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Clostridium virulence cysteine protease

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Summary

Clostridium perfringens secretes a cysteine protease in a VirR/VirS-dependent manner

Significance and context

Clostridium perfringens is a Gram-positive, anaerobic, food-borne pathogen that infects animals and humans. Symptoms include sudden onset of colic followed by diarrhea, fever, which is mild and short in duration, and abdominal pain. Type A strains can also contaminate wounds and cause gas gangrene in humans. *C. perfringens* is transmitted through ingestion of food that has been contaminated by soil or feces and held under conditions that permit multiplication of the organism - for instance inadequately cooked or reheated meats; the incubation period is usually 12 to 18 hours. Although *C. perfringens* infections are local, the bacterium produces extracellular toxins that cause extensive tissue destruction. It has been shown previously that the production of particular virulence factors, including, for instance, α-toxin (a phospholipase C), κ-toxin (a collagenase), protease and hemagglutinin, is controlled by the two-component system VirR/VirS. Using two-dimensional gel electrophoresis Shimizu *et al*. have now analyzed *C. perfringens* proteins whose secretion is dependent on the VirR/VirS system.

Key results

Proteins present in culture supernatants of wild-type *C. perfringens* and a *virR* mutant strain were separated by two independent rounds of two-dimensional gel electrophoresis, first at pH 5-8 and second at pH 3-10. Protein spots that differed between the two strains were analyzed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Seven proteins, including a perfringolysin O, a cysteine protease (P4), a collagenase, a probable surface protein (P6) and two unknown proteins, were found exclusively in the wild-type strain, and eight proteins, including a probable cell-wall-binding protein, an iron-sulfur cofactor synthesis protein (N3), a ribokinase (N4), an NAD-dependent β-hydroxybutyryl-coenzyme A dehydrogenase, a fructose-biphosphate aldolase (N6), an elongation factor G, and an unknown protein, were produced exclusively by the *virR* mutant. A VirR/VirS-dependent proteolytic activity in *C. perfringens* culture supernatants has previously been described. Indeed, the proteolytic activity found in *C. perfringens* was approximately 200-fold higher than that in the *virR* mutant culture. Because this protease activity might have affected the secreted proteins, culture supernatants were also analyzed after inhibiting protease activity with a variety of

protease inhibitors. This showed that the production of N3, N4 and P6 was not dependent on the VirR/VirS system, whereas the production of N6 was. In addition, the protein responsible for the proteolytic activity in *C. perfringens* cultures was purified and further characterized. This protein of approximately 40 kDa had significant similarity to clostripain of *Clostridium histolyticum*; it was identical to P4, a cysteine protease, and its production was VirR/VirS dependent. This cysteine protease activity was strongly inhibited by leupeptin, antipain and benzamidine, but not by phenylmethylsulfonyl fluoride, pepstatin, soybean trypsin inhibitor and phosphoramidon, consistent with the reported properties of cysteine proteases.

Links

The complete genome sequence of *C. perfringens* is available from the TIGR%20microbial%20database.

Reporter's comments

The next step will be the identification of the genes that encode these proteins. A subsequent mutant and phenotype analysis should illustrate whether they are of importance for pathogenesis. The cysteine protease is a good candidate for involvement in inducing responses in host cells that stimulate infection and/or survival of the pathogen in tissues. Subsequent screening of potential protease inhibitors could throw up new inhibitors that might, together with known inhibitors, have a role in treating *C. perfringens* infections.

Table of links

Journal%20of%20Bacteriology

TIGR%20microbial%20database

References

1. Shimizu T, Shima K, Yoshino K, Yonezawa K, Shimizu T, Hayashi H: Proteome and transcriptome analysis of the virulence genes regulated by the VirR/VirS system in *Clostridium perfringens*. J Bacteriol. 2002, 184: 2587-2594.