

# In vitro bioassays simulating infectious diseases

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## DESCRIPTION

In this project, two different subjects regarding microorganisms have been investigated using *in vitro* bioassays. The first one investigated the potential antiviral properties of metal-based particles. The second one investigated the influence of the emetic toxin contained in the spores of *Bacillus paranthracis* on the viability of L929 cells. Every part of the poster will be subdivided into subparts that are addressing the two different projects

### Investigation of the antiviral properties of metal-based particles

Since a long time, metals such as copper, silver and gold have been used to treat infectious diseases in both humans and animals with silver as the most frequently used on because of its recognized antimicrobial properties. The antimicrobial properties of metal nanoparticles have recently been demonstrated to be linked not only to the release of metal ions and the subsequent formation of ROS in solution but also to their small size and high surface volume ratio allowing them to interact with microbial membranes. The mechanism of antiviral activity by metal nanoparticles is linked to their ability to bind to the different viral components.

### Investigation of the influence of the emetic toxin cereulide contained in *Bacillus paranthracis*' spores on L929 cells

63'000 cases of food poisonings each year are attributed to the multiple species of Gram-positive, spore producing bacteria *Bacillus cereus sensu lato*. The food poisonings provoked by *B.cereus s.l.* can be separated into two types: the diarrheal type, induced by diarrheal enterotoxins, and the emetic type, induced by the emetic toxin cereulide produced by *B.paranthracis*. The ionophoric toxin cereulide is structured as a ring of 1.191 kDa composed of three repeats of two hydroxy acid residues and two amino acids very similarly to the antibiotic valinomycin. It will induce the uptake of potassium ions into the mitochondria resulting in a collapse of the mitochondrial membrane potential which can in turn lead to mitochondrial swelling and eventually cell death.

## OBJECTIVES

### Investigation of the antiviral properties of metal-based particles

The antiviral properties of metal-based particles are far from completely understood. The main mechanism which is attributed to the possible antiviral properties of metal-based particles is their ability to bind to viral components. The aim of this part of the project was, preliminary, to investigate the ability of the beads to bind to viral components. In a second time, the ability of the beads to produce an inhibition on phages was assessed using bacteriophages with the corresponding *E.coli* host. Inhibitions assays made in liquid cultures as well as phage susceptibility assessments were conducted to fulfill this goal.

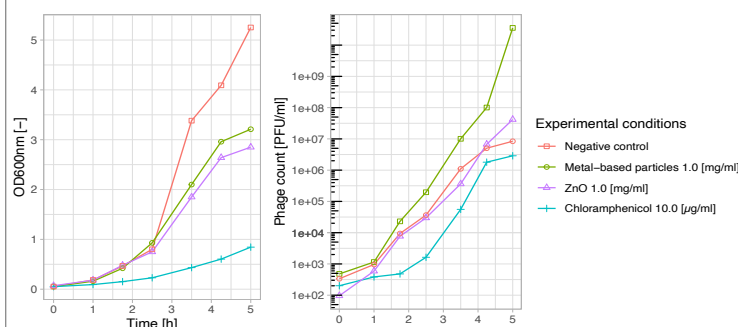
### Investigation of the influence of the emetic toxin cereulide contained in *Bacillus paranthracis*' spores on L929 cells

The presence of cereulide on *B.paranthracis*' spores discovered in a previous work by a HES-SO student is of great concern as *B.paranthracis*' spores are among the most resistance in existence. The question that remains to be answered is whether the presence of cereulide on the spores can affect the viability of mammalian cells. The main aim of the first part of this project was to answer this question using a cell-based *in vitro* bioassay with L929 cells and WST-8 colorimetric cell viability analysis. In a second time, a study of potential antidote candidate to cereulide was conducted using the same L929 model. The potential candidates investigated were dichloroacetic acid (DCA), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and bile salts.

## RESULTS

### Investigation of the antiviral properties of metal-based particles

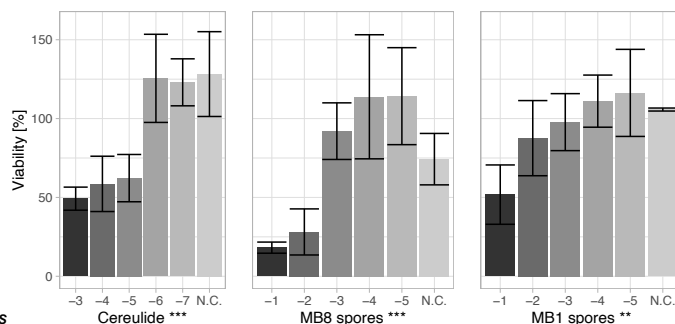
Addition of the metal-based particles to a liquid co-culture of *E.coli* DSM613 and phages induced an inhibition of bacterial growth by ~20% and an increase of the phage count by ~50 fold. This experiment showed clear antibacterial properties of the metal-based particles but no antiviral effect, rather an increase in the phage growth.



Phage growth inhibition assay using the metal-based particles – a) optical density at 600nm [-] and b) phage count [PFU/ml] against time [h] of phage growth inhibition assay using the metal-based particles and *E.coli* DSM613

### Investigation of the influence of the emetic toxin cereulide contained in *Bacillus paranthracis*' spores on L929 cells

Incubating the spores of the MB8 and MB1 strain induced a significant cytotoxic effect. Furthermore, the cereulide positive spores managed to reduce the cell viability further, even increasing the ANOVA degree of significance (\*\*\*). This shows that the cereulide present on *B.paranthracis*' spores is active and creates an increased cytotoxic effect.



WS (cereulide negative) – a) cereulide decimal dilution from  $10^{-3}$  (-3) to  $10^{-7}$  (-7) ng/ml and spores decimal dilution from  $10^{10}$  (-1) to  $10^6$  (-5) spores/ml with b) MB8 spores and c) MB1 spores. N.C. stands for negative control in all of the conditions. Degree of significance of two-way ANOVA analysis are: \*\*\*\* corresponding to a degree smaller than 0.01, \*\*\*\*\* corresponding to a degree smaller than 0.001

## CONCLUSION

### Investigation of the antiviral properties of metal-based particles

The investigation of the potential antiviral properties of the metal-based particles did not show any antiviral activities but rather highlighted the antibacterial properties of the beads either by a direct effect on bacteria or by an indirect effect due to the increase in killing by phages. Further experiments using other viruses or a human cell line for cytotoxicity could be pursued in the future

### Investigation of the influence of the emetic toxin cereulide contained in *Bacillus paranthracis*' spores on L929 cells

The cereulide contained in *B.paranthracis* spores showed an increased cytotoxic effect on L929 cells compared to the cereulide negative spores which indicates that the toxin is active on the spores. This is concerning regarding the underestimated pathogenic potential of *B.paranthracis*. A research of potential antidotes against cereulide should be a perspective for further experiments.