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Identification of *Batrachochytrium dendrobatidis* inhibiting isolates via challenge against salamander skin microbiota.

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Abstract

Rapid declines in global amphibian populations can be partially attributed to chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (Bd). Due to how quickly it has spread worldwide, and the number of amphibians affected, this fungus is of increasing concern in both the herpetological and mycological communities. We characterized the cutaneous microbiome of Appalachian salamanders, which persist in the presence of the fungus despite high rates of infection in co-occurring frog populations. The cutaneous microbiome of vertebrates is known as the first line of defense against invading pathogens. We hypothesized that these salamanders might have resident bacteria on their skin with the ability to inhibit Bd growth. Our objectives were to 1) isolate resident bacteria from salamander skin swabs, 2) challenge these isolates against Bd, and 3) genotype all inhibitory isolates. During the summer of 2016, 82 swabs were collected from three salamander genera including *Desmognathus* (n=36), *Eurycea* (n=20), and *Plethodon* (n=26). To isolate cutaneous bacteria, swabs were streaked on Reasoner's 2A agar plates that we checked daily for two weeks. Colonies with unique morphology were isolated onto secondary plates until pure cultures were obtained. To challenge the isolates against Bd, zoospores were harvested from cultures that had been aged 5–7 days, counted with a hemocytometer, and diluted to approximately 1,000,000 cells/milliliter (mL). Tryptone agar plates were then flooded with 1.0 mL of standardized zoospore solution and dried for 45 minutes in a laminar flow hood. Bacterial isolates were streaked onto each plate in co-culture with Bd. Plates were incubated at room temperature for four days, examined for noticeable zones of inhibition (ZOI), measured in millimeters. Isolates that produced a measurable ZOI were photographed and will be screened in further testing against Bd. We used direct colony PCR and Sanger sequencing to identify all inhibitory isolates. Presently, 63 of the 472 acquired isolates have been challenged, 16 of which produced a ZOI. The challenge phase is ongoing and we anticipate discovery of additional candidate probiotic species. In addition, we plan to test for possible synergistic inhibition by challenging candidates in polyculture.

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