Temperature dependent toxin expression in *B. cereus* G9241, the causative agent of anthrax-like illness

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Background

- The bacteria *Bacillus cereus* G9241 is closely related to the anthrax agent *B. anthracis*.
- Members of the *B. cereus* group express **PlcR**, a transcriptional regulator of secreted toxins and proteases, which is **activated by the peptide PapR**.
- However, in all *B. anthracis* isolates, the *plcR* gene is truncated.
- It has been proposed that in *B. anthracis,* the acquisition of **AtxA**, which regulates anthrax toxins, was **incompatible with the activity of PlcR**, leading to PlcR inactivation, and consequently restriction to mammalian hosts. Interestingly, G9241 encodes intact copies of both *atxA* and *plcR*.
- We suggest that, at least partial PlcR suppression is essential to facilitate the anthrax-like disease state in humans.

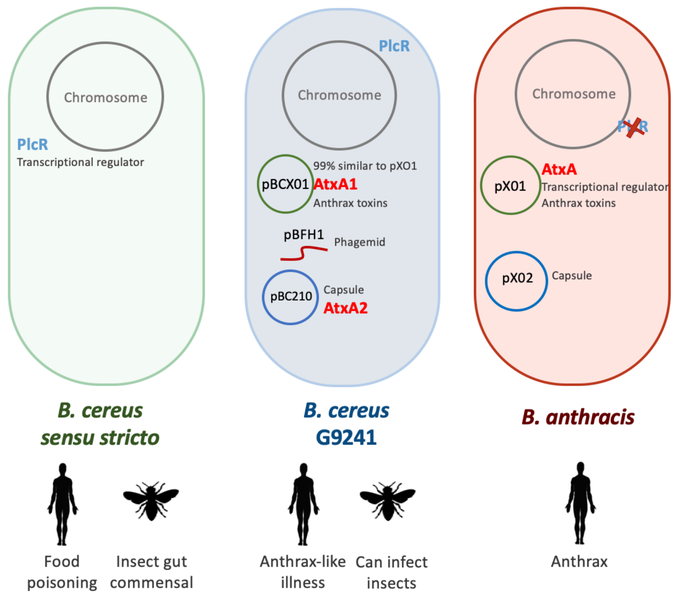


Figure 1: **The *Bacillus cereus* group** consists of many species which includes *Bacillus cereus sensu stricto* and *Bacillus anthracis.*

Aim

Investigate whether the activity of the PlcR-PapR regulatory circuit and PlcR-regulated toxins in *B. cereus* G9241 are **temperature dependent**, in order to accommodate the activity of AtxA.

1. *B. cereus* G9241 supernatant is more toxic to sheep red blood cells, when extracted from 25 °C compared to 37 °C growing culture.

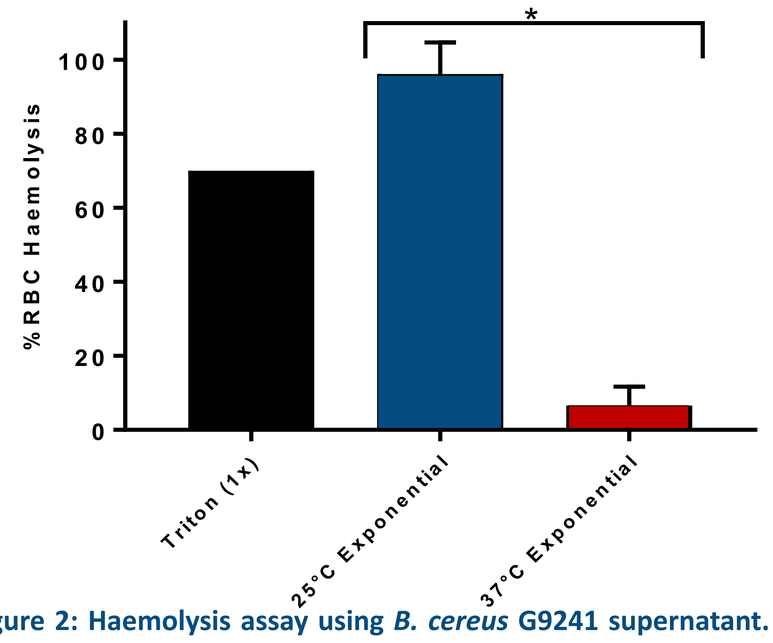
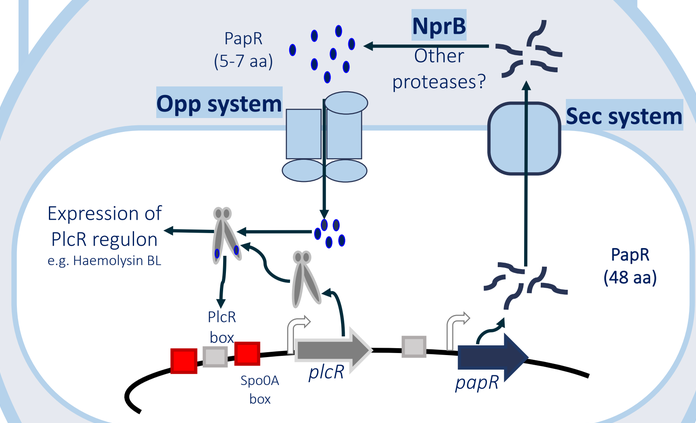


Figure 2: Haemolysis assay using *B. cereus* G9241 supernatant. *B. cereus* G9241 supernatant is significantly more toxic to sheep red blood cells, when extracted from 25 °C growing culture compared to 37 °C growing culture (Brooker *et al.*, unpublished data). * denotes an unpaired t-test with a p-value of 0.0232. Error bars denote one standard deviation and all samples were to an n=3.



The PlcR-PapR regulatory circuit

2. Expression of PlcR-regulated toxins are temperature dependent.

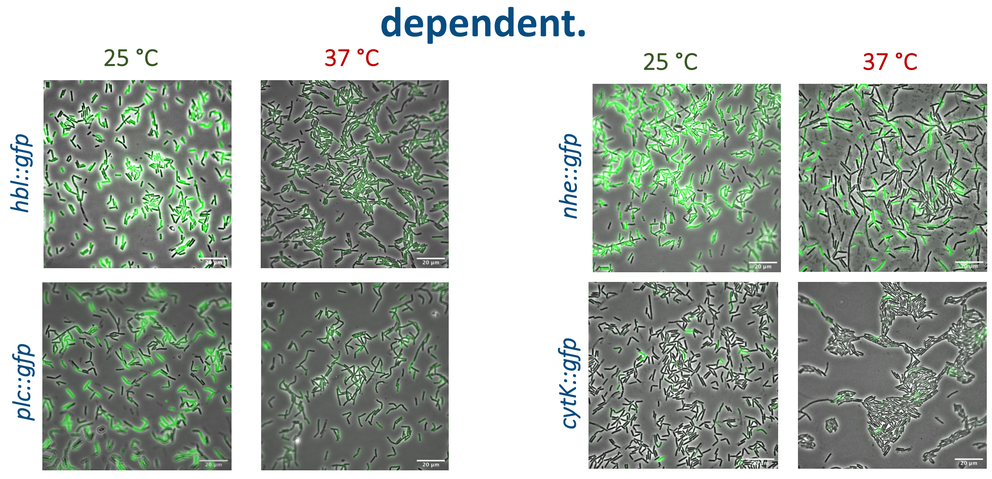


Figure 3: Microscopy images of *B. cereus* G9241 at 24 hours harboring plasmid-based GFP reporter constructs of PlcR-regulated toxins.

3. Population heterogeneity observed with the expression of PlcR and PapR

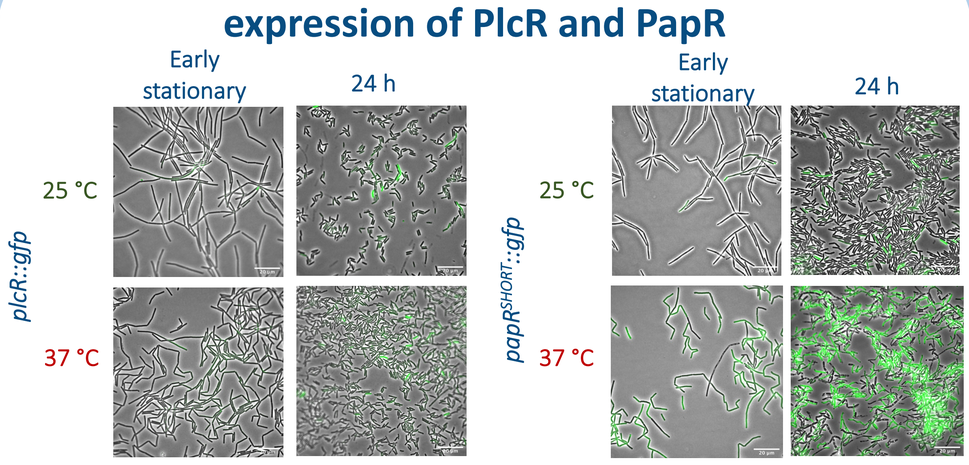


Figure 4: Microscopy images of *B. cereus* G9241 harboring *plcR::gfp* or *papR^{SHORT}::gfp.* Plasmid-based GFP reporter constructs.

4. Oligopeptide permease (Opp) system, which is involved in importing active PapR peptide, is functional at 37 °C

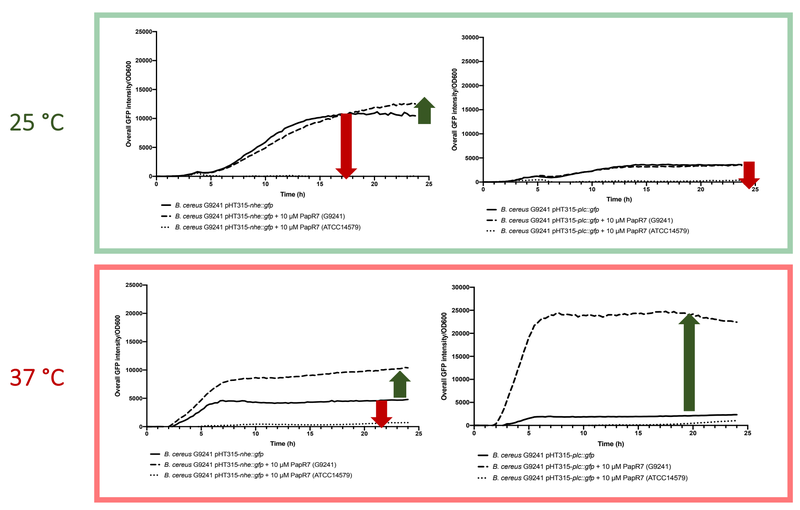


Figure 5: Overall GFP intensity per cell of *B. cereus* G9241 containing plasmid-based toxin reporters over time grown at 25 °C and 37 °C in LB media, with or without the addition of synthetic PapR$_7$. N=3

Conclusion

- The expression of PlcR-regulated **toxins are higher at 25 °C** compared to 37 °C, which indicates why we **see haemolytic activity at 25 °C.**
- The **export** of **unprocessed PapR** (data not shown) and **import** of **processed PapR** is **functional at both temperatures**, suggesting that they do not play a role in the temperature dependent expression of toxins.
- This suggests that the protease(s) needed in processing PapR is possibly involved in the temperature dependent toxin expression.
- Similar results is observed in *B. cereus* G9241 lacking the pBCX01 plasmid (data not shown), which suggests that the **AtxA does not modulate the activity of the PlcR-regulated toxins.**

REFERENCE

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