Toxin Synthesis and Mucin Breakdown Are Related to Swarming Phenomenon in \textit{Clostridium septicum}

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\textit{Clostridium septicum} is responsible for several diseases in humans and animals. The bacterium is capable of a simple kind of multicellular behavior known as swarming. In this investigation, environmental and physiological factors affecting growth and swarm cell formation in \textit{C. septicum} were studied over a range of dilution rates (\(D = 0.02\) to 0.65 h\(^{-1}\)) in glucose-limited, glucose-excess, and mucin-limited chemostats. Cellular differentiation was observed at low specific growth rates, irrespective of the carbon and energy source, showing that swarming occurred in response to nutrient depletion. Differential expression of virulence determinants was detected in swarm cells. Hemolysin was secreted by short motile rods but not swarm cells, whereas in cultures grown with glucose, only swarm cells formed DNase, hyaluronidase, and neuraminidase. However, neuraminidase and, to a lesser degree, hyaluronidase were induced in short motile rods in mucin-limited cultures. Both swarm cells and short rods were cytotoxic to Vero cells. Mucin was chemotoxic to \textit{C. septicum}, and large amounts of mucin-degrading enzymes (\(\beta\)-galactosidase, N-acetyl \(\beta\)-glucosaminidase, and neuraminidase) were produced. Synthesis of these enzymes was catabolite regulated. In chemostat experiments, glycosulfatase secretion occurred only in swarm cells at low dilution rates in mucin-limited cultures. Determinations of oligosaccharide utilization demonstrated that N-acetylgalactosamine, galactose, and N-acetyl-galactosamine were the main carbon sources for \textit{C. septicum} in mucin. Neuraminic acid was not assimilated, showing that neuraminidase does not have a direct nutritional function in this pathogen.

\textit{Clostridium septicum} is the etiologic agent of several diseases in humans and animals (7, 20, 33). Unlike many clostridia, which are indigenous to the large intestine, \textit{C. septicum} is not part of the normal microbiota in humans (16). Despite this, the main route of nontraumatic infection is the large bowel (11). Infection is associated primarily with mucosal damage, resulting from a number of causes including neutropenia, radiation therapy, cancer, and chemotherapy (7, 29, 41). \textit{C. septicum} infections of the bowel are rapidly fulminating, and mortality is high (29).

Like many other bacterial pathogens (17), virulence in \textit{C. septicum} is multifactorial, and the organism secretes several toxins and spreading factors that take part in destruction of the bowel wall (38, 51). Four major toxins are recognized, including alpha-toxin and delta-toxin, which are hemolysins, beta-toxin (DNase), and gamma-toxin (hyaluronidase) (38, 46). \textit{C. septicum} also produces a variety of other secretory products, including neuraminidase (22, 45).

Alpha-toxin is hemolytic and has delayed action on red blood cells, whereas delta-toxin is oxygen labile and rapidly acting (45). Beta-toxin is one of the main extracellular proteins formed by \textit{C. septicum} (46). Few studies have been performed on this enzyme, but DNases secreted by other bacteria are often highly toxic, and beta-toxin is thought to act as a spreading agent in \textit{C. septicum} infections (50). More is known about the role of hyaluronidase in this bacterium, which is involved in tissue breakdown (22). The enzyme is secreted by \textit{C. septicum} after intramuscular injection in experimental animals (22). A similar role has been linked to hyaluronidase in \textit{Treponema pallidum}, where it is involved in cell adhesion as well as dissemination of the organism through the body (18).

An interesting characteristic of \textit{C. septicum} is its ability to differentiate into giant hyperflagellated swarm cells, which can participate in rapid and concerted population migrations across surfaces (51). Swarm cell formation also occurs in other clostridia, such as \textit{C. tetani}, \textit{C. sporogenes}, and \textit{C. bifermentans}, as well as in species belonging to the genera \textit{Vibrio}, \textit{Proteus}, \textit{Serratia}, \textit{Bacillus}, \textit{Chromobacterium}, and \textit{Salmonella} (3). Swarm cell formation in \textit{Proteus mirabilis} has been directly linked to virulence (6), and swarming forms of \textit{C. septicum} have been observed in necrotic tissue in the large bowel (28, 52), suggesting that pathogenicity may also be associated with cellular differentiation in this bacterium.

The aims of this study were to use the chemostat to investigate multicellular behavior in \textit{C. septicum}, in relation to the synthesis and secretion of toxins and other virulence factors, and the mucinolytic capabilities of the organism, since in the large intestine, penetration of the mucus layer is a prelude to invasion of the underlying mucosa.

**MATERIALS AND METHODS**

\textbf{Bacteria.} \textit{Clostridium septicum} NCTC 282 was supplied by the National Collection of Type Cultures, Public Health Laboratory Service, London, United Kingdom.

\textbf{Microscopy.} Swarming of \textit{C. septicum} over surfaces was studied by removing a 15-mm section of agar containing the swarm zone edge from a culture of \textit{C. septicum} grown anaerobically on Wilkins-Chalgren agar. This was stained with 200 ml of Baclight Live/Dead viability stain, comprising 1.5 ml of SYTO 9 and 1.5 ml of propidium iodide in 1 ml of anaerobic distilled H\(_2\)O (Molecular Probes Europe BV, Leiden, The Netherlands), and placed in an anaerobic jar in the dark for 10 min. A Nikon Eclipse E800 microscope attached to a Nikon PCM 2000 confocal system with a 488-nm argon laser (green fluorescence, live) and a 543-nm helium-neon laser (red fluorescence, dead) was used to visualize the...
section. Images were captured using C. Imaging software (Compix Inc., Cranberry Township, Pa.). Cell morphology of bacteria grown in chemostats was studied by phase-contrast microscopy using a Zeiss Axioskop photomicroscope. For transmission electron microscopy of negatively stained preparations, the cells were centrifuged for 30 s (13,000 g) onto Formvar or carbon, cyanogen bromide-treated copper grids (Agar Scientific Ltd., Stansted, United Kingdom), and stained with uranyl acetate (0.5% (wt/vol)).

Chemotaxis of \textit{C. septicum} to mucin. Chemotaxis studies were done using 25-μL glass capillary tubes (Camlab Ltd., Cambridge, United Kingdom). A 5-μL volume of a 1% agar slurry of porcine gastric mucin (1% (wt/vol) in anerobic 50 mM potassium phosphate buffer (pH 6.8) was added to the tubes, which were then attached to universal bottles (BDH Ltd., Poole, United Kingdom) containing either \textit{C. septicum} swarm cells (9.4 × 10^4 ± 4.5 × 10^4 cells ml^{-1}) or short rod forms (2.1 ± 1.9 × 10^5 ml^{-1}). Control tubes contained phosphate buffer. The capillaries were incubated anaerobically at 37°C for 60 min. Following incubation, the contents were ejected and serial dilutions (10^{-2} to 10^{-7}) were made in half-strength peptone water (pH 6.8). Samples (0.1 ml) were spread onto Wilkins-Chalgren agar plates containing 4% (wt/vol) purified agar to inhibit viable counts of the organism and concentrations of short-chain fatty acids in the controls. Cell-free culture supernatants from the chemostats were incubated for up to 18 h, to detect both hemolysins. Hyaluronidase and neuraminidase were determined using colorimetric methods described by Linker (31) and Warren (49), respectively. One unit of neuraminidase activity is defined as 1 μg of N-acetylneuraminic acid released h^{-1} (sensitivity, 5 μM h^{-1}), and 1 unit of hyaluronidase is defined as 1 μmol of \(N\)-acetylglucosamine liberated h^{-1} (sensitivity, 3 μM h^{-1}). N-Acetyl-β-glucosaminidase and β-galactosidase levels were measured by monitoring the release of chromogen from \(p\)-nitrophenyl-\(N\)-acetyl-\(β\)-glucosaminide and \(p\)-nitrophenyl-\(β\)-d-galactopyranoside (32). One unit of glucosidase activity corresponds to 1 μmol of \(p\)-nitrophenol released h^{-1} (sensitivity, 1 μM h^{-1}). DNase measurements in cell-free culture supernatants were made using the method of Bergmeyer et al. (10). One unit of DNase activity was taken as a reduction in the absorbance at 260 nm of 0.001 min^{-1} (sensitivity, 5 μM h^{-1}). Glycosulfatase activities were detected by monitoring the liberation of free sulfate from partially purified porcine gastric mucin, as described above. One unit of sulfatase activity defined as the release of 1 μmol of \(SO_4^{2}\) h^{-1}.

Cytotoxin assay. The cytolytic effect of culture supernatants was demonstrated using African Green Monkey (Vero) cells (Public Health Laboratory Service, Cambridge, United Kingdom). Cell-free culture supernatants (0.1 ml) were added in duplicate to 96-well microtiter plates containing confluent Vero cells and incubated at 37°C for 12 h before being subjected to microscopic examination for cytolytic effects. Cytotoxin-positive samples were serially diluted in phosphate-buffered saline (pH 7.0) and the toxin titer was determined as the dilution at which less than 50% of the monolayer showed evidence of cytolytic effect. Uninoculated culture medium was used as a control.

Carbohydrate measurements. The chemical compositions of the oligosaccharides in the mucin preparations were determined by hydrolyzing uninoculated medium and culture samples in 2 M \(H_2SO_4\) for 2 h at 100°C. Sugars were then separated by high-pressure anion-exchange chromatography with pulsed amperometric detection, on a Dionex CarboPac PA 10 column, using 15 mM \(NaOH\) as the eluant (42). The \(N\)-Acetyl-D-glucosamine was determined by hydrolyzing samples in 0.05 M \(H_2SO_4\) for 1 h at 80°C. The neuraminic acid released was then detected colorimetrically using the periodate method (49). Residual glucose in the glucose-excess chemostats was also measured using the Dionex system. Glucose was not detected in effluent from the glucose-limited cultures.

Culture dry weights. Culture dry weights were measured as described by Degnan and Macfarlane (14). All the chemicals for bacterial culture media were obtained from Oxoid. All fine chemicals were purchased from the Sigma Chemical Co. (Poole, United Kingdom).

RESULTS

Swarming in \textit{C. septicum}. After inoculation onto plates containing 1% agar, \textit{C. septicum} spread rapidly over the surface, covering the entire plate within hours. Figure 1 shows a fluorescent light micrograph of an advancing raft of giant multinucleate swarm cells at the edge of a swarm zone. Figure 2A shows a hyperflagellated \textit{C. septicum} swimmer taken from the edge of the swarm zone on a 1/8% agar plate. These cell forms varied considerably in size, ranging from 10 to 40 μm long. Figure 2B shows \textit{C. septicum} short motile rods, taken from a colony on a 4% agar plate, where swarming was inhibited.

Mucin as a chemotactic substance. Measurements of mucin as a chemotactic substance were made using capillary tubes attached to liquid suspensions of either short motile rods or \textit{C. septicum} swarm cells. The results demonstrated that mucin was
stron glycosulfatase, and neuraminidase (Table 1). This was particularly evident with neuraminidase, β-glucosaminidase, and β-galactosidase, which were increased during growth on mucin and partially or completely suppressed by a readily fermentable carbon source (55 to 84% utilization) while N-acetyl-β-glucosamine (20 to 34%) and galactose (24 to 42%), together with small amounts of glucose in the preparation, were also fermented. Fucose and neuraminic acid were not assimilated to a significant degree under any growth conditions.

Utilization of mucin oligosaccharides by C. septicum. Measurements of mucin oligosaccharide carbohydrate in the chemostat feed medium and residual glycoprotein in spent culture fluid showed that quantitatively, N-acetylglucosamine served as the principal carbon source for C. septicum, with utilization ranging from 53 to 64%. Mannose was also a preferred carbon source (55 to 84% utilization) while N-acetylgalactosamine (20 to 34%) and galactose (24 to 42%), together with small amounts of glucose in the preparation, were also fermented. Fucose and neuraminic acid were not assimilated to a significant degree under any growth conditions.

DISCUSSION

Bacterial swarm cells are capable of swift concordant movement over surfaces, but individual organisms are incapable of swarming by themselves. This form of multicellular behavior proceeds only when groups of bacteria aggregate and act in

strongly chemotactic to C. septicum swarm cells ($R_{chem} = 5.9 \pm 1.4$ [standard deviation]; $n = 5$), compared to short motile rod forms ($R_{chem} = 1.7 \pm 0.6$).

Induction and repression of mucin-degrading enzymes. Batch culture experiments showed that the activities of a number of enzymes involved in mucin breakdown (N-acetyl-β-glucosaminidase, β-galactosidase, glycosulfatase, and neuraminidase) were increased during growth on mucin and partially or completely suppressed by a readily fermentable carbon source such as glucose. This was particularly evident with neuraminidase and glycosulfatase (Table 1).

Desulfation of carbohydrates. Cultures of C. septicum grown on low concentrations of mucin secreted glycosulfatase. The specificities associated with this enzyme activity (in micromoles of $SO_4^{2-}$ released per hour per milligram [dry weight] of cells ± standard deviation; $n = 4$) against different sugar sulfates were as follows: mucin, 0.201 ± 0.026; glucose-6-sulfate, 0.125 ± 0.002; N-acetylgalactosamine-6-sulfate, 0.120 ± 0.009; galactose-6-sulfate, 0.055 ± 0.004; and N-acetylglucosamine-3-sulfate, 0.013 ± 0.001. Thus, free sulfate was released most rapidly from mucin, while the location of sulfate on the C-3 position of the sugar molecule markedly reduced the rate of hydrolysis.

Expression of virulence determinants in glucose-excess and glucose-limited continuous cultures. Changes in the dilution rate did not markedly affect bacterial viability in these chemostats (Table 2). However, cell counts did increase with specific growth rate under carbon limitation, while the reverse occurred in glucose-excess fermentors. Cell morphology was strongly affected by dilution rate, irrespective of glucose availability. At low dilution rates, essentially all of the bacteria observed in the chemostats were found to be swarm cells, whereas at $D = 0.36$ and 0.65 h⁻¹, C. septicum reverted to short rods (results not shown). Table 2 also shows the effects of nutrient limitation and dilution rate on the synthesis of toxins and mucinolytic enzymes. Hemolysin was detected only in cultures containing short motile rods, especially during glucose-excess growth. Conversely, DNase, hyaluronidase, and neuraminidase were formed only by swarm cells at low specific growth rates. Furthermore, DNase secretion occurred principally under glucose limitation, while cell-associated and extracellular neuraminidase and hyaluronidase were formed mainly in glucose-excess culture vessels.

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DISCUSSION

Bacterial swarm cells are capable of swift concordant movement over surfaces, but individual organisms are incapable of swarming by themselves. This form of multicellular behavior proceeds only when groups of bacteria aggregate and act in
concert (9, 25). In *Proteus*, swarming is a cyclical phenomenon during growth on agar plates, because differentiated cells periodically revert to short rods and concentric swarm zones form (3); however, this type of consolidation process does not take place in *C. septicum*. For most bacteria, little information is available concerning the environmental factors and intercellular signaling processes involved in controlling swarming, but studies with *P. mirabilis* show that glutamine both induces cellular differentiation and is a chemoattractant for swarm cells (5) while studies with *Vibrio parahaemolyticus* show that swarming is induced by iron limitation (35).

Swarm cells often differ biochemically and metabolically from short motile forms (8), and studies described in this paper demonstrated that major changes occur in virulence characteristics during swarming in *C. septicum*. Hemolysin formation was principally an attribute of short rods, either on blood agar plates or during growth in chemostats (Tables 2 and 3). In contrast, the production of DNase and hyaluronidase, as well as that of putative spreading factors such as neuraminidase, was associated primarily with swarm cells in the absence of carbon source. This was not an effect of growth rate per se, because swarming bacteria on agar plates grow very rapidly.

Instead, the results suggest that swarming in *C. septicum* is associated, to some degree, with nutrient (energy) depletion, which has also been linked to swarming in *Proteus* (12).

The chemostat experiments undertaken in this investigation showed that cellular differentiation was induced at low dilution rates, irrespective of whether glucose or mucin served as the carbon source. This was not an effect of growth rate per se, because swarming bacteria on agar plates grow very rapidly.
mucin (Tables 2 and 3). This mucin glycoprotein can therefore modulate the virulence characteristics of *C. septicum*.

Marked differences have been reported in the expression of several virulence attributes in *P. mirabilis* swarm cells, including increased urease, hemolysin, and flagellin synthesis, while swarm cells were found to be more invasive to epithelial cells than the short rod forms (4). However, the reverse is true in *C. septicum*, where short motile rods are more hemolytic and are more cytotoxic and invasive to Caco-2 and HEp-2 cells than are swarming bacteria (51).

Not all bacterial pathogens are motile, but motility frequently favors survival and enhances colonization in the host (17). A number of motile intestinal bacteria exhibit chemotaxis (5, 30) or possibly viscotaxis in response to mucin. Motility also promotes virulence in many organisms, including *Pseudomonas* (26) and *Salmonella* (44), and is often controlled by chemotaxis. The present study shows that this occurs in *C. septicum*, where mucin is a chemotactant, as well as in *Campylobacter jejuni*, which is chemotactic toward serine and fucose residues in mucus (27). Nonmotile *C. jejuni* mutants are unable to invade the gut because they cannot pass through the mucus layer (37), while nonchemotactic but motile *C. jejuni* mutants are similarly deficient in invasive qualities (48). Virulence is also related to chemotaxis in *Brachyspira* (*Serpulina*) *hyodysenteriae*, where pathogenic strains are considerably more chemotactic toward mucin than are nonvirulent isolates (36).

Mucus plays a protective role against enteric pathogens such as *Veronella enterocolitica* by reducing binding of the organisms to brush border membranes (34), while sulfomucins prevent colonization of the gastric mucosa by *Helicobacter pylori* (40). Conversely, the abilities of some gram-negative pathogens to colonize the mouse gut are specifically related to their abilities to adhere to mucus (13). For example, campylobacters do not degrade mucus, but they bind to the glycoprotein before gaining access to cell membrane receptors (47).

*N*-Acetylglucosamine, *N*-acetylglactosamine, galactose, fucose, and neuraminic acid are the principal constituents of mucin oligosaccharide side chains; however, small amounts of glucose and mannose are also present in mucin preparations (2). Although *α*-fucosidase and *N*-acetyl-α-glucosaminidase production was never detected in *C. septicum* (results not shown), the bacterium synthesized several mucinolytic enzymes that, in principle, would allow extensive digestion of this chemically complex, highly sulfated glycoprotein. Their formation was catabolite regulated, being induced by mucin and repressed by glucose (Table 1). The glucose effect was reduced at low dilution rates in the chemostats (Table 2), where the carbon source was strongly limiting, whereas the cell growth rate had little effect on glycoprotein-degrading enzymes in the mucin-limited chemostats, due to the continuous presence of the inducer (Table 3). These results show that carbon availability will affect the expression of virulence determinants in *C. septicum* invading the large bowel while the occurrence of high levels of cell-associated enzymes, especially in the presence of mucin glycoprotein (Table 3), may confer a significant competitive advantage in the mucus layer lining the bowel.

Although the major cytotoxin produced by *C. septicum* is believed to be alpha-toxin, which is hemolytic, necrotizing, and lethal (45), it has also been observed that neuraminidase is important in cell damage and spread of the bacterium through

### Table 1. Induction and repression of extracellular mucin-degrading enzymes in *C. septicum*

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Activity (U/mg [dry wt] of cells) of:</th>
<th>Neuraminidase</th>
<th>Glycosulfatase</th>
<th>β-Galactosidase</th>
<th>N-Acetyl β-glucosaminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>[ND]</td>
<td>[ND]</td>
<td>[0.03 ± 0.01]</td>
<td>[0.18 ± 0.02]</td>
<td></td>
</tr>
<tr>
<td>Mucin</td>
<td>[620 ± 22.1]</td>
<td>[5.1 ± 0.3]</td>
<td>[0.41 ± 0.02]</td>
<td>[0.48 ± 0.02]</td>
<td></td>
</tr>
<tr>
<td>Glucose and mucin</td>
<td>[0.8 ± 0.3]</td>
<td>[0.38 ± 0.01]</td>
<td></td>
<td>[0.25 ± 0.02]</td>
<td></td>
</tr>
</tbody>
</table>

*a* Results are means and standard deviations of data obtained in three separate experiments.

### Table 2. Effect of glucose availability on the production of toxins and spreading factors in continuous cultures of *C. septicum*

<table>
<thead>
<tr>
<th><em>D</em>&lt;sup&gt;a&lt;/sup&gt; (h&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th><em>T</em>&lt;sub&gt;0.5&lt;/sub&gt; (h)</th>
<th>Culture conditions</th>
<th>Cell count (ml&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Activity (U/mg [dry wt] of cells) of:</th>
<th>Hemolysin</th>
<th>DNase</th>
<th>Hyaluronidase</th>
<th>Neuraminidase</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cell-bound Extracellular</td>
<td>Cell-bound Extracellular</td>
<td>Cell-bound Extracellular</td>
<td>Cell-bound Extracellular</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>34.7</td>
<td>Glucose excess</td>
<td>(4.0 ± 1.2) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.5 ± 2.7</td>
<td>14.2 ± 3.8</td>
<td>55.8 ± 3.4</td>
<td>360 ± 20.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose limited</td>
<td>(2.3 ± 1.2) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.2 ± 3.6</td>
<td>14.5 ± 2.1</td>
<td>89.6 ± 6.6</td>
<td>55.8 ± 4.8</td>
</tr>
<tr>
<td>0.10</td>
<td>6.9</td>
<td>Glucose excess</td>
<td>(3.6 ± 0.5) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.1 ± 0.4</td>
<td>2.1 ± 1.1</td>
<td>22.7 ± 2.9</td>
<td>200 ± 18.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose limited</td>
<td>(5.9 ± 0.8) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3 ± 1.9</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.36</td>
<td>1.9</td>
<td>Glucose excess</td>
<td>(9.2 ± 1.1) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>34.5 ± 5.6</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Glucose limited</td>
<td>(6.7 ± 2.1) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>15.2 ± 3.4</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.65</td>
<td>1.1</td>
<td>Glucose excess</td>
<td>(8.8 ± 1.4) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>108 ± 12.7</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose limited</td>
<td>(6.8 ± 0.7) × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>82.6 ± 8.5</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

*a* Results are means ± standard deviations of data obtained in three separate experiments.

*b* Dilution rate.

*c* Cell doubling time, calculated as 0.693/D.

*d* ND, not detected.
body tissues when cytolytic activities are low or undetectable (22). This was also shown in the present study, where nonhemolytic cell-free culture supernatants were found to be highly toxic to Vero cells. C. septicum neuraminidase seems to be particularly active in lyophores proteins (45, 53). Unlike in other bacteria such as C. perfringens (39) or Bacteroides fragilis (42), the release of neuraminic acid residues by C. septicum neuraminidase does not serve a direct nutritional function. However, it is likely to provide access to the mucin molecule for other digestive enzymes. Moreover, through affecting its gel-forming properties, neuraminidase may enhance invasiveness in a similar way to that in Vibrio cholerae (23). Invasive clostridia, such as C. septicum and C. perfringens, form large amounts of neuraminidase, whereas the noninvasive C. tetani and C. botulinum do not (21). Secretion of neuraminidase by C. septicum was demonstrated by Gadalla and Collee (22) in animal experiments, as well as in studies of gas gangrene in humans (21). In contrast to neuraminic acid and fucose, C. septicum was able to utilize other mucin oligosaccharide constituents, particularly N-acetylglucosamine, showing that the high levels of N-acetyl-β-glucosaminidase and other mucin-degrading glycosidases formed were nutritionally important. Catabolite regulation undoubtedly plays a role in virulence in the bowel, and substrate induction of mucinolytic enzymes is likely to facilitate the movement of C. septicum through the viscous mucus layer and underlying gut mucosa.

Relatively few studies have been performed on bacterial glycosulfatases, their substrates, or their modes of activity. It was therefore of interest to find that C. septicum cell-free culture supernatants desulfated partially purified porcine gastric mucin, together with a number of sulfated monosaccharides. Sulfate residues on the mucin molecule are believed to have a protective effect, in that through steric hindrance they assist in preventing its destruction by bacterial glycosidases (43). Galactose and N-acetylglucosamine are the principal sulfated moieties in mucin, with the hydrophilic sulfate group usually occurring in the 6’ position (15). Extracellular sulfatase activity in C. septicum was active against glucose-6-sulfate, N-acetylglucosamine-6-sulfate, and galactose-6-sulfate but not N-acetylglucosamine-3-sulfate, showing a high degree of steriospecificity in function.

In conclusion, pathogenicity in C. septicum is multifactorial, and physiologic studies in the chemostat show that the organism secretes several toxins and spreading factors under a variety of growth conditions, while evidence has been obtained for the differential expression of virulence determinants in swarming bacteria. Further studies are now needed to elucidate the environmental and molecular mechanisms that regulate these processes.

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