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Introduction:

- What is *Coprinopsis cinerea*?

Coprinopsis cinerea is also known as the gray shag mushroom and is commonly studied in interest towards fungal mating and meiosis (Atlas). The mushroom is depicted in Figure 1. *Coprinopsis cinerea* is a basidiomycete fungus that secretes lactonases from its mycelium, made up of hyphae (Stöckli et al. 2017).

- What are lactonases, and what do they do?

Lactonases from *Coprinopsis cinerea* belong to the metallo- β -lactamase family. The lactones inactivate the quorum sensing molecule acyl-homoserine lactone (HSL), depicted in Figure 2. This works by targeting and hydrolyzing the ester bond between the homoserine lactone and fatty acid groups of the lactone (Stöckli et al. 2017). For these reasons, lactonases are great biofilm inhibitors.



Figure 1. *Coprinopsis cinerea* (Coprinopsis cinerea (Schaeff.) Redhead, Vilgalys & Moncalvo. (2008))

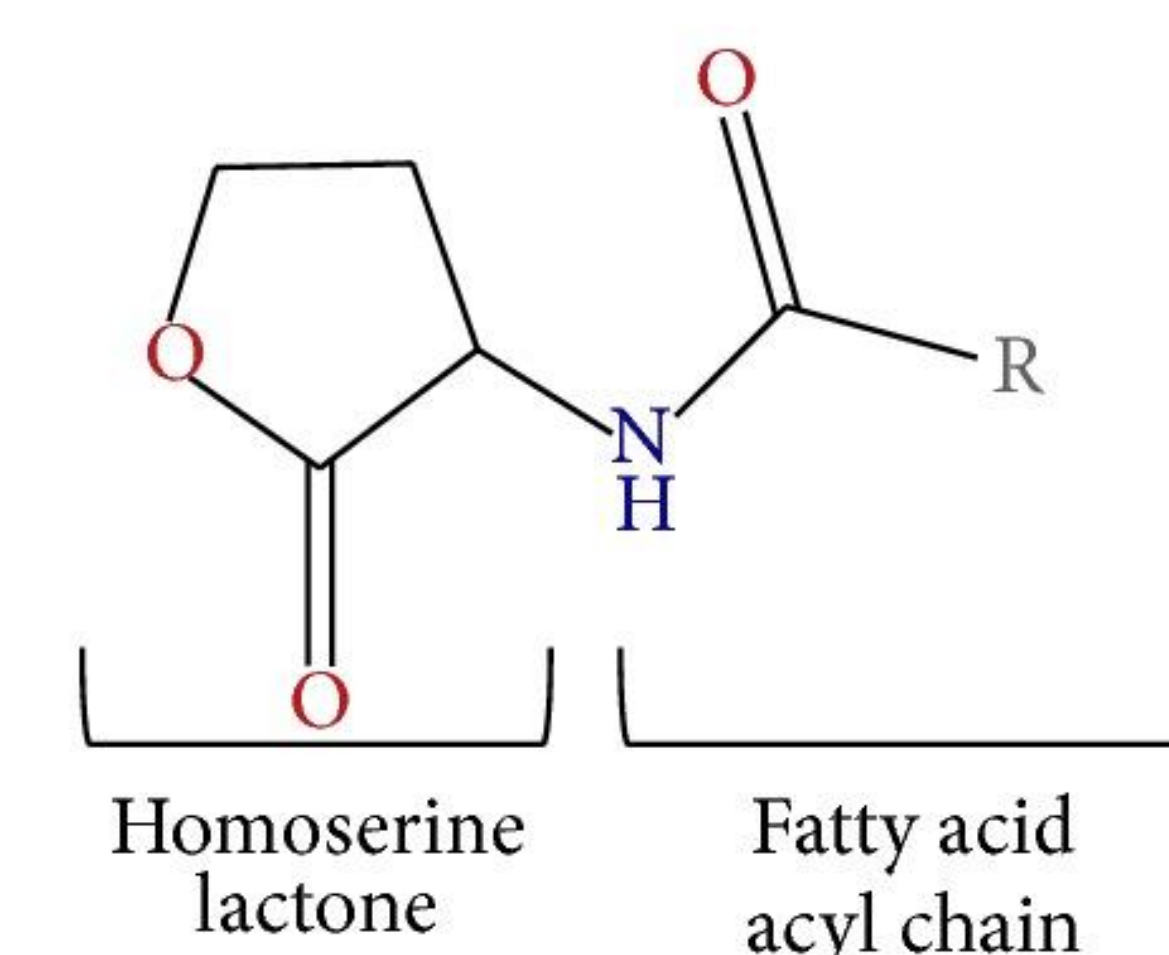


Figure 2. HSL (Chan, K., Cheng, H., Chen, J., Ngeow, Y., Castillo-Juárez, I., López-Jácome, E., . . . Atkinson, S. (2020, September 09). General structure of n-acyl homoserine lactone (ahl))

Materials and Methods:

The method of obtaining lactonase is as follows:

- Grow up the mycelium of *Coprinopsis cinerea* so that the mycelium can be harvested. Harvest the mycelium and centrifuge it with Tris-HCl buffer to extract whole cell proteins, which should contain lactonase.

The method of using lactonase as a biofilm inhibitor is as follows:

- Plate *Chromobacterium violaceum* with a serial dilution of whole cell proteins. The lack of violet coloring over time indicates inhibition of quorum sensing and biofilms.
- Plate *Pseudomonas fluorescens* grown in iron limited succinate minimal medium with a serial dilution of whole cell proteins. The lack of coloring under UV light indicates inhibition of quorum sensing and biofilms.
- In a liquid culture, bacteria can be cultured and measured with a spectrophotometer to measure absorbance, indicating the amount of biofilm. Absorbance can be measured after the addition of lactonase to measure the impact of lactonase on absorbance. If absorbance decreases, the biofilm is broken down. If absorbance increases, the biofilm is still growing. If neither, the biofilm is inhibited from growth.

Anticipated Results:

It is anticipated that the QS molecules produced by the bacterial strains *Chromobacterium violaceum* and *Pseudomonas fluorescens* will be inhibited by the whole cell proteins collected from *Coprinus cinerea*. It is expected that biofilms will not only be inhibited but will also break down in the presence of lactonase (Stöckli et al. 2017).

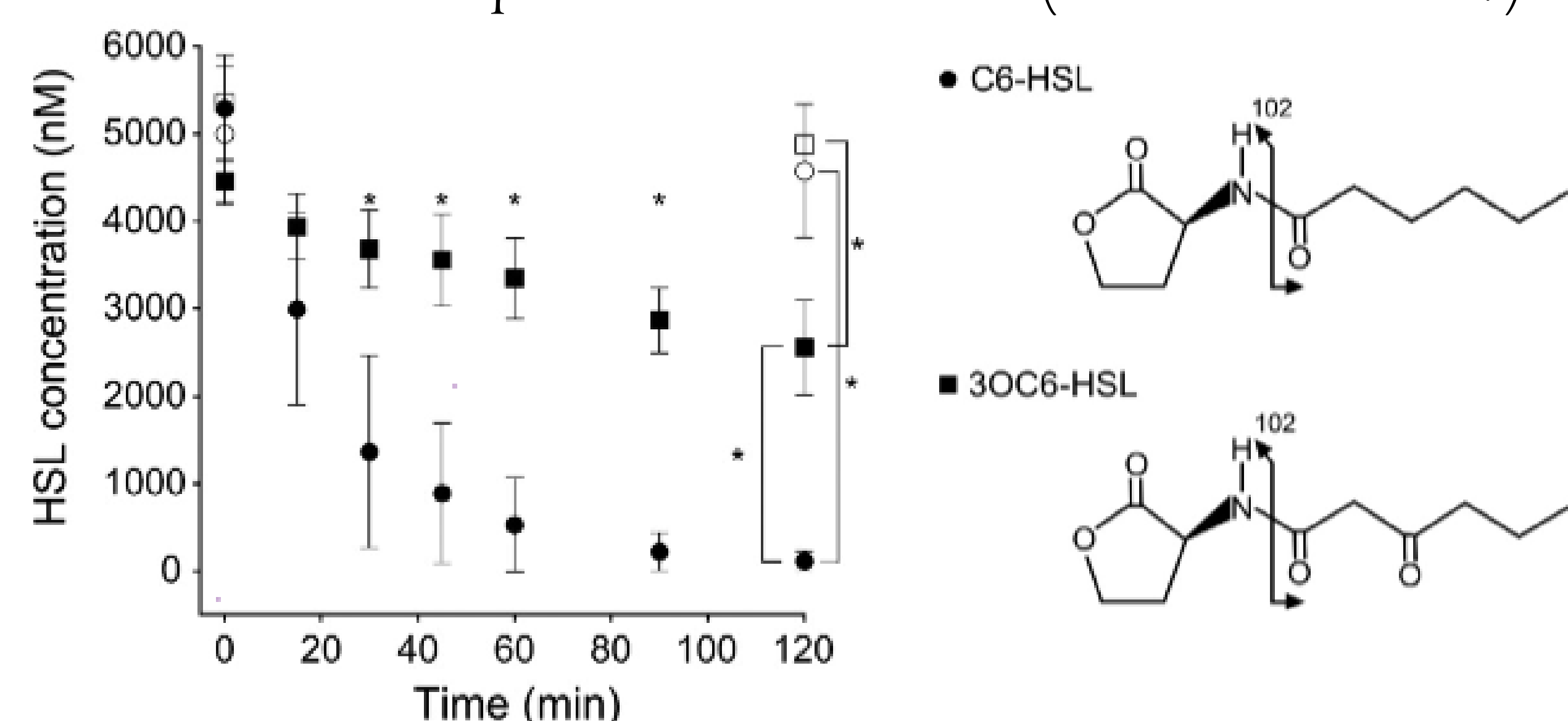


Figure 3. The amount of HSL present in culture with lactonases from *Coprinopsis cinerea*

In Figure 3, the amount of homoserine lactone (HSL) within a culture is shown to have decreased over time due to exposure to lactonases from *Coprinopsis cinerea* significantly ($p < 0.05$). The closed points represent the amount of HSL detected in the sample with lactonase, and the blank points represent the amount of HSL detected where lactonase is not present. If HSL is the quorum signaling molecule used in the creation and sustenance of biofilms, lactonase then should impact this by breaking down and inhibiting biofilms (Stöckli et al. 2017).

Figure 4. Phylogenetic Tree:

Made up of lactonase-producing fungi

(Stöckli et al. 2017)

- [x] = produces 0 lactonases
- [x] = produces 1 lactonase
- [x] = produces 2 lactonases
- [x] = produces 3 lactonases
- [x] = produces 5 lactonases
- [x] = produces 6 lactonases

Anticipated Results:

It is shown here in Figure 4 that *Coprinopsis cinerea* is not the only lactonase-producing fungus, but one of many. There are even fungi that produce more lactonases than *Coprinopsis cinerea* does. The effects of these lactonases can be studied more closely when there are known amounts of lactonases being made and studying these other lactonase-producing fungi can lead to even more discovery pertaining to the role of lactonase in biofilm inhibition and more.

Literature Cited:

1. Atlas, N. (n.d.). *Coprinopsis cinerea* (Schaeff.) REDHEAD, VILGALYS & Moncalvo. Retrieved April 14, 2021, from <https://species.nbnatlas.org/species/NHMSYS0020061856>
2. Stöckli, M., Lin, C. W., Sieber, R., Plaza, D. F., Ohm, R. A., & Künzler, M. (2017). *Coprinopsis cinerea* intracellular lactonases hydrolyze quorum sensing molecules of Gram-negative bacteria. *Fungal Genetics and Biology*, 102, 49-62.

