Nutritionally Limited Pectinolytic Bacteria from the Human Intestine

NEIL S. JENSEN and EROCLE CANALE-PAROLA*

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003

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A selective procedure was used to isolate pectinolytic intestinal bacteria from human subjects. The three isolates with the greatest pectinolytic activity utilized pectin and a few related compounds as fermentable substrates for growth but did not utilize any other compound tested. Thus, their substrate utilization pattern was markedly different from that of previously described intestinal pectinolytic isolates. The three isolates are representatives of a nutritionally defined group of bacteria for which the term pectinophilic is proposed.

Pectin in food ingested by humans is extensively metabolized in the intestinal tract, even though human enzymes do not break down pectin (10, 11). Bacteria are responsible for the digestion of pectin in the intestine (2, 6, 10, 11). However, very little is known about the identity and characteristics of the bacteria that participate in pectin degradation in the human intestinal tract, and only limited information is available on the enzymes and metabolic steps used by these bacteria for pectin breakdown.

During the first stage of a study on the anaerobic breakdown of pectin in the intestinal tract, we isolated 42 strains of pectinolytic bacteria from the feces of two people, one on a vegetarian diet and the other on a nonvegetarian diet. Neither person was taking antibiotics. The following isolation procedure was used. Immediately after collection, a small amount (approximately 0.5 g) of fecal material was diluted serially into test tubes containing 10 ml of prerredded PFP broth (see below) in an N2 atmosphere. Then a sample from the 10⁻¹, 10⁻³, and 10⁻⁵ dilutions was either spread onto PFP agar plates (0.1 ml per plate) or mixed into melted PFP agar medium, which was then poured into sterile petri dishes (0.1 ml of sample per 30 ml of melted medium at 47°C). Some of the samples were heated at 70°C for 10 min to select for sporeformers. Inoculation of PFP agar with the dilution samples and incubation of the cultures (37°C) was done in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) containing an N2-H2-CO2 (80:10:10, vol/vol/vol) atmosphere.

Medium PFP had the following composition (grams per 100 ml of distilled water): sodium polygalacturonate (grade II; Sigma Chemical Co., St. Louis, Mo.), 0.5; Trypsitcase (BBL Microbiology Systems, Cockeysville, Md.), 1.0; yeast extract (Difco Laboratories, Detroit, Mich.), 0.5; MgSO₄ - 7H₂O, 0.25; CaCl₂ - 2H₂O, 0.015; FeSO₄ - 7H₂O, 0.002; (NH₄)₂SO₄, 0.14; L-cysteine hydrochloride, 0.1; resazurin, 10⁻⁶; NaHCO₃, 0.2; agar (when needed), 1.5; NaHCO₃ was filter sterilized separately as a 20% (wt/vol) solution and then was added to the rest of the medium, which had been previously sterilized by autoclaving.

After 4 to 5 days of incubation, cells from colonies that had developed on the PFP isolation plates were transferred, using sterile toothpicks, to duplicate PFP agar plates. Pectin (polygalacturonate) degradation by cells growing on these plates was detected by flooding one of the duplicate plates of each set with a solution of a polysaccharide precipitant (hexadecyltrimethylammonium bromide, 1%, wt/vol; Fisher Scientific Co., Fair Lawn, N.J.) (5). A few minutes after flooding, a clear zone was visible around colonies that degraded polygalacturonate or pectin. Pure cultures of the pectinolytic strains were obtained by standard procedures. The samples that were heated did not yield sporeformers. A total of 20 pectinolytic strains were obtained from the vegetarian donor, and 22 strains were obtained from the nonvegetarian donor. No attempt was made at enumerating the pectinolytic bacteria in the samples because such enumeration will not be meaningful until information is obtained on the diverse types of pectinolytic bacteria present in the human intestine and on media and conditions appropriate for their growth. The 42 isolates obtained were divided into three groups on the basis of their gram reaction, cell size, and colonial morphology. The isolate that exhibited the greatest pectinolytic activity (the largest zone of clearing around colonies on flooded plates) from each group was chosen for further investigation.

The three representative isolates (strains N3, N6, and N9; all from the vegetarian donor) were obligately anaerobic, rod-shaped, nonsporeforming, nonmotile bacteria. Strains N3 and N6 were gram negative (Table 1) and were identified as strains of Bacteroides according to current taxonomic criteria (4). However, as discussed below, their substrate fermentation patterns (Table 1) are markedly different from those of recognized species of Bacteroides (4), and thus they may represent a new species of that genus. Identification of the gram-positive isolate (strain N9) (Table 1) was not attempted.

All three isolates produced extracellular enzymatic activities that functioned in pectin depolymerization. One of these enzymatic activities was pectin methylesterase (EC 3.1.1.11), and the other was a polygalacturonate lyase with an exo action pattern. The hydroxamic acid assay, the 2-thiobarbituric acid assay, and the Nelson-Somogyi reducing-sugar assay were used to detect these enzymes, as previously described (9).

The most striking feature of the three isolates was that they utilized pectin and a few related compounds as energy and carbon sources, but they did not utilize other carbohydrates or amino acids or any other compound tested (Table 1). Thus, they differed markedly from intestinal pectinolytic isolates previously described, which ferment a relatively wide range of carbohydrates (1, 7). Since our isolation method was selective for strains with strong pectinolytic ability, it is possible that high pectinolytic...
### TABLE 1. Phenotypic characteristics of pectinolytic strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N3</th>
<th>N6</th>
<th>N9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram reaction</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.5 by</td>
<td>0.6 by</td>
<td>0.8 by 3-8</td>
</tr>
<tr>
<td>Fermentation products from polygalacturonate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A, F, L, E</td>
<td>A, F, L, E</td>
<td>A, F, L, E</td>
</tr>
<tr>
<td>Fermented:&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pectin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polygalacturonate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Galacturonate</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucuronate</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> A, Acetate; F, formate; L, lactate; E, ethanol. Fermentation products were determined as described previously (3, 9). All strains formed methanol when grown in pectin-containing medium.

<sup>b</sup> +, Fermented (utilized as energy source for growth); -, not fermented (no growth). The ability of the isolates to ferment the substrates listed above was tested as described previously (9), except that the substrates were added to PFP broth lacking polygalacturonate. Final yield growth values ranged from 1 x 10<sup>9</sup> to 3 x 10<sup>8</sup> cells per ml, as determined by correlating turbidometric measurements to direct cell counts made with a Petroff-Haussler bacteria counter. Initial culture pH was 7.1; pH at the end of growth in polygalacturonate-containing medium was 5.3. Not fermented by any of the strains: L-arabinose, D-ribose, D-xylose, D-fructose, D-galactose, D-glucose, D-mannose, cellulbiose, lactose, maltose, sucrose, L-rhamnose, sodium pyruvate, glycerol, Casamino Acids (Difco), sodium alginate, amylose, arabinogalactan, carboxymethyl cellulose, cellulose, chondroitin sulfate, dextran, hyaluronic acid, inulin, starch, xylan. Occasionally, strain N3 fermented D-fructose after a lag of several days.

Activity in bacteria from the human intestine (and from other environments) is associated with nutritional specificity for pectin and related compounds. It may be surmised that because of their lack of nutritional versatility the three pectinolytic isolates must possess effective strategies that allow them to survive during periods of starvation when the few substrates they ferment are not available in their natural environment. Pectinolytic strains N6 and N9 utilized D-glucuronate (Table 1), a component of mucopolysaccharides that may be present in the intestinal secretions and sloughed cells of the host. It is possible that D-glucuronate released in the intestine by mucopolysaccharide-degrading bacteria serves as a fermentable substrate for these pectinolytic strains when pectin is not available.

Pectinolytic spirochetes that have nutritional limitations similar to those of the intestinal strains described above have been isolated from the bovine rumen (12) and from human subgingival plaque (8, 9). These spirochetes and our intestinal isolates are representatives of a nutritionally defined group of bacteria for which we propose the term pectinophilic, to indicate their nutritional specificity for pectin and a few related compounds. To date, pectinophilic bacteria have been found only in the alimentary tracts of humans and ruminants. Most likely, these bacteria are important components of pectin-based food chains that play a significant role in the nutrition of the host.

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**LITERATURE CITED**