11th YU-RCTMR Symposium
6th Seminar of Priority Universities

International Symposium

"Bioactive Molecules from Microorganisms"

Friday, November 15, 2019
Media Center Lecture Hall
Yamaguchi University

Research Center for Thermotolerant Microbial Resources (RCTMR), Yamaguchi University

Priority Universities for Cooperation between Chulalongkorn University, Kasetsart University and Yamaguchi University

Opening Remarks

Prof. MD. Dr. Masaaki Oka

It is a great pleasure and privilege for Yamaguchi University to hold the "Bioactive Molecules from Microorganisms" International Symposium, which is a joint meeting of the 11th YU-RCTMR (Research Center for Thermotolerant Microbial Resources, Yamaguchi University) Symposium and the 6th Seminar of Priority Universities for Cooperation between Chulalongkorn University, Kasetsart University and Yamaguchi University. Our speakers today have gathered from four different universities. They come from Kasetsart University and Chulalongkorn University in Thailand as well as Hiroshima University and our University in Japan. I would like to express our sincere appreciation to the invited speakers for presenting at this symposium. I would also like to thank Prof. Shinichi Ito and everyone who helped to make this seminar possible.

Yamaguchi University has encouraged advanced science and innovation research centers within the University since 2014. There are currently 4 research cores, and RCTMR is one of the most successful. This symposium is an important event for RCTMR and will be an excellent opportunity to exchange opinions on the advanced research presented by the invited speakers. The RCTMR is a unique and vital center in the microbiology research world, focusing on the foundations for research on thermotolerant microbes and their applications. We wish to make it a gateway for access to microorganisms for scientists both in Japan and other countries, such as Thailand.

Yamaguchi University has designated several overseas partner universities since 2013. We started the overseas partnerships with Kasetsart University and Chulalongkorn University as "priority universities for cooperation" in 2013. Our partnership grew from the extremely active research collaboration via three international core programs over 20 years, which were mainly supported by the Japan Society for the Promotion of Science (JSPS) and the National Research Council of Thailand (NRCT). Since then, seminars have been regularly held at the three Universities every year. I am very grateful to Associate Prof. Dr. Gunjana in Kasetsart University and Prof. Dr. Alisa in Chulalongkorn University for their continuing efforts to hold seminars at their respective universities.

Congratulations on the opening of this international symposium and I hope that your discussion will be successful.

Masaaki Oka
President, Yamaguchi University

Message from Thai Coordinator

Assoc. Prof. Dr. Gunjana Theeragool,
Department of Microbiology, Faculty of Science,
Kasetsart University

It is my great honor to welcome all of the distinguished guests and participants to 11th YU-RCTMR Symposium and 6th Seminar of Priority Universities on "Bioactive Molecules from Microorganisms".

Kasetsart University and Yamaguchi University established the Core University Program on "**Development of Microbial Resources and Their Applications**" with financial support from the Japan Society for the Promotion of Science (JSPS). It took place over 10 years (April 1998 - March 2008). The success of the ten-year core university program had the potential to be extended to the Asian Core Program on "**Capacity Building and Development of Microbial Potential and Fermentation Technology toward New Era**". This program was created with financial support from JSPS and the National Research Council of Thailand (NRCT), ran for 5 years (April 2008 - March 2013), and received collaboration from 4 active teams from Japan, Vietnam, Laos and Thailand, respectively. Following on this fruitful collaboration, we have established the Core to Core Program A. Advanced Research Networks on "**Establishment of an International Research Core for Bio-research Fields with Microbes from Tropical Areas (World-class Research Hub of Tropical Microbial Resources and Their Utilization)**". This five-year (April 2014 - March 2019) program received financial support from JSPS, NRCT, the Vietnam Ministry of Science & Technology (MOST), the National University of Laos, The University of Brawijaya (Indonesia), Beuth University of Applied Sciences (Germany) and The University of Manchester (England).

The 11th YU-RCTMR Symposium is one of the successful academic activities under Core to Core Program. The 6th Seminar of Priority Universities is also one of the fruitful academic cooperation between Yamaguchi University, Kasetsart University and Chulalongkorn University since 2014. This seminar will provide a good opportunity for the participants to discuss and summarize their output/outcome of collaboration in order to obtain the most fruitful results. In addition, I hope that the presentation and discussion which take place during this seminar will spur the participants towards the development of new research opportunities and productive collaboration.

I would like to express my sincere appreciation to Yamaguchi University for holding 11th YU-RCTMR Symposium and 6th Seminar of Priority Universities. My thanks also go out to the speakers for contributing their research work to this seminar. Last, but not least, I would like to express my sincere gratitude to JSPS, NRCT, MOST in Vietnam, The National University of Laos, The University of Brawijaya, Beuth University of Applied Sciences and The University of Manchester for their continuing financial support.

On this seminar

Prof. Dr. Mamoru Yamada

It is our great pleasure to hold the International Symposium entitled "Bioactive Molecules from Microorganisms", jointing the 11th YU-RCTMR (Research Center for Thermotolerant Microbial Resources, Yamaguchi University) Symposium and the 6th Seminar of Priority Universities for Cooperation between Kasetsart University, Chulalongkorn University and Yamaguchi University. I would like to take this opportunity to appreciate the enormous efforts of Professor Dr. Shinichi Ito as a chief organizer of this symposium as well as the financial support of Yamaguchi University.

Research Center for thermotolerant Microbial Resources was launched 2009 as a research institute belonging to Faculty of Agriculture and became a research institute in Yamaguchi University 2014. The YU-RCTMR consists of three sections, Division of Fermentation Microorganisms, Division of Environmental Microorganisms and Division of Patogenic Microorganisms. Due to such three sections, it is an integrated institute, which seems to be the only program of its kind globally and mainly focuses on thermotolerant or semi-thermophilic microorganisms including viruses.

Yamaguchi University has designated several overseas partner universities including Chulalongkorn University and Kasetsart University as "Priority Universities for Cooperation." We see great potential for the university's research level to grow through connection with these universities and have set goals of providing educational support, contributing internationally through academic exchange, and increasing the overall level of research by strengthening research-focused international cooperation activities. This is the second time of this Seminar in Yamaguchi University with scientists invited from the two Universities. Previously, we had Seminar of Priority Universities two times in Kasetsart University and two times in Chulalongkorn University

Finally, I would like to thank all attendees and their contributions to this seminar. I hope this seminar will be fruitful and successful.

Prof. Dr. Mamoru Yamada
Director, Research Center for Thermotolerant Microbial
Resources, Yamaguchi University (YU-RCTMR)
Senior Presidential Advisor, Yamaguchi University

COMMITTEE and others

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11th YU-RCTMR Symposium and 6th Seminar of Priority Universities for Cooperation

International Symposium "Bioactive Molecules from Microorganisms"

Date: Friday, November 15, 2019

Venue: Media Center Lecture Hall, Yamaguchi University

Time	Program	Chairperson
14:00	**Opening Remarks** Dr. MD. Masaaki Oka (President, Yamaguchi University)	
14:15-14:45	**Analysis of *Francisella* effector interacting with centrosome** Dr. Takashi Shimizu (Joint Faculty of Veterinary Medicine, Yamaguchi University)	Dr. Kazunori Sasaki (Yamaguchi University)
14:45-15:15	**Assembly of a fungal macrocyclic polylactone is catalyzed by two iterative polyketide synthases** Dr. Pakorn Wattana-Amorn (Faculty of Science, Kasetsart University)	Dr. Toshiharu Yakushi (Yamaguchi University)
15:15-15:45	Coffee break (**FAVO**)	
15:45-16:15	**Genosensor: when nucleic acid assay was used as a biomolecular tracer in microbial biotechnology** Dr. Piyasak Chaumpluek (Faculty of Science, Chulalongkorn University)	Dr. Hisashi Hoshida (Yamaguchi University)
16:15-16:45	**Chemotaxis involved in plant infection in *Ralstonia solanacearum* and control of plant infection by intervening chemotaxis** Dr. Jun-ichi Kato (Graduate School of Integrated Sciences for Life, Hiroshima University)	Dr. Gunjana Theeragool (Kasetsart University)
16:45	**Closing Remarks** Dr. Kenji Hori (Vice-President (Academic Research), Yamaguchi University)	

Invited speaker

Name: Takashi Shimizu

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Short Biography:

Yamaguchi University

2012-present Joint Faculty of Veterinary Medicine, Associate Professor

2011-2012 Faculty of Agriculture, Associate professor

Kurume University

1999-2011 School of Medicine, Research Associate

Osaka City University

1998-1999 Graduate school of Science

1995-1998 Faculty of Science

Analysis of *Francisella* effector interacting with centrosome

Takashi Shimziu[1], Kenta Watanabe[1], Akihiko Uda[2] and Masahisa Watarai[1]

[1] Laboratory of Veterinary Public Health, Joint Faculty of Veterinary Medicine, Yamaguchi University. [2]Department of Veterinary Science, National institute of Infectious Disease.

Francisella tularensis is facultative intracellular bacteria and causes tularemia in human. *F. tularensis* possess *Francisella* pathogenic island (FPI) that is responsive for intracellular growth. FPI encodes type VI secretion system (T6SS), but the molecular mechanisms by which *F. tularensis* survives intracellular is still unclear. In this study we focused on one of T6SS effectors, IglE and analyzed its function.

Deletion mutant of *iglE* showed decreased intracellular growth and cytotoxicity. Confocal microscopic analysis revealed that *IglE* was localized around Golgi-derived membranes and autophagosomes. WT *F. tularensis* proliferated in autophagosomes, whereas *iglE* deletion mutants were not observed within autophagosomes. To determine IglE binding proteins of host cells, immune precipitation assay was carried out. As the result, β-tubulin was identified. The association was confirmed by pull-down assay. Although co-localization of IglE and β-tubulin was not observed in host cells, IglE was localized with centrosomes. In addition, *iglE* deletion mutant localized within the area around centrosomes where lysosomes are usually distributed. Taken together, these data suggested that IglE located at *F. tularensis* containing membrane and inhibited membrane transport toward centrosome and fusion with lysosomes.

Invited speaker

Name: Pakorn Wattana-Amorn

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Photo:

Short Biography:

Kasetsart University

2016-present Assistant Professor of Chemistry

2009-2015 Lecturer

Univeristy of Bristol

2007-2009 Postdoctoral Fellowship (Research: Biological NMR)

2003-2007 PhD in Chemistry (Research: Type II polyketide synthases)

Assembly of a fungal macrocyclic polylactone is catalyzed by two iterative polyketide synthases

Waraporn Bunnak,[1] Passorn Wonnapinij, [2] Ajaraporn Sriboonlert, [2] Colin M. Lazarus[3] and Pakorn Wattana-Amorn *[1]

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Menisporopsin A is a bioactive macrocyclic polylactone produced by the fungus, *Menisporopsis theobromae* BCC 4162. Reducing (R) and nonreducing (NR) polyketide synthases (PKSs) are believed to catalyze the formation of each menisporopsin A subunit, and a non-ribosomal peptide synthetase (NRPS)-like enzyme is predicted to perform multiple esterification and cyclolactonization reactions.[1] Transcriptome analysis of *M. theobromae* identified an R-PKS gene, *men1*, and an NR-PKS gene, *men2*, that both showed highest expression levels during the menisporopsin A production phase. These genes were cloned into separate vectors for heterologous expression in *Aspergillus oryzae* NSAR1. Interestingly, co-expression of the two PKSs was found to be sufficient to catalyze the formation of ascotrichalactone A, a structural derivative of menisporopsin A.[2] The unanticipated enzymatic activities could reside in the unusual thioesterase domain of the NRPKS, which is similar to that of the NRPS in enterobactin biosynthesis and that of modular PKSs catalyzing macrodiolide formation in elaiophylin and conglobatin biosyntheses

References

1) Bunnak, W., Wonnapinij, P., Sriboonlert, A., Lazarus, C.M., Wattana-Amorn, P. (2019) Heterologous biosynthesis of a fungal macrocyclic polylactone requires only two iterative polyketide synthases. *Org. Biomol. Chem.* 17, 374-379.

2) Wattana-Amorn, P., Juthaphan, P., Sirikamonsil, M., Sriboonlert, A., Simpson, T.J., Kongkathip, N. (2013) Biosynthetic Origins of Menisporopsin A. *J. Nat. Prod. 76*, 1235-3.

Invited speaker

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Chulalongkorn University

1996-present Assistant Professor

Head of Plant Transgenic Technology and Biosensor Laboratory, Faculty of Science

2001- present Advisor of Molecular Biology Unit,
Food Research and Testing Laboratory, Faculty of Science

Iwate Biotechnology Research Center

1993-1996 Researcher (Plant Virology)

Kyoto University

1993 PhD in Agricultural Biology (Molecular Characterization of Cucumber Mosaic Virus Isolates Occurring on Gentian Plants and Production of Satellite RNA-mediated Virus Resistant Transgenic Plants)

Genosensor: when nucleic acid assay was used as a biomolecular tracer in microbial biotechnology

Piyasak Chaumpluk[1,2]

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In microbial molecular analysis, nucleic acid plays an important clue since tracing through or by it definitely reveals the secrets underneath any attributes of hidden diversity of microbial life. Nucleic acid assay, marking either on gene or gene regulation or using genetic materials as biomolecular tracer, thus provides a way to solve conundrum in our microbial biotechnological system. Recently, advent of genosensors have changed the way for nucleic acid assay, answering a desire to develop rapid, specific, robust and highly sensitive methods for the detection or identification of genetic basis of life. Genosensors are analytical devices or tools, based on genetic analysis, incorporating a biological sensing element for nucleic acid assessments. They harness the exquisite sensitivity and specificity of DNA or RNA in conjunction with physicochemical transducers to deliver complex bio-analytical measurements with simple, rapid, and easy-to-use formats, answering those prompted demanding.

The first category of genosensor harnesses a physical phenomenon, exhibited by electrons translocation upon chemical transduction occurred during nucleic acid binding interactions. Either the current (rate of flow of electrons is proportional to the nucleic acid concentration) at a fixed potential or the voltage (measured at zero current) can be assessed. The second approach relies on a light emission through the assessment of fluorescence light upon binding of labels or dyes during nucleic acid hybridization, allowing extending the capability of a conventional instrument to detect and quantify the binding phenomena simultaneously. The third is based on size and distance dependent surface plasmon resonance (SPR) properties and ultra-high molar extinction coefficients of metal nanoparticles which could exhibit observable color change upon aggregation or growth, even at nanomolar concentrations.

In practical, target nucleic acid can be directly analyzed if it is provided with sufficient signals. And if this is not the case, alternative signal amplification via polymerase based enable to increase signals to a measurable level specifically. With these, attempts to detect target nucleic acid can go from tissue through even a single cell event.

In this talk, several microbial models using a combination of gene markers with either electrochemical principle or fluorescence signal illumination or colorimetric method via metal nanoparticle basis, are demonstrated for their tracing of the genes expression in microbial biosynthesis pathways, for the detection of particular gene in one strain from others, for microbial monitoring, and for tracing target metabolite with novel nucleic acid aptamer approach. Challenges to be overcome for the applications of genosensor to microbial biotechnology are also discussed.

References

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2) Chaumpluk, P. et al. Rapid detection of aflatoxygenic *Aspergillus sp*. in herbal specimens by a simple, bendable, paper-based lab-on-a-chip. Biotechnology Journal, 11:768-779 (2016).

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Invited speaker

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Short Biography:

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2001-present Professor of Molecular Biotechnology

1995-2001 Associate Professor of Fermentation Technology

1990-1995 Assistant Professor of Fermentation Technology

University of Illinois at Chicago

1988-1990 Postdoctoral Researcher (Microbiology)

University of Tokyo

1988 PhD in Agricultural Chemistry (Metabolic engineering of anaerobes)

Chemotaxis involved in plant infection in *Ralstonia solanacearum* and control of plant infection by intervening chemotaxis

Akiko Hida[1], Tunchai Mattana[2], Takahisa Tajima[1] and Junichi Kato*[1]

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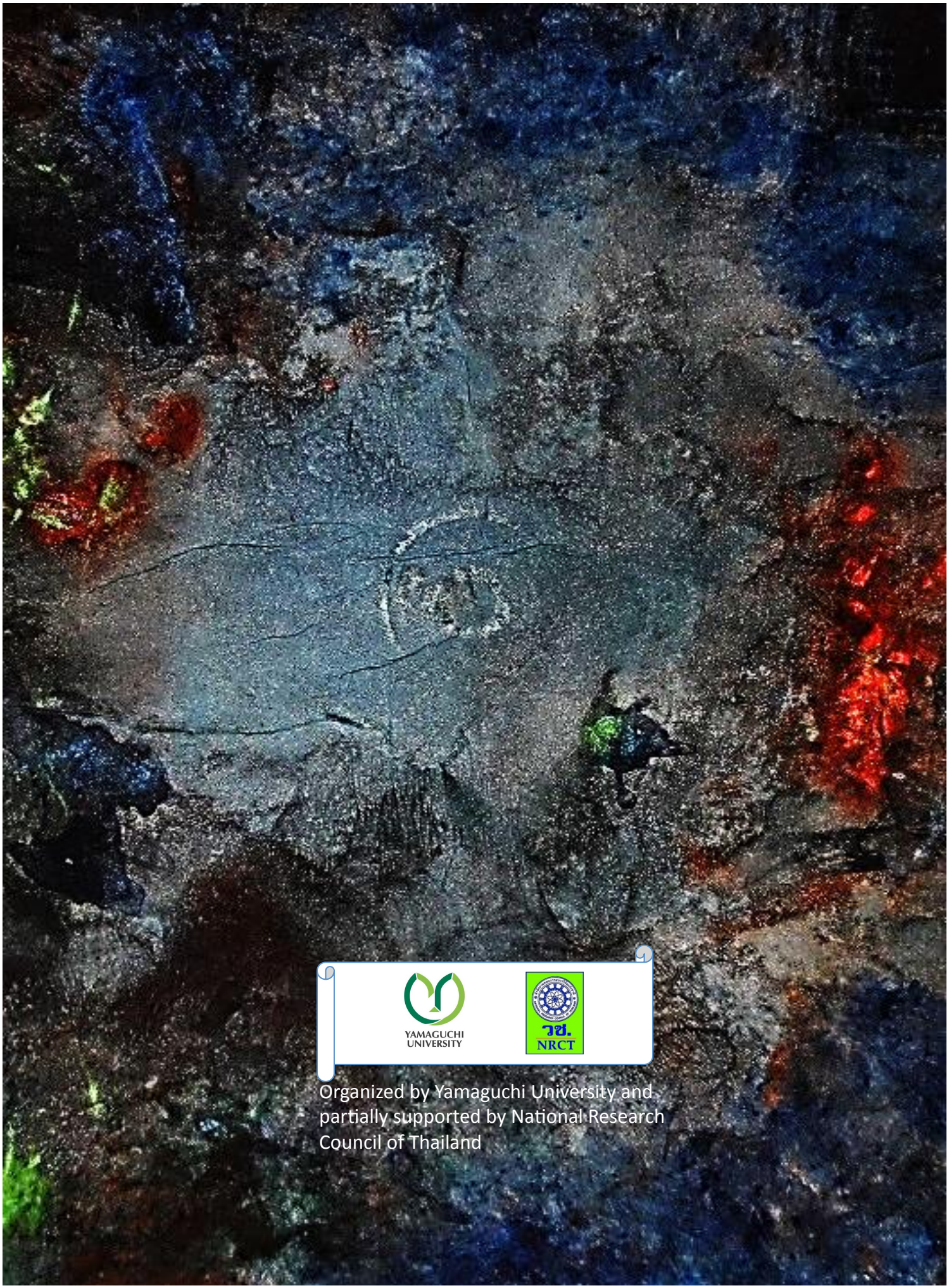
Motile bacteria always sense changes of chemical concentrations surrounding their cells, control their swimming based on the sensing data, and swim toward their "favorite" environments and away from "unfavorite" environments. The behavioral responses are called chemotaxis. It is known that chemotaxis plays important roles in searching growth substrates in environments. It is also believed that chemotaxis assists ecological interaction including symbiosis, infection, and prey-predator relationship. *Ralstonia solanacearum* is a soil bacterium causing bacterial wilt in many economically important crops. We demonstrated that chemotaxis, especially chemotaxis to L-malate, is involved in the very early stage of its plant infection, that is, approach to plant roots in soil. This finding has inspired us to a possibility to control plant infection of *R. solanacearum* by disturbing its chemotaxis. In this presentation, I will talk about molecular biology of chemotaxis, especially chemotaxis sensors of *R. solanacearum* and trials to control its plant infection based on disturbance of chemotaxis.

References

1) Hida, A., et al. Identification of the *mcpA* and *mcpM* genes, encoding methyl-accepting proteins involved in amino acid and L-malate chemotaxis, and involvement of McpM-mediated chemotaxis in plant infection by *Ralstonia pseudosolanacearum* (Formerly *Ralstonia solanacearu*m phylotypes I and III). Appl. Environ. Microbiol., 81:7420-7430 (2015).



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