

Engineering new biocatalysts

Dr Hirofumi Hara has been investigating *R. jostii* RHA1 genome for more than a decade. His work shows that changing the environment of this bacterium can deliver metabolites with desirable properties



You are currently investigating the production of secondary metabolites from *Rhodococcus*. What do you hope to achieve?

Analysis of *Rhodococcus jostii* RHA1 revealed that secondary metabolite biosynthesis genes, such as putative polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) exist in their genome. Most of the secondary metabolites so far, such as antibiotics, are mainly produced from the *Streptomyces* species, another genus of actinomycetes. My objective is to obtain novel secondary metabolites from PKS or NRPS in *R. jostii* RHA1.

Why did you induce secondary metabolite production genes in different environmental situations?

We know that the expression pattern of secondary metabolites biosynthesis gene was regulated differentially under various environmental conditions. This suggests *R. jostii* RHA1 produces different kinds of secondary metabolite under specific circumstances. Each secondary metabolite produced during a particular condition might have a precise physiological or chemical role of this organism under these conditions. We are now investigating this point. This information might help us

understand why actinomycetes need to produce secondary metabolites.

Could you discuss the methodology you are using?

There are seven PKSs and 24 NRPSs located across the RHA1 genome; we developed methods to investigate all of these secondary metabolite production genes under various environmental conditions. For example, one method involved inserting the red fluorescent protein under the promoter of each secondary metabolite biosynthesis gene.

Why does *Rhodococcus* have high industrial importance? How will your findings affect the commercial application of *Rhodococci*?

Rhodococcus is well suited for industrial purposes and bioremediation applications because it can resist against any kind of environmental stress, including osmotic, temperature and desiccation stressors.

It shows a high capability of removal of some environmental pollutants, such as polychlorinated biphenyl (PCB) or phthalate esters. After the genome analysis of RHA1, we annotated a lot of primary metabolism pathways, so there are many genetic and protein materials that can be used for removal of harmful chemical compounds from the environment.

Moreover, the production of secondary metabolites, from not only *R. jostii* RHA1 but also any kind of actinomycetes, depends on various environmental conditions. Therefore, it might be possible that the induction conditions we have found from this project can be used for the production of novel secondary metabolites from any kind of actinomycetes, without any kind of omics (genomics, transcriptomics, proteomics or metabolomics) analysis.

Have you come across any obstacles during this project?

Since the beginning of the genome project of *R. jostii* RHA1, it has taken more than 10

years to reach this point. There were many problems and I really appreciate all of the support from related researchers.

Do you have plans to develop your research further, perhaps moving into other actinomycetales?

I am now working at the Malaysian-Japan International Institute of Technology in Universiti Teknologi Malaysia (UTM), Kuala Lumpur, Malaysia. As we know that actinomycetes contain high capabilities of primary and secondary metabolism, we are now isolating actinomycetes from the environment in Malaysia. One of our projects will be biomass conversion into energy and chemical materials by enzymes that might contain the unique capability of both primary and secondary metabolism.

Additionally, one reason my team originally selected the *Rhodococcus* species is that it is easy to manipulate genetically, especially on gene recombination – easier than *Streptomyces*. Now, I am planning to use other actinomycetes, such as *Streptomyces*, for the production of secondary metabolites under different environmental conditions.

Finally, what do you consider to be the most novel aspect of your research?

The fact our findings demonstrate we can produce more than 20 secondary metabolites from one microorganism depending on the cultivation conditions. It suggests that we still have a chance to get novel chemical compounds from microorganisms that have already been isolated and characterised.

To obtain chemicals such as novel antibiotics, most researchers are now focused on omics analysis to identify biosynthesis pathway genes and enzymes. However, our research will give us a simple method that could be effective for producing useful and novel chemicals.

Maximising microbial resources

Researchers at the **Malaysia-Japan International Institute of Technology**, Universiti Teknologi Malaysia in Kuala Lumpur are showing they can steer *Rhodococcus* bacterium gene expression and so tailor its secondary metabolites to specific industrial needs

THE ACTINOMYCETES ARE a group of mostly aerobic, non-motile fungus-like bacteria, many of which have pathogenic effects, for example *Mycobacterium tuberculosis*; metabolites of the pathogenic members of the group are used for antibiotics, such as streptomycin which is sourced from *Streptomyces griseus*. Among the actinomycetes, however, the widely prevalent genus *Rhodococcus* is mostly benign. Various strains are able to catabolise organic compounds, converting them into other products, such as from harmful pollutants into harmless compounds or from valueless compounds into products of value. For example, members of the *Rhodococcus* genus have been applied to bioactive steroid production, fossil fuel biodesulphurisation and the production of acrylamide and acrylic acid. Apart from the use of microbes in the antibiotic industry, *Rhodococcus* represents the most commercially successful actinomycetes microbial biocatalyst.

***R. JOSTII* RHA1**

One of the largest bacterial genomes completely sequenced belongs to *R. jostii* RHA1, a potent soil microbial organism that catabolises a wide range of compounds. It is known for its ability to use aromatic compounds, carbohydrates, nitriles and steroids as energy sources. However, it is perhaps best known for its ability to degrade and convert polychlorinated biphenyls (PCBs), which were produced through some industrial processes in the 20th Century. Classified as 'persistent organic pollutants', PCBs are notoriously difficult to remove from contaminated sites, as they are resistant to environmental degradation. Since their widespread production, scientists have demonstrated their toxic effects which can accumulate in human and animal tissues, making their removal from the environment all the more important.

This *R. jostii* RHA1 species has been the subject of recent analyses in the laboratory of Dr Hirofumi Hara, Associate Professor in the Environmental Engineering and Green Technology Department of the Malaysia-Japan International Institute of Technology (MJIIT) at the Universiti of Teknologi Malaysia (UTM). Hara's focus is dual – he is studying the possible applications of *R. jostii* RHA1 and other tropical actinomycetes in bioremediation contexts, and he is researching the rich, unexploited resource base that the actinomycetes as a group represent.

SECONDARY METABOLISM IN *R. JOSTII* RHA1

While there was no prior record of any *Rhodococcus* species producing secondary metabolites – organic compounds inessential to an organism's ability to grow, develop or reproduce during secondary metabolism – Hara thought it may be possible, and sought to prove it. He obtained support from the Japanese Society for the Promotion of Science for a project to this end, as Hara explains: "From its genome sequence, we were sure that it bore a higher possibility of primary metabolism than other bacteria. From the fact that its genome showed nonribosomal peptide synthase (NRPS) genes and polyketide synthase (PKS) genes, we thought it would harbour an extensive secondary metabolism, like other actinomycetes".

The PKS and NRPS gene clusters are those mostly responsible for biosynthesis of secondary metabolites. For example, PKS produces compounds including macrolides, aromatic polyketides and polyphenols, while NRPS produces cyanotoxins and the compounds used in the antibiotics Vancomycin and Thiostrepton.

TAPPING INTO PRESERVED BIOACTIVE CHEMICALS

Since actinomycetes have provided two-thirds of the 12,000 bioactive chemicals isolated from microbes to date, Hara evaluated what effects environmental conditions had on actinomycete expression of secondary metabolite biosynthesis genes. From this, he sought to prove that an actinomycete could produce several types of secondary metabolites according to its environmental



Scanning electron microscopy (SEM) image of *R. jostii* RHA1 grown on biphenyl.

INTELLIGENCE

SEARCH AND ITS APPLICATION OF INDUCTION CONDITIONS OF SECONDARY METABOLITE BIOSYNTHESIS GENES IN BACTERIA OF THE GENUS *RHODOCOCCUS*

OBJECTIVES

To clarify whether *Rhodococcus jostii* RHA1 can produce a secondary metabolite; to elucidate whether the gene expression of secondary metabolite biosynthesis genes depend on environmental conditions in actinomycetes; to prove that actinomycetes can produce many kinds of secondary metabolites under various environmental conditions.

KEY COLLABORATORS

Professor Datin Dr Zuriati Zakaria; **Professor Ir Megat Johari Megat Mohd Noor;** **Professor Dr Norio Sugiura;** **Professor Dr Masafumi Goto;** **Dr Rory Padfield;** **Ms Effie Papargyropoulou,** Department of Environmental Engineering and Green Technology; **Dr Nor' azizi Bin Othman,** Department of Mechanical Precision Engineering, Malaysia-Japan International Institute of Technology

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HIROFUMI HARA received his PhD in Information Science and Control Engineering from Nagaoka University of Technology, Japan, in 2003. He then completed two postdocs: one in the Department of Microbiology and Immunology at the University of British Columbia and the other in the Graduate School of Agricultural and Life Science at the University of Tokyo. Hara is now working as Associate Professor at the Malaysia-Japan International Institute of Technology where he focuses on finding useful chemicals and enzymes in tropical actinomycetes.

Positioning Malaysia for sustainable growth

The Malaysia-Japan International Institute of Technology (MJIT) was inaugurated in June 2011 as a centre of excellence in leading edge engineering research and development in South East Asia

Based at the Universiti Teknologi Malaysia, MJIT functions as a hub of research institutes and industry for Malaysia and other members of the Association of South East Asian Nations (ASEAN) and Japan.

As a joint venture of the Malaysian and Japanese Governments, the objective of MJIT is to infuse Malaysian engineering with Japanese work ethics and skills in technological innovation and entrepreneurship. It provides an affordable Japanese-style education to students not only from Malaysia but also the rest of the world – 40 per cent of student intake will be international. Programmes offered at MJIT include BSc, MSc and PhD courses in several engineering specialisations. The Institute also boasts a R&D centre on precision engineering, electronics, environmental and green engineering and management of technology.

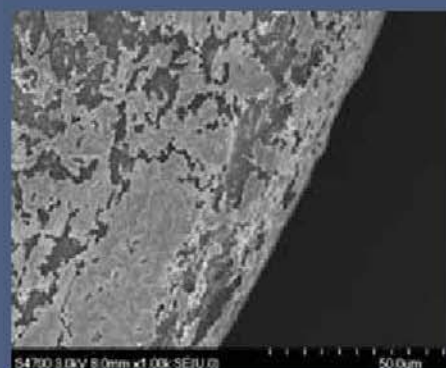
Alongside their technical courses, undergraduate students at MJIT take two courses in the Japanese 'Ningen Ryoku' way. This seeks to inculcate 'soft' human skills, such as communication, teamwork and leadership, with strong environmental values and business awareness, so graduates will be better equipped to deal with complex challenges in business settings.

By simply varying environmental factors such as nutrient content or availability, additional novel secondary metabolites can be obtained from any actinomycete already known to be capable of producing one metabolite product

conditions. Hara met this goal; he found that the expression pattern of streptomycin biosynthesis gene clusters varied over time and according to whether they were in liquid and solid environments.

The researcher then explored whether osmotic stress, temperature, culture conditions or nutrients had any effect on bacteria. To go on this exploration, Hara constructed and inserted a mutant plasmid to encode red fluorescent protein in several *R. jostii* RHA1 PKS and NRPS genes. He then cultivated each under various environmental stress conditions and compared the results against wild-type *R. jostii* RHA1 gene expression patterns. The genes selected for evaluation were PKS genes ro04065, ro04231, ro00739, ro01201, ro0125 and ro05206; a PKS-NRPS hybrid ro02209; and NRPS genes ro08547 and ro08649.

The results clearly showed that the secondary metabolite biosynthesis gene regulation in *R. jostii* RHA1 varies according to stressors such as deprivation of nutrients – carbon, nitrogen and phosphates – and the effects of osmosis when cultured in a liquid environment rather than soil. In effect, *R. jostii* RHA1 'recognises' its situation and adjusts its behaviour to cope with stress.



Scanning electron microscopy (SEM) image of *R. jostii* RHA1 grown on hexadecane.

FUTURE DIRECTIONS

Based on these results Hara is now building a clearer picture of why actinomycetes produce secondary metabolites – information that will hopefully enable the scientific community to exploit these attributes further and in new ways. Furthermore, by simply varying environmental factors such as nutrient content or availability, future experiments can obtain additional novel secondary metabolites from any actinomycete already known to be capable of producing one metabolite product.

Hara is now preparing a manuscript on the results of the project and plans to extend his studies to include topics such as the physiological role of secondary metabolites in actinomycetes. He is also already in the process of isolating further actinomycetes with desirable properties, such as resistance to severe environmental conditions, from a variety of soils in Malaysia and other South East Asian countries. For example, enzymes from some of these actinomycetes will shortly be tested for their capabilities for both primary and secondary metabolism in a new project at MJIT, which has the objective of converting biomass into energy or specific chemical materials. Through these future endeavours, Hara hopes to interest other institutions and industrial partners in productive collaborations.

