. This indicates that the equol-producing bacteria from EPC4 are able to survive in a complex microbial community and beyond that, maintain their metabolic activity. They were able to proliferate efficiently in the simulated colon because equol production was maintained up to 2 wk after ending the treatment.

Equol was detected only in the transverse and descending colon compartments. The net production was limited to the transverse colon compartment, but it cannot be excluded that this could also take place in the descending colon compartment because all of the daidzein was already consumed in the former compartment. This was confirmed in batch tests with suspensions drawn from the descending colon compartment to which daidzein was supplemented and in which equol was produced after 24 h of anaerobic incubation (data not shown). The equol present in the descending colon compartment at concentrations similar to those in the previous reactor was the result of hydraulic overflow and further substantiated the resistance of equol to further bacterial breakdown (13,26). The manifestation of equol production not earlier than the transverse colon is probably due to the environmental conditions that are necessary for growth and metabolic activity of equol-producing bacteria. The most plausible determining factor would be the redox potential which decreases progressing in the colon to −250 mV in vivo (43) and from −190 mV in the ascending colon compartment to −240 mV in the descending colon compartment in the SHIME (this study). The strictly anaerobic character of equol-producing bacteria was suggested earlier (44), and a recently reported equol-producing isolate was also strictly anaerobic (25). The fact that equol was produced only in the distal colon compartments is in good agreement with in vivo data on plasma concentrations of equol. Zubik et al. (45)
found that peak plasma concentrations appeared 24 h postprandially after a single soy challenge; this is the time in vivo after ingestion in which food reaches the distal parts of the colon (46). In addition, data on peaks in urinary excretion of equol suggest formation of the compound in more distal parts of the colon (47,48).

The inter-regional differences in colonic environmental conditions were well reflected in the daidzein metabolism in both SHIME-runs. In the proximal colon compartment (ascending colon), daidzein metabolism was limited. This part of the colon is characterized by a higher redox potential, higher carbohydrate concentration, and lower pH than the distal parts (27). The high redox potential could be a limiting factor for reductive metabolism, and carbohydrates were already shown to inhibit daidzein degradation (49) and equol production (26,50). Like equol, O- DMA was produced only in the distal colon compartments. Because all bacterial strains able to form O-DMA isolated to date are anaerobic (51,52), this is probably due to the lower redox potential in distal colon regions.

Thus far, bacteria that target specific transformations of certain compounds related to beneficial health effects have not been considered for human consumption. However, because knowledge about the complex processes in the colon is increasing, the latter could be next-generation bacterial supplements. Because EPC4 could not maintain its equol-producing capacity under the acidic and biliary stress conditions in the stomach and small intestine, it could be limited in its potential application as an oral supplement. However, this could be circumvented by specific encapsulation of the strains, releasing them not earlier than the colon (53). Although all of the strains present in EPC4 are normal inhabitants of a healthy intestinal microbiota, the safety of supplementing these bacteria, which are not generally recognized as safe (GRAS) to humans, must be investigated thoroughly. As far as it can be derived from the data obtained in this work, administration of EPC4 did not have adverse effects on the general composition and activity of the microbial community. On the contrary, inoculation with EPC4 increased the Lactobacillus population, probably as a consequence of the presence of Lactobacillus mucosae EP1 in EPC4, and also of Enterococcus faecium EP2, which can lower the intestinal bioactivation or degradation of phytoestrogens and similar compounds. This effect was maintained beyond the period of inoculation (54). Because all bacterial strains able to form O-DMA isolated to date are anaerobic (51,52), this is probably due to the lower redox potential in distal colon regions.

In conclusion, we found that administration of equol-producing bacteria to a dynamic in vitro model of the gastrointestinal tract, inoculated with a nonequol-producing fecal sample, resulted in the formation of equol in the distal colon parts. This effect was maintained beyond the period of inoculum administration. Although further mechanistic studies and experiments with in vivo models are necessary to explore the efficacy and safety of EPC4, these results comprise a first validation of the concept that equol-producing bacteria can be used as a novel means for the induction or enhancement of equol production in situ. Furthermore, the SHIME was an excellent tool for the study of the intestinal metabolism of daidzein, revealing inter-regional differences in colonic biotransformation processes that can be explicable for in vivo data on urinary and plasma concentrations of daidzein metabolites. This suggests that the SHIME can be used successfully for mechanistic studies on the intestinal bioactivation or degradation of phytoestrogens and similar compounds.

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