



Assessing *Batrachochytrium dendrobatidis* (*Bd*) infections in Jamaican frogs and antifungal properties of *Pseudomonas* bacteria



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Abstract

Chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (*Bd*), is a major driver of global amphibian declines. However, some populations exhibit resistance attributed to skin-associated microbes such as *Pseudomonas* that inhibit *Bd* growth. This study assessed *Ba* prevalence in Kingston, Jamaica, a tropical region where its impact on endemic and invasive frogs remains understudied. Skin swabs from 33 frogs were analyzed using qPCR, with only 3 individuals (9.09%) testing positive for *Bd*. Two of these were found at higher elevations, suggesting environmental conditions may influence *Bd* distribution. Additionally, *in vitro* inhibition assays identified two *Pseudomonas* strains with anti-*Bd* activity not previously reported, highlighting their potential for bioaugmentation-based conservation strategies.

Bd infections in frogs

- The fungal disease Chytridiomycosis caused by *Batrachochytrium dendrobatidis* (*Bd*) is the leading cause of amphibian death worldwide.
- Amphibian skin is responsible for respiration, thermal regulation and electrolyte balance. *Bd*'s growth into the skin induces thickening of keratin layers leading to muscle loss and cardiac **arrest**.
- Skin microbiome (mucosome) contains bacteria that produce anti-fungal metabolites to inhibit *Bd* replication

Bd invasion and replication

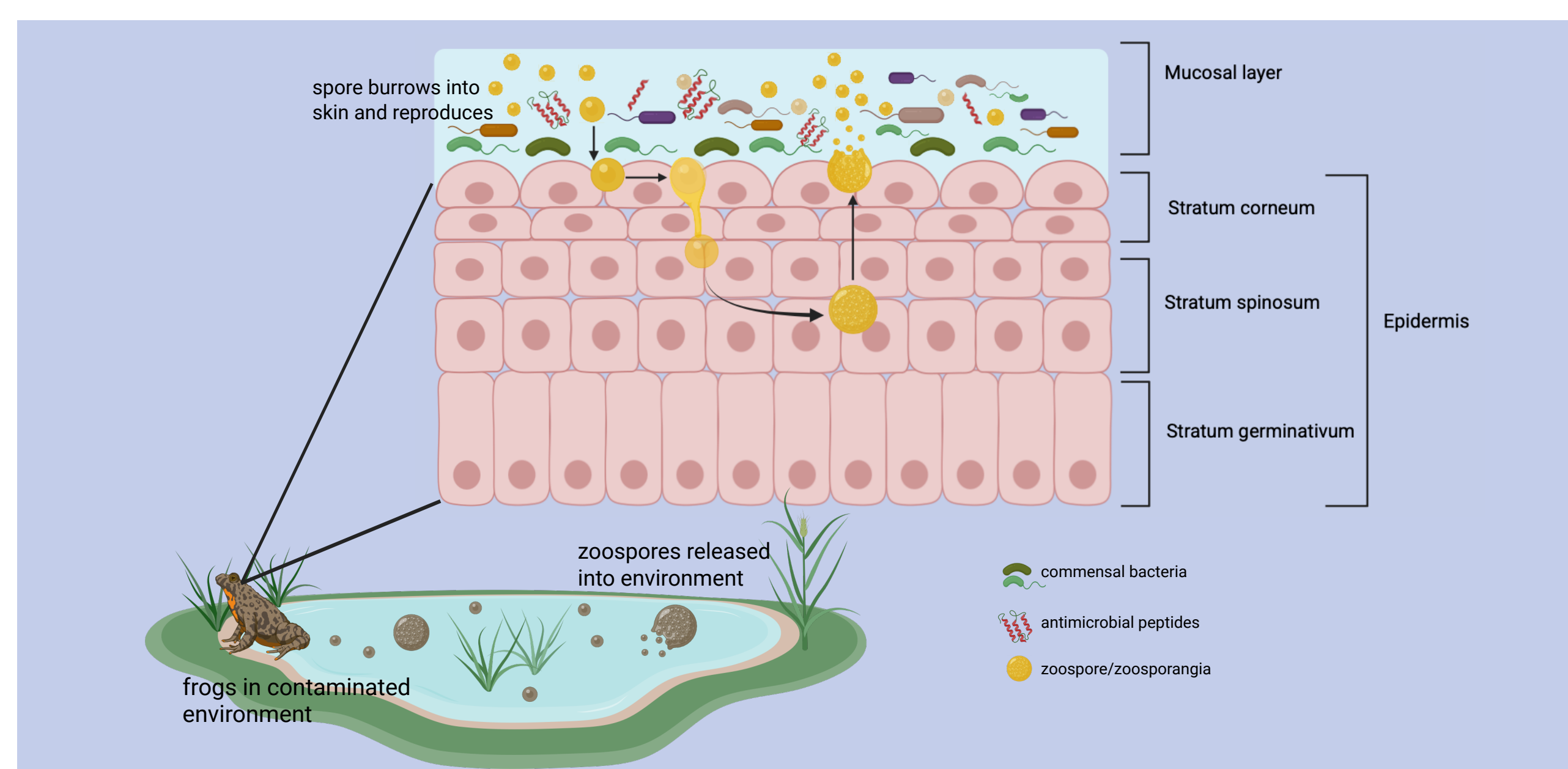


Figure 1. *Bd* exposure and life cycle in host skin

References

Campbell et. al (2012). Frog skin epithelium: Electrolyte balance and chytridiomycosis. *Int'l Journal of Biochemistry and Cell Biology* 44(3). ; Van-Rooij et. al (2015) Amphibian chytridiomycosis: review with focus on fungus-host interactions. *Vet. Research* (46).

Are there differences in infection rates between native and invasive frogs?

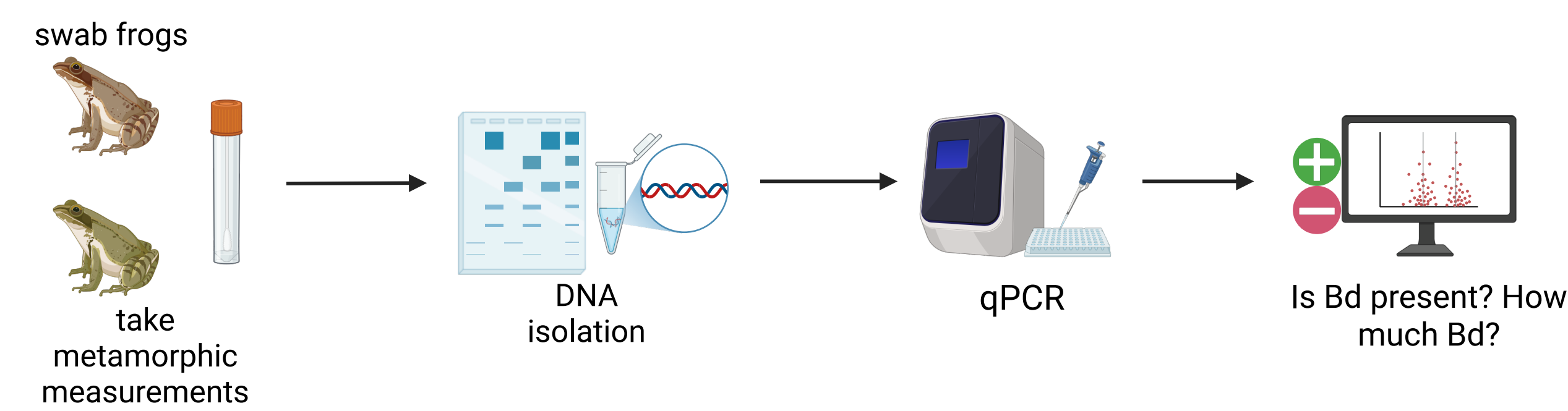


Figure 2. Method for assessing infection rates using qPCR

Table 1. qPCR analysis of *Bd* infection of frogs from St. Andrew, Jamaica.

Frog Species	n	no. <i>Bd</i> +	% <i>Bd</i> +	<i>Bd</i> load
Native				
<i>Eleutherodactylus gossei</i>	13	1	7.79	970
<i>E. cundalli</i>	1	0	0	-
<i>E. andrewsi</i>	2	0	0	-
<i>E. alticola</i>	1	1	100	386
<i>Osteopilus ocellatus</i>	1	0	0	-
Invasive				
<i>E. planirostris</i>	6	1	33.3	53
<i>E. johnstonei</i>	10	0	0	-

Bd load is the number of DNA copies for whole swab values

- All positive samples were taken from 800m and higher
- Frogs at lower altitudes may be protected from *Bd* due to high temperatures and *Bd* heat sensitivity
- Only one sample displayed symptoms of chytrid with a deformed jaw and its tongue could not be retracted

Does *Bd* infection affect body condition?

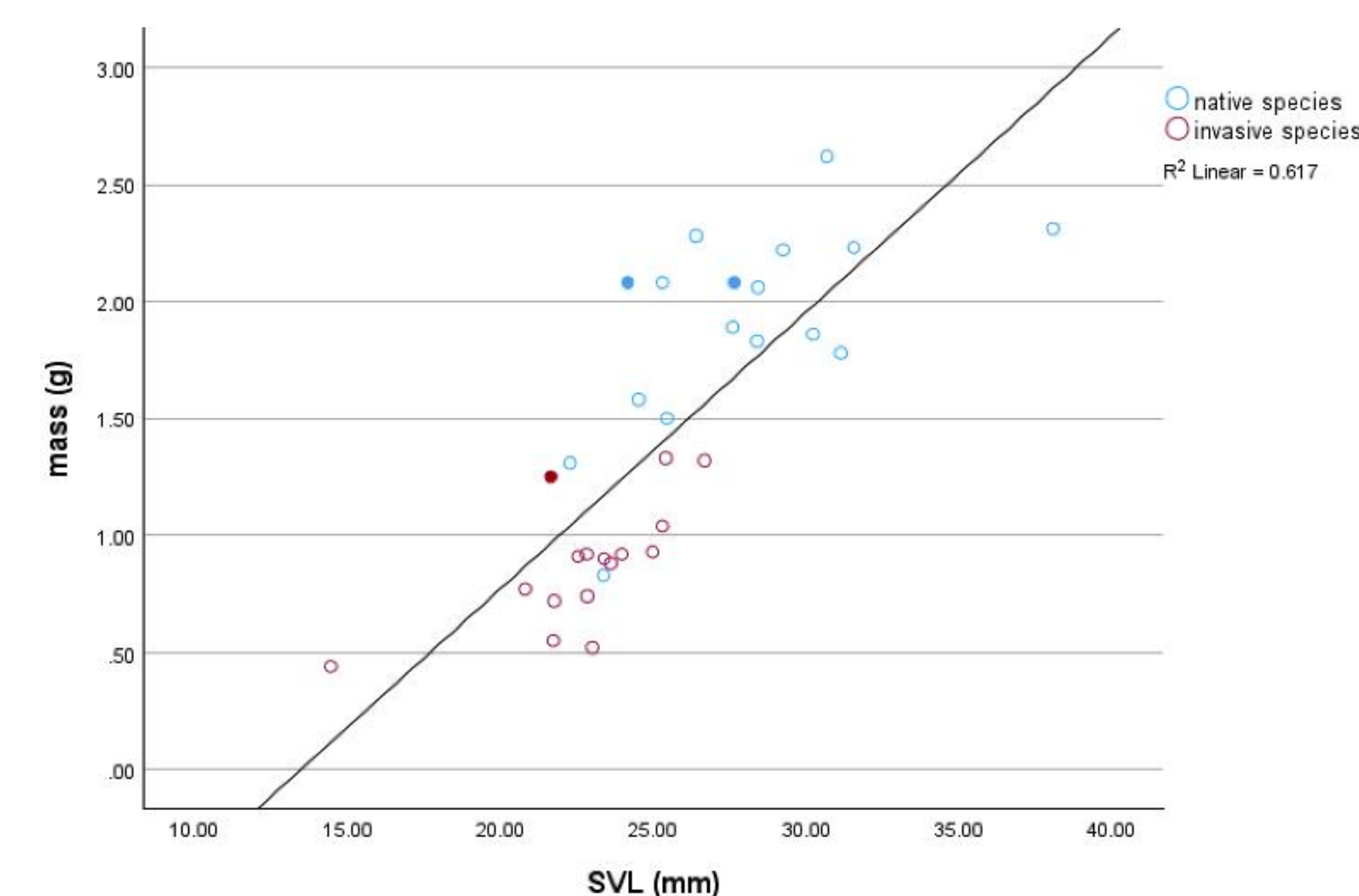


Figure 4. Linear regression plot of mass against length/SVL in *Eleutherodactylus* species indicate *Bd* infected native and invasive species are not in poor body condition.

- No positive samples appear to be in poor body condition
- Invasive species could be exhibiting smaller body condition scores due to heat stress and other environmental stressors

Which *Pseudomonas* species display inhibitory effects?

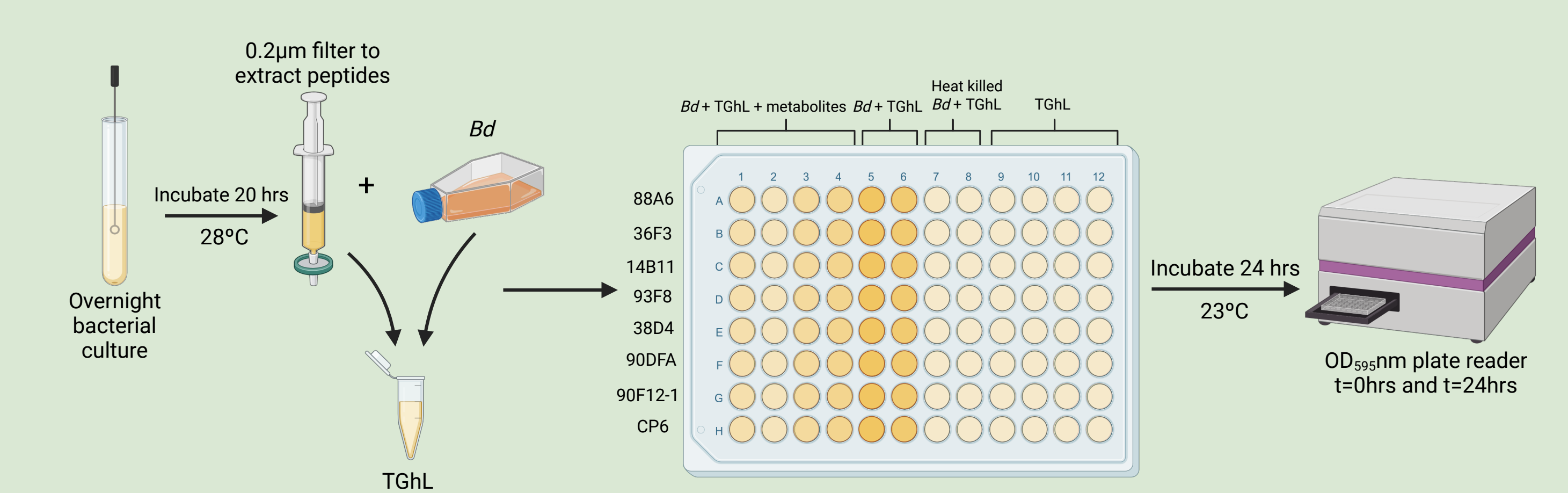


Figure 6. Method for liquid cell-free supernatant inhibition assay

Bd Inhibition Assay

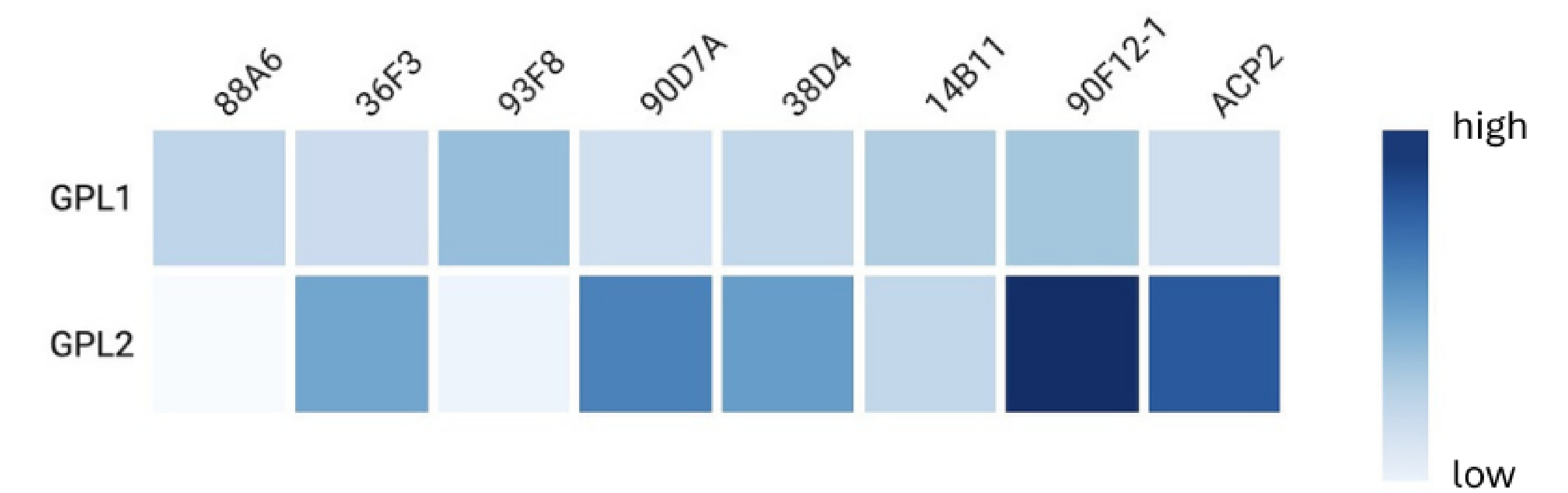


Figure 7. Heatmap displaying which bacterial strains displayed the most inhibition. Darker colors indicate higher inhibition.

- P. rhodesiae* 90F12-1 displays the most inhibition in GPL2
- P. brassicacearum* displays moderate inhibition in both GPL1 and GPL2
- GPL2 appears to be more sensitive to inhibitory species

Future Works

- Shotgun metagenomic sequencing to determine if there are microbiome differences that may be attributed to lower *Bd* loads
- Mass-spectrometry to identify the specific anti-fungal metabolites that inhibit *Bd* growth
- More cell-free supernatant inhibition assays to determine other potential inhibitory strains

Acknowledgments

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