Lectin Typing of *Campylobacter concisus*

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A total of 44 clinical isolates and the type strain of the putative pathogen *Campylobacter concisus* were grouped based on their reactions with plant lectins. The optimized lectin typing system used *C. concisus* strains proteolytically pretreated and subsequently typed by using a panel of four lectins. The system grouped all 45 strains into 13 lectin reaction patterns, leaving no strain untypeable due to autoagglutination. Lectin types were both stable and reproducible.

In contrast to other members of its genus, a clear pathogenic role for *Campylobacter concisus* has not yet been established. *C. concisus* has been isolated as gingival pockets of patients with periodontal disease (1, 15), foot ulcers (6), and gastrointestinal disorders (7a, 8, 11, 17) and has been isolated in predominantly pediatric cases of bacteremia (7a). However, other investigators have reported conflicting results (18). Moreover, previous studies have demonstrated that *C. concisus* has a large degree of genotypic and phenotypic diversity (12, 17). Bastyns et al. (2) developed two different PCR primers in order to ensure identification of clinical *C. concisus* isolates. Considerable variability exists, both taxonomically and clinically, among the strains, and a simple method of subclassifying *C. concisus* strains is required. Lectins are proteins with binding specificities for certain carbohydrate moieties and have been used previously for typing bacteria, including other campylobacters (4, 14, 19, 20) and the closely related *Helicobacter pylori* (5). As with other gram-negative bacteria, lipopolysaccharide (LPS) contributes to the pathogenesis of campylobacters (9, 10). The present study sought to develop a simple and reproducible lectin typing system for *C. concisus* based on the binding of lectins to LPS extracts of *C. concisus*.

In total, 44 *C. concisus* strains were isolated at the National University Hospital, Copenhagen, Denmark, from stool samples of the same number of patients presenting with a wide spectrum of clinical conditions, including malignancies (n = 13), human immunodeficiency virus (n = 7), inflammatory bowel disease (n = 11), and others (n = 13). All patients complained of diarrhea and upper gastrointestinal dyspepsia. Isolates were selected by laboratory surveillance of all culture-positive patients, and other enteric pathogens were absent. *C. concisus* strains were isolated from stool samples by filtration onto blood agar plates and subsequently incubated in hydrogen-enriched microaerobic conditions for 48 h at 37°C (3). Initial identification was by microscopic morphology and Gram staining and was followed by conventional biochemical testing (13). Cultures were maintained in ox broth media containing glycerol at −80°C. The type strain, *C. concisus* ATCC 33237, was obtained from the American Type Culture Collection (Manassas, Va.). Harvested bacterial biomass was prepared as described by Hynes et al. (5). Briefly, bacteria were harvested in 10 ml of 0.1 M phosphate-buffered saline (PBS), pH 7.2, and washed once in PBS. Biomass was stored at 4°C for a maximum of 3 weeks before use.

Stored samples were washed once in PBS, resuspended in 5 ml of PBS (pH 4), and incubated for 30 min at room temperature to induce gentle lysis of the cells. Subsequently, treated cells were washed twice and (i) resuspended in 5 ml of PBS containing 0.1 mg of protease K (Sigma Chemical Co., St. Louis, Mo.) per ml and incubated at 56°C for 1 h, (ii) resuspended in 5 ml of PBS containing 0.1 mg of pronase E (Sigma) per ml and incubated at 37°C for 15 h, or (iii) resuspended in PBS containing a combination of both. The suspensions were heated at 100°C for 5 min to denature the respective enzymes and then centrifuged (5,000 × g, 15 min). The resultant pellet of cell debris was resuspended in PBS to an optical density of 0.9 at A550 or McFarland standard 4 before lectin typing. Also, representative strains were tested in lectin agglutination assays as untreated whole-cell samples suspended in PBS (A550 = 0.9).

Native lyophilized lectins were obtained from Sigma: *Bauhinia purpurea* lectin (BPA), specific for N-acetyl-galactosamine; concanavalin A (ConA; *Canavalia ensiformis*), specific for α-mannose; *Erythrina cristagalli* agglutinin (ECA), specific for β-galactose and β-N-acetyl-galactosamine; *Maackia amurensis* lectin (MAA), specific for sialic acid; peanut agglutinin (PNA; *Arachis hypogaea*), specific for β-d-galactose-(1,3)-N-acetylglactosamine; and *Solanium tuberosum* agglutinin (STA) and wheat germ agglutinin (WGA; *Triticum vulgaris*), specific for β-N-acetylglucosamine. The lectins were dissolved in PBS to a final concentration of 0.5 mg/ml. Lectin typing was carried out by both slide agglutination and microtiter well assays.

Whole-cell samples were tested in the slide agglutination assay by mixing 20 μl of bacterial sample and 10 μl of lectin solution (0.5 mg/ml) on a slide. Subsequently, the slides were gently tilted, and results were determined by visual inspection after 5 min. In the microtiter well assay, bacterial samples (50 μl) were mixed with 15 μl of lectin solution (test) or PBS (negative control) in U-shaped microtiter wells and allowed to
settle overnight at room temperature (5). Results were read by visual inspection; a positive reaction was recorded when a carpet of aggregated cell debris had formed on the bottom of the well, whereas a negative reaction was evidenced by a dot of cellular debris on the bottom of a well. Negative results were confirmed by tilting wells at an angle of >45°C and observing the movement of cell debris.

When whole-cell samples were used for lectin typing, 4 of 10 isolates tested did not react with any lectins in the panel used and another 2 strains were untypeable due to autoagglutination in the control well. Identical results were found when either slide agglutination or microtiter wells were used. The remaining four strains that did not autoagglutinate and reacted with lectins did not have stable lectin reaction patterns that were reproducible, indicating nonspecific interactions between whole cells and lectins.

The discriminatory power of lectin typing varied according to the pretreatment protocols used. Biomass from two clinical isolates was not available for all four treatments, and therefore, results for 42 isolates are shown in Table 1. For proteinase K-treated cells, 29 of 42 (69%) isolates did not react with any of the lectins. A total of eight lectin patterns (including no agglutination) were observed. Following pronase E treatment alone, a greater number of lectin patterns (12 in total) were observed and a smaller number of isolates (23 of 42; 55%) were unreactive with any of the lectins. One strain remained untypeable following either treatment due to autoagglutination.

Subsequently, cells were treated with both enzymes prior to lectin typing with seven lectins (Table 1). Treatment with proteinase K followed by pronase E yielded a total of 10 lectin reaction patterns. The majority (26 of 42; 61%) of the isolates did not react with any lectins. However, unlike with single enzymatic treatments, no isolate was untypeable. Conversely, treatment with pronase E and then proteinase K improved the typeability of isolates and the degree of discrimination. Only 8 of 42 strains (19%) remained unreactive with any lectins. Moreover, 19 separate lectin patterns were observed, and no strain autoagglutinated.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7) with silver staining (16) or Coomassie blue staining was carried out with (i) whole-cell lysates, (ii) proteinase K-treated cells, (iii) pronase E-treated cells, (iv) proteinase K-plus-pronase E-treated cells, and (v) pronase E-plus-proteinase K-treated cells. Silver-stained sodium dodecyl sulfate-polyacrylamide gels of proteolytically treated C. concisus whole-cell samples exhibited profiles consistent with those of smooth LPS, composed of a low-M₆ band (lipid A and core polysaccharide) and also a slower-migrating high-M₆ band (lipid A, core polysaccharide, and an O side chain). The silver-stained profiles varied according to the enzymatic treatment of bacterial cells. Compared with no treatment, proteinase K or pronase E treatment alone enriched the LPS content, but protein contamination was still observed with Coomassie blue staining (data not shown), whereas the combined pretreatment protocols gave similar profiles consistent with LPS enrichment and a decreased protein content (data not shown). Importantly, combined enzymatic treatments, particularly that with pronase E and then proteinase K, allowed discrimination of more strains and detection of more lectin patterns than single treatments (Table 1), which suggests strong interaction between LPS and proteins in C. concisus which, when overcome, allows optimal exposure of sites reactive for lectins.

When the latter pretreatment system was used, all 45 clinical isolates were tested in duplicate from separately cultured batches over a period of several weeks, with identical results. Of the 45 strains, the following percentages reacted with the specified lectins: ECA, 54%; STA, 51%; BPA, 41%; ConA, 19%; WGA, 14%; MAA, 5%; and PNA, 5%. Although with the original seven lectins, 19 different lectin types were obtained, three lectins (MAA, PNA, and WGA) were subsequently excluded, as they reacted at lower frequencies. Using the remaining four lectins (BPA, ConA, ECA, and STA) would potentially yield 16 reaction patterns, of which 13 were observed (Table 2).

No consistent correlation between lectin reaction pattern and patient clinical category was observed (Table 2).

The typing system allowed grouping of 45 strains of C. concisus, including the type strain, into 13 lectin reaction patterns. Moreover, the effective use of pretreatment with two proteolytic enzymes left no strain untypeable due to autoagglutination, but despite optimizing the pretreatment, about 20% of strains remained unreactive with the lectin panel (reaction pattern 1). Nevertheless, the method produced stable and reproducible results, and in contrast to previous reports, no difficulties were encountered with the microtiter well format (20). Furthermore, the microtiter format was considered superior to slide agglutination as it was less laborious and allowed a higher throughput of strains. In general, lectin typing is simple to perform and does not require specialized equipment or the use of laboratory animals for raising antisera, as does serotyping. Lectins are inexpensive, commercially available, and stable in storage. This contrasts with the vigorous quality control and the expense associated with reagents and equipment required for DNA-based methods of strain typing, which is also true for serotyping. These advantages coupled with the ease of use of the lectin typing system make it a potentially useful approach worthy of further investigation.

### Table 1. Reactivity of seven lectins with 42° C. concisus isolates following different proteolytic pretreatments of cell samples

<table>
<thead>
<tr>
<th>Enzymatic treatment</th>
<th>No. of lectin reaction patterns</th>
<th>% of strains not reacting with lectins</th>
<th>No. of strains autoagglutinating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase K</td>
<td>8</td>
<td>69</td>
<td>1</td>
</tr>
<tr>
<td>Pronase E</td>
<td>12</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>Proteinase K and then pronase E</td>
<td>10</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>Pronase E and then proteinase K</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

* Biomas from two clinical isolates was not available for all four treatments, and therefore these strains were excluded from the results.
The lectins used in the panel were chosen based on previous compositional analysis of LPS from related Campylobacter spp., combined with others to react with other potential carbohydrates. The lectin reactivity indicates that C. concisus expresses terminal β-galactose and 1,4-linked N-acetylgalcosamine residues, N-acetylgalcosamine disaccharide, and N-acetylgalactosamine residues, consistent with previous reports on C. concisus (14, 20). However, the lack of reactivity of C. concisus combined with others to react with other potential carbohydratesexpressed on the surfaces of Campylobacter jejuni and Campylobacter coli (14, 20). However, the lack of reactivity of a proportion of C. concisus isolates with any of the lectins in the panel indicates that certain C. concisus strains have a unique LPS composition. Detailed chemical analysis of C. concisus LPSs is required to confirm this, which in turn will aid the choice of additional lectins for improving the typing panel.

REFERENCES