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Master of Science HES-SO in Life Sciences

BIOASSAYS SIMULATING HOST-PATHOGEN-INTERACTIONS

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DESCRIPTION

Bacillus cereus produces the emetic toxin cereulide, which is responsible of many food illnesses and represents a food safety issue. As the bacteria is a very complex organism and this toxin is also difficult to avoid during food production, it is important to investigate the mechanism of action and better understand everything that is involved.

This work aimed to investigate the effect of the spores from cereulide positive strains of *B. cereus*. For that, coculture with positive and negative cereulide strains were performed under different initial cer+ concentrations and conditions. The addition of salt such as NaCl and KCl was also tested. After 24h of coculture and the isolation of colonies, a PCR was performed to detect the presence of the gene producing cereulide.

The other aim of this work was to observe the influence of cereulide on the inflammatory response from Caco-2 cells. These endothelial cells were exposed to cereulide, and the production of IL-6 was then determined after 24h of incubation. Cell viability was also studied with WST-8.

OBJECTIFS

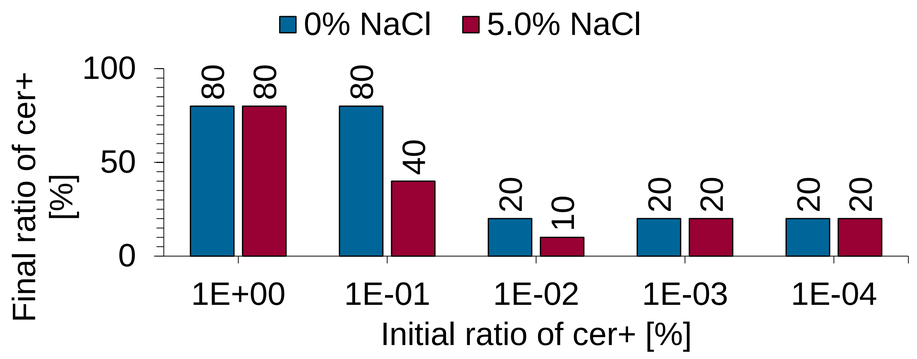
The master thesis had two main objectives.

The first one was to study the pathogen *B. cereus* with different cultures in-vitro, under different conditions. Cocultures with spores of different strains were tested with different starting cereulide positive spores concentrations or different medium.

The second one is to simulate host-pathogen-interaction via in-vitro bioassays. For that, a model using Caco-2 cells was used. They were be exposed to the emetic toxin cereulide, extracted from the spores of *Bacillus cereus* and the inflammatory response as well as the cell viability was determined.

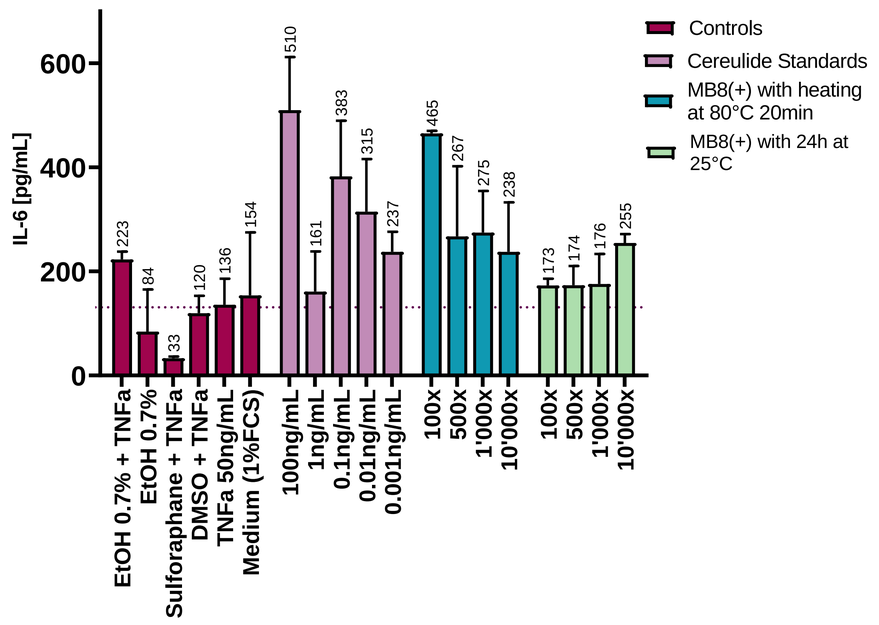
RESULTS

It was observed that even at very low initial cer+ concentration (10^{-4} spores/mL), the cer+ strain was able to over-compete with the cer- strain, resulting in the presence of a lot of colonies producing cereulide. The addition of NaCl seemed to disturb the gene formation sometimes, and to lower the final ratio of cer+. The PCR method gives a good indication of detection of positive cereulide colonies, but that it still has its limits, and while the gene might be present, the physiological part shows that the cereulide is not produced.



Result of cer+ colonies after 24h of coculture, with MB1/MB8

An inflammatory effect was observed. The production of IL-6 was increased when cereulide was added. However, the cell viability test showed that the cells had a high viability (above 70%) after exposition to the toxin. Thus, it is complicated to say with assurance if the cereulide is harming the cells or not. Another point is that the positive controls had a high variability between the assays, which make the comparison more difficult. More tests need to be done to investigate further the effect of the cereulide on the inflammation and to also observe the inflammation at different times.



Effect of cereulide on the production of IL-6 after induced inflammation

CONCLUSION

In conclusion, this project showed that cereulide has in impact in many ways. First, the coculture with spores of B. cereus showed that emetic strains were able to compete with non-emetic ones, even at low concentration. However, the addition of salt like NaCl or KCl disrupted their cereulide production, which reassuring in finding solutions to food poisoning by this toxin. Other tests more focused on the genetic part could also be of interest. The comparison with MALDI/TOF is also very useful to obtain accurate results.

The other discovery was that cereulide has a proinflammatory effect on the Caco 2 cells, by increasing the production of IL-6 after inducing an inflammation. Other investigations can be done with the addition of the effect of IL-6 at different times. The addition of other compounds like cholesterol or LPS could also be interested to test.

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