The Relationship Between Refrigerating Temperature and the Growth of *Nostoc commune* Measured by Dry Weight and the Methylene Blue Staining Method.

IKEDA Neo SHINTANI Saya SUGIMORI Haruka IKEDA Kousei TAKABATAKE Taiki

Abstract

We were interested in the terraforming of Mars and wanted to understand whether organisms on the earth could survive on Mars. Therefore, focusing on the harsh environment of Mars, we investigated whether microorganisms on the earth can resist low temperatures like that of Mars.

In this study, *Nostoc Commune* which is a kind of Cyanobacteria, was exposed to the low temperatures of -20℃ and -40℃ to examine the resistance to low temperature stress. We used Indophenol blue absorptiometry to conduct the Methylene Blue method.

The experiment results showed that there was no significant difference between the activity of the cells and the nitrogen fixing ability between the low temperature treated *Nostoc commune* and the Nostoc Commune kept at normal temperature, thus *Nostoc commune* is resistant to extreme low temperature stress.

The Study's Purpose

To research whether bacteria on earth can resist stress from low temperature like Mars in.

We focused on the Earth bacteria, *Nostoc Commune*.

Nostoc Commune belongs to the Nostocules of Cyanobacteria and they can photosynthesis and perform dinitrogen fixation.

Some Cyanobacteria live in cold places, so we thought they could withstand low temperature environments.

We expected if we performed low temperature treatment of the bacteria, their cells would be injuried by ice crystals and some of their works decreased –like a cell's work, and their ability of dinitrogen fixation.

So we researched the change in the state of *Nostoc Commune* put in low temperature treatment.

Experiment method

We collected Nostoc Commune from our school's outdoors areas and used it for our experiments. When we used it, we stained water more than twelve hours. Then, we left it at -40℃, -20℃, and room temperature without light. We performed three different experiments and checked the changes in bacteria activity.

<The Experiment 1: weigh dry weight>

We washed *Nostoc Commune* and dried it. And we left it in a desiccator for a day and weighed it. Then, we stained it in water and left it at -40℃, -20℃, and room temperature

for fourteen hours and incubated it for six weeks. We incubated it at room temperature on cotton in a petri dishes. We weighed *Nostoc Commune's* dry weight every two weeks and calculated its growth rate and drew a graph.

<The Experiment 2: Methylene Blue Staining Method>

We stained cells blue with Methylene Blue, to find out the activity of *Nostoc commune*. If is the activity of *Nostoc Commune* is high, Methylene Blue doesn't change and the color of cells stays blue.

On the other hand, if the activity of *Nostoc Commune* is low, Methylene Blue becomes Leucomethylene Blue and the color of the cells changes from blue into clear because Methylene Blue is deoxidized.

By using this Methylene Blue staining method, we were able to check the activity of Nostoc Commune at different temperature. We counted the number of cells whose Methylene Blue was deoxidized, and calculated the reduction rate. Then we showed it graphically (fig, 2).

<The Experiment 3: Indophenol Blue Absorptiometry>

Indophenol blue absorptiometry is the method to determine the absorbance of Indophenol blue. We estimated the degree of the nitrogen fixation of *Nostoc Commune*. We calculated the concentration of ammonia nitrogen by the following formula which was based on the analytical curve. Then, we translated it in to a graphic form (fig, 3).

$$\text{The concentration of ammonia nitrogen} = 84.55 \times \text{absorbance}$$

Result

<The Experiment 1: Weigh Dry Weight>

We put *Nostoc Commune* at -40℃, -20℃, and room temperature. The amount of their dry weight increased. Fig.1.shows this result.

We set up an equation. It shows the growth rate of *Nostoc Commune*.

$$\text{Growth rate} = \frac{\text{Nostoc Commune's dry weight after incubation}}{\text{Nostoc Commune's dry weight before incubation}}$$

<The Experiment 2: Methylene Blue Staining Method>

We put *Nostoc Commune* at -40℃, -20℃, and room temperature and stained it with Methylene Blue. An hour after we stained it, Methylene Blue was deoxidized and become Leucomethylene Blue in sixty percent of those cells. Fig.2. shows this result.

We set up an equation. It shows the percentage of those cells which were stained with Leucomethylene Blue.

$$\text{Percentage of Methylene Blue deoxidized} = \frac{\text{The number of cells which Methylene Blue changed from blue into clear by deoxidizing}}{\text{The number of cells which we stained with Methylene Blue}}$$

<The Experiment 3: Indophenol Blue Absorptiometry>

We put *Nostoc Commune* at -40℃, -20℃, and room temperature several times. The concentration of ammonia nitrogen which *Nostoc Commune* fixed dwindled. We couldn't

find that the variation which is the concentration of ammonia nitrogen has a large difference.

Consideration

We did three experiments which compared the growth rate, the percentage of Methylene Blue, and the concentration of ammonia nitrogen in Nostoc Commune. We found that there wasn't a difference between *Nostoc Commune* put at low temperature and normal temperatures in these experiments.

In conclusion, our research shows that *Nostoc Commune* can resist stress at low temperatures of -40℃.

References

1) 「栄養塩類の測定　アンモニア態窒素の測定」
2) Ichikawa Takahiro, Takahashi Terumasa, Asano Yoshito, Kobayashi Tatsuaki (2002) インドフェノール青法によるアンモニア態窒素の簡易訂量法の検討,『日本緑化工学会誌』,p623-626
3) Ozeki Yuika, Shiomi Yui, Syirakami Riko, Murakawa Kaho (2015)「イシクラゲの生育抑制について」,『平成２７年度理数科課題研究論文集』岡山県立一宮高等学校理数科
4) 「火星基礎知識」,(2018/3/15)

Key words

Dry weight, low temperature, *Nostoc Commune*, Methylene Blue staining method,