


Surface Colonization Area was Associated with Phylogenetic Group and Hemolysin BL Production in *Bacillus cereus sensu lato*

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Background and Objectives



Bacillus cereus sensu lato (*s.l.*) are a group of Gram-positive, spore-forming bacteria¹. Some strains of this group are cytotoxic and can cause foodborne illness while others are harmless and can be used in the agricultural industry.



Some strains of *B. cereus s.l.* from phylogentic group IV secrete Hemolysin BL (Hbl), a pore-forming enterotoxin that causes foodborne illness².



B. cereus s.l. can persist in some environments, competing for nutrients and space with other microorganisms to colonize surfaces³.

Objective: Assess if Hbl-producing strains are associated with increased surface colonization area when grown in competition with non-Hbl producing strains.

Significance

B. cereus s.l. strains can persist through surface colonization via nutrient acquisition and microbial competition, therefore understanding how Hbl may provide a competitive advantage can provide better strain prediction for persistence and biofilm formation.

Materials and Methods

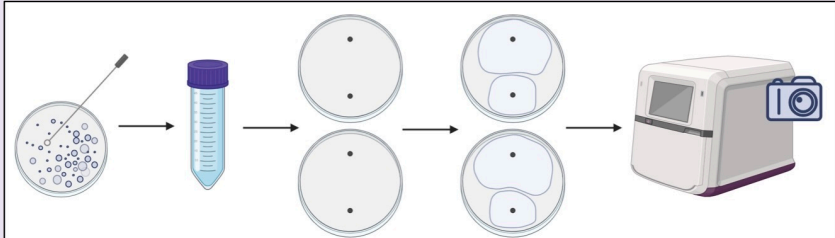


Figure 1: 18 strains **originating from dairy environments** were chosen and screened for motility and Hbl production using 0.3% Reasoner's 2A agar (R2A) soft agar and a latex agglutination kit for Hbl respectively. Nine isolates from Group IV were Hbl+, eight from Group III were Hbl-, and one isolate from Group IV was Hbl-. Each strain was grown on R2A agar and then R2A broth before being **spot inoculated** on 0.3% R2A agar in **competition pairs in duplicate**. Each Hbl+ isolate was tested in competition with each Hbl- isolate as pairs, as well as in self-pairs (Hbl+ with Hbl+ or Hbl- with Hbl-) and alone. The competition plates were incubated for **48 hours at 25°C**. Images were collected and analyzed using ImageJ to calculate the **surface colonization area**, which was then analyzed using a **paired t-test**.

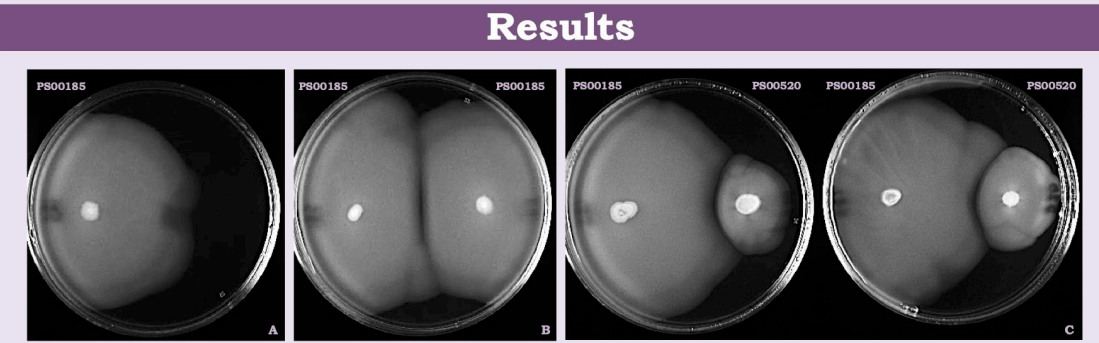


Figure 1: Subset of images taken of Pair competition plates, with Self pair and Alone counterparts after 48 hours of incubation at 25°C. PS00185 (Hbl+) alone (**Panel A**), PS00185 (Hbl+) self pair (**Panel B**), and PS00185 (Hbl+) vs PS00520 (Hbl-) competition pair replicates (**Panel C**). Photos were taken on a BioRad Gel Doc XR+ imaging station.

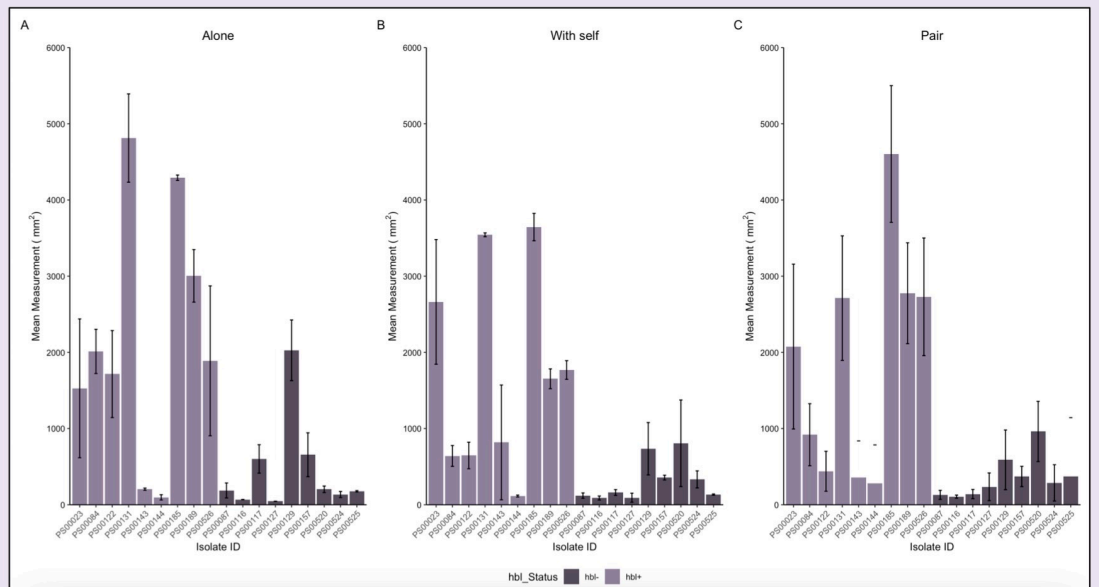


Figure 2: Mean growth **surface area** (mm²) for each **growth condition** (Alone, With Self, and Pair) of all isolates, separated by **Hbl status**. For the Pair condition, the average represents the growth area of all competition pairs for each isolate. The With Self condition's averages are derived from pairing the same isolate with itself across a replicate plate. Lastly, the Alone condition's mean is calculated as the average of two replicate measurements. Standard deviation bars are indicated, however, several standard deviations are larger than the mean measurement, so a negative is used instead. Using a paired t-test, the surface area for Hbl+ isolates were compared to that of the Hbl- isolates. There was a **significant difference** in the growth between Hbl+ isolates and Hbl- isolates when grown in competition **pairs** (**p<0.01**), in **self pairs** (**p<0.01**), and **alone** (**p<0.01**).

References

Conclusions

Future Directions

(1) Carroll, Laura M et al. Critical reviews in food science and nutrition vol. 62,28 (2022); 7677-7702. doi: 10.1080/10408398.2021.1916735

(2) Kovac, Jasna et al. BMC genomics vol. 17,581 (2016). doi: 10.1186/s12864-016-2883-z

(3) Alonso, Vanessa P.P. et al. Frontiers in microbiology vol 11 (2020). doi: 10.3389/fmicb.2020.00177

On average, Hbl+ isolates had a significantly larger surface colonization area when compared to Hbl- isolates. Although these results seem straightforward, it is likely that there are some confounding variables that could contribute to the trends in surface colonization area that were found.

The role of Hbl in surface colonization needs to be further evaluated with Hbl gene knockouts to determine if the growth advantages are due to Hbl or another factor associated with a phylogenetic group.

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