The endosymbiotic bacterial community of the causative agent of white-nose syndrome (*Pseudogymnoascus destructans*) of bats.

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Introduction

- White-nose syndrome (WNS) is an aggressive and fatal disease currently affecting bat populations in North America. It causes bats to behave abnormally by waking early from hibernation, resulting in depletion of crucial fat reserves. This abnormal behavior is also accompanied by changes to the infected bat's physiology, such as elevated CO_2 levels and changes in pH associated with respiratory acidosis (Verant et al., 2014). The causative agent of WNS has been determined to be a pathogenic fungus known as *Pseudogymnoascus destructans* (*Pd*) [Lorch et al., 2011]. It is a white-gray fungus that can be seen on the skin of infected bats, most predominately the wings and nose.
- Our research focuses on developing an understanding of the microbial community that associates with *Pd*. The long-term goal is to determine if the presence of bacteria, living within fungal cells of P. destructans (endohyphal), alters the virulence of this pathogenic fungus. Although previous studies (Hoffman, M. Gunatilaka, Wijeratne, L. Gunatilaka, & Arnold, 2013) have been performed to show intracellular bacterial presence within fungi, those studies have predominantly focused on plant fungal pathogens, not animal fungal pathogens.

Objectives

- Measure the relative protease activity (Figure 3A), lipase activity (Figure 3B), and growth rates (Figure 4) of *Pd* isolates with and without bacterial symbionts.
- Determine bacterial-fungal relationships between Pd, Nocardia spp., and other bacterial symbionts.

Methods

- 18 wildtype Pd isolates were cultured on PDA, and 18 Pd antibiotic treated isolates were cultured on PDA +6 prior to enzymatic assay media inoculation

  PDA+6 antibiotics: ciprofloxacin (20 µg/mL), kanamycin (60 µg/mL), chloramphenicol (8 µg/mL), penicillin (25 µg/mL), streptomycin (25 µg/mL), and amoxicillin (64 µg/mL)
- A 3 mm plug was used to inoculate skim milk agar (SMA) and tributyrin agar (TRI) plates with Pd to ensure uniformity among samples. SMA was used to test protease activity, while TRI was used to test lipase activity. Each isolate had 5 replicates.
- Data was collected throughout the 14-day enzymatic assays for plug growth size and agar clearing size. This data was used to determine relative enzymatic activity (REA) and fungal growth rate.
- qPCR and high-throughput 16S sequencing was used to determine bacterial residency status. This data was used to assign post-hoc designations to all isolates: no bacteria present, bacteria present without Nocardia spp., bacteria present with Nocardia spp.

Figure 1: SMA protease assay for CCB 87.2 Wildtype/NT (top) and Treated/T (bottom)

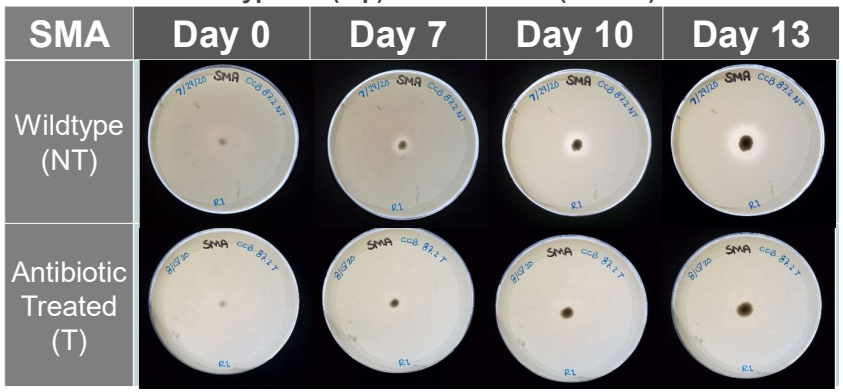


Figure 2: TRI lipase assay for CCB 87.2 Wildtype/NT (top) and Treated/T (bottom)

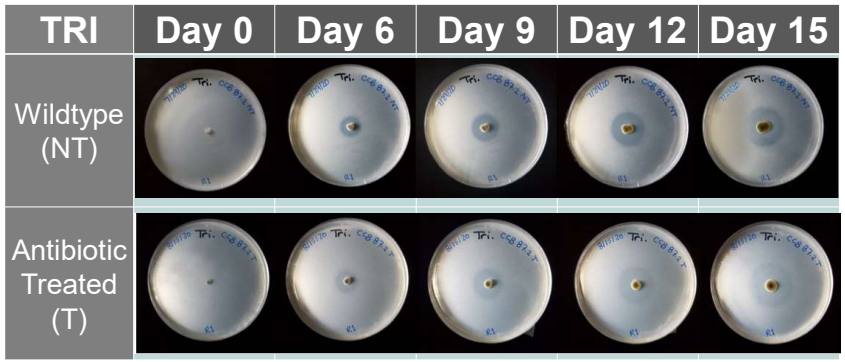
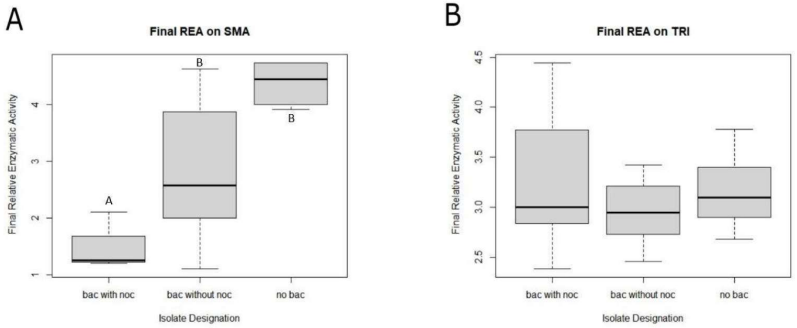
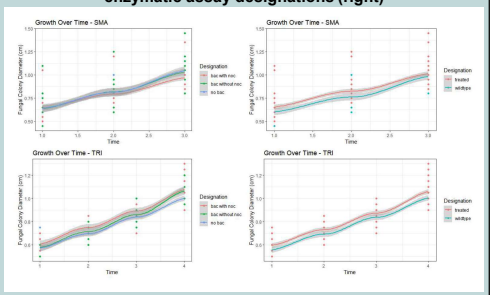


Figure 3: Relative Enzymatic Activity (REA) of protease (A) and lipase (B) based on post-hoc designations.



Results

Figure 4: Growth rates for *Pd* isolates on SMA (top) and TRI (bottom) relative to post-hoc designations (left) and enzymatic assay designations (right)



- Protease activity between wildtype and antibiotic treated isolates was insignificant (GLMM, t-value = -1.432, p > 0.05).
- Protease activity between post-hoc designations was significant (Figure 3A) (GLMM, t-value = -2.441, p < 0.05).
- SMA isolate growth rates between post-hoc designations were significant (Figure 4) (GLMM, t-value = -2.797, p < 0.005).
- Lipase activity between wildtype and antibiotic treated isolates was insignificant (GLMM, t-value = 0.175, p > 0.05).
- Lipase activity between post-hoc designations was insignificant (Figure 3B) (GLMM, t-value = 0.704, -0.890, p > 0.05).
- TRI isolate growth rates between post-hoc designations were insignificant (Figure 4) (GLMM, t-value = 1.914, -1.280, p < 0.05)

Discussion

- Neither SMA or TRI assays showed significant differences in REA or growth rates between wildtype and antibiotic treated isolates.
- SMA assay isolates with bacteria present but were absent of *Nocardia spp.* had significantly higher protease activity and growth rates than isolates in the other two post-hoc designations (without bacteria present, with bacteria and *Nocardia spp.* present).
- TRI assay isolates did not show any significance in difference between post-hoc designations in regards to lipase activity or growth rate.
- These results show a potential antagonistic relationship between *Pd* and *Nocardia spp.* regarding protease activity. More data is needed to confirm bacterial-fungal relationships between other bacterial symbionts.

Literature Cited

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