In vivo antagonism of *Lactobacillus gasseri* against the Group B *Streptococcus* hypervirulent clone

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Abstract

Background: The precise mechanisms involved in the Group B *Streptococcus*(GBS) transition from colonizer to pathogen remain unclear, however microenvironmental interactions may be pivotal to determine bacterial invasion and to address control measurements. We have used the moth *Galleria mellonella* to analyze the virulence and *in vivo* interactions of invasive and noninvasive human GBS isolates from our region.

Methods: *G. mellonella* model was adapted to laboratory conditions and larvae killing after GBS(ATCC 12403) infection was determined under different conditions of temperature, pH and bacterial competition. The effects of infection with invasive and noninvasive GBS isolates were also assessed. The survival of *G. mellonella* larvae inoculated with GBS strains (ATCC 12386 and ATCC 12403) was determined. Temperature, pH and bacterial competition effects were examined as well as the response of *Galleria* hemocytes to GBS infection.

Results: GBS was able to infect *G. mellonella* in a dose dependent manner with a (half-lethal dose) LD_{50} 1×10^7 CFU after 24h. Larval killing increased with temperature (37°C) and pH (≥5.5–7.2). Bacterial interaction assays showed a remarkable antagonistic effect of *L. gasseri* (cells and filtrates) on GBS infection and significantly improved *Galleria* survival. A multidrug-resistant hypervirulent clone (III/ ST17) showed higher killing rates in *G. mellonella* (100% after 48h). However, co-incubation of *L. gasseri* acidic filtrates with the hypervirulent clone before larva infection, induced growth inhibition and prevented larval killing (only 30% after 48h). Although to a lower level, a protective effect was also seen after exposure to neutralized filtrates.

Conclusions: Given the potential effects over the hypervirulent strain, our findings support the use of *L. gasseri* in the GBS control strategies based on *Lactobacillus* formulations. We showed that mechanisms mediating these effects are mainly pH dependent (~40%), however other mechanisms may have a role depending on inoculum.

Introduction

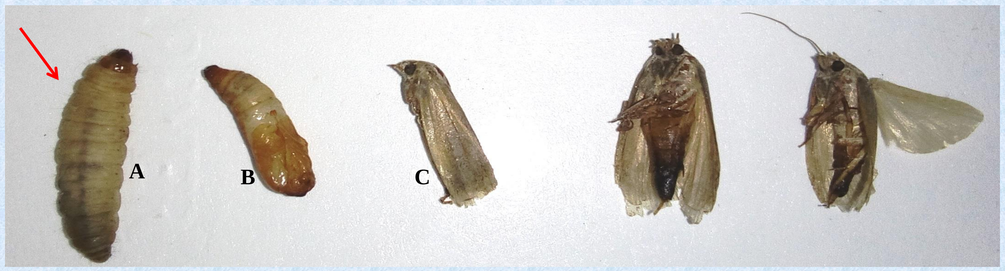
Pathogenesis of GBS is conferred by virulence factors associated with adherence to the cell surface or extracellular matrix, persistence, invasion and immune evasion or modulation (1). Nevertheless, when and how the colonizing GBS becomes invasive still remain to be elucidated.

DNA typing and virulence gene profile analyses have found not difference between invasive and colonizing isolates. This suggests that conditions in the microenvironment in the host seem to control the expression of invasion determinants.

Lately **an invertebrate model, the wax worm larva** *Galleria mellonella* has been used to study pathogenesis of a wide range of microorganisms, including bacteria, fungi and parasites. One main advantage of this model is the ease establishment and maintenance, low cost and feasibility for high throughput studies (2).

The aim of this study was to analyze the virulence and *in vivo* interactions of invasive and noninvasive human GBS isolates using G*alleria mellonella*.

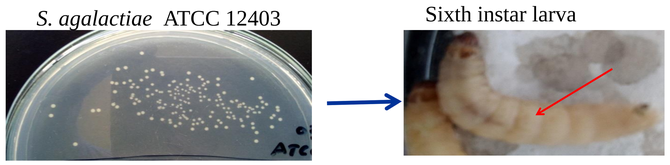
The *G. mellonella* model



G. mellonella (Lepidoptera) is a moth of the family Pyralidae, also known as the greater wax moth or honeycomb moth. This insect is found worldwide and lives in beehives causing severe damage to honeybee populations.
The life cycle lasts 60 days and goes through four developmental stages: egg, caterpillar(A) pupa (B) and adult (C). The caterpillar (arrow) has been used as an alternative model for host- pathogen interactions. The larva is easy to inoculate and handle at different laboratory conditions and temperatures. The model also allows to study immune response to microbial infections (2).

Experimental approach

Establishment and validation of *G. mellonella* as a model



Experiment set up

- Temperature 28°C vs 37°C
- pH 4.5 - 7.2
- Bacterial interactions/ *Lactobacillus gasseri* ATCC 19992

Experiments were performed in duplicate at least three times. Survival data were analyzed using Kaplan-Meier curves. Comparison of curves and signficance were determined by the log-rank test. Statistical analyses were performed using the GraphPad Prism 8.2.0 software.

Results

1. *G. mellonella* is infected by GBS strains

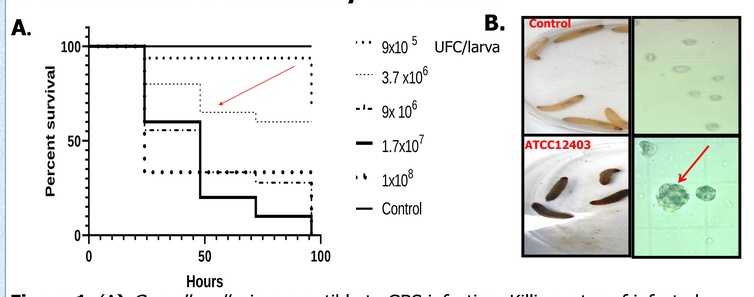


Figure 1. (A) *G. mellonella* is susceptible to GBS infection. Killing rates of infected larvae are dose-dependent (arrow) (B) After GBS infection hemocyte depletion and vacuolation were seen (arrow).

2. Temperature increases mortality after 24 h

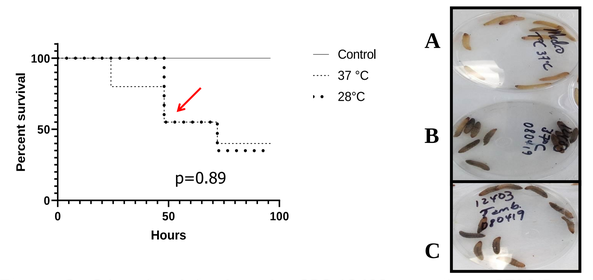


Figure 2. 24 h after infection with GBS 12403 mortality at 37°C(B) was higher than at 28°C (C) although the lethal dose (LD) was the same after 48h (arrow). A control group with only culture medium was included (A).

3. pH effects on killing rates depend on inoculum

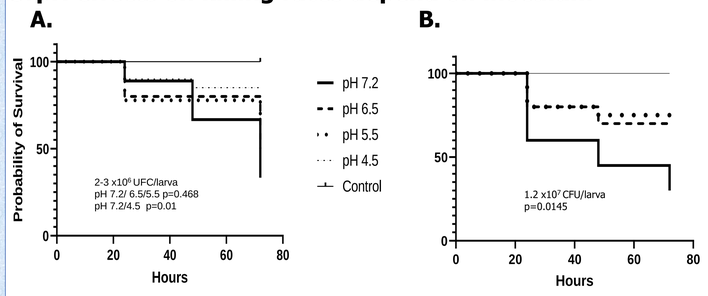


Figure 3. **(A)** Larval mortality showed no difference at pH 5,5, 6,4 and 7.2 but it was significantly lower at pH 4.5 at sublethal inoculum. However, killing rates were significantly higher at pH 7.2 as the inoculum increases **(B)**. Groups of larvae were injected with control media adjusted to each pH, all the control larvae survived over time.

4. *L. gasseri* improves survival of GBS infected larvae

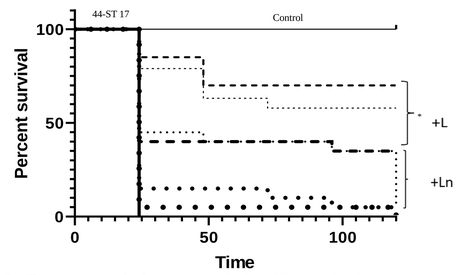


Figure 4. **Filtrates of *L. gasseri* significantly improved survival of *G. mellonella* after infection with a GBS hypervirulent isolate**. *L. gasseri* antagonism is dose-dependent. Solid lines represents killing curves after infection with GBS 44-ST17 in culture media. GBS 44-17ST inocula + *L. gasseri* filtrate (44 ST 17/L) is shown in dashed lines. GBS 44-ST17 inocula + neutralized filtrates of *L. gasseri* (44- ST17/Ln) is indicated in dotted lines. Inocula density is represented by line thickness. *p< 0.001

5. Mechanisms of *L. gasseri* – GBS antagonism

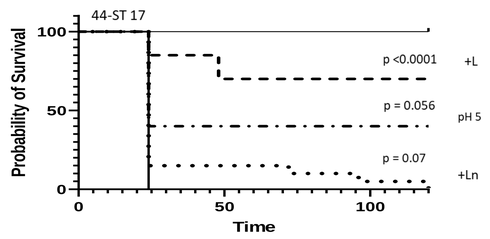


Figure 5. Attenuation of GBS virulence by *L. gasseri* may be mediated by pH dependent and pH independent mechanisms. Data of GBS44 ST17 (2.2×10^8 CFU/mL) are shown in solid lines. Killing curves of GBS 44-ST17+ neutralized filtrates of *L. gasseri*, (Dotted lines) are contrasted with GBS 44-ST17+ acidified media to pH 5 (Dashed/dotted line) and GBS 44-17ST inocula + *L. gasseri* filtrate (dashed lines).

Summary

- *G. mellonella* model for GBS infection was adapted in local laboratory conditions and a reproducible manner. GBS induced melanization and depletion of hemocytes.
- Killing rates were higher at 37°C and pH 7.2, conditions seen in blood and tissue invasion.
- *L. gasseri* filtrates remarkably prevent larvae mortality induced by GBS hypervirulent clone. Inhibition of virulence seems to be mainly mediated by pH dependent mechanisms, however pH independent mechanisms may be also involved to a leeser extent.

REFERENCES

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2. Tsai CJY, Loh JMS, Proft T. *Galleria mellonella* infection models for the study of bacterial diseases and for antimicrobial drug testing. *Virulence*. **2016**;7(3):214–29.