Biosynthesis and mechanism of action of a sideromycin class antibiotic from *Streptomyces* sp

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Research aims

Infectious diseases caused by multidrug-resistant bacteria are anticipated to become a major cause of death within the next 30 years; therefore, there is a strong demand for the development of antibiotics with new mechanisms of action. Microbes such as Actinomycetes are important resources for the production of antibiotics, and their metabolites have contributed to the fight against human infectious disease for nearly 80 years. Furthermore, with the rapid development of genome analysis technology since 2000, it is strongly suggested that a huge number of untapped potential secondary metabolites still exist.

In an approach to activate the potential secondary metabolic ability of Actinomycetes, studies demonstrating the efficiency of using the interaction between microorganisms (co-culture) have gradually accumulated. In this study, we screened for antibiotics specifically produced in a combined-culture of Actinomycetes and mycolic acid-containing bacteria (MACB), and characterized a newly discovered sideromycin class antibiotic.

Methods and results

In 2014, soil samples were collected from the Forest of the University of Tokyo in Furano, Hokkaido, and 59 species of Actinomycetes were isolated. A combined-culture of these strains and the MACB, *Tsukamurella pulmonis* TP-B0596, was performed. Culture extracts showing growth inhibition against *Staphylococcus aureus*, which was specifically induced during the combined-culture, were investigated. As a result of screening, the HOK021 strain, identified as *Streptomyces hygroscopicus* by 16S rDNA analysis, was found to produce a substance exhibiting growth inhibition against *S. aureus* specifically during combined-culture.

Based on the data obtained by ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (MS) analysis, the active substance contained in the combined-culture extract was estimated, and the bacterial fatty acid synthesis inhibitor

platensimycin (PTM) [1] was identified. Furthermore, MS network analysis revealed that many unknown PTM analogues were included; thus new analogues were purified using MS information as an index. From 10.8 L of the combined-culture, 8.7 g of crude extract was obtained by ethyl acetate extraction, and 16.7 mg of the target compound was purified with a low-pressure octadecylsilyl column and two cycles of preparative HPLC. As a result of structural analysis using ^{1}H, ^{13}C, and 2D NMR data, the purified compound was revealed to be a substance in which the antibiotic PTM was linked to the siderophore enterobactin (ENT) [2] dehydrated dimer (DED) through a thioester bond. This novel sideromycin-like antibiotic was named harundomycin (HDM). [3]

To obtain knowledge on the mechanism of HDM synthesis, especially the thioester bond formation that links PTM and the DED, the newly purified thioPTM [4] and DED were incubated under physiological buffer conditions, but HDM formation could not be confirmed. This suggested that some intracellular catalyst was required for bond formation. Further, the draft genome sequence of the HOK021 strain was acquired and analyzed. Specific genes with high homology to known PTM biosynthetic genes and ENT biosynthetic genes were identified in the HOK021 genome. Future plans involve acquiring more accurate genome sequence data so that the identification of cellular factors involved in bond formation can continue.

In collaboration with Prof. Yoshichika Arakawa, Graduate School of Medicine, Nagoya University, MIC measurements on various bacteria were performed to clarify the antibacterial spectrum of HDM. HDM showed an MIC of 16-32 μM against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). [3] However, it did not show antibacterial activity against *E. coli*, which produces ENT as a siderophore, or other Gram-negative bacteria. This was consistent with the fact that PTM itself does not exhibit antibacterial activity against Gram-negative bacteria. In addition to HDM having an ENT dimer unit, new analogs that bound an ENT monomer or trimer were recently isolated. Currently, MIC measurements of these analogs under conditions where iron is depleted from the medium are planned to investigate whether there is a change in antibacterial activity against Gram-positive bacteria, such as MRSA, in iron-depleted conditions.

Conclusion

In this study, our group discovered a novel sideromycin-like antibiotic, HDM, from a

combined-culture of *S. hygroscopicus* HOK021 and *T. pulmonis*, and analyzed the production mechanism and biological activity. "Trojan horse" antibiotics are a group of antibiotics that enter the cell using the transporters of target bacteria and exert antibacterial activity through cleavage of the antibiotic unit, mostly by hydrolysis. Among them, a group of antibiotic conjugants using a siderophore as a “carrier cargo” are called sideromycin class antibiotics, which when connected to an antibiotic warhead, are transported into the cell *via* a siderophore-specific transporter, resulting in enhanced antibacterial activity. [5)]

The presence of the “Trojan Horse” natural antibiotics can be thought to reflect the existence of a natural logic that actively attacks the competitor in a microbial interaction. As HDM is produced specifically in combined-culture, the regulation of the mechanism of production would be also interesting. I would like to analyze the biosynthetic mechanism and mechanism of action of HDM in more detail at the molecular level, which could lead to the development of new research areas. In the future, using methodologies such as MS network analysis, I will screen for new sideromycin antibiotics in the extracts of Actinomycetes and MACB combined-culture to identify naturally-occurring sideromycin class compounds and explore a new direction for drug discovery.

References

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