Development of a bioluminescent reporter system to monitor neonatal Group B Streptococcal infection

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Introduction

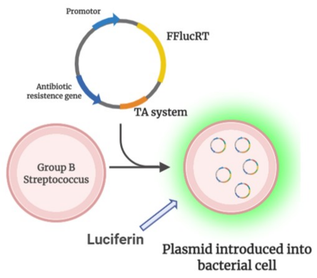
Group B *Streptococcus* (GBS) is the main bacterial cause of severe neonatal invasive disease, including meningitis[1]. Morbidity is high and up to 50 % of surviving infants experience long-term neurologic sequalae[2]. To define new therapeutic and neuroprotective strategies, we must gain deeper insights into the pathophysiology of disease. The classical methods for quantifying infection do not allow longitudinal studies as it entails the animal death[3].

Objective

To develop a bioluminescent reporter system using a plasmid containing the red-shifted firefly luciferase to monitor GBS dissemination.

Methods

1st Transformation of the hypervirulent GBS strain BM110 with plasmid containing the red-shifted firefly luciferase, FFluc(P_{TA})



Plasmid pLZ12Km2-P23R:TA:fflucRT [FFluc(P_{TA})] created by Loh and Proft, 2013[4]

2nd Validation of bioluminescent reporter system in GBS BM110

- ✓ Assess the correlation between bioluminescence signal, bacterial numbers and optical density (OD) on Synergy 2
- ✓ Control the growth monitoring on Spectrophotometer
- ✓ Test the plasmid stability
- ✓ Verify the GBS virulence *in vitro* and *in vivo*
- ✓ Confirm the bioluminescence in living pups using an *in vivo* imaging system

Results and Discussion

1. The presence of the plasmid does not affect GBS fitness and the plasmid remains stable in the absence of antibiotic selection pressure

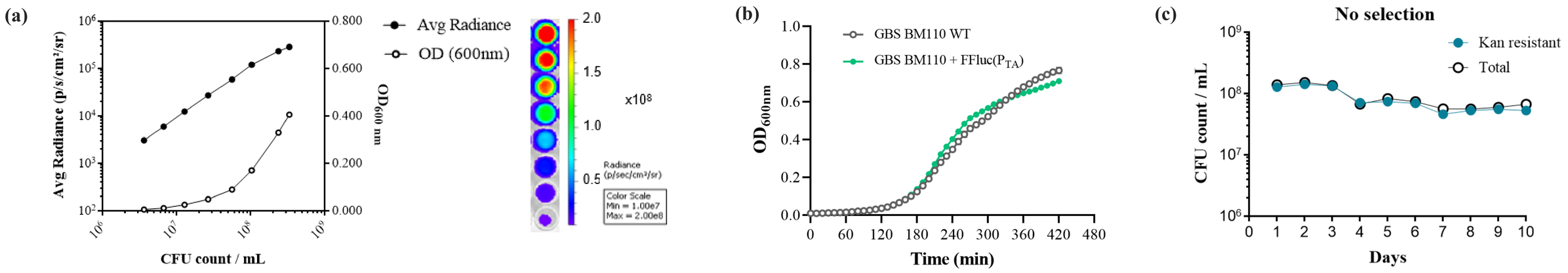


Figure 1- (a) Correlation of bioluminescent signal, OD and colony-forming units (CFU) count; **(b)** Growth curves of GBS BM110 WT and GBS BM110+Ffluc(P_{TA}); **(c)** GBS-FFluc stability evaluation. Numbers of Kanamycin (Kan) resistant GBS and total GBS cells grown in medium without Kan selection.

2. The plasmid does not affect the virulence of GBS *in vitro*

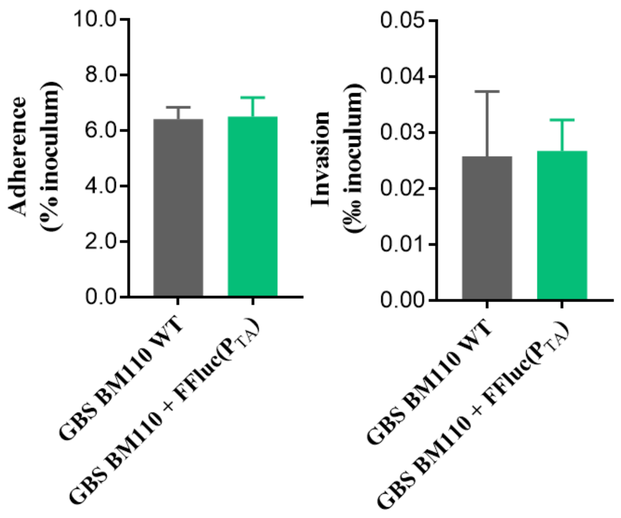


Figure 2- Bacterial adhesion and invasion of human epithelial cell line Caco-2. Cells were infected at a multiplicity of infection 30, for 3 h. The percentages are expressed relative to the initial inoculum.

- ❑ No significant differences were found in the percentage of invasion and adhesion.

3. The *in vivo* virulence is not affected by the presence of the plasmid and the bioluminescence is detected in living pups

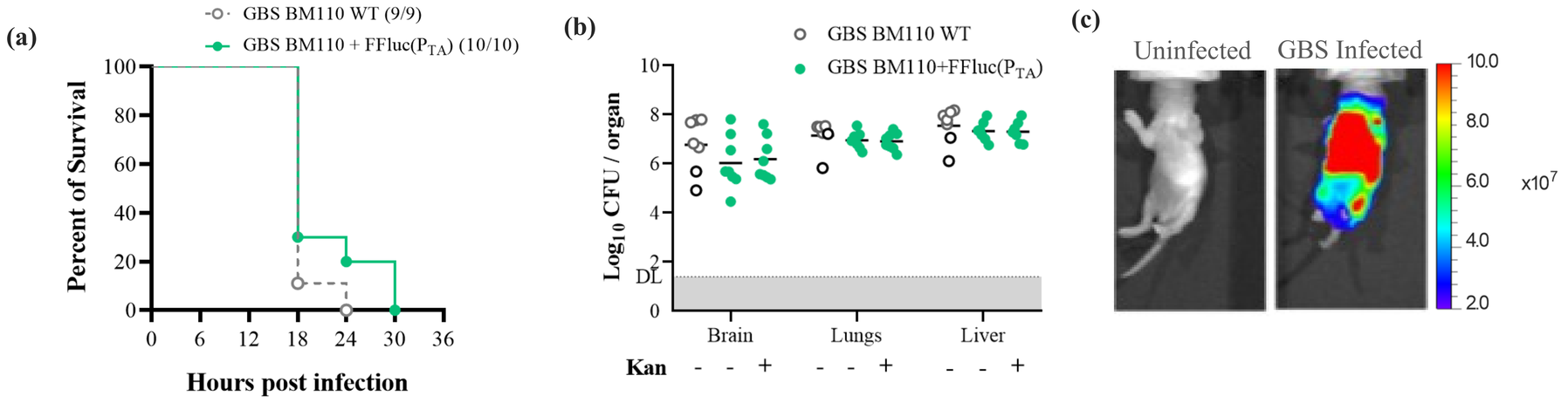
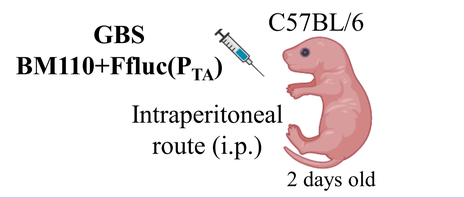


Figure 3- (a-b) Two-day-old C57Bl/6 mice i.p. inoculated with 5x10^5 CFU of GBS BM110 WT or GBS BM110+FFluc(P_{TA}); **(a)** Kaplan-Meier survival curve monitored for 30 h. Numbers in parenthesis represent pups that survived versus the total number of infected pups; **(b)** Bacterial load in the brain, lungs and liver 18 h post-infection; **(c)** Bioluminescence imaging using IVIS Lumina LT of C57Bl/6 mice infected with 1x10^5 CFU of GBS BM110+FFluc(P_{TA}) 18 h post-infection.

- ❑ No differences was obtained in the survival curve and the organ colonization. The results also showed that the stability of the plasmid was maintained through the infection process (neonatal mice did not receive Kan).



Conclusion

GBS BM110 can be efficiently transformed with plasmid, FFluc(P_{TA}):

- ✓ Bioluminescence signal and OD were proportional to GBS cells;
- ✓ GBS fitness was not affected;
- ✓ Plasmid stability was maintained in the absence of selective pressure;
- ✓ GBS virulence was not affected *in vitro* and *in vivo*;
- ✓ The bioluminescence was detectable in living pups.

FFluc(P_{TA}) plasmid is a promising option for studying GBS disease

- ❖ Offering information about the infection process
- ❖ Assessing bacterial dissemination *in vivo*
- ❖ Determining when GBS invades the CNS

References

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4. Loh, J.M. and Proft, T.,Toxin-antitoxin-stabilized reporter plasmids for biophotonic imaging of Group A streptococcus. Applied microbiology and biotechnology. 2013;97(22):9737-45.

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