プレスリリース

Keio University

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Host–pathogen interaction activated silent genes
-Production of novel natural products by microbial-animal cell co-culture-

A research group led by Professor Midori A. Arai, Associate Professor Shun Saito, and third-year doctoral student Yukiko Ujie (at the time of the research) of the Department of Biosciences and Informatics, Faculty of Science and Technology, Keio University, discovered that interactions between pathogenic fungi and macrophages activate the fungi's ability to produce secondary metabolites, resulting in the production of novel natural products. They also successfully elucidated the detailed mechanism of this interaction.

Activating silent genes in microorganisms can produce many novel natural products, accelerating natural product drug discovery. In this study, they discovered that novel natural products can be obtained using a unique co-culture system of pathogenic fungi and immune cells and further revealed that the competition for survival between pathogenic microorganisms and macrophages is key to activating secondary metabolite production. The results of this study were published in the American Chemical Society journal, *Journal of Natural Products* on August 28, 2025. This article will be included in a special issue entitled "Natural Product Signals – from microbiomes to the environment."

1. Points of this research

- Co-culture system of pathogenic fungi *Aspergillus fumigatus* IFM60237 and mouse macrophage cells RAW264 activated silent genes to produce a new natural product fumigatinolactone (**1**).
- The co-culture-specific natural products **1** and fumigatin (**2**) inhibited the production of nitric oxide (NO), a mediator of immune responses, from macrophages, which is thought to be a survival strategy for microorganisms.
- We found that strain IFM60237 activates silent genes in response to iron starvation and NO produced by macrophages. This is the first example of elucidating the mechanism by which host-pathogen interactions activate secondary metabolic production in microorganisms.

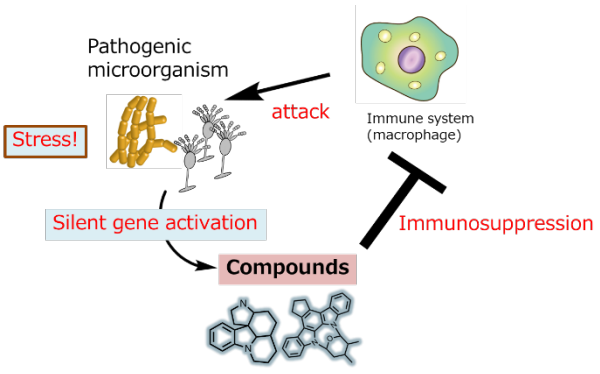

Figure 1 Host–pathogen interaction activated silent genes

2. Research background

Secondary metabolites (natural products) (※1) produced by microorganisms and plants have made a significant contribution to humanity as numerous medicines. However, in recent years, it has become increasingly difficult to obtain new natural products. Meanwhile, advances in genome science have revealed that a lot of the

compound biosynthetic genes in microorganisms, particularly actinomycetes, are silent. As a result, efforts are being made around the world to develop methods for activating silent genes, but currently there is no versatile method with an elucidated mechanism.

3. Results of the research

Isolation, structure determination, and biosynthetic mechanism of the novel natural product fumigatinolactone (1)

We isolated a compound specifically produced in the co-culture of *A. fumigatus* IFM60237 and mouse macrophage RAW264. Structural analysis by nuclear magnetic resonance (NMR) and mass spectrometry (MS) revealed the new natural product, designated as fumigatinolactone (**1**). Furthermore, we demonstrated that the expression of the fumarylalanine biosynthetic gene *IFM 60237_sidE* and the biosynthetic gene *IFM 60237_orsA* for fumigatin (**2**), one of the co-culture-specific compounds, increased after co-culture. It clearly suggested that silent genes were activated during co-culture. Furthermore, we expressed and purified the IFM 60237_sidE protein in *Escherichia coli and* demonstrated *in vitro* synthesis of **1** by coupling between the biosynthetic intermediates fumarylazlactone (**3**) and fumigatin (**2**).

NO inhibitory activity of fumigatinolactone (1) and fumigatin (2)

Macrophages produce NO to combat pathogens upon detection. Fumigatinolactone (**1**) and fumigatin (**2**) exhibited NO inhibitory activity. This suggests that pathogens stimulated by macrophages activate silent genes and produce immunosuppressants to survive.

Interaction between *A. fumigatus* IFM60237 and macrophage RAW264

What makes fungi produce compounds? To combat pathogens, macrophages take up the iron ions needed by pathogens and produce NO to kill them. The resulting "iron starvation" stimulates the production of fumarylazlactone (**3**) in strain IFM60237, while "NO stimulation" activates the production of fumigatin (**2**), which then leads to the nonenzymatic coupling of **2** and **3** to produce **1**.

4. Future developments

We have successfully elucidated, for the first time, the mechanism of the interaction between microorganisms and animal cells by activating silent genes in microorganisms using the microorganism-animal cell co-culture method. This result reaffirms the usefulness of this co-culture method and sheds light on the phenomenon by which microorganisms produce natural products to survive. By further improving and refining this co-culture method, we hope to discover new natural products and contribute to the further development of natural product chemistry.

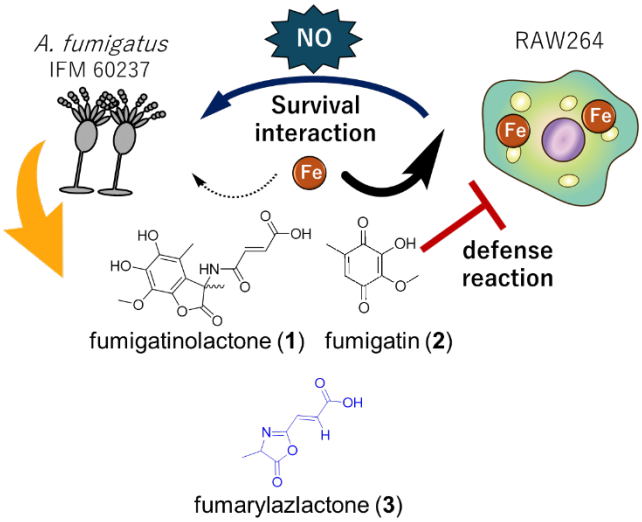

Figure 2 Elucidation of interactions between pathogenic microorganisms and immune cells

＜Publication Details＞

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＜Glossary＞

※1 Secondary metabolites

Metabolites that do not directly contribute to the growth of the organism and are produced as branches of primary metabolism.

*Please direct any requests or inquiries to the contact information provided below

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