

## Research Project Mid-term Evaluation Result

The following research project underwent a mid-term evaluation in accordance to Clause 10 and 11, Chapter 2 of the *Regulations for Research and Development Evaluations* (Regulation No. 74, October 1, 2003.)

### Evaluation system:

Out of five reviewers, two experts from outside of RIKEN and three RIKEN Science Council Research Programs Committee members were appointed as reviewers for the following research project. The reviewers evaluated the project based on the reporting session held on December 21, 2017.

### Reviewers list:

#### External experts (alphabetical order)

- 1) Kiyoshi TAKEDA, Professor, Osaka University
- 2) Nobuhiko NOMURA, Professor, University of Tsukuba

#### RIKEN Science Council Research Program committee member (alphabetical order)

- 3) Shigeru KURATANI, Chief Scientist, Evolutionary Morphology Laboratory
- 4) Yasushi SAKO, Chief Scientist, Cellular Informatics Laboratory
- 5) Makoto TAJI, Group Director, Laboratory for Computational Molecular Design

### Research project brief overview

**Project name:** Biology of Symbiosis

**Project Leader:** Hiroshi OHNO

**Project duration:** April, 2015~March, 2020 (5 years)

**Budget allocated:** Total of 430,000 thousand Yen (past 3 years)

#### Research overview:

All living organisms, do not live by themselves, but are closely associated with a huge number of bacterial community, from birth to death. These “symbiotic” microbiota are thought to profoundly impact the physiology and pathology of host organisms. However, the knowledge on these symbiotic microbiota is virtually marginal; we do not know exactly what kinds of bacteria exist and how they metabolically behave, in a steady state condition as well as in the disease state, and so on. In this project, we aim to first create an overhead view of symbiosis, by making a summary review of the participating members of bacteria and their metabolic products. We then examine the effects of a certain bacterial species or a metabolite(s) on the host by reconstitution in germ-free and/or gnotobiotic models.

### 1. Comprehensive Evaluation (To be disclosed)

1) Evaluation on five-grade scale	S	A	B	C	D
(1) Research objective:	4	1	0	0	0
(2) Implementation of research plan:	1	4	0	0	0
(3) Research achievement:	1	4	0	0	0
(4) Future research plan:	1	4	0	0	0

S Outstanding / A Excellent / B Good / C Acceptable / D Not acceptable

## **2) Evaluation details (reviewer's number is different from the order of the above list)**

### **< Reviewer 1 >**

#### **(1) Research objective**

Symbiosis is a phenomenon widely seen in the living world, and its research is one of the important tasks of current biology. This project has a spectacular goal of comparing symbiotic systems in different organisms, including animals and plants, aiming for a unified understanding of symbiosis phenomena in general. This kind of research topics are unique to the RIKEN Pioneering Project jointly conducted by researchers in various fields of inside and outside RIKEN, derived from curiosity of each project member. The research goal of this project is highly appreciated.

#### **(2) Implementation of research plan**

A large number of groups inside and outside RIKEN engaged in this project to conduct comprehensive research of symbiosis. As the result, this project is performing fundamental and wide-ranging research including development of new methods of omics analysis and theoretical study of symbiotic dynamics. Participation of researchers outside RIKEN is another important effort in the implementation of this project to build a broad research system. It seems that sufficient attention is paid to promote information sharing and collaborative research.

#### **(3) Research achievement**

Studies in each three subgroups are proceeding smoothly. For example, elucidation of the metabolic interaction in the multiple term symbiotic system of termites, protozoa, and bacteria, roles of the microbiota in the differentiation of M-cells in human intestine, and interactions between host plants and various symbiotic species such as bacteria, fungi and plants through metabolic products are the remarkable achievements in this project. In addition, a number of collaborative research results have been announced. Over all, this project has enough research achievement as the intermediate stage.

#### **(4) Future research plan**

Up to now, this project has conducted research on static omics analysis as the main method. In the future, they are planning to conduct dynamics analysis. Needless to say, research on symbiosis necessitates a viewpoint of dynamics, however, it means that it is necessary to acquire and analyze enormous amounts of data. Dynamics analysis with narrowed targets will be necessary.

### **< Reviewer 2 >**

#### **(1) Research objective**

This research project intends to reveal the mechanisms by which "symbiotic" microbiota influences the physiology and pathology of the host. The possible outcome of this research may create a new research field of biology of "symbiotic" bacteria, which will definitely have a high impact on a variety of research areas as well as the society.

#### **(2) Implementation of research plan**

This research project is composed of three groups (Ohkuma's, Ohno's, and Shirasu's groups), each of which conduct their original and excellent researches. Particularly, Ohno's group is now leading the research field worldwide on microbiota in mammals. Ohkuma's group is essential to the project by establishing technologies that are required for the analysis of microbiota biology. Shirasu's group is conducting a really unique research on microbiota in rhizosphere, which will absolutely be an important since the achievements of this field will be beneficial for several areas on agriculture, farming, and forestry and so on.

#### **(3) Research achievement**

In each group, many distinguished achievements have so far been obtained. Ohkuma's group developed several technologies that are required for the analyses of microbiota. Particularly, this group is trying to develop a single cell analysis technology, which will greatly enhance the quality of the analysis on microbiota. The reviewer asks to consider a good strategy to integrate "single cell" analysis and "community" analysis. The research achievements of Ohno's group are so excellent, and the reviewer does not have any comment on it. Shirasu's group obtained spectacular

accomplishments by identifying rhizosphere microbiota as well as chemical compounds influencing the plant physiology. What is noteworthy is that there are many publications of collaborative studies between members of this project. This means that the integration of different research fields within the project is arising on their own. Furthermore, this research group is involved in the establishment of “the Japanese Society of Symbiosis” in 2017. Thus, this research project has already been contributing to the creation of a new research field on “symbiosis”. “Symbiosis” is a really complicated system, which is hard to be understood. Therefore, the reviewer asks Dr. Ohno to take the leadership more actively for the integration of different research fields on symbiosis, for example, interaction of young researchers between different research groups.

#### **(4) Future research plan**

Based on these three-year research achievements, the researchers make a reasonable research plan, which will generate a new concept of microbiota robustness and homeostasis and disclose a universal mechanism for microbiota commensalism.

### **< Reviewer 3 >**

#### **(1) Research objective**

This project aims at understanding the biological mechanism of various types of symbiosis by describing ecological networks mainly involving single-celled organisms that make the whole system functional at molecular and genetic levels. This is quite an extensive and important area of biology, since the phenomena of symbiosis is one of the most basic and ubiquitous features of any biological systems. This theme ranges from pathological phenomena to ecological systems in nature. The major challenge of the project is to unite analyses of organisms that have never been possible to culture and analysis at the single cell level, for which comprehensive genomic studies are being applied. Thus the project is potentially capable of providing a new concept of biology from unique view point of organismal interactions at various levels.

#### **(2) Implementation of research plan**

The research plan contains several different fields of researches, ranging from plants to human diseases, making the whole project very profound and extensive. The research plan is well prepared, and RIKEN facility is capable of supporting the entire project.

#### **(3) Research achievement**

So far, this project has seen a great deal of achievement, as exemplified by a number of research papers published in major international journals. To be noted would be that many of the papers are written as the result of collaborations performed among different laboratories involved in this project, showing that this project is significantly enhancing the joint research activities among the laboratories.

#### **(4) Future research plan**

As stated in (1), this research project is extensive and thus it will potentially be expanded into various different fields at various levels. However, I would recommend for the rest of the term to continue the current levels of studies as has been done, since there remains a lot more to be elucidated. Also this project has to be allowed to have enough time to spend for their researches as far as possible.

### **< Reviewer 4 >**

#### **(1) Research objective**

This project is intended to understand the whole image of symbiosis. Until now, symbiosis research projects have been done around the world in each individual field: animals-microorganisms, and plants-microorganisms. However, there has been no study to deeply explore animals-microorganisms, and plants-microorganisms all together in one project under the concept of symbiosis. In each symbiosis mechanism of animal-microorganisms and plant - microorganisms, building a fundamental common understanding leads to an understanding of true symbiosis. An understanding of these combined symbioses greatly contributes to the fields of human and animal

health, food production, and the environment. Therefore, this project offers valuable contributions to the RIKEN's mission: the realization of a healthy and safe life of people who live with the future, and continuous developments of science and civilization.

This project consists of three sub-projects. Dr. Ohno is the leader of the animal-microorganisms subproject, and Dr. Shirasu leads plants-microorganisms subproject. However, in nature, 90 to 99% of microorganisms is unculturable, and many microorganisms of symbionts are also difficult to culture, which has been a large hurdle in the research of symbiosis. To overcome this hurdle, it was selected Dr. Ohkuma as the third subproject leader as he leads the world in the analysis of unculturable microorganisms in symbioses. Therefore, in this project, the analysis of uncultured microbial symbionts, which are researched by Dr. Ohkuma, is the key to the project. The leaders are pioneers in each field. Moreover, each subproject has members from a wide range of scientific research, such as engineering, etc. This project is unique in that researchers of different field enter in each subproject and all work together on a shared objective. Thus, the researchers who are global leaders in their fields respectively have gathered and work with researchers from different research fields to collaborate on the key issue of symbiosis. Thanks to the wide resources of RIKEN, they have been able to realize this project and expand the frontiers of science.

## **(2) Implementation of research plan**

The three subprojects have fully attained each research program, respectively.

## **(3) Research achievement**

(Subproject1)

By cooperating with different fields such as IMS, device, single cell genomics, etc., they have succeeded in the analysis of uncultured microbial symbionts. Therefore, it can be judged that the construction of the platform of the analysis was attained. Thus, it can be evaluated enough that the preparation of cooperation with other subprojects was completed.

(Subproject2)

Advanced results of the mechanism of differentiation of M cell in interactions between intestinal mucosa of mammals and bacteria have been obtained. Furthermore, the advanced results of the effects of metabolites of gut microbiota of hosts have also been obtained.

(Subproject3)

Advanced results of interactions in the rhizosphere of plants and microorganisms have been obtained. The compounds metabolized from symbiosis microbes only at the time of an interaction with a plant were discovered. Furthermore, they succeeded to identify some compounds from plants which induce the compounds of microorganisms. It can be evaluated that the results are obtained from both plants and microorganisms. This valuable result was achieved by the close cooperation of the fields of chemistry and microbiology.

## **(4) Future research plan**

It is expected to achieve each future research plan considering the research potential of each subproject.

The biggest feature of this project is to lead a universal law of symbiosis. Therefore, after the results of animals-microorganisms, and plants-microorganisms are accumulated, they should reach for a fundamental and common understanding of symbiosis and /or extraction of universality for symbiosis. For example, it will also be necessary to add theoretical researchers. World leading researchers of animals, plants and microbes, and also of chemistry, theory and engineering have gathered at RIKEN so RIKEN should have a long range perspective and this project should be promoted.

## **<Reviewer 5>**

### **(1) Research objective**

The research objective is timely, but the common goal or idea that keep the research program consistent is a bit unclear.

**(2) Implementation of research plan**

The research group is well organized to include the appropriate researchers in RIKEN.

**(3) Research achievement**

Excellent achievements have been reported. We expect more results that is realized by this collaborative research program.

**(4) Future research plan**

It was not very clear what is the common goal of the research groups, and it looks like a combination of various researches that are related with the concept of symbiosis. Discussions on common aspects of symbiosis, and its theoretical understanding will stimulate exploration of new directions of the symbiosis.

RIKEN Science Council Research Programs Committee