

BACILLUS CEREUS IRON TRANSPORTERS AND IRON SOURCES USED FOR GROWTH AND BIOFILM FORMATION

• Hasmik Hayrapetyan^{1,2}, Masja Nierop Groot^{1,3}, Tjakko Abbe^{1,2}

Introduction

Iron is an important element for bacterial viability, however it is not readily available in most environments where it is bound to complex compounds. Microorganisms developed mechanisms to scavenge iron from complex compounds, which is one of the prerequisites of a successful pathogen.

We studied the ability of 2 reference strains and 20 undomesticated food isolates of *Bacillus cereus* to use different iron sources including host-specific iron complexes for growth and biofilm formation. Furthermore the links between growth and the presence of putative iron transport systems in the genome sequences were analysed.

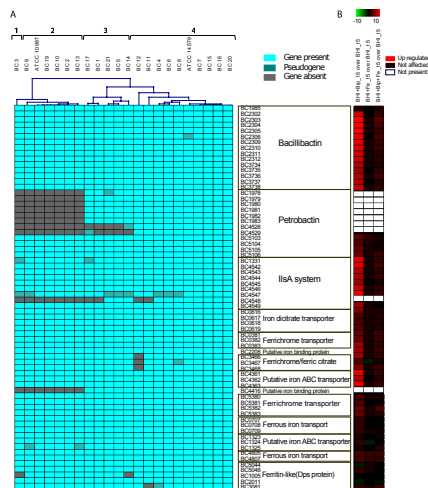


Figure 1. A. Comparative analysis of genomes of 22 *B. cereus* strains for genes encoding iron transporters. B. Expression of iron transporter genes in *B. cereus* ATCC 10987 in iron deplete (BHI+Fe and BHI+Bip+Fe) and iron replete (BHI+Bip) conditions.

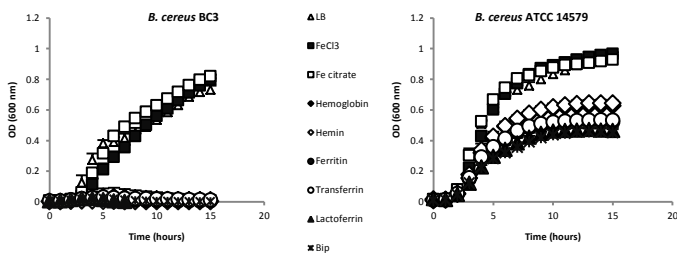


Figure 2. Growth of the strains BC3 and ATCC 14579 in LB and LB supplemented with iron scavenger (Bip) with and without addition of different iron sources.

Results

Genomes of all 22 strains encoded genes for biosynthesis of the siderophore bacillobactin, whereas 7 strains lacked genes for petrobactin. Iron starvation caused overexpression of most predicted iron transporters in *B. cereus* ATCC 10987. All strains effectively used Fe citrate with the exception of strain BC3 (lacking both functional petrobactin and IIsA systems), all could use haemoglobin. Ferritin, transferrin and lactoferrin could be used only by a minority of strains, with all functional iron transporters present (Fig.1 and 3, cluster nr.4). Biofilm formation was strongly dependent on the type of iron available (Fig. 4).

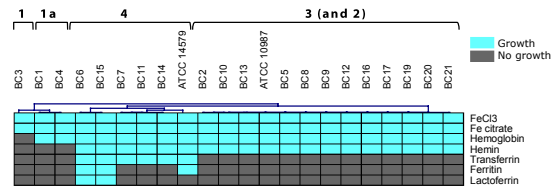


Figure 3. Phenotypic hierarchical clustering of 22 *B. cereus* strains based on their ability to grow on different iron sources.

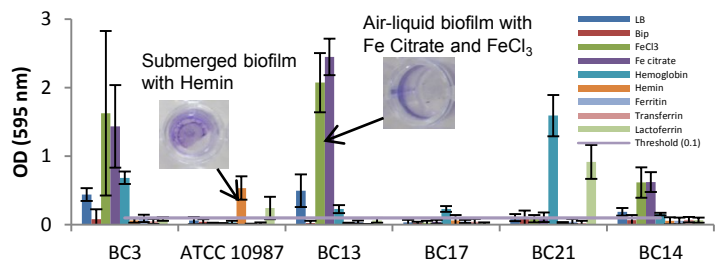


Figure 4. Biofilm formation for selected strains. Biofilms were formed in 96-well polystyrene plates in LB supplemented with Bipyridine, with or without addition of different iron sources. The biofilm was measured using CV staining after 24 h incubation at 30 °C.

Conclusions

Growth

- Preferred iron sources were FeCl₃ and Fe citrate followed by Hemoglobin>Hemin>Transferrin>Ferritin>Lactoferrin
- Genotypic and phenotypic hierarchical clustering revealed appr. 70 % matching between gene content and ability to use complex iron sources

Biofilm formation

- Biofilm formation was most effectively restored by FeCl₃ and Fe citrate
- Some iron sources, such as Hemin and Hemoglobin, triggered submerged biofilm formation.