Science and Technology Group
Annual Report FY2019

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1 Introduction

I continued to engage in two independent research projects during FY2019, namely, engineering of catalytic RNAs (ribozymes) and investigations on natural fibers (e.g. Bashofu). This year I gave invited talks on our Bashofu projects inside[3A] and outside[3B] Okinawa. In collaboration with another STA Dr. Moriyama, I organized a new children summer class offered by OIST/Onna village as a part of my outreach activities.

2 Activities and Findings

1) Engineering of Catalytic RNAs (as a member of the Nucleic Acid Chemistry and Engineering unit)

Template-directed RNA ligation catalyzed by an RNA enzyme (ribozyme) is a plausible and important reaction that could have been involved in transferring genetic information during prebiotic evolution. Laboratory evolution experiments have yielded several classes of ligase ribozymes, but their minimal sequence requirements remain largely unexplored. We used large-scale DNA synthesis and high-throughput ribozyme assay enabled by deep sequencing to systematically minimize a previously laboratory-evolved ligase ribozyme. We already had identified a small catalytic core consisting of only 18 contiguous bases that catalyzes template-directed regiospecific RNA ligation in 2018, and we characterized the ligation mechanism of this small catalytic core through biochemical analysis in 2019. Furthermore, we continued to search for smaller catalytic sequence and concluded that this 18-nt sequence was the minimum sequence under our conditions. The fact that such a short sequence can catalyze this critical reaction suggests that similarly simple or even simpler motifs may populate the RNA sequence space which could have been accessible to the prebiotic ribozymes. The outcome from our research was presented in an international conference [4A] and published [1A]. We plan to identify smaller ligase RNA sequences using selection methods next year.

As another contribution to the NACE unit in 2019, we designed RNA nanomachines that can detect an RNA sequence, amplify signal, and produce an optical output, all encoded in a single RNA transcript, self-powered by a metastable structure. I prepared all experiments of this metastable RNA for paper submission, and the outcome was published [1B].

2) Natural fiber projects

Kakenhi project (Kiban C, 19K02308, FY2019-2021, 3 years)[2A]

I was awarded a Kakenhi grant for the Bashofu project. Bashofu producers have serious production problems due to the reduction of high-quality Bashofu fiber called Nahagu. The goal of this Kakenhi project is to utilize our scientific findings to improve the productivity of high-quality Bashofu fibers. During the first year, we investigated the traditional methods for Bashofu production in the literature (e.g. local history records), and we analyzed the records on fiber extraction methods for Bashofu in the 213 references we collected. Another important goal of this Kakenhi project is an estimation of quantity of Bashofu raw material *Itobasho* plant in Okinawa. *Itobasho* and other banana plants are indistinguishable from their appearances. For preparation of genetic identification of *Itobasho* plant, Dr. Koizumi (OIST Imaging Section) and I optimized the PCR protocol. We plan to collect plant samples in the field in Okinawa and to identify *Itobasho* plant.

Scientific evaluation of traditional Bashofu fibers (collaborator: OIST Imaging Section, University of the Ryukyus)

Bashofu fibers are categolized into three classes: Waha (hardest fiber), Nahau, and Nahagu (high-quality fibers) dependending on the hardness of the raw materials. We observed cross-sections of both the raw materials (*Itobasho* plant leaf sheaths) and the corresponding fibers derived from the materials with optical microscopes. We also compared the mechanical properties of the fibers by tensile test. Interstingly, fiber force (N) of Nahagu is smaller than that of Waha, but after normalization by cell wall area, the tensile strength (MPa) is comparable to that of Waha (Figure). Futhermore, AFM analysis showed that the surface of Nahagu is smoother than that of Waha. We predicted that the high-quality fiber Nahagu is thin, has smooth surface, and shows a similar tensile strength as that of Waha[4B].

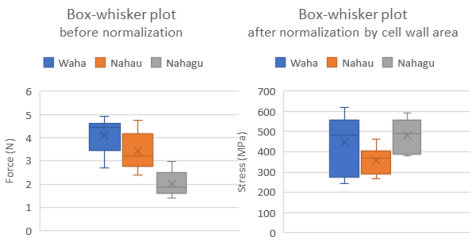

Figure. Mechanical properties of Bashofu fibers.

Furthermore, we are trying to produce Nahagu-like fiber from Waha. We found that an imporatnt difference between Waha and Nahagu is the thickness of the cell walls resulting from the accumulation of lignin. we isolated several interesting strains of bacteria from *Itobasho* fields in Kijoka. In a preriminary test using one of the isolated strains, we confirmed degradation of Waha materials after traditional alkaline degumming.

Confirmation of Bashofu cooling model (collaborator: Japan Women's University)

Bashofu was an excellent textile in summer in the Ryukyu Kingdom. Evaporation of moisuture in textiles requires heat form the human skin surface resulting in a drop in the skin temperature. The ability of the fiber to retain moisture is important for the cooling mechanism of textiles. We measured the mositure contents of Bashofu fibers and compared them to other fibers. Bashofu moisture content (12 %) was equivalent to that of the other bast fibers (e.g. ramie), and it was twice as much as that of cotton (6 %) [4C].

Natural fiber research with industry[2B]

We continued working on a research project on natural fibers in collaboration with one of the largest textile/fiber companies in Japan, Teijin Frontier Co. Ltd. This collaborative research on natural fibers is based on our Bashofu study. In this collaboration, we anaylze and evaluate natural fibers other than Bashofu.

Outreach activity based on silk materials

We organized a new class for elementary school children at Onna Village/OIST Children's School of Science 2019 (2019 Aug 20th, 21st) titled "*Miracle gifts for our future scientists from Okinawan silkworms"*. The Kumejima Tsumugi Association and Okinawa UKAMI Sericulture Co. Ltd. fully supported this class. In class, we used yellow cocoons of the Okinawa native species *Kumejima Tasanken* that are used for the traditional Okinawan silk textile Kumejima Tsumugi. Dr. Yohsuke Moriyama (STA) and I introduced this Okinawan traditional silk cocoon to participating children and they enjoyed making transparent films from these yellow cocoons used for biomedical applications. Several other volunteers from the NACE unit and other OIST employees contributed to the successful class. Furthermore, this event led to a new collaboration between the OIST Imaging Section and UKAMI Sericulture Co. Ltd.

3 Collaborations

NACE (Yokobayashi) unit, OIST Imaging Section, University of the Ryukyus (Prof. Ryuichi Suwa), Japan Women's University (Department of clothing), Teijin Frontier Co. Ltd

4 Publications and other output

1) Publications:

1A. Y. Nomura and Y. Yokobayashi, Systematic minimization of RNA ligase ribozyme through large-scale design-synthesis-sequence cycles, Nucleic Acids Research, 47, 8950-8960, 2019.

1B. S. Kobori, Y. Nomura and Y. Yokobayashi, Self-powered RNA nanomachine driven by metastable structure, Nucleic Acids Research, 47, 6007-6014, 2019.

2) Grants:

2A. FY 2019-2021: JSPS Kakenhi Kiban C, 19K02308, 4,420,000 yen over 3 years, "Contribution of scientific analysis to Bashofu production".

2B. FY 2019: Teijin Frontier Co. Ltd., 3,000,000 yen, "Collaboration research on natural fiber".

3) Invited talks:

3A. Luncheon Seminar Lecture, Okinawa Association of Radiological Technologists Academic Conference, (ランチョンセミナー講演, 沖縄県放射線技師会学術大会), OIST, 2019 May 19th.

3B. Special Lecture in English, Presentation in Conjunction with the Graduate Student Course: Science and Technology in Japan, Shizuoka University, 2019 July 16th (Hamamatsu) and 17th (Shizuoka).

4) Presentations:

4A. Y. Nomura and Y. Yokobayashi, International Symposium on Nucleic Acids Chemistry, Poster, Tokyo, 2019 Oct 28th, 29th, 30th.

4B. K. Koizumi, T. Sasaki, C. Mitani, H-B. Kang, N. Uehara, R. Suwa, B. Humbel, and Y. Nomura*

4C. C. Mitani, K. Koizumi, T. Sasaki, H-B. Kang, B. Humbel, Y. Nomura*

4B&4C: Comfort and Smart Textile International Symposium, Poster, Nara, 2019 Sep 6th, 7th.

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