

Toxigenic *Bacillus cereus* in food crops and in recycled food waste



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Introduction

Toxigenic bacteria are common in food products (17%) and rare in the environment (<1%; Shaheen 2009). What happens to the toxin producers when the food waste containing toxic bacteria are recycled and used in agriculture? Are there cereulide producers in food crops and if so, where are they coming from? I focus here on potato crops and anaerobically digested food waste from a biogas plant (BGP).

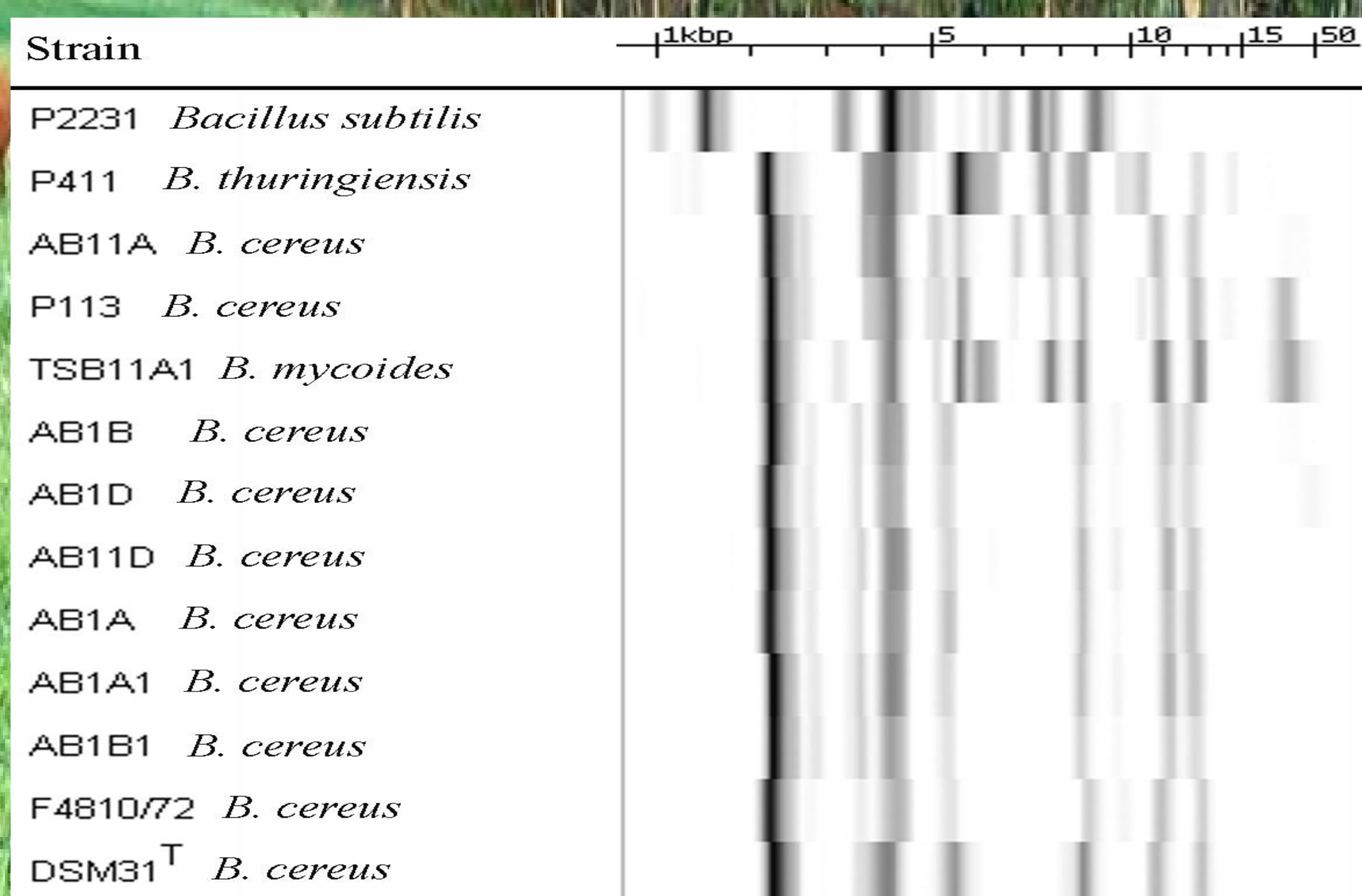


Figure 1. Ribopatterns of the *Bacillus* strains isolated from potatoes and of the reference strains *B. cereus* DSM31^T (not producing cereulide) and F4810/72 (produces cereulide). The measure bar shows the fragment sizes in kb of those fragments that contain sequences hybridizing with the ribosomal operon.

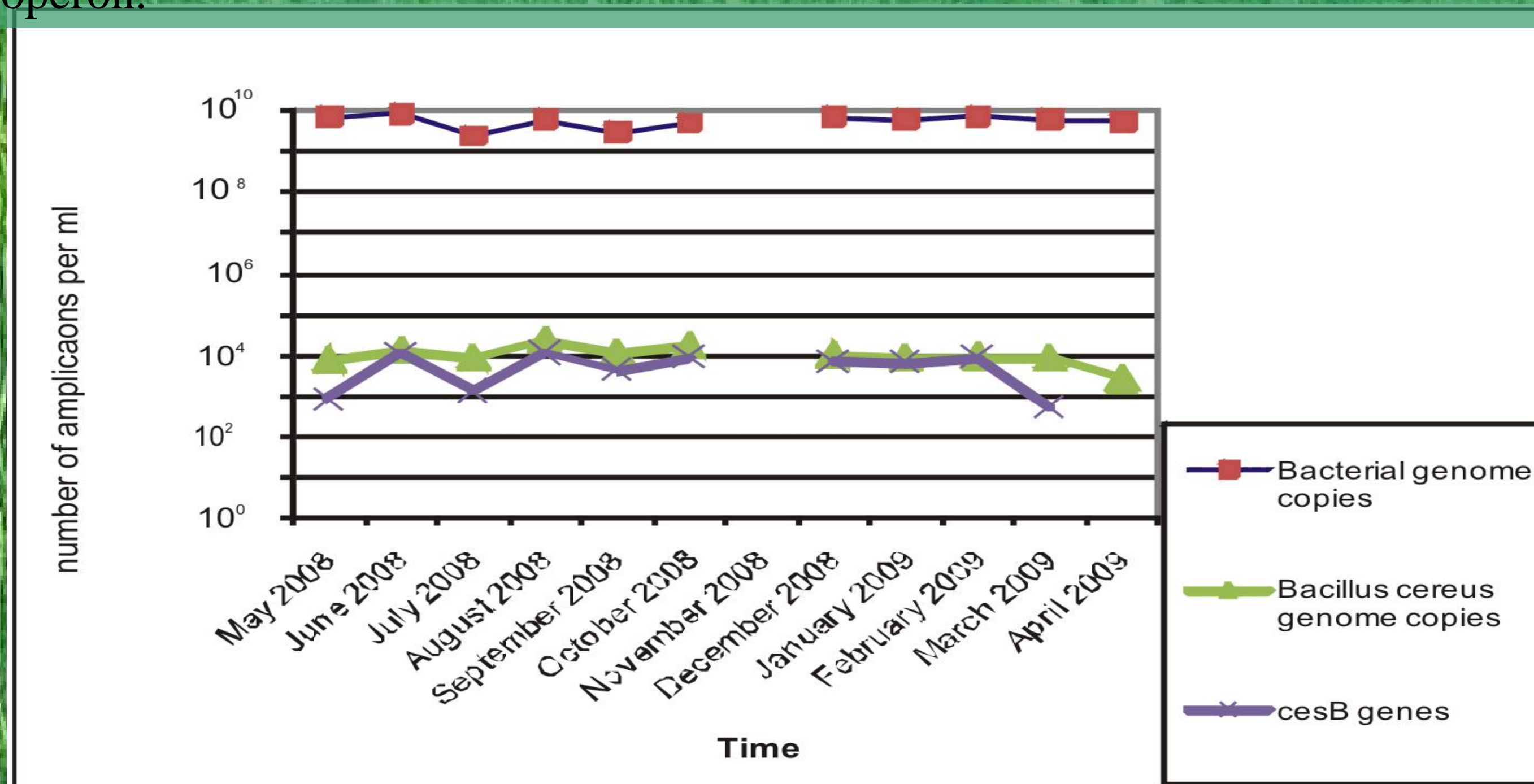


Figure 2 *B. cereus* genomes in the anaerobically digested food waste. The *B. cereus* genome content of the digestate were counted by quantitative PCR with primers specific for the species *B. cereus*.

Future plans

Next the digestate will be used in a small scale field experiment to assess the microbiological risks. To answer the question: Does the additional bacteria available in the digestate cause risks to the products obtained from the animals grazing on those fields fertilized with the digestate?

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Results and conclusions

Cereulide producing *B. cereus* in field crops

I isolated 11 *Bacillus sp.* from apparently healthy field potatoes. Six of these produced cereulide. The cereulide producing isolates from the potato crop were identical in ribopatterns with the most common ribopattern among cereulide producers isolated from processed foods (*B. cereus* 4810/72; Fig. 1). **My results showed that *B. cereus* cereulide producers can readily be isolated from field potatoes. The genotyping results suggested that the origin of the cereulide producers in foods could be the crops.**

I investigated the prevalence of *B. cereus* and cereulide producers in anaerobically digested food waste. The biogas plant treated municipal collected food waste in South of Norway. I analysed samples taken from the biogas digestate monthly from May 2008 to April 2009.

I used the sperm motility inhibition assay to identify the cereulide producers among the viable *B. cereus* in the digestate. The density of *B. cereus* in the digestate was measured by quantitative PCR (Fig 2) using primers targeted at *B. cereus* group and by plate counting (Fig. 3). The potential for cereulide production was measured by *cesB* gene specific PCR.

I found that the digestate contained an average of 10^4 copies of *B. cereus* genomes per ml of which average 10^2 were viable. DNA isolated from the digestate contained 10^3 to 10^4 copies of the *cesB* gene per ml in all samples. Viable cereulide producers I only found in 2 monthly samples of the digestate. **The digestate contained recycled food waste and high amounts of DNA from cereulide producing *B. cereus*, but only few viable cereulide producers. This result shows that the digesting process in the BGP was successful in inactivating (> 99%) the cereulide producers as well as all *B. cereus*.** The high prevalence (>10% of all *B. cereus*) of DNA, specific for cereulide synthesis, in digested food waste is in line with the results of a large European study which showed that 17 % of the isolates from foods were cereulide producers (Shaheen 2009).

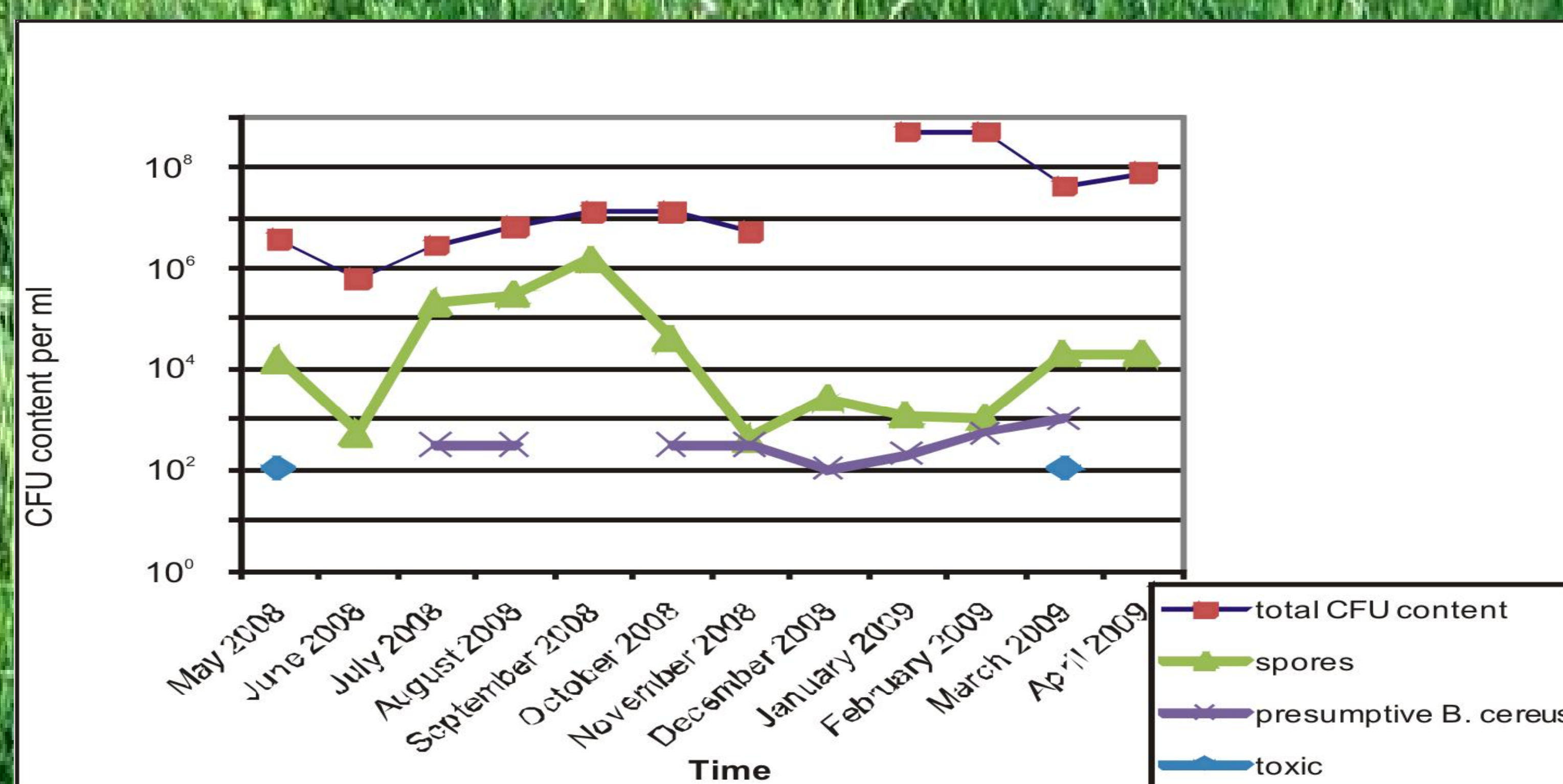


Figure 3 Viable count of bacteria, of heat stable spores and of presumptive *B. cereus* in the digestate. It was assumed that colony count obtained from samples heated for 10 min at 80 ° C represented spores.

My publication activity 2008-2010

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2. Bradley EL, Honkalampi-Hämäläinen U, Weber A, Andersson MA, Bertaud F, Castle L, Dahlman O, Hakulinen P, Hoornstra D, Lhuguenot JC, Mäki-Paakkanen J, Salkinoja-Salonen M, Speck DR, Severin I, Stamatii A, Turco L, Zucco F, von Wright A. 2008. The BIOSAFEPAPER project for in vitro toxicity assessments: Preparation, detailed chemical characterisation and testing of extracts from paper and board samples. *Food Chem Toxicol.* 46(7):2498-509.
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4. Bradley EL, Stamatii A, Salkinoja-Salonen M, Andersson M, Bertaud F, Hoornstra D, Zucco F, Weber A, Turco L, Traussnig H, Hakulinen P, Speck DR, Von Wright AJ, Honkalampi-Hämäläinen U, Mäki-Paakkanen J, Severin I, Lhuguenot JC, Dahlman O. Test procedures for obtaining representative extracts suitable for reliable in vitro toxicity assessment of paper and board intended for food contact. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2010 Feb;27(2):262-71.