Exploration of Strigolactone-Like Molecules in Phytopathogenic Fungi

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Research objective

Strigolactones (SLs) were initially identified as germination-inducing molecules for root-parasitic weeds, which cause significant damage to crop production. By sensing this molecule, root-parasitic weeds recognize the presence of a host plant and germinate to initiate parasitism. This is a survival strategy for root-parasitic weeds that cannot survive in the absence of a host. In 2005, it was revealed that SL is a signaling molecule that induces symbiosis with arbuscular mycorrhizal fungi, which are important symbionts for plants. In 2008, it was discovered that SLs are a class of new hormone molecules that regulate shoot branching. Therefore, SL plays an extremely important role in the regulation of plant growth, and root-parasitic weeds exploit such molecules for their own germination.

Many phytohormone molecules have been found not only as plant metabolites but also as metabolites of phytopathogenic microorganisms. However, no such case has been reported for SLs to date. In terms of root parasitic weed control, a suicide germination induction method was devised in which SL molecules were sprayed on the field before planting crops. However, this method has not been put to practical use because of the difficulty in the quantitative production of SL. If we can find a microorganism that produces a molecule with the same activity as SL, it may be possible to produce a suicidal germination inducer using a fermentative method. Therefore, the aim of this study was to search for molecules with SL-like activity, especially in phytopathogenic fungi.

Methods

SLs in plants are biosynthesized from carotenoids. When carotenoids are irradiated with light to induce non-enzymatic degradation, germination-inducing molecules against root-parasitic weeds are produced (Teramoto et al., 49th Annual Meeting of the Society for Chemical Regulation of Plants). Since carotenoids are found not only as plant metabolites, but also as metabolites of various microorganisms, we focused on plant pathogenic fungi that are known to produce carotenoids. We screened for SL-like molecule-producing fungi via a germination-inducing assay using the root-parasitic plant, *Orobanche minor*. At the time of application for this project, we had already found phytopathogenic fungi (Fungus A) that

produce SL-like molecules. Therefore, we isolated several more pathogenic fungi of the same genus as Fungus A and tested their germination-inducing activity using their culture broth. We then narrowed down to one strain that showed the highest activity and examined the culture and purification conditions for the active molecule. DWARF14 (D14), the SL receptor in plants, belongs to the α/β-hydrolase family and has been reported to perceive and hydrolyze SLs. To investigate whether the root parasitic plant germination-inducing molecules from pathogenic fungi have SL-like chemical structures, we conducted an experiment to determine whether the active molecules are degraded by the SL receptor, D14.

We speculated that fungi that produce SL-like active molecules may respond to SL. Therefore, we examined SL responsiveness by growing fungi on medium supplemented with GR24, a synthetic SL analog.

Results

1. As mentioned above, seven fungi belonging to the same genus as Fungus A were obtained. Ethyl acetate extracts of the culture filtrate of each fungus were further purified using a silica gel cartridge column to obtain several fractions. Using each fraction, we performed a germination assay with *O. minor* seeds and found that almost all fungal metabolites showed germination-inducing activity (Fig. 1). Therefore, it is possible that the active molecules are metabolites common to the same family of fungi, or analogous molecules with similar skeletons.

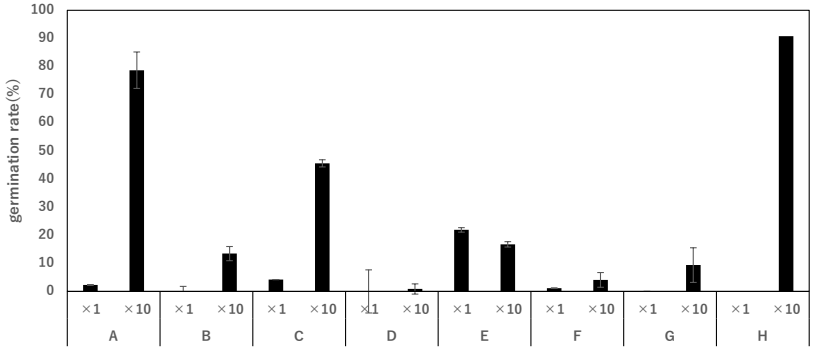

Fig. 1. Germination assay results of *Orobamche minor* using ethyl acetate extract of pathogenic fungus A to H（×1, ×10 indicates the concentration which is compared with the original culture broth）。

2. Strains with strong activity were selected through screening, as described above, and various culture conditions were examined. Interestingly, when the active fraction was concentrated, germination-inducing activity did not increase significantly, but radicle elongation was inhibited. This result suggests that the metabolites may contain not only germination-inducing substances, but also components that inhibit germination or post-radicle growth. Considering these points, we decided that ICI medium, a synthetic medium, was suitable for the production of bioactive molecules. Various purification conditions were examined prior to mass culture and isolation of active molecules. The crude extract was fractionated using silica gel and octadecyl-silica reversed-phase high-performance liquid chromatography columns, and the activity of each fraction was tested. The results showed that in both cases, activity was observed in several fractions, suggesting the

presence of multiple active molecules.

Next, we performed an experiment using the SL receptor, D14. One active fraction obtained using the silica gel normal-phase column described above was pre-incubated with D14 and subjected to a germination assay. GR24, a synthetic SL analog, was used as a control, and its activity was completely lost upon incubation with D14. In contrast, in the sample after treatment of the active fractions of fungal metabolites with D14, the activity was predominantly, though not completely, reduced. In the case of the D14 mutant protein, in which a catalytic His residue was replaced with Ala, the activity was not reduced, indicating that the active molecule was most likely degraded in a D14-dependent manner.

3. We applied a synthetic SL analog, GR24, to a fungus that produces this active compound. When GR24 was administered at final concentrations of 1 and 10 μM, colony growth was inhibited in a concentration-dependent manner. Furthermore, abnormalities in mycelial growth were observed, with severe branching following GR24 treatment.

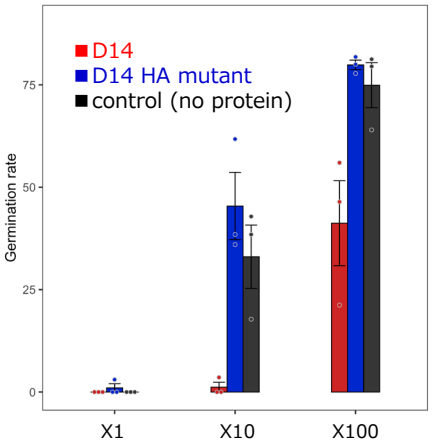

Fig. 2. Germination assay results after incubation of the active fraction with D14.

Conclusion

The objective of this study was to identify the metabolites with SL-like activity in phytopathogenic fungi. Although we were unable to isolate and identify the active molecules, we found that several pathogenic strains belonging to one genus produced the active molecules. Furthermore, it was strongly suggested that the active molecule was degraded by the SL receptor, D14. This result indicates that the active molecule may have a chemical structure similar to that of SL. Since we were able to examine the purification conditions of the active molecule to some extent, we proceeded with its mass culture and purification to identify the active molecule. Since this strain was observed to respond to SL, it is possible that the active molecules found in this strain are involved in its growth control. In the future, we aim to investigate the biological significance of the production of SL-like molecules in detail.