Evaluation of the AnaeroPack Campylo System for Growth of Microaerophilic Bacteria

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Growth of microaerophilic bacteria in the AnaeroPack Campylo (Mitsubishi Gas Chemical America, Inc., New York, N.Y.) atmosphere generation system was compared to growth in the CampyPak Plus jar and CampyPak pouch (Becton-Dickinson Microbiology Systems [BDMS], Cockeysville, Md.). Growth in the AnaeroPack Campylo system was considered equivalent to or better than growth obtained in the CampyPak Plus and CampyPak pouch systems for 48 of the 50 Helicobacter pylori strains and for all 28 Campylobacter species tested. All of the 78 organisms tested were recovered in each system in equivalent colony counts. Two strains of H. pylori grown in the AnaeroPack Campylo system were observed to have colony morphology growth discrepancies when compared to growth in the two BDMS systems. Atmosphe failure with the AnaeroPack Campylo was not detected with Campylobacter jejuni ATCC 33291 used as a growth control. The AnaeroPack Campylo system is easy to use and supports the growth of campylobacters and H. pylori.

Helicobacter and Campylobacter often cause human gastrointestinal infections (3, 4, 7). These organisms require reduced oxygen concentrations of approximately 5 to 10% and enhanced CO2 concentrations of approximately 10% for optimal growth (3, 4, 7). These organisms are relatively slow growers and require atmosphere generation systems that rapidly achieve and maintain the appropriate microaerobic atmosphere. Culture methods to obtain a reduced-oxygen atmosphere have often relied on microaerobic gas generator systems and gas evacuation replacement methods (4, 7). The AnaeroPack Campylo system (Mitsubishi Gas Chemical America, Inc., New York, N.Y.) is a new microaerobic gas generator system for the culture of microaerophilic microorganisms. The AnaeroPack Campylo system is placed directly into a jar or resin pouch without the need either for catalyst or for the addition of water. Upon exposure to air, the sachet rapidly removes oxygen to produce a final atmosphere of approximately 6% ± 2% oxygen and 14% ± 2% CO2 (6).

We compared the AnaeroPack Campylo sachet in a 2.5-liter jar system to the CampyPak Plus envelope in a GasPak jar and to the CampyPak pouch (Becton-Dickinson Microbiology Systems, Cockeysville, Md.) for the ability of each system to support growth of Helicobacter pylori and Campylobacter species.

(A preliminary report of this work was presented previously [9].)

A total of 78 microaerophilic strains were tested for growth in a 2.5-liter Mitsubishi AnaeroPack jar with an AnaeroPack Campylo sachet, in a 2.5-liter GasPak jar with a CampyPak Plus envelope, and in a CampyPak pouch. The test organisms included 50 recent clinical isolates of H. pylori, 24 recent clinical isolates of Campylobacter jejuni (17 obtained from Children’s Hospital, Ontario, Canada), C. jejuni ATCC 33291, Campylobacter fetus subsp. venerealis ATCC 33561, Campylobacter lari ATCC 35221, and Campylobacter coli ATCC 43477. All clinical isolates were identified by conventional methods (4, 8). C. jejuni ATCC 33291 was used as an atmosphere control indicator in each test system.

A suspension of each freshly grown organism was prepared in sterile saline to a turbidity measurement equivalent to a 0.5 McFarland standard. Each suspension was further diluted in saline to achieve a final organism concentration of approximately 106 to 107 CFU/ml. For each organism tested, the inoculum was plated to the appropriate blood-based medium with a 10-μl calibrated loop to achieve an approximate final inoculum of 30 to 500 CFU per plate. All H. pylori organisms were plated to brucella blood agar with TVP (Remel, Lenexa, Kans.) and all campylobacters were plated to Campy Blood agar (Remel). All tests were performed in duplicate. Test plates were placed into each of the three atmosphere-generating systems, and the systems were charged according to the manufacturer’s instructions. A plate of C. jejuni ATCC 33291 was added to each system as an atmosphere control indicator. Each system was closed securely and incubated at 35°C for all organisms except for the 25 C. jejuni isolates, which were incubated at 42°C. The H. pylori isolates were incubated for at least 5 days up to a maximum of 7 days. The Campylobacter species were incubated for at least 48 h up to a maximum of 96 h.

Upon removal from each test system, and prior to examination and scoring, all media were marked with a code designating the appropriate test system to minimize any reading bias. All plates were examined and interpreted independently by two microbiologists. Organism colony counts were obtained for each plate incubated in each system. Colony counts were considered discrepant between systems only if there was greater than a 90% reduction of CFU in one system when compared to the system with the highest CFU for the strain tested. The quality of growth for each organism was scored as described previously (10) and based on typical colony morphology characteristics. A growth characteristic value (GCV) from 0 (no growth) to 5 (most typical morphology) was observed based on the quality of growth as scored by the average of two independent readings. The quality of growth in one system was considered equivalent to that of the other systems if the average of the GCV readings for growth in one system was within...
TABLE 1. Number of *H. pylori* strains and *Campylobacter* spp. yielding equivalent growth in the three microaerobic atmosphere systems

<table>
<thead>
<tr>
<th>Organism (no. of strains tested)</th>
<th>No. (%) of strains with equivalent growth&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CFU</th>
<th>GCV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em> (50)</td>
<td>50 (100)</td>
<td>47</td>
<td>94</td>
</tr>
<tr>
<td><em>C. jejuni</em> (25)</td>
<td>25 (100)</td>
<td>23</td>
<td>92</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp. (3)</td>
<td>3 (100)</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Total (78)</td>
<td>78 (100)</td>
<td>73</td>
<td>94</td>
</tr>
</tbody>
</table>

<sup>a</sup> CampyPak Plus jar, CampyPak pouch, and AnaeroPack Campylo.

<sup>b</sup> Colony counts were assessed by CFU, and growth was assessed by GCV scores.

2 GCV of the highest average GCV scored for growth in another system.

The colony counts for all 50 *H. pylori* strains and all 28 *Campylobacter* species were considered equivalent among the three systems tested (Table 1). None of the *H. pylori* or campylobacter strains failed to grow in any of the test systems. A total of five strains (three *H. pylori* and two *C. jejuni*) exhibited growth quality discrepancies (Table 1). Only two of those five were observed with the AnaeroPack Campylo system (Table 2). The quality of growth of *H. pylori* 14265 and 10715 was scored lower (GCV, 2.5) in the AnaeroPack Campylo system than in the two CampyPak systems (both GCVs, 5). The quality of growth of *H. pylori* 51687 was observed to be optimal in both the AnaeroPack Campylo and CampyPak Plus jar systems (GCV, 5) while the quality of growth in the CampyPak pouch was observed to be lower (GCV, 3). The growth qualities of *H. pylori* 25094 (GCV, 2.5) and *C. jejuni* 8396 (GCV, 3) were observed to be lower in the CampyPak Plus jar than in the AnaeroPack Campylo system and the CampyPak pouch system.

To our knowledge, this is the first extensive evaluation of the AnaeroPack Campylo microaerobic gas generation system for growth of microaerophilic organisms. The AnaeroPack Campylo sachet for a pouch system was not evaluated in this study. All three atmosphere generation systems appear to satisfactorily support the growth of microaerophilic organisms, since none of the strains failed to grow in any of the systems. Atmosphere failures, as indicated by growth of the *C. jejuni* ATCC 33291 indicator control strain, were not observed in any test system. The previously reported failures of the GasPak jar systems with anaerobic atmosphere generators (1, 2, 5, 10) were not observed in this study with the CampyPak Plus system and could have been due to the anaerobic generator itself. It is also possible that leakage of small amounts of oxygen into a jar system may not affect the microaerophilic organisms as much as it might affect the growth of anaerobes. This is more likely, since GasPak jar failures with an AnaeroPack sachet added for the growth of anaerobes have been reported (1, 10). The atmosphere for isolation of microaerophilic organisms is considered ideal at 5 to 10% oxygen and 10% CO₂ (3, 4, 7). The production of approximately 14% CO₂ by the AnaeroPack Campylo system does not appear to adversely affect the growth of either *H. pylori* or the *Campylobacter* species tested, since all strains grew well, with only two discrepant *H. pylori* strains observed. High humidity is also reported to be required for good growth of *H. pylori* (3), and recent AnaeroPack system instructions recommend adding moisture for better growth. In this study, methods to add moisture were not used. Based on these results, added moisture may not be necessary for growth of *H. pylori* and *Campylobacter* species in the AnaeroPack Campylo system. Further studies with variable levels of humidity during incubation might be warranted to obtain optimal incubation conditions for these organisms.

The AnaeroPack Campylo sachet, when used in a 2.5-liter jar, is an acceptable method for the growth of *H. pylori* and *Campylobacter* species. The system is easy to use and does not require the addition of water, reagents, or a catalyst. Since this study challenged the AnaeroPack Campylo system only with known organisms, future studies might include direct testing of clinical specimens. Further testing to include the AnaeroPack Campylo pouch system is also needed.

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REFERENCES