Effect of snail rearing temperature on protein content in mucus

Hyogo Prefectural Kobe High School　Science Course　First Grade
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Motive＆Purpose

How can snails walk faster?According to previous researches, their walking speed is related to the concentration of mucin.Mucin is a kind of protein, that holds water strongly. We think that if we raise temperature, snails can produce more put a space mucin.

About snails used

Species: Euhadra brandtii,
in Japanese Hitachi-MaiMai
Shell diameter: Average 2.46cm
Shell height: Average 1.59cm
Room: Cubic aquarium, cardboard roof
Food: Carrot, cabbage, egg shell

Experiment Methods

1. Breed snails at a constant temperature.(experiment1: 22°C ,experiment2 : 18°C, 22°C, 26°C)
2. Place two snails in the same 50ml tube along with 2ml of water and let them walk in it for 4 hours. During this time, keep the temperature at the same level as in experiment 1.(Figure 1)
3. Pick out the snails from the tube and mix the mucus and water for 30 seconds with Vortex Mixer.
4. Add 2ml of ninhydrin solution (1g/L) in the tube and mix for 30 seconds.
5. Warm it with boiling water to accelerate the ninhydrin reaction for 5 minutes. After that, mix for 10 seconds.
6. Using a spectrophotometer,measure the absorbance of the liquid at the wavelength of the complementary color of the color of the ninhydrin reaction using a spectrophotometer.
GENESYS 150 UV-visible spectrophotometer (Figure 2)

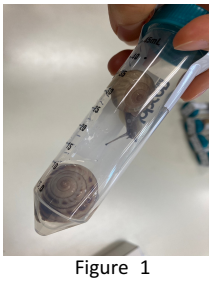
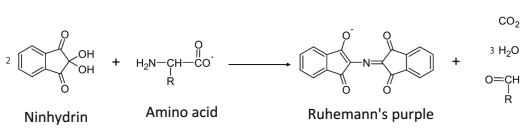


Figure 1

Figure 2

Measuring principle

We used the ninhydrin reaction because we believed that the concentration of mucin in mucus is proportional to the concentration of proteins. The ninhydrin reaction is the reaction between amino groups and ninhydrin, which produces a purple color. Protein concentrations can be compared by comparing the absorbance of the complementary colors exhibited by the ninhydrin reaction in an absorbance spectrophotometer.
The reaction mechanism of ninhydrin is shown below.



It would have been nice to measure the viscosity of the mucus or the concentration of mucin only, but these were difficult to measure because of very little mucus and the impossibility to extract mucin only.

References

(i) J. Technology and Education , Vol.27, No.1, pp15-19(2020)
http://bigjohn.ce.fukui-nct.ac.jp/journal/V271/JTE27115TT.pdf
(ii) Masao Higashi, "A Catalogue of Shellfish from Japan," Conservation Society, 1985
(iii) Matsumoto, Yosuke, Skuea, The Newest Chemistry, Daiichi Gakusha, 2022

Experiment①

All snails were kept in an incubator set at 22C for one week and tested according to the experimental method.
Liquid was collected from a total of five tubes and absorbance was measured.

Result①

A peak in absorbance was seen at a wavelength of 570 nm.
The average absorbance was 0.164.

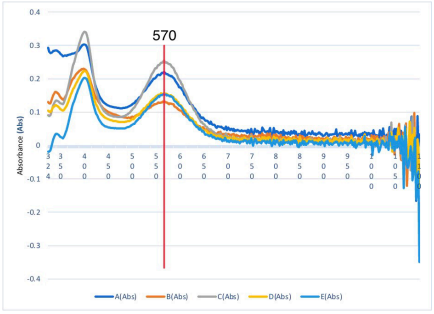


Figure 3：Graph obtained from absorbance measurements

Experiment②

The snails were divided into three groups and kept in incubators set at 18°C, 22°C, and 26°C, respectively, for one week, for a total of four experiments over two weeks.
Liquid was collected from six tubes, two at each temperature, in one experiment, and the absorbance of each was measured.

Result②

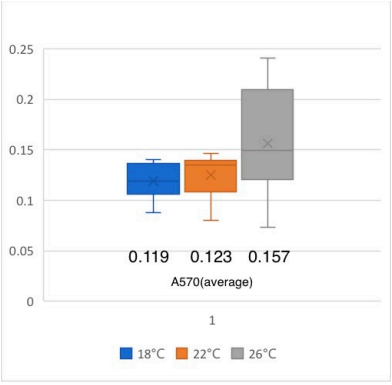


Figure 4：All data are summarized in a box-and-whisker plot.

Consideration

Result of experiment 1 showed that the amount of mucin corresponds to a 570nm wavelength.
Also,the results of experiment 2 showed that the more the temperature rises the more amount of mucin can increase. We think that snails prevent more water from changing into vapor by temperature rising. But we can't conclude that for these experiments are not easily reproducible.

Prospects

The amount of mucus produced differed from one individual to another, there were differences in the way each individual crawled inside the tube, and we were unable to conduct experiments that did not take into account the differences between individuals that defecate and those that do not. In the future, we would like to improve the reproducibility of the experiment by reconsidering the experimental method to solve the above problems, examining the characteristics of each individual, such as size and type, and using individuals with similar characteristics. We would also like to investigate the temperature at which the concentration of mucin is highest.