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Master of Science HES-SO in Life Sciences

# Screening for resistances to treatment of toxin-producing subpopulation of *Bacillus cereus*

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## CONTEXT

The increase in antibiotic resistance among *Bacillus cereus* (BC) strains is becoming a topic of concern in medicine. Despite all the current treatments to control bacterial contamination in food industry, they may be bacterial and toxin accumulation. Some resistant toxins hence contaminate the consumer. Moreover, malpractices, lack of knowledge and inappropriate equipment might lead to nosocomial infections. Therefore, both definition of better industrial and clinical practices and characterization of microorganism are of interest. A focus on the latter was done in this thesis by studying a subpopulation of BC producing a resistant and harmful toxin.

## OBJECTIVES

The aim of this thesis was to determine whether BC producing a specific toxin X (hereafter mentioned as X+) had different resistances compared to non-producing strains X-.

The segregation between the two states of production was previously done by toxin detection. The subpopulation determination allowed to study the long-term effects of X production regarded as a common genetic background. Moreover, addition of synthetic toxin allowed to study the short-term effects on bacterial response to treatments.

The potential changes in the resistance due to X production capability or its addition was assessed for three treatments :

- The commonly used antibiotics known to be dead end paths
- Bacteriophages infection which are promising and versatile biocontrol treatments
- Phagocytosis in immune control of bacterial infections

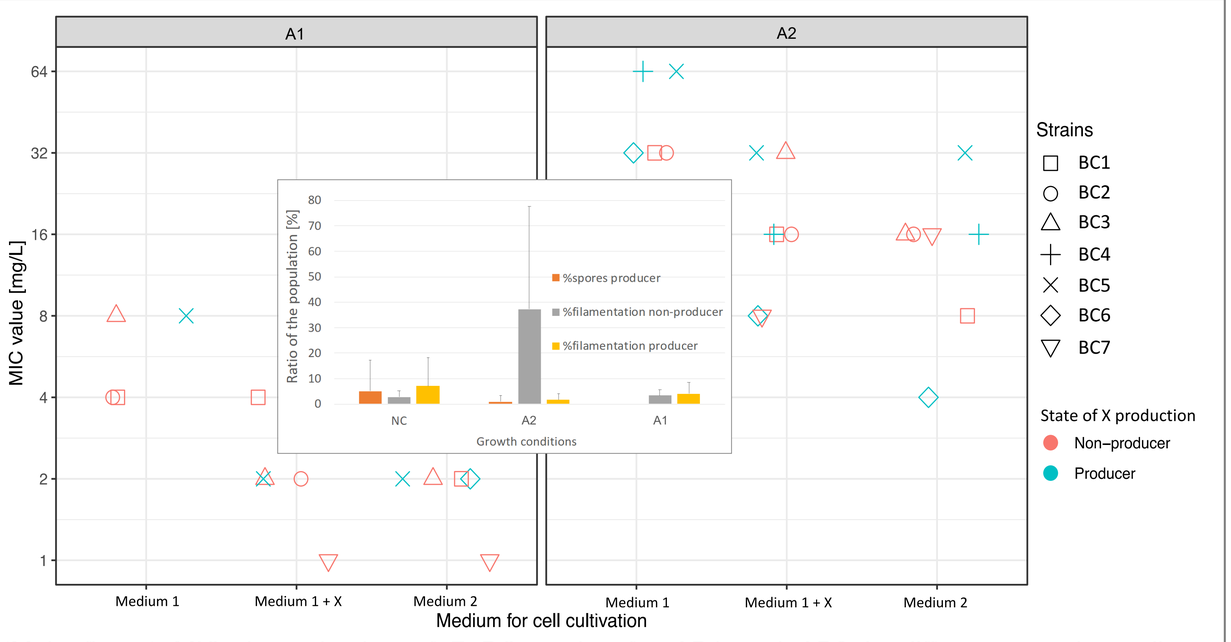
## METHODS

To study antibiotic resistance, the determination of the minimal inhibitory concentration (MIC) was performed according to the gold standard at the HES-SO for *Escherichia coli*. Two antibiotics (AB) were used at various concentration, namely AB1 and AB2, both inhibiting protein synthesis. The MIC value was the lowest concentration at which the growth was totally inhibited by visual assessment. Moreover, a microscopy analysis considering the filamentation and sporulation ratio of the population was conducted. The effects of the two subpopulations, addition of the toxin in the medium as well as two growth media (1 and 2) were studied.

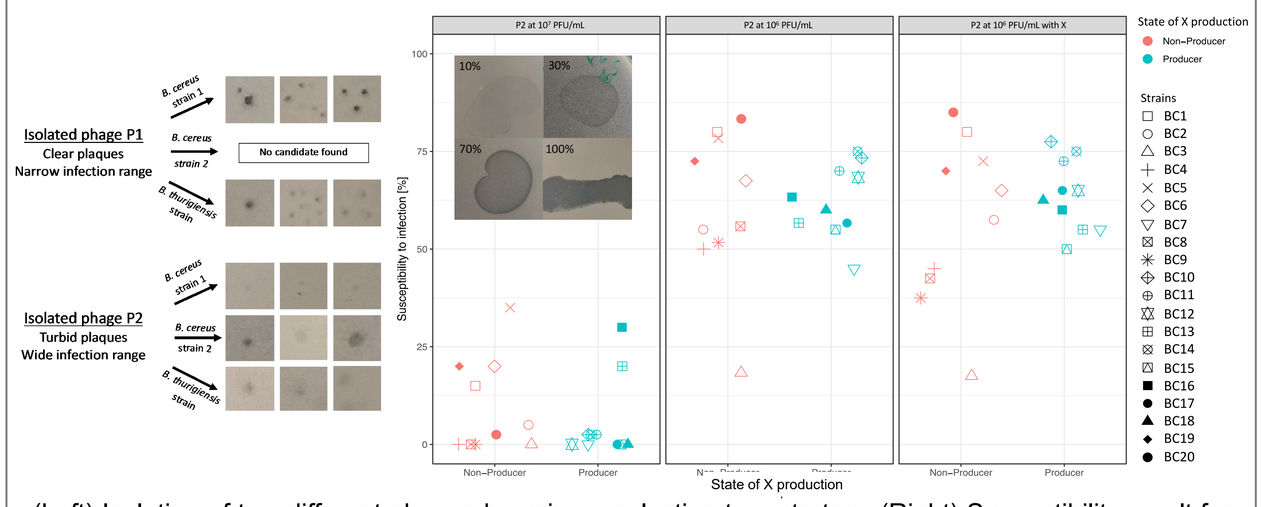
The BC susceptibility to bacteriophage infection was also studied. To do so, two bacteriophages were isolated from a wastewater treatment plant by using a selection tree strategy. This consisted mainly of viral plaques morphology and infection range studies in a double layer agar assay (gold standard). Then, the concentration of each phage was scaled-up to produce high titer stocks. Phage stocks (with or without spiking of the toxin X) were spotted on the lawn of each bacterial strain and the percentage of inhibition at the location of the droplet was visually assessed compared to the surrounding, fully grown, lawn of bacteria.

The phagocytosis efficiency required the isolation of neutrophils from fresh blood. Then, the neutrophils were put in contact with a strain of BC under vegetative and sporulating forms with a multiplicity of infection (MOI) of 1. A centrifugation step was added to synchronize the infection. A MOI of 10 was tested as well as other lysis methods, namely passaging through a needle and sonication. The number of logarithmic reduction was defined for each conditions to determine what are the optimal parameters to perform phagocytosis.

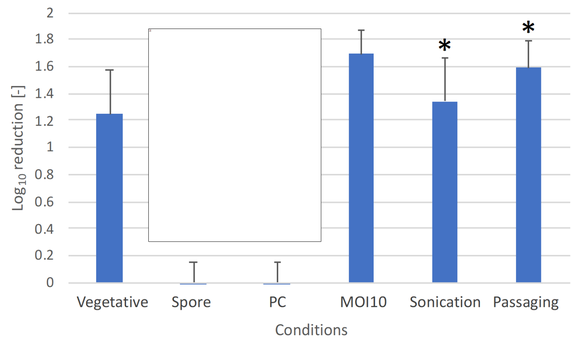
## RESULTS




(Main figure) MIC determination of 7 BC strains for AB1 and AB2 in different growth medium (Embedded figure) Filamentous and sporulating state of each subpopulation against different selective pressure in medium 1. No sporulation was observed for X- in all conditions (NC) Negative control




(Left) Isolation of two different phages by using a selection tree strategy (Right) Susceptibility result for the BC library against the two isolated phages at the indicated concentration with addition of the toxin X (Embedded) Typical inhibition range obtained during microspotting




(Left) Determination of the logarithmic reduction by neutrophil phagocytosis in different conditions. The stars indicate that the remaining number of bacteria was estimated (Embedded Figure) Typical neutrophil and Giemsa staining

## CONCLUSION AND PERSPECTIVES

The MIC method was successfully adapted to BC. Tested subpopulations indeed showed discrepancy in their morphological response after stress with antibiotics such as filamentation of X- and repression of spore formation in X+ strains. The selection tree strategy was efficient to isolate bacteriophages with different morphotypes and infection range. Nevertheless, phage scale-up was difficult due to low yield of extraction in agar and controlled liquid propagation. Great leads towards phagocytosis method optimization were found during this thesis that need to be implemented. Overall, no significant difference were found between subpopulation resistances. Nevertheless, great variation from a strain to another were found.
There is a strong need for automation of the method that speeds up the possibility for repetition and the definition of the critical parameters to explain toxin involvement. Moreover, a reliable analytical support is needed to quantify the toxin production by strain rather than having a qualitative assessment. It would help to refine the result in function of the highest producers to the lowest. Omics analyses could explain the genetic and metabolic similarities among each subpopulations. The toxin and their producers might also be involved in other *in vitro* systems such as the gastrointestinal barrier where occur most of the infections by *Bacillus cereus* spores and the toxin.

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