Spotlight on *Bacillus cereus* and its food poisoning toxins

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**MY INTEREST FOR *BACILLUS CEREUS* STARTED IN 1983**

The first publication I published internationally was on *Clostridium perfringens* enterotoxin (CPE) in 1977, and my PhD is on CPE. At the ‘First European Workshop on Bacterial Protein Toxins’ (Seillac, France, June 1983), I got an update on the food poisoning caused by *Bacillus cereus*, and was surprised how little that was known about both the emetic- and diarrhoeal syndrome. I actually decided then that I would like to follow up on the *B. cereus* toxins, but I mostly focused on the CPE for many years to come.

Two years later at the ‘Second European Workshop on Bacterial Protein Toxins’ (Wepion, Belgium, July 1985), I was invited speaker, and gave the talk: ‘Structure and mechanism of action of the enterotoxin from *Clostridium perfringens* type A’. At this conference, I talked with Dr David Ellar from University of Cambridge, UK, and members of his group. Dr Ellar’s group was working on *B. thuringiensis* δ-endotoxins. The following year the ‘bacterial genetic revolution’ hit our laboratory at Norwegian Food Research Institute, and as a microbiologist/protein chemist, I needed to make a choice: Weather or not to follow up on microbial genetics. I applied for a sabbatical year, which I got (1986/87) and contacted Dr Ellar about working in his laboratory on *B. thuringiensis* δ-endotoxins. The following year the ‘bacterial genetic revolution’ hit our laboratory at Norwegian Food Research Institute, and as a microbiologist/protein chemist, I needed to make a choice: Weather or not to follow up on microbial genetics. I applied for a sabbatical year, which I got (1986/87) and contacted Dr Ellar about working in his laboratory on *B. thuringiensis* δ-endotoxins. I was accepted and moved to Cambridge late June 1986, for a year. My plans was to work on *B. cereus* after returning home and with the very close relationship between *B. cereus* and *B. thuringiensis*, I got to the best possible environment in Cambridge. This year also resulted in my first paper using microbial genetics (Granum, Pinnavaia and Ellar 1988).

**MY WORK ON *BACILLUS CEREUS* STARTED SLOWLY IN 1988**

Back in Norway, I started slowly working on *B. cereus*, partly in collaboration with John Kramer (Public Health Laboratories Central, London, UK) that was the expert on the topic at the time. However, it took long before it ‘took off’ because I moved from Norwegian Food Research Institute to Norwegian College of Veterinary Medicine in September 1988. As usual, it took more than a year to set up a new laboratory and to get new grants for further research. In 1989, Kramer and Gilbert (1989) published their extensive review on *B. cereus* food poisoning, and the toxins were still a mystery, although they knew that the diarrhoeal toxin(s) were proteins and the emetic toxin was not. They also had made antiserum from a partly purified extract of the enterotoxin(s), which they shared with me. First in 1993, we published the first paper on *B. cereus* (Granum, Brynestad and Kramer 1993), and two more came the same year, although we had six papers/posters presented at national and international conferences between 1989 and 1993. To my surprise, it took a relatively short time before I was acceptance as a *B. cereus* expert, probably because of my previous work on CPE that also continued alongside with our new pathogen.

By the time I was asked to write the review, by Professor Fergus Priest (at that time the editor FEMS Microbiology Letters) we had published eight papers on the topic and had one in press, and had discovered a new *B. cereus* enterotoxin Nhe (Lund and Granum 1996). At this time, I had one postdoc, one PhD student and on technician (Kristin O’Sullivan, through all years) working with *B. cereus* enterotoxins. Early during these years, I also discovered that the first proof that *B. cereus* caused food poisoning...
was discovered in Norway by Hauge in 1948 (Hauge 1955), making the B. cereus the most ‘Norwegian food poisoning’ organism.

WHAT IS TODAY STILL CORRECT AND WHAT IS NOT IN THE 1997 MINIREVIEW?

The abstract and the first three headlines of the minireview are mainly still correct, although one of the enterotoxins mentioned (enterotoxin T) does not exist. Instead, cytotoxin K (CytK) was discovered and characterised in 2000 (Lund et al. 2000). In section 4, ‘Infection dose’, the lowest number of 200 cfu/g is giving a wrong impression, although correct, because the count was due to spores that formed large aggregates of about 50 spores in each. This makes the correct number about 10^4/g of spores.

What we wrote about the emetic toxin (section 5.1) is still correct, but we might include that Agata in 2002 published (Agata, Ohta and Yokoyama 2002) that foods causing emetic food poisoning contained between 10 and 1280 ng of toxin per gram of food. The following section (5.2) on the enterotoxins contains several mistakes (also included in table 2). First, enterotoxin T does not exist (it was an unintended fusion protein made during cloning), but the third enterotoxin is CytK, which is a single protein β-barrel pore forming toxin (Lund et al. 2000). The last thing that is wrong is that collagenase although a virulence factor is not a part of the non-haemolytic three-component enterotoxin (Nhe). The reason for this mistake was that the fraction containing NheC is a dimer between NheB and NheC with a molecular weight of about 80 kDa, and ran together with collagenase during purification. This fraction contained much more collagenase than NheB-C, because B. cereus also produces so little NheC that this dimer is hardly detected on an SDS gel.

Another interesting observation we have done later is that the Nhe is actually haemolytic, although so much weaker than Hbl (haemolysin AB) that we did not detect the haemolysis of Nhe in our initial studies.

The regulation of enterotoxin production (section 5.5) has mainly still corrected, although much more complicated, also involving CodY. It is interesting to see that the conclusions (Headline 6) are mainly still correct.

CITATIONS OF THE MINIREVIEW

This minireview is my second most cited paper and have been cited 355 times according to ISI (Web of Science) by the end of 2016, and was even cited 23 times in 2016. However, according to Google Scholar it is cited 584 times. I think that the reason for why it is still cited so many times is that it is short, easy to read and does not contain unnecessary details. It is great to be cited so many times but this minireview is now outdated and should now only be cited for historical reasons.

THE SECOND AUTHOR OF THE MINIREVIEW

Dr Terje Lund worked with me from 1994 to 2001 as a research scientist. He was magic with proteins including protein purification. He was the author on nine papers on Bacillus cereus enterotoxin, and a key to characterise both Nhe and CytK. The highlight of his work in my laboratory was the paper: ‘A new cytotoxin from Bacillus cereus that may cause necrotic enteritis’, published in Molecular Microbiology in 2000 (Lund, De Buyser and Granum 2000). In 2001, he was offered a position at the Norwegian Radium Hospital as protein chemist (cancer research). He had skills that not many scientists have today. Dr Lund has recently retired from science.

WHAT HAS BEEN THE OUTCOME OF THE BACILLUS CEREUS WORK AFTER THE MINIREVIEW?

The discovery of Nhe (Lund and Granum 1996) and 10 papers on B. cereus, including the minireview, gave me new contacts after John Kramer moved away from B. cereus research to Campylobacter spp. at PHLC in London. The first collaborator was Dr Ulf Rönner at SIK in Gothenburg, Sweden. We shared a PhD student four years—that mainly worked on B. cereus spore adhesion. A little later, I met Professor Erwin Märtelbauer and Dr Richard Dietrich (Ludwig-Maximilians-Universität München) at a conference, and we have collaborated continuously since 1999. During the same period, I was also invited to give talks at INRA in Avignon (Dr Christophe Nguyen-The) and at Technische Universität München (Professor Siegfried Scherer and Dr Monika Ehling-Schulz) that resulted in fruitful collaboration for several years. The last important collaborator was Professor Peter Artymiuk (University of Sheffield, UK). This collaboration lead to the crystallisation of NheA (Ganash et al. 2013). Together with several of the people mentioned above, we also got a big EU grant: ‘Preventing Bacillus cereus foodborne poisoning in Europe’ (2002–2005). For this project I employed Dr Toril Lindbäck that has been very important for our B. cereus research ever since. She is now senior lecturer in our department.

Apart from discovering Nhe and CytK, we have also come very close to a full model on how the pore of Nhe is formed in the membranes of epithelial cells during food poisoning, mainly in collaboration with Professor Märtelbauer’s group.

THE SCIENTIFIC OUTCOME OF THE BACILLUS CEREUS WORK

Over the 24 years I have worked with B. cereus our group has published 70 papers on the topic (53 in peer reviewed international journals, 13 in book chapters and 4 in printed proceedings). In addition, I have friends for life from many countries. What we had done up until 2008 is well covered in our review in FEMS Microbiology Reviews (Stenfors Arnesen, Fagerlund and Granum 2008). After then I would like to specifically mention two papers: crystallization of NheA (Ganash et al. 2013) and the binding order of the Nhe components to membranes (Heilkenbrinker et al. 2013).

References


