

How to scape from a killer fungus: a study of the epithelial microbiota as the main barrier to *Batrachochytrium dendrobatidis* infection.

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Amphibians are the world's most threatened class of vertebrates. Habitat loss and the emerging fungal infectious disease, chytridiomycosis, have been singled out as the causes that inflict the most damage on amphibian biodiversity. Chytridiomycosis is causing mass mortality and population declines worldwide. This disease is the greatest challenge for amphibian conservation as no proven implementable strategy in the field exists that can combat this disease. The community composition of the cutaneous microbiota has a strong effect on the morbidity caused by chytridiomycosis and is essential in the survival of the populations. The experiments proposed later try to recognize if different species of wild amphibians can promote beneficial anti-Bd bacteria when they are exposed to the fungus and acquire more information about the microbiota's role in the resistance of amphibians to chytridiomycosis.

PREVIOUS KNOWLEDGE

About the fungus:

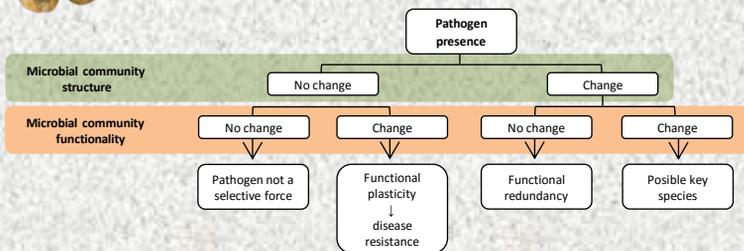
- Batrachochytrium dendrobatidis* (Bd) causes the disease chytridiomycosis in amphibians.
- Bd colonizes skin cells of adults and the keratinized mouth parts of tadpoles but does not invade other tissues.
- Death and sub-lethal effects have been observed depending on the specie, life stage and season.
- It's being hypothesized that death results from impaired retention of essential ions by the skin resulting in eventual cardiac arrest.



About the epithelial microbiota communities:

- Innate defenses of amphibians include antimicrobial peptides and symbiotic skin bacteria, both of which have been found to inhibit Bd growth.
- The community composition of the cutaneous bacteria is essential in the survival of the populations.
- This microbiota differs between species, populations and individuals.
- The differences in the microbiota composition may have a role in the species-specific resistance to fungal pathogen.
- A plausible future conservation strategy is the use of probiotic anti-Bd bacteria to combat chytridiomycosis.

PREDICTED RESULTS



It's expected to observe changes in the communities
Bd acts as a selective force in these communities

In relation to Bd treatment:

- No changes are expected in richness.
- Changes are expected in the diversity and relative abundance of promoting immunity species → increasing densities of some them with anti-Bd capability.
- A drop of weight is expected by Bd infection or by the energy costly changes in the epithelial flora.
- It has been found that the zoospores load does not have to be related with mortality rate → may neither be with weight loss.

In relation to the comparison of the two species (more and less sensitive):

- It's expected to find differences both in richness and diversity → it is expected to find a greater increase of any anti-Bd bacteria with high capacity in the most resistant specie.

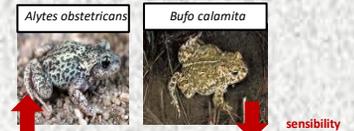
MATERIAL AND METHODOLOGY



1. AFTER CAPTURE



- Body mass
- Check for possible infections (Bd mainly) → Duplex real-time TaqMan PCR



2. TREATMENT GROUPS

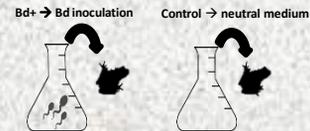


3. PRETREATMENT



- Body mass
- Bacterial community analysis:
 - Anti-Bd bacterial richness → Epithelial bacteria culture, Bd-challenge assay and identification
 - Relative abundance of bacterial species → TRFLP
 - Diversity index → Shannon and Simpson

4. Bd TREATMENT



6. STATISTICAL ANALYSIS

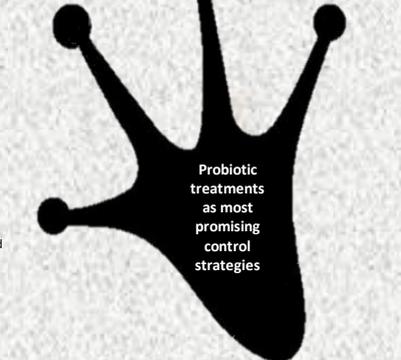


5. POST-TREATMENT

- Body mass
 - Bacterial community analysis:
 - Anti-Bd bacterial richness → Epithelial bacteria culture, Bd-challenge assay and identification
 - Relative abundance of bacterial species → TRFLP
 - Diversity index → Shannon and Simpson
 - Pathogenic load (in Bd+ group) → Taqman-PCR
- 1, 2 and 3 weeks after treatment

IMPORTANCE AND IMPACT

Climate change will get the situation worst
Knowledge of epithelial microbiota is critical for effective conservation management



SELECTED REFERENCES

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