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Report of "Research Award of Oral Sciences"

Major : Oral Sciences
Grade : 4th Grade
Department : Oral Microbiology
Name : Muhammad Reza Pahlevi

Title: *pruR* and PA0065 genes are responsible for decreasing antibiotic tolerance by Autoinducer Analog-1 (AIA-1) in Pseudomonas aeruginosa

1. Aim of research and results obtained (Approximately 400 words):

Pseudomonas aeruginosa is classified as a serious threat to public health due to its antibiotic resistance phenotype. It regulates its virulence by a cell-to-cell communication mechanism called the quorum sensing (QS) system. The regulation of QS involves the emission of N-acyl homoserine-lactone, an autoinducer (AI) to regulate gene expression. An analog of this autoinducer, the Autoinducer Analog-1 (AIA-1), has been demonstrated to decrease the antibiotic tolerance of *P. aeruginosa*, however its mechanisms are still unknown. My study aimed to investigate the mechanisms by which AIA-1 decreases antibiotic tolerance, including the genes that are affected by this compound and their dynamics in the antibiotic tolerance mechanisms of *P. aeruginosa*.

Previously, I screened more than 3700 transposon mutants to isolate high antibiotic tolerant mutants even after exposure to antibiotics and AIA-1. Finally, I found two genes that are related to AIA-1 mechanisms in decreasing antibiotic tolerance, *pruR* and PA0066-65-64. Deletion mutant of *pruR* (Δ*pruR*) and PA0066-65-64 (ΔPA0066-65-64) displayed high antibiotic tolerance after being exposed to antibiotics and AIA-1 and complementation of each gene suppressed the antibiotic tolerance. Based on these findings, I found that *pruR* and PA0066-65-64 are related to AIA-1 mechanisms in decreasing antibiotic tolerance.

Furthermore, I found that biapenem and AIA-1 significantly inhibited the expression of both genes in the wild-type *P. aeruginosa* (PAO1). Meanwhile, the combination of biapenem and AIA-1 restored the expression of both genes, although

only to a level comparable to that of the control. These findings demonstrated that *pruR* and PA0066-65-64 has a role in antibiotic tolerance suppression, hence, considered as the members of the antibiotic tolerance suppressors.

I assumed other genes may be involved in antibiotic tolerance suppression. Therefore, I investigated whether *pruR* and PA0066-65-64 also regulate antibiotic tolerance through stress response pathway. For that purpose, the expression of stress response gene, catalases, and superoxide dismutases including *rpoS*, *katA*, *katB*, *sodM*, and *sodB*, in Δ*pruR* and ΔPA0066-65-64 were analyzed. I used this award to support my research in elucidating the relation between the antibiotic tolerance suppressors and these stress response genes.

Quantitative Real Time PCR (qRT-PCR) analysis revealed that the deletion of PA0066-65-64 upregulated the expression of *rpoS*, *katB*, and *sodM*. However, the expression of those genes was almost the same between PAO1 and Δ*pruR*. These results demonstrated that PA0066-65-64 has the function to inhibit the stress response gene, namely *rpoS*, as well as its dependent genes. Inhibition of *rpoS* by PA0066-65-64 resulted in a higher killing effect of antibiotics. Meanwhile, *pruR* regulates antibiotic tolerance through another pathway.

2. Self-evaluation of research achievement:

I think this Research Award for Oral Science is a very good way to encourage graduate students to pursue more achievement in their academic life by supporting research that will be published in any academic meeting or journal. I am very thankful that I was able to get this award and it really helped me to progress my research. With this award, I was able to find that the gene I studied, the PA0066-65-64, had the function to inhibit the stress response gene in order to decrease the antibiotic tolerance of *P. aeruginosa*.

As for the self-evaluation of my research achievement, I think I should have increase the capacity of the transposon mutant screening. In the beginning of my study, I screened a transposon mutant library of *P. aeruginosa*. During that phase, I successfully screened more than 3700 transposon mutants, and in the end, I obtained 2 genes that are related to AIA-1 mechanisms in decreasing antibiotic tolerance. I think this was just a minimal number of mutants due to time constraints. If I had more time, I could have screened more mutants and by doing that I can get more genes that are related to AIA-1 mechanisms.

Elucidating the gene-related mechanisms of AIA-1 in decreasing antibiotic tolerance is very important. So as the elucidation of AIA-1 effectiveness in mouse infection

model. For me, the next challenge is to increase the level of my study to *in vivo* mouse infection model study.

3. Meeting presentation:

   * Title, conference, venue, date, co-author, presentation (oral/ poster).
   (Underline the speaker.)

   a. *pruR* and PA0065 genes are responsible for decreasing antibiotic tolerance by Autoinducer Analog-1 (AIA-1) in Pseudomonas aeruginosa, 2022 Tokushima Bioscience Retreat, online via Zoom at 2nd – 3rd September 2022, Muhammad Reza Pahlevi, Keiji Murakami, Yuka Hiroshima, Akikazu Murakami, Hideki Fujii, Oral presentation.

   b. Autoinducer Analog-1 (AIA-1) decreases antibiotic tolerance of Pseudomonas aeruginosa through *pruR* and PA0066-65-64 expression, The 64th Annual Meeting of the Japanese Association for Oral Biology (第 64 回歯科基礎医学会学術大会), held on 17th – 19th September 2022 at Tokushima University, Kuramoto Campus. Muhammad Reza Pahlevi, Keiji Murakami, Yuka Hiroshima, Akikazu Murakami, Hideki Fujii, Oral Presentation

   c. *pruR* and PA0065 genes are responsible for decreasing antibiotic tolerance by Autoinducer Analog-1 (AIA-1) in Pseudomonas aeruginosa, 2022 The 142nd Fall Conference of The Korean Society for Microbiology and The 15th Japan-Korea International Symposium on Microbiology, held on 13th-14th October 2022 at The Ocean Convention Center, Yeosu, Republic of Korea, Hideki Fujii, Muhammad Reza Pahlevi, Keiji Murakami, Yuka Hiroshima, Akikazu Murakami, Poster presentation.

      The Research Award prize was used to purchase the equipment to support my research presented in these academic meetings.

4. Journal publication:

   * Title, journal, volume, number, paragraph, date, co-author.
   (Underline the speaker.)

   None.