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The effects of *Sceloporus virgatus* cloacal microbiota on the growth of pathogenic fungi

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**Background**

- Female *Sceloporus virgatus* (Figure 1) lay their eggs at the start of monsoon season in damp dirt nests, then provide no parental care to their offspring.
- Pathogenic fungi from the dirt can grow in the nest environments and kill the embryos of unhatched eggs (Figure 2).
- To protect against fungal diseases some oviparous species pass beneficial bacterial from their cloaca (Figure 3) to the eggshells of their offspring via oviposition (Sarmiento-Ramírez et al. 2014).
- When female *S. virgatus* lizards lay their eggs, bacteria from their cloaca is transferred to the eggshells of their offspring and can increase hatch success (Bunker et al. 2021).

**Prediction:** *S. virgatus* cloacal bacteria will inhibit the growth of pathogenic fungi through secreting some byproduct detrimental to fungal growth.

**Objective**

- Determine how bacteria species isolated from the cloaca of *S. virgatus* affect the growth of pathogenic fungal strains:
  - *Neocosmospora rubicola*
  - *Purpureocillium lilacinum*
  - *Aspergillus sp.*
  - *Fusarium sp.*

**Methods**

- Cloacal bacteria and environmental fungal cultures were grown separately from previously sequenced vouchers.
- Plate-based challenges tested the growth of each fungal strain when exposed to different cloacal bacteria (Figure 4).
- Zones of clearing around the bacteria treatments (Figure 5) were traced in ImageJ to quantify the amount of inhibited fungal growth in cm².

**Results**

- In challenges where the bacteria swarmed (Figure 6), zones of clearing could not be quantified. So, the percent of disturbed fungal growth was estimated by comparing the fungal growth on the control plate (Figure 7) to the fungal growth on each challenge plate.

**Future directions**

- Increase sample size and find ways to quantify the amount of fungal growth inhibited by swarming bacteria.
- Determine whether the growth of other fungal strains are inhibited by *S. virgatus* cloacal bacteria.
- Challenge *S. virgatus* cloacal bacteria against fungus directly on eggshells to see if the bacteria contributes to a fitness advantage.
- Examine the chitinase antifungal mechanisms used by cloacal bacteria.
- Use transposon mutagenesis to pinpoint antifungal genes in the cloacal bacteria.

**Conclusions**

- In many of the challenges the bacteria swarmed in the top agar (Figure 6), making it difficult to quantify zones of clearing.
- *Enterococcus faecalis*, *Citrobacter amalonaticus*, and *Serratia* 31 swarmed the least, compared to the other bacteria strains, and consistently created circular zones of clearing, inhibiting growth in the fungal lawns (Figure 8).
- All bacteria treatments disrupted fungal growth to some degree, through forming circular zones of clearing, or through outcompeting fungi for nutrients and space when swarming (Figure 9).
- Taken together, these findings imply that *S. virgatus* cloacal microbiota can inhibit and disrupt the growth of different pathogenic fungal strains.

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**References**


