Fighting a Deadly Fungus with Bacteria Harvested from Salamander Skin

Fantasia Erdman, Gabrielle Russell, Aubree J. Hill, and Donald Walker, Department of Biology, Tennessee Technological University

Figure 1. A deceased fire salamander in the Netherlands infected with a chytrid fungus. Photo credit: Susan Walker

Introduction

Amphibian populations across the globe have been rapidly declining as a result of the emerging fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Fig. 1).[1-6] This is particularly concerning to local biologists, as salamanders are vital members of forest ecosystems, and Southeastern Appalachia, U.S., is the most biodiverse location for these amphibians on the planet. The most promising option for developing an antifungal treatment is to harvest fungus-fighting bacteria (known as probiotics) directly from the skin of salamanders. [2, 5]

Hypothesis and Objectives

Numerous studies have isolated antifungal bacteria from skin of amphibians, the so-called cutaneous microbiome.[2, 5] We predicted that probiotics could be isolated from the skin of native salamander species. The objectives of our research were to (1) culture bacteria from skin swab samples collected from wild salamanders, and (2) challenge these bacterial isolates against Bd in co-culture to determine which isolates can inhibit growth of the fungus.

Methods

Skin swab samples were collected from wild salamanders in Tennessee (Fig. 2), using an established rinsing and swabbing protocol.[2, 5, 7] From 11 swabs, a total of 98 bacterial isolates were grown in pure culture and cryopreserved. A donated strain of Bd (isolate #JEL423) was propagated in tryptone broth at 23˚ C. A carefully standardized concentration of chytrid zoospores (844,000 cells / mL) was measure using a hemocytometer for consistency across all assays. Challenge assays were prepared on tryptone agar plates. One milliliter of standardized zoospore solution was added to each tryptone agar plate and allowed to dry for 30 minutes in a laminar flow hood. Fresh cultures of two bacterial isolates (grown 24 hours prior) were then streaked down the center of both sides of each plate (Fig. 3). Plates were incubated at room temperature for five days, and zones of fungal inhibition were measured.

Results

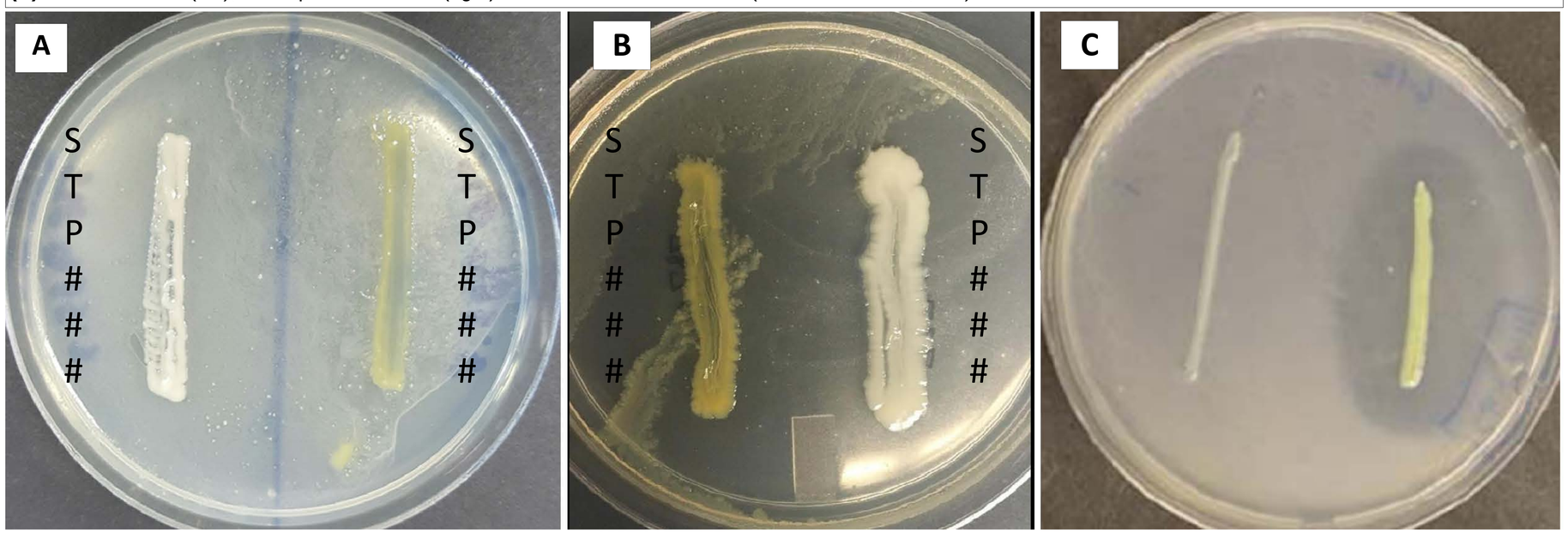
Table 1. Summary of Challenge Assay Results

Isolate Number	Salamander Host Species	Inhibited Bd Growth	Motility
STP783.2	Plethodon glutinosus	No	No
STP783.3	Plethodon glutinosus	No	No
STP784.3**	Eurycea longicauda	Yes	No
STP785.1**	Eurycea longicauda	Yes	No
STP785.3	Eurycea longicauda	No	No
STP785.5	Eurycea longicauda	No	No
STP785.7	Eurycea longicauda	No	No
STP786.4**	Plethodon glutinosus	Yes	No
STP787.1	Eurycea cirrigera	No	Yes
STP787.5	Eurycea cirrigera	?	Yes
STP787.6	Eurycea cirrigera	Yes	Yes
STP787.10	Eurycea cirrigera	?	Yes
STP787.11	Eurycea cirrigera	Yes	Yes
STP787.15	Eurycea cirrigera	No	Yes
STP787.17	Eurycea cirrigera	Yes	Yes
STP789.3**	Eurycea longicauda	Yes	No
STP790.6**	Desmognathus. sp.	Yes	No
STP791.1**	Eurycea longicauda	Yes	No

**candidate probiotic

Figure 2. Gabrielle Russell helping collect salamander skin swab samples.

Figure 3. Growth of Bd challenged against bacterial isolates on tryptone agar plates. **(A)** Two bacterial streaks, isolated during this study from a long-tail salamander (*Eurycea longicauda)*, showing no inhibition of Bd. (B) Two bacterial streaks, isolated during this study, showing bacterial motility that is masking possible antifungal activity. **(C)** A control streak (left), and a probiotic streak (right) with a zone of Bd inhibition (from Harris et al., 2006).



Discussion

Thus far, all bacterial isolates that have been tested (n = 18, Table 1) grew in the presence of Bd. Some did not form measurable zones of inhibition (Fig. 2A), and others produced zones that were masked by bacterial motility (Fig 2B). Non-inhibitory isolates have been discounted as candidate probiotics, and motile isolates will be retested in the future, using a modified protocol. Work is still ongoing for both objectives and we anticipate discovery of probiotic bacteria in future challenges. Candidate probiotics which meet additional screening criteria will be genotyped (meaning their taxonomic identification will be obtained) and recommended for future therapeutic trials. Results may directly impact future conservation efforts for valuable North American wildlife.

References

1. Belden LK, RN Harris. In *Front Ecol Env* 5.10 (2007): 533-9.
2. Bletz MC, et al. In *Ecol Letters* 16.6 (2013): 807-20.
3. Bosch J, et al. In *Bio Conserv* 97.3 (2001): 331-7.
4. Garner TWJ, et al. In *Biol Letters* 2.3 (2006): 455-59.
5. Harris RN, et al. In *EcoHealth* 3.1 (2006): 53-6.
6. Martel A, et al. In *Science* 346.6209 (2014): 630-31.
7. Walker DM, et al. In *HerpReview* 46 (2015): 349–353.

Acknowledgments: Great Smoky Mountains Conservation Association (funding), Tennessee Herpetological Society (funding), Calvin Hall (lab assistance), and Joyce E. Longcore (donated strain of Bd)