Project number 57

Discovery of novel RNA modifications from long-lived, cold-adapted species

［1］ **Research group**
Principal Investigator (PI) :
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Host researcher at IDAC :
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Expenditure report of research funds :
Consumables 100,000 Yen
Travel cost 0 Yen

［2］ **Research setup**

The long-term goal of this study is to identify and characterize RNA modifications that are associated with adaptation to cold environments in long-lived organisms. As target organisms, we selected three understudied eukaryotes spanning millions of years: yeast, elasmobranchs, and amphibians (**Table I**). As a first step, to establish the basic methodology for discovery of novel RNA modifications, two species of yeast from the genus Naganishia were investigated. One of them, *Naganishia antarctica*, is a cryophilic organism, which cannot survive temperatures above 15ºC. The other one, *Naganishia uzbekistanensis*, is cryotolerant, meaning that it can also grow at lower temperatures, but prefers temperatures above 15ºC.

Table I. Organisms considered in this study

#	Scientific name	Type	Range (ºC)
1	*Naganishia antarctica*	Yeast	4 – 16ºC
2	*Naganishia uzbekistanensis*	Yeast	4 – 24ºC
3	*Scyliorhinus torazame*	Shark	10 – 20ºC
4	*Ambystoma mexicanum*	Amphibian	10 – 20ºC

Before applying to this grant, Galipon's team had obtained preliminary data to determine the growth range of these target organisms (**Table I**). Especially, the yeast growth had been evaluated by automation of image scanning of yeast growing on solid agar.

In order to obtain a sufficient amount of total RNA to detect RNA modifications, liquid culture is needed. To assess the growth range of Naganishia yeasts in liquid medium, yeast growth in M25 medium (1 % glucose, 0.5 % peptone, 0.3 % yeast extract, 0.3 % malt extract) was measured by optical density at 660 nm (OD660) every 30 min for 6 days after dilution of pre-cultures to OD660 = 0.1. To establish the optimal growth range, acidity (pH 4, 5, 6, 7) and temperature conditions (8 ºC, 12 ºC, 16 ºC, 20 ºC, 24 ºC) were tested, with 3 replicates per condition. The doubling time (Dt) during logarithmic growth and maximum OD values were then calculated. Dt is shown in **Fig. 1-2**.

Naganishia yeast growth is slower than standard yeast such as baker's yeast or fission yeast. It takes 10-14 days to grow colonies on agar, 2-3 days for the pre-culture, and 6-7 days for the main experiment. Due to equipment limitations, only one temperature can be assessed at the same time. Therefore, it took four months to obtain this basic data. However, this is essential because almost nothing is known about Naganishia. Obtaining this data was absolutely essential to define the conditions for "cold-shock" and "heat-shock" for these organisms.

After determining the optimal growth range, as well as determining the OD value corresponding to logarithmic growth as being generally between 0.3 and 0.4, the "cold-shock" condition was set to 1 h at 0 ºC, and the "heat-shock" condition to 1 h at 30 ºC. RNA extraction was carried out in four replicates for each condition, with the "before-stress" sample as a control for both. High quality RNA (RIN: RNA Integrity Number > 7) was obtained for all samples in all conditions (**Fig. 3**). The samples are being prepared to be sent to the Wei lab for LC-MS/MS analysis and identification of RNA modifications.

Exchange of information between the Galipon lab and Wei lab was done regularly by e-mail and Zoom. Dr. Galipon and the student involved in the study also had the opportunity to meet and talk with Prof. Wei in person during the 26th Annual Meeting of the RNA Society of Japan, organized by Prof. Wei.

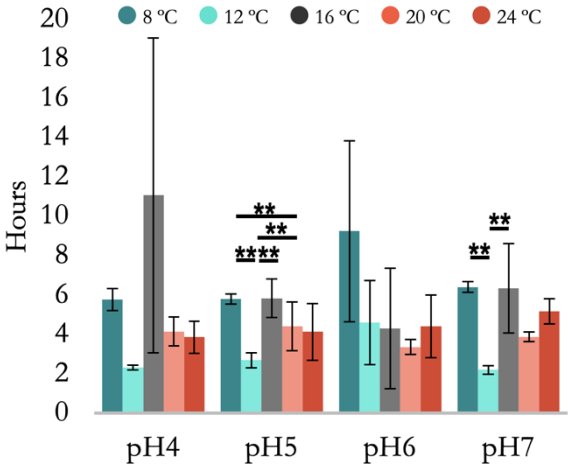

Fig. 1. Doubling time of *N. antarctica* (n=3) ANOVA with post-hoc Tukey HSD ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***)

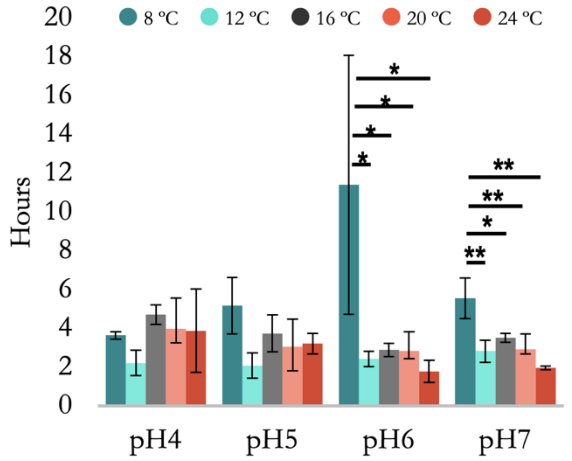

Fig. 2. Doubling time of *N. uzbekistanensis* (n=3) ANOVA with post-hoc Tukey HSD ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***)

［3］Research outcomes

（3－1）Results summary

★*N. antarctica*, a cryophilic yeast isolated from Antarctica, could grow between 4 – 16 ℃.

★*N. uzbekistanensis*, a cryotolerant yeast isolated from desert soil in Uzbekistan, could grow between 4 – 24 ℃.

★For both yeasts, logarithmic growth was observed between $0.3 < OD\ 660 < 0.4$ (data not shown).

★For both yeasts, the standard growth temperature was 12 ℃, pH 5 (**Fig. 1-2**), and the cold- shock and heat-shock responses were speculated to happen at 0 ℃ and 30 ℃, respectively.

★The above conditions were applied when sampling for RNA modifications, and total RNA was extracted.

★Cold- and heat-shock for 1 hour did not have any significant effect on RNA integrity (**Fig. 3**).

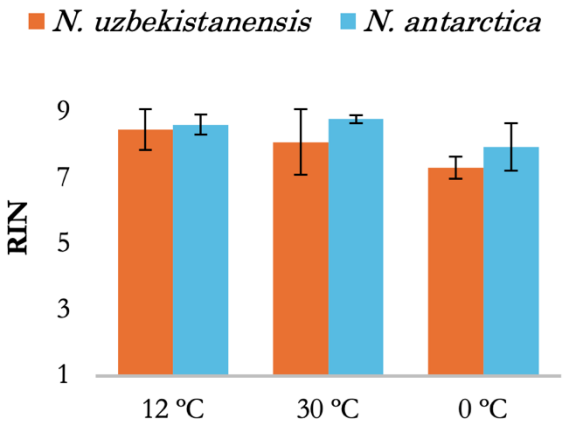

Fig. 3. RIN before and after heat- and cold-shock stress (n=4); Paired Student t-test (all $p > 0.05$)

（3－2）Future perspectives

Thanks to the preliminary data obtained in this study, the following has become possible.

★RNA modifications specific to the cold- or heat-shock responses are currently being analyzed at the Wei lab.

★The role of such RNA modifications will be investigated, especially focusing on their effect on RNA and protein synthesis at cold temperatures.

★This successful preliminary work may be useful to secure other sources of funding in the future.

★The study will be expanded to more cryophilic and cryotolerant organisms, including long-lived sharks and amphibians.

［4］List of research achievements

Part of this research was mentioned during an oral presentation at the following local conference.

開催日：2025 年 11 月 8 日
学会名：第 33 回 山形分子生物学セミナー
会場名：山形大学米沢キャンパス
課題名：好冷酵母が持つ低温適応メカニズムの解明に向けて
著者名：○佐坂佑菜（山形大学）、吉田天音（山形大学）、成田大輝（慶應義塾）、Galipon Josephine（山形大学）