Okayama University Medical Research Updates （OU-MRU） 2021.06 Vol.91

Source: Okayama University (JAPAN), Public Relations Division

For immediate release: 23 June 2021

Okayama University research: Meeting high demand: Increasing the efficiency of antiviral drug production in bacteria.

(Okayama, 23 June) **In a study published in the journal *Bioscience, Biotechnology, and Biochemistry*, researchers from Okayama University induce mutations in a bacterial strain to increase the production of an antiviral chemical it secretes.**

The COVID-19 pandemic has shed light on the need for antiviral drugs which are effective in suppressing viruses. Sinefungin is one such antibiotic produced by the bacteria *Streptomyces incarnatus* NRRL8089 (*S. incarnatus*) and has shown efficacy against multiple viruses including the SARS coronavirus. However, to understand its full potential in clinical studies large quantities of the compound are required. Now, Professor TAMURA Takashi and his Okayama University research team have found a way to increase to triple the production efficiency of sinefungin by inducing multiple mutations in *S. incarnatus*.

Molecular biologists have shown that antibiotic synthesis in certain bacteria can be increased by mutating an enzyme called RNA polymerase (RNAP). RNAP plays a role in running the genetic machinery inside a cell. Thus, to start off their study the researchers picked a segment of RNAP that is typically mutated for this purpose. Using biostatistical modeling, they then identified four potential sites of mutation. Subsequently, six mutant strains with varying combinations of mutations were created. Changes in sinefungin synthesis were then investigated.

While the production efficiency of sinefungin was higher in three mutants, a strain termed 'dsRC' showed three times the usual sinefungin release after 8 days. What's more, the total bacterial mass remained similar in all the strains, suggesting that antibiotic production alone (and not cell growth) was altered.

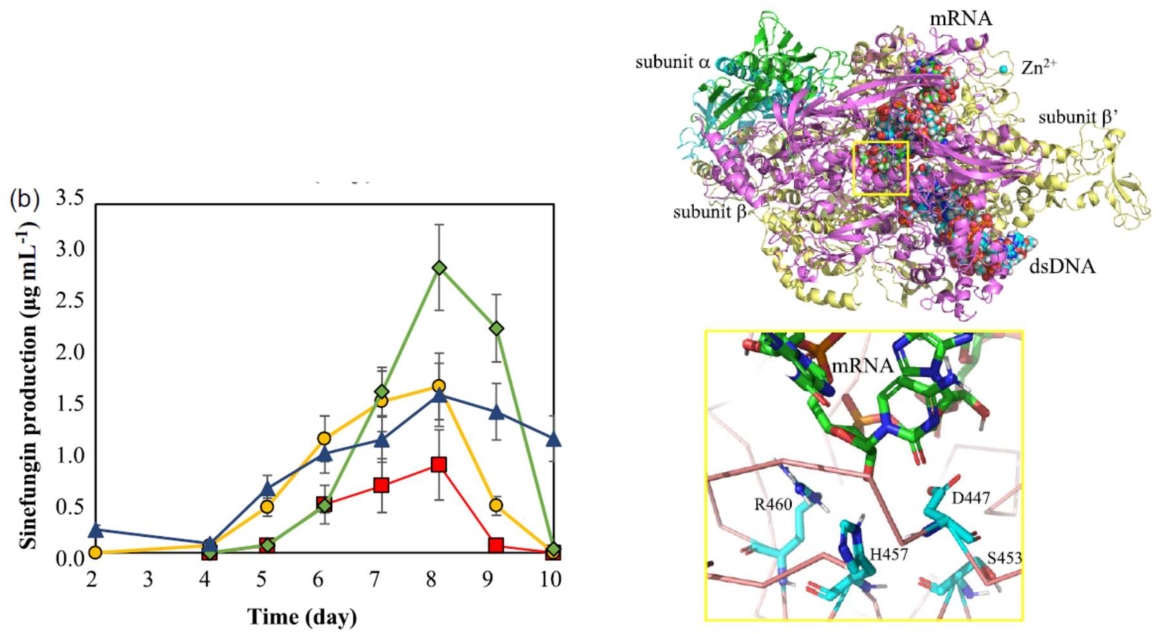
The structure of biochemical molecules always determines changes in their functionality. Therefore, the team used computational modeling to better depict the structure of RNAP—particularly the spatial arrangement of the mutation sites. RNAP is responsible synthesizing RNA strands from DNA (a process that goes on to form the basis for all biological functions). When the spatial configuration of RNAP and its associated DNA/RNA complex was examined, the four sites on RNAP were found to be in close proximity to the newly growing RNA strand. Three of these sites also seemed to be interacting with the RNA. The mutations could likely be involved with RNA synthesis thereby impacting the production of sinefungin. In an unexpected observation, four additional sites on RNAP intercalated with the RNA strand were found. The effects of mutations at these sites, however, remain to be understood.

This study shows a relatively simple and fast method of increasing the production of sinefungin in bacterial cells. “Our genome editing technique altering rif cluster residues arbitrarily now allows any point mutation at the target residues D447, S453, H457, and R460, or at new target candidates F445, R619, R495, and I503,” conclude the researchers. With the issue of large scale production partially solved, procuring sinefungin for testing in animal and human studies should be relatively easy.

Background

RNA polymerase and transcription: All cells undergo a set process to execute biological functions. The DNA within cells contains a set of instructions which are first transcribed onto an RNA strand by a process called transcription. The RNA then goes on to create protein molecules which are the primary facilitators of cellular functions. RNA polymerase is an enzyme that helps synthesize the RNA strand during transcription. The orientation of the mutation sites suggested their interference with the transcription process. Perhaps proteins related to sinefungin production were subsequently impacted.

Mutations: A mutation is a change caused in the sequence of the DNA. Thus, a specific set of instructions within the DNA strand are altered which lead to a change in the chemical structure of the end product: the protein. In this case, mutations at the four sites on the RNA polymerase protein facilitated its close interaction with the nascent RNA strand.



Caption

Left. Production of sinefungin was the highest in the dsRC strain (green), followed by the GsRf (yellow) and GLhC (blue) strains.

Right. Computational models showing the close interactions between the four sites of mutation (R460, H457, D447, and S453) and the RNA strand.

Reference

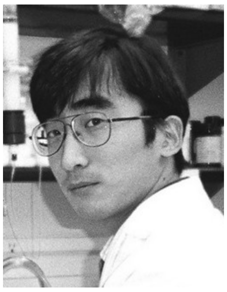
Saori Ogawa, Hitomi Shimidzu, Koji Fukuda, Naoki Tsunekawa1, Toshiyuki Hirano, Fumitoshi Sato, Kei Yura, Tomohisa Hasunuma, Kozo Ochi, Michio Yamamoto, Wataru Sakamoto, Kentaro Hashimoto, Hiroyuki Ogata, Tadayoshi Kanao, Michiko Nemoto, Kenji Inagaki, and Takashi Tamura. Multiple mutations in RNA polymerase β-subunit gene (*rpoB*) in *Streptomyces incarnatus* NRRL8089 enhance production of antiviral antibiotic sinefungin: modeling rif cluster region by density functional theory. *Bioscience, Biotechnology & Biochemistry*, 2021, Vol. 85, No. 5, 1275-1282.
DOI: https://doi.org/10.1093/bbb/zbab011

Reference (Okayama Univ. e-Bulletin): Professor TAMURA's team

OU-MRU Vol.65 : Game changer: How do bacteria play Tag?

Correspondence to

Professor TAMURA Takashi, Ph.D.
Department of Bioresources Chemistry,
Okayama University Graduate School of Environmental and Life Science, 1-1-1, Tsushima-naka, Kita-ku, Okayama 700-8530, Japan.
E-mail: tktamura@okayama-u.ac.jp



Professor TAMURA Takashi

Further information

Okayama University
1-1-1 Tsushima-naka , Kita-ku , Okayama 700-8530, Japan
Public Relations Division
E-mail: www-adm@adm.okayama-u.ac.jp
Website: http://www.okayama-u.ac.jp/index_e.html
Okayama Univ. e-Bulletin: http://www.okayama-u.ac.jp/user/kouhou/ebulletin/
We love OKAYAMA UNIVERSITY:
https://www.youtube.com/watch?v=7cXlttQIk3E
Okayama University Image Movie (2020):
https://www.youtube.com/watch?v=vQxeL0ztSLA
Okayama University supports the Sustainable Development Goals: https://sdgs.okayama-u.ac.jp/en/

OKAYAMA
UNIVERSITY

Okayama University Medical Research Updates (**OU-MRU**)

The whole volume : OU-MRU (1-)

OKAYAMA University
1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530 Japan

「Pergola」 Kazuyo Sejima + Ryue Nishizawa / SANAA
Okayama University (Tsushima Campus, Okayama City)
http://www.okayama-u.ac.jp/eng/access_maps/Tsushima_Campus.html

Okayama University supports the Sustainable Development Goals

OKAYAMA University
1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530 Japan

◆About Okayama University

Okayama University is one of the largest comprehensive universities in Japan with roots going back to the Medical Training Place sponsored by the Lord of Okayama and established in 1870. Now with 1,300 faculty and 13,000 students, the University offers courses in specialties ranging from medicine and pharmacy to humanities and physical sciences.

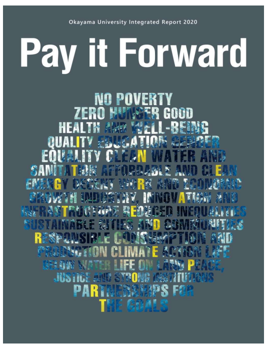
Okayama University is located in the heart of Japan approximately 3 hours west of Tokyo by Shinkansen.

Website: http://www.okayama-u.ac.jp/index_e.html

Japan（日本）

Hirofumi Makino, M.D., Ph.D.
President , Okayama University

"Okayama University supports the Sustainable Development Goals"



Okayama University Integrated Report => [click]()

An integrated report is intended to explain how an organization creates value over time through an organic integration of the vision and the combination of financial information and other information. Through this report we hope to promote greater interest in Okayama University among readers everywhere. In order to help us make improvements in future editions, we encourage you to contact us with any comments and suggestions you may have.