Microbial combat and the race to save the frogs: The effects of Bacillus thuringiensis secondary metabolites on Batrachochytrium dendrobatidis

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Background

- Batrachochytrium dendrobatidis (Bd) is a fungal pathogen that infects the skin of amphibians leading to chytridiomycosis.
- Chytridiomycosis causes symptoms of appetite loss, lethargy, excessive skin shedding, and cardiac arrest resulting from ion imbalance.
- Amphibian skin is more than just a protective barrier, it is used for ion exchange, nutrient absorption, and breathing.
- The keratinized skin of amphibians attracts Bd zoospores, the zoospores burrow into the skin, develop into sporangia and release new Bd zoospores.
- Bacillus thuringiensis (Bt) is a common soil bacteria commonly used as an agricultural pesticide.
- The strain B. thuringiensis israelensis (Bti) is used to control larval growth of mosquitoes and flies. B. thuringiensis kurstaki (Btk) is used to control the growth of caterpillars, particularly in corn farming.
- Bti and Btk also produce antifungal secondary metabolites.
- Secondary metabolites are produced by many organisms, including bacteria, and some have antifungal properties.
- Some amphibians are protected from Bd infection by antifungal secondary metabolites from bacteria they acquire in their environment.

Methods

- Bti and Btk were grown separately on 1% tryptone agar plates
- Btk and Bti was transferred to 1% tryptone broth, incubated at 37°C for 48 hours, and a standard growth curve was created.
- Secondary metabolites were extracted from bacterial liquid cultures and plated with the Bd in a 96 well plate.
- Experimental Design consisted of:
  - Bd only (n=20)
  - Bd + 2° metabolites (n=20)
  - Heat killed Bd + 2° metabolites (n=20)
- Bd vs. Bd assay was read with spectrophotometer each day for 1 week. Day 0 absorbance reading was subtracted from Day 7 reading to determine change.
- Growth enhancement calculated from: \[ \frac{Bd \text{ and metabolites}}{Bd \text{ only}} \times 100 \]
- Data analyzed with two sample t-test

Results

- Bti metabolites significantly enhanced the growth of Bd by 230.5%\[ t_{18} = -10.59, p < 0.001 \]
- Btk metabolites significantly enhanced the growth of Bd by 201.5%\[ t_{18} = -21.50, p < 0.001 \]

Discussion

- The growth of Bd was significantly enhanced by the presence of Bacillus thuringiensis secondary metabolites.
- Bd is commonly used as an organic pesticide. Organic pesticides are typically thought of as better for the environment, but we do not know all their affects.
- Humans release harmful chemicals into water all the time that directly effect amphibian health.
- Bt, if grown in liquid, releases secondary metabolites that may increase the growth of Bd in the environment if Bt gets into the water through crop run off.

References


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