Ecotoxicology of different bacteria to *Daphnia magna*

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Abstract

- The ecotoxicity of seven different bacteria was investigated *in vivo* on the freshwater crustacean *Daphnia magna* (Straus, 1820). The effect of the bacteria *Bacillus cereus* (Frankland & Frankland, 1887), *Bacillus megaterium* (Bary, 1884), *Escherichia coli* (Migula, 1895), *Micrococcus luteus* (Cohn, 1872), *Pseudomonas fluorescens* (Flügge, 1886), *Staphylococcus epidermidis* (Winslow & Winslow 1908) and *Serratia marcescens* (Bizio, 1823) was tested according to ISO 6341 (2012) standard procedures. The most active bacteria have been studied using *in silico* methods to find possible target proteins, namely chitinases from *Serratia marcescens.*

Keywords: Toxicity; Ecotoxicity; Bacterial agents; *Daphnia magna*

Materials and methods

- *Daphnia magna:*

The use of *Daphnia magna* for this kind of experiments is largely extended because of its short lifespan and its fast-reproductive capabilities. Their transparent aspect allows the scientists to study the internal organs in live specimens. The *Daphnia magna* specimens have been donated by CESIRE (Dept. Ensenyament, Generalitat de Catalunya).

- *Bacteria:*

The seven-different species of bacteria have been living in different pots which have been conserved at 5ºC. The bacteria have been donated by CESIRE (Dept. Ensenyament, Generalitat de Catalunya).

- *Oxygen Pump:*

An oxygen pump has been used to keep the medium where the *Daphnias* were living oxygenated. The oxygen pump's power is 2.2W and its frequency is 50/60 Hz.

- *pH meter:*

A pH meter was used to measure the acidity or basicity of the water in the different water tanks. The pH meter have been donated by CESIRE (Dept. Ensenyament, Generalitat de Catalunya).

- *Swissdock Software:*

The Swissdock software by the Swiss Institute of Bioinformatics[(1)] have been used to study the different interactions between the *Daphnia* and the bacteria.

- *R programming language and Rstudio:*

R professional software was used to analyse the data arising from the experiments and the *in silico* procedures.

Literature cited

- *(1):* SwissDock. Swiss Institute of Bioinformatics. Available on: http://www.swissdock.ch/
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- *(5):* Gustav Vaaje-Kolstad, Svein J. Horn, Morten Sørlie, Vincent G. H. Eijsink, 2013. The chitinolytic machinery of Serratia marcescens – a model system for enzymatic degradation of recalcitrant polysaccharides. The FEBS Journal 280: 3028-3049.

Results

- Taking into account the 21 experiments (3 experiments for each bacterium) which were performed between August and October 2017, the only bacterium which surpassed the 75% rate of mortality were *Micrococcus luteus* and *Serratia marcescens* (**Figure 1**). Therefore, it was decided to study the interactions between *Daphnia magna* and *Serratia marcescens (Sm)* because the mortality caused by these infections was higher than the one caused by *Micrococcus luteus* and because *Serratia*'s organism has been studied in depth since it was first discovered (Bizio, 1823).
- The main hypothesis which could explain why this bacteria caused such mortality on *Daphnia magna* was based on the belief that the *Serratia*'s chitinases could destroy the *Daphnia*'s chitin carapace, which separates the animal's internal medium from the external environment. *Serratia marcescens* major chitinases are ChiA, ChiB[(2)] and ChiC, the last one being divided in ChiC1 and Chi C2[(3)].
- This hypothesis was tested using *in silico* methods, more specifically, the SwissDock fuction from the Swiss Institute of Bioinformatics' web page. This function is capable of predicting the more stable ways the enzymes and ligands can connect themselves and the energy of these links (**Figure 2**). The most stable interaction was the one between Chitobiose and its enzyme, Chitobiase[(4)]. It may be because the chitobiose is in fact dimers of chitin and, therefore, the enzyme has to interact with smaller and more organised molecules. With this information, the hypothesis would report that Chitinase A, B and C1 from *Sm* would act first reducing long chains of chitin to dimers (Chitobiose) and Chitobiase would act last turning the dimers into monomers. This would be the theoretical way that the *Sm* would use to destroy the *Daphnia*'s carapace, causing the death of the specimens.

Molecule	Family	Uniprot ID	PDB ID	Interacts with…
Chitinase A	GH-18	P07254	1CTN	Chitin polymers from their reducing ends
Chitinase B	GH-18	Q54276	1E15	Chitin polymers from their non-reducing ends
Chitinase C1	GH-18	Q700B8	4AXN	Amorphous regions in the chitin polymers
Chitobiase	GH-20	Q54468	1QBA	Chitobiose
CBP21[(5)]	CBM33	O83009	2BEM	Crystalline regions in the chitin polymers

Table 1. *Families and IDs of the chitinolytic machinery of Serratia marcescens and the molecule they interact with.*

- To see if the *Serratia marcescens* could affect the heart of the *Daphnia magna*, the Daphnia's heart rate was calculated on healthy specimens and on specimens who were infected for 8 hours. It was observed that the heart rate decreased a 5,25% afer 8 hours of infection **(Table 2)**.

Daphnia's health condition	Heart beat counted in 4 different specimens in 60 seconds				Mean
Healthy *Daphnia*	200	202	198	195	199
Daphnia infected with *Serratia marcescens* for 8 hours	177	186	174	183	180

Table 2. *Difference in the heart beats of healthy Daphnia magna and infected Daphnia magna at a temperature of 20ºC. The number of heart beats are counted in periods of 20 seconds and then multiplied by 3. The infected Daphnia was in contact with Serratia marcescens for 8 hours. We can observe that the mean was reduced from 199 beats/minute to 180 beats/minute, that is to say that the heartbeat decreased a 5.25%.*

Graphics

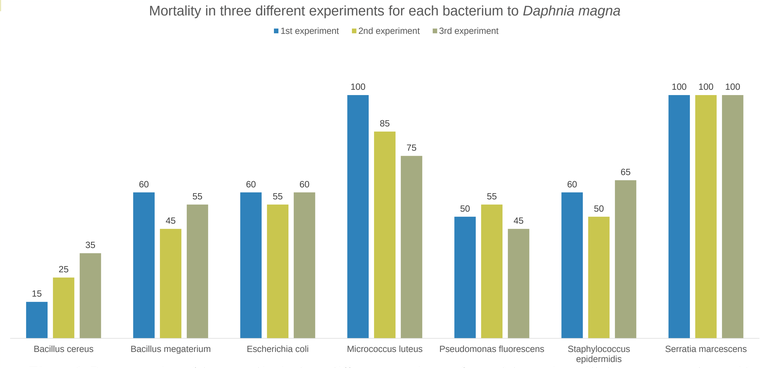

Figure 1. *Representation of the mortality in three different experiments for each bacterium. All the cases were done with 20 individuals of Daphnia magna, at room temperature and with direct sunlight during the day. The 20 specimens were infected with 2mL of a certain bacterium (1:9 proportion). They were left together for 24 hours. After this time, the living Daphnia were counted and noted down on an Excel table.*

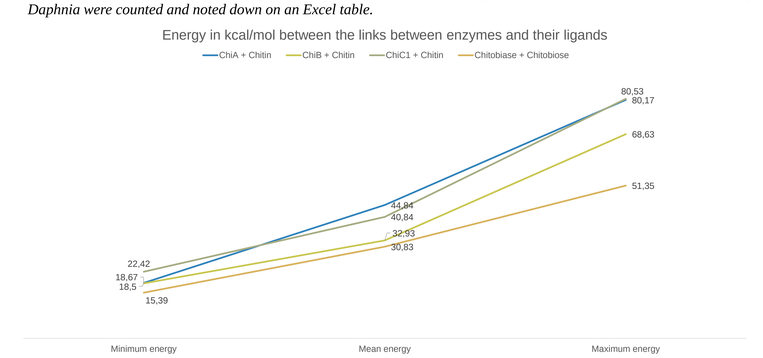

Figure 2. *Outcomes of SwissDock for the assessment of the interactions of Chitin with Serratia marcescens' chitinases A, B and C1 and Chitobiose with Serratia marcescens' chitobiase. The data was analysed with RStudio.*

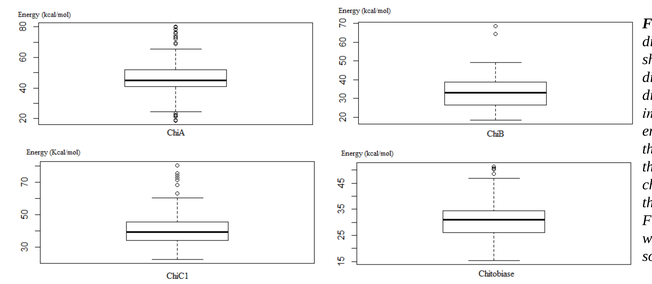

Figure 3. *4 different boxplots showing the distribution of the different interaction's energy between the chitinases and the chitin, and the chitobiase with the chitobiose. Figures made with RStudio software.*

Conclusions

- *Serratia marcescens* is the Bacterium which causes the highest mortality on *Daphnia magna* among the 7 which were tested.
- This mortality rate is possibly caused by the chitinolytic machinery of *Serratia marcescens* which could destroy the *Daphnia*'s carapace. Without its exoskeleton, the animal could not keep its internal medium apart from the external environment.
- The failure of these protection mechanism would have an impact on a decrease in the *Daphnia*'s heart rate, finally causing its death.