(Form4-2)

JOINT RESEARCH PROJECT

FINAL REPORT
For Japan-Korea Joint Research Project

AREA	1. Mathematics & Physics 2. Chemistry & Material Science 3. Biology 4. Informatics & Mechatronics 5. Geo-Science & Space Science 6. Medical Science 7. Humanities & Social Sciences

1. Research Title:

Conversion system of sucrose (released from plant-root) to novel saccharides by soil microorganisms and its application

2. Term of Research: From 2010.7.1 To 2012.6.30

3. Total Budget

a. Financial Support by JSPS: Total amount: 2.400 thousand yen

1st Year 900 thousand yen 2nd Year 1,200 thousand yen

3rd Year 300 thousand yen

b. Other Financial Support : Total amount: 0 thousand yen

4. Project Organization

a. Japanese Principal Researcher	
Name	Atsuo KIMURA
Institution / Department	Research Faculty of Agriculture, Hokkaido University
Position	Professor
b. Korean Principal Researcher	
Name	Doman KIM
Institution / Department	School of Biological Sciences and Technology, Chonnam National University,
Position	Professor

c. List of Japanese-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Takahiro NODA	Crop Functionality and Utilization Research Subteam (Hokkaido Region), National Agricultural Research Center for Hokkaido Region	Head of Subteam
Kazumi FUNANE	National Food Research Institute, National Agriculture and Food Research Organization	Head of Unit
Keitaro KIMURA	National Food Research Institute, National Agriculture and Food Research Organization	Senior Researcher
Weeranuch LANG	Research Faculty of Agriculture, Hokkaido University	Postdoctoral Fellowship
Hee-Kwon KANG	Research Faculty of Agriculture, Hokkaido University	Ph.D.-Course 3rd-Grade Student
Lukana NGIWSARA	Research Faculty of Agriculture, Hokkaido University	Ph.D.-Course 2nd-Grade Student
Juri SADAHIRO	Research Faculty of Agriculture, Hokkaido University	Ph.D.-Course 1st-Grade Student
Takeyoshi TAGAMI	Research Faculty of Agriculture, Hokkaido University	MS-Course 2nd-Grade Student

d. List of Korean-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Hwa-Ja RYU	Research Institute for Catalysis, Chonnam National University	Research Professor
Young-Min KIM	Molecular Bioprocess Research Center, KRIBB	Researcher
Hee-Kyoung KANG	Research Institute for Catalysis, Chonnam National University	Research Professor
Go-Eun KIM	Graduate Student of Interdisciplinary Program for Bioenergy and Fine Chemicals, Chonnam National University	Master Program Student
Hae-Jin WU	Graduate Student of Biological Science and Biotechnology, Chonnam National University	Master Program Student
Sang-Il YOON	Graduate Student of Biological Science and Biotechnology, Chonnam National University	Master Program Student

5. Number of Exchanges during the Final Fiscal Year*

a. from Japan to Korea *Japanese fiscal year begins April 1.

Name	Home Institution	Duration	Host Institution
Takahiro NODA	Crop Functionality and Utilization Research Subteam	6 days	Research Institute for Catalysis, Chonnam National University
Kazumi FUNANE	National Food Research Institute	4 days	Research Institute for Catalysis, Chonnam National University
Keitaro KIMURA	National Food Research Institute	4days	Research Institute for Catalysis, Chonnam National University

Kazumi FUNANE	National Food Research Institute	8 days	Naples Convention Center (Italy)
Atsuo KIMURA	Research Faculty of Agriculture, Hokkaido University	8 days	Research Institute for Catalysis, Chonnam National University
Atsuo KIMURA	Research Faculty of Agriculture, Hokkaido University	12 days	Research Institute for Catalysis, Chonnam National University
Takeyoshi TAGAMI	Research Faculty of Agriculture, Hokkaido University	3 days	Research Institute for Catalysis, Chonnam National University
Atsuo KIMURA	Research Faculty of Agriculture, Hokkaido University	8 days	Research Institute for Catalysis, Chonnam National University

For Final Fiscal Year(FY2012)	For Final Fiscal Year(FY2012)
Total: 8 persons	Total: 53 man-days

Numbers of Exchanges during the Past Fiscal Years

FY2010: Total 3 persons
FY2011: Total 3 persons

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution

For Final Fiscal Year(FY2012)	For Final Fiscal Year(FY2012)
Total: 0 persons	Total: 0 man-days

Numbers of Exchanges during the Past Fiscal Years

FY2010: Total 0 persons
FY2011: Total 0 persons

6. Objective of Research

(1) Purpose: Plant photosynthesis produces starch, which is converted to sucrose (**Suc**). Suc moves to storage organ, like seeds (rice). It is well known that plants secret Suc from their roots to soil, and use Suc for cross-talking with soil-microorganisms (**SM**). Very recently, it was discovered that plant-roots released the huge amount of Suc, corresponding to 20 to 40% of one day's photosynthesis. We call this system as a "Sucrose-World". **Purpose of this project** is **the analysis of Sucrose-World members [SM and their enzymes** (**SM-E**)**] and their application**.

(2) Significance of the proposed project

1) Research ability about polysaccharide (**PS**): Biochemistry textbook mentions that the binding energy of sucrose (**Suc**) is extremely high, almost identical to UDP-Glc, so that there are two types of enzymes to synthesize PSs: i) Glucansucrase forming glucan from glucose-unit of Suc; ii) Fructansucrase forming fructan from fructose-unit. All members of Korean team are specialists for glucansucrase and fructansucrase, contributing to researches about novel PS-forming enzymes. Korean team is also familiar to oligosaccharide (**OS**) production by acceptor reactions of those enzymes, also contributing to OS study in this project.

Currently we expect that many types of PSs are produced from Suc by soil microorganisms (**SM**s), since the preliminary results, obtained by Japanese team, suggest that several SMs generate β-glucan from Suc. One of SMs produces a novel β-glucan, meaning that dextran and fructan are not sole products from Suc.

2) Isolation of valuable SMs: Finding of PS-forming SM is a "key" in this project, since PS will be used for a carbon-source in medium for screening of further SMs, which convert PS into the first OS (**1-OS**). Korean team is rich in experiences to isolate PS-forming SMs, by which the novel SMs is quickly obtained. The 1-OS is also important, due to a carbon-source for screening of SMs to form the second OS (**2-OS**).

3) Function analysis of PS and OS: The function-analyzing system of PS and OS is available in Korean team, enabling us to find novel functions of PS and OS, so that the collaboration is essential.

(3) Past research activities

There were many studies to isolate PS-forming MSs using Suc (e.c. dextran- or fructan-forming MSs). But, no researcher has thought about Sucrose-World (plant-Suc→PS→1-OS→2-OS), even in Japan, Korea, and in other countries. By our new idea (Sucrose-World), Japanese team has already found MSs to produce two novel enzymes (i.e. PS-synthase to form PS from Suc; transferase to form 2-OS), proving the significance of sucrose-world. We strongly believe that valuable PSs and OSs will be found by our project.

7. Methodology

In FY 2010 (9 months):

1. Isolation of SMs to produce PS (both teams): Both teams will screen SMs from soil near plant roots

using agar medium plates containing Suc as a sole carbon-source. Formation of PS [making viscous materials (PS) around colony] is easily detected by visible observation. After single colony isolation, 16S rRNA analysis identifies its species. After a large amount of PS is produced, we study the structure of PS by monosaccharide-analysis, methylation-analysis, and NMR. Physicochemical properties of PS are also analyzed.

2. SMs to form 1-OS (both teams): We will isolate 1-OS-forming SMs with the same agar medium plates [described in section 2)-1], containing PS instead of Suc as a sole carbon-source. PS-containing agar medium is opaque, so that SM, displaying clear halo around its colony, becomes a candidate to form 1-OS (If a certain dye binds PS, it can be also used for screening, since a 1-OS-forming SM makes the clear halo). Single colony isolation will be done, followed by16S rRNA analysis to identify the species. We produce 1-OS and study its structure by monosaccharide-analysis, methylation-analysis, MS, and NMR. Large amount of 1-OS is necessary for obtaining 2-OS.

3. PS- and 1-OS-forming enzymes (both teams): PS- and 1-OS-forming enzymes are purified from SMs and characterized. Thermal- and pH-stabilities are important for enzymatic production of PS and 1-OS.

4. PS-synthase and transferase (Japanese team): Japanese team purifies and characterizes novel PS-synthase. This team also isolates a gene of novel transferase.

In FY 2011 (12 months):

5. SMs and SM-Es to form PS and 1-OS (both teams): Both teams continue isolation and characterization of MSs and enzymes by the same approaches as mentioned in FY 2010.

6. SMs to form 2-OS (both teams): We will isolate 2-OS-forming SMs with the same agar medium plates as described in section 2)-1, but a carbon-source is 1-OS. SM to grow on 1-OS-plate is cultivated in liquid- culture in small scale. Formation of 2-OS in medium is analyzed by TLC. Single colony isolation will be done, and 16S rRNA analysis identifies the species. After 2-OS production in the large scale, its structure is studied by the same procedure as shown in section 2)-2.

7. 2-OS-forming enzymes (both teams): We will purify and characterize 2-OS-forming enzymes.

8. PS-synthase and transferase (Japanese team): Japanese team will isolate and express a gene of novel PS-synthase. The gene-expression of novel transferase will be also performed.

9. Functions of PS, 1-OS, and 2-OS (Korean team): Functions of PS, 1-OS and 2-OS will be analyzed by Korean team: i.e. stability test at high temperature and acidic condition; test for sweeteners; anti-oxidant test; inclusion ability; growth-test of probiotic organisms such as *Bifidobaterium*; inhibitory effects on the formation of dental caries.

In FY 2012 (3 months):

10. Continuous researches (both teams): The identical researches in FY 2010 and 2011 continue this year. Both teams exchange the information, enabling us to learn the climate-effects on Sucrose-World.