Influence of Temperature on Short-Chain Fatty Acid Production by Pig Cecal Bacteria In Vitro

Daisuke KOBAYASHI and Takashi SAKATA*

Department of Basic Sciences, Ishinomaki Senshu University,
1 Minamisakai Shinmito, Ishinomaki, Miyagi 986–8580, Japan
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Summary We studied the effect of incubation temperature on the production of short-chain fatty acids (SCFA) by pig cecal bacteria in vitro in order to assess short-term influences of body temperature on bacterial metabolism in the large intestine. We employed a 200 mL scale continuous culture system using cecal bacteria from commercially slaughtered pigs as inoculum. The culture was maintained at 30, 37, 40 or 42°C and continuously diluted by continuous feeding of bicarbonate buffer (pH 7.4) added with lactose (10 g/L) and by simultaneous continuous efflux both at 4.17 mL/h. We monitored SCFA concentration of the culture for 12 h, which represents their production rate. Concentrations of SCFA increased during the first several hours and plateaued at around 11 h of incubation. Incubation temperature significantly affected mean concentrations from 1 to 12 h of acetic (40°C > 37°C > 30°C), propionic (40°C > 42°C > 30°C), n-butyric (42°C > 37°C > 30°C, 40°C > 30°C) and n-valeric (42°C > 40°C > 37°C > 30°C) acids, and total SCFA (40°C > 42°C > 37°C > 30°C) (p<0.05). These results indicate that both hyperthermia and hypothermia depress the microbial breakdown of carbohydrates.

Key Words pig, cecum, bacteria, temperature, short-chain fatty acids

A dense and complex bacterial population occupies the lumen of the cecum and proximal part of the colon in mammals (1). Mammalian large-intestinal bacteria ferment carbohydrates that have not been absorbed in the small intestine, and produce organic acids and gases. Short-chain fatty acids (SCFA) such as acetic, propionic and n-butyric acids are the major products of such bacterial metabolism (2). SCFA are the predominant energy source for colonic epithelial cells (3), contribute to the systemic energy budget (4), and affect various gastrointestinal functions (5). Therefore, the effects of SCFA represent many effects of large-intestinal bacteria and their substrates, i.e. indigestible carbohydrates.

That temperature affects the rate of bacterial metabolism was shown for gut bacterial populations in ectothermic teleosts such as carp (6) and rainbow trout (7); the rate of production of organic acids from various carbohydrates by bacterial populations taken from the gut of these teleosts was higher when cultured at 25°C than that at 15°C. Although mammals are endothermic, their body temperature may vary in a circadian rhythm (8), during the female menstrual cycle (8), with food intake (9) and during fever (10) and hibernation (11). Such changes in body temperature may affect the metabolism of large-intestinal bacteria and the rate of SCFA production in endothermic species as well. If this is the case, then changes in body temperature should affect mammalian gut function via altered bacterial production of SCFA in the large intestine. We studied the effect of incubation temperature on the production of SCFA by pig cecal bacteria in vitro in order to assess short-term influences of body temperature on bacterial metabolism in the large intestine. We employed a continuous culture technique in order to avoid the exhaustion of substrates and excess accumulation of products and the resultant lowering of pH.

Materials and Methods Preparation of inoculum. We pooled the cecal contents of four to five commercial castrated male meat pigs of approximately 100 kg body mass at a local slaughter-house (Miyagi Meat Inspection Center, Tomeshi, Miyagi, Japan) immediately after veterinary inspection and under the surveillance of veterinary officers, and stored them on ice. The cecal contents were mixed with an equal volume of bicarbonate buffer (pH 7.2, 300 mM/L) and lactose (10 g/L final). We used lactose as a representative carbohydrate that enters the large intestine of lactose-intolerant adult humans and is a normal component of pig feed. Two hundred milliliters of the mixture (inoculum) were dispensed into each of four thermo-controlled Wheaton Celstir spinner double side arm flasks with water jackets of 250 mL capacity (No. 356949, Wheaton Science Products, Milville, NJ, USA). The gas space of the culture was filled with carbon dioxide. Care was taken to maintain the air-
tightness of the culture vessel. The temperature of the culture was adjusted to 30°C (lower lethal core temperature for most mammals), 37°C (normal human core temperature), 40°C (normal pig core temperature) or 42°C (higher lethal core temperature for most mammals) using four independent water heaters (Thermo Max TM-3, AS ONE, Tokyo, Japan). We infused the bicarbonate buffer including lactose (10 g/L) into the culture and removed the culture from the culture flask using eight independent peristaltic pumps (MP-3, EYELA, Tokyo, Japan) both at 4.17 mL/h (dilution rate=0.5/d). This dilution rate was based on the published mean retention time in the large intestine of healthy adult humans (1.85 d for males, 2.84 d for females) (12). Cultures were continuously mixed at approximately 120 rpm using synchronous magnetic stirrers (SR-306, Advantec, Tokyo, Japan).

One milliliter of the culture was taken from each culture flask hourly from 0 to 12 h of incubation. We measured organic acid concentration in these samples with HPLC using crotonic acid as an internal standard (13). As the culture was fed with a fluid containing no organic acid at a constant rate, concentrations of these acids in the effluent, i.e. in the culture, represented the production rate of these acids.

The experiment was replicated four times using different sets of donor pigs.

Calculations and statistical methods. To estimate the overall rate of microbial fermentation of carbohydrates, we calculated the concentration of total SCFA as the sum of mass concentrations (g/L) of each individual SCFA at each sampling time. Data were expressed as the mean and standard error of the mean. Differences between means were tested by analysis of variance (ANOVA) and subsequent Tukey’s multiple comparison using JMP 5.0.1 J software (SAS Inc.) based on the following model:

Observed value
= (culture time effect) + (culture temperature effect) + [(culture time) × (culture temperature) interaction effect] + (replication effect) + error.

The effect was considered significant at an error probability of less than 0.05.

Results

We detected acetic, propionic, n-butyric and n-valeric acids. Concentrations of individual and total SCFA at 0 h did not vary significantly (p>0.78, error DF=9) (Figs. 1 and 2). There was no significant 2-way interaction effect (culture time × culture temperature) on the concentrations of individual and total SCFA, while effects of culture time, culture temperature and replication were significant (Table 1). Thus, we compared overall means involving data from 1 to 12 h of culture among different temperature settings.

Concentrations of SCFA increased during the first several hours and plateaued at around 11 h (Fig. 2).

Incubation temperature affected (p<0.05) concentrations of acetic (40°C > 42°C > 37°C > 30°C), propionic (40°C > 42°C > 30°C), n-butyric (42°C > 37°C > 30°C, 40°C > 30°C) and n-valeric (42°C > 40°C > 37°C > 30°C) acids (Fig. 2), and total SCFA (40°C > 42°C > 37°C > 30°C) (Fig. 1).

Discussion

Pigs are omnivorous colonic fermenters, as are humans. The large volume of the cecum and colon of pigs enabled us to collect large amounts of contents, which allowed the use of less diluted inoculum than is possible with smaller animals such as rats. Excessive dilution of the inoculum results in the proliferation of bacteria during the early phase of the incubation, which inevitably reduces the net production of organic acids. Thus, the use of a denser inoculum should minimize any underestimation of SCFA production rate. Further, the use of slaughterhouse material negated the need to kill animals specifically for experimental study.

We infused lactose as the carbon/energy source of the culture to mimic the more-or-less continuous entry of undigested carbohydrates from the small intestine into the large intestine. Thus, the present results should reflect close to physiological conditions, in which undigested polymeric and oligomeric carbohydrates are present in the large intestinal lumen. We presented data as mass total of all detected SCFA (total SCFA) or molar concentrations of each acid. This was simply to facilitate readers’ comparison of the present results with other studies. However, multiplication of these concentration data by dilution rate (4.17 mL/h) provides the production rate of total or individual SCFA.

The SCFA concentrations at 0 h did not vary among experimental groups (Fig. 2). This supported the random allocation of inoculum, and enabled direct comparison of changes in SCFA concentrations over time as
a measure of organic acid production.

The rate of production of total SCFA was highest at 40°C (Fig. 1). This indicated that both hyperthermia and hypothermia depress the microbial breakdown of carbohydrates. Thus, the temperature of the pig cecal bacterial ecosystem must be optimal for carbohydrate breakdown. It is not known if this is the case for human large bowel bacteria, for which the host has a normal core temperature that is lower than in the pig. The lower rate of microbial metabolism in hypothermia has some similarity with findings from reptiles, in which both energy consumption and the rate of digestion are lower at lower body temperatures (1), and with results from fish gut microbes, for which metabolic activity was higher at 25°C than at 15°C, independent of the temperature of their natural habitat (6, 7).

It is interesting that the rate of production of the two dominant acids, i.e. acetic and propionic acids, was maximal at 40°C, the normal body core temperature of pigs. This cannot be explained by the Q<sub>10</sub> effect (1) alone, because the rate of acetic acid production was depressed at 42°C.

The influence of incubation temperature on n-valeric acid concentration took several hours to appear. The highest rate of production of n-butyryl and n-valeryl acids was at 42°C, suggesting that the bacteria responsible for their production have optimal temperatures that differ from those for acetate- or propionate-producing bacteria. Alternatively, the optimal temperature for the rate-limiting enzyme(s) in the production of n-butyric and n-valeric acids may differ from that for acetic or propionic acid production.

Different responses in production of different acids to temperature suggest that changes in body temperature may modify the proportion of each SCFA produced in the large intestine, i.e. more longer chain acids at higher temperature. Knowing that the effects of SCFA on host animal metabolism vary among acids (14), changes in body temperature should affect body functions via changes in the production of bacterial metabolites in the large intestine.

Table 1. Summary of analysis of variance on concentrations of individual and total SCFA.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>Acetate</th>
<th>Propionate</th>
<th>n-Butyrate</th>
<th>n-Valerate</th>
<th>Total SCFA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour of culture (A)</td>
<td>12</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Culture temperature (B)</td>
<td>3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0004</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interaction (A×B)</td>
<td>36</td>
<td>&gt;0.9924</td>
<td>&gt;0.9233</td>
<td>&gt;0.7546</td>
<td>&gt;0.2511</td>
<td>&gt;0.9467</td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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Acknowledgments

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REFERENCES


