Production of new biologically active compounds by a combined culture of fungi and investigation of their activation mechanism

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Research aims

Secondary metabolites produced by microorganisms have been widely used in medicine to date. Recent techniques in genome analysis revealed that most genes coding for secondary metabolites are not expressed and that fungal cultures can provide only a few of those metabolites. Therefore, a variety of techniques to activate dormant genes has been developed. We have been searching for drug candidates from marine organisms using a bioassay screening and have identified new compounds with unique structures. We have also isolated 3000 fungi from sediments, marine organisms, and plants. The activation of dormant genes may lead to the identification of unprecedented metabolites. In this study, we aimed to obtain new metabolites from a combined culture of fungi and to study their mechanism of production. Our results may significantly extend the metabolite profile of fungi.

Methods

Our experiments showed that fungi produce more metabolites in solid cultures than in liquid cultures. To obtain new metabolites we conducted a combined culture of fungi on agar plates.

(1) Screening of a combined culture of fungi.

Two fungi species with various combinations were cultured on malt agar plates and the contact sections were extracted by MeOH. The MeOH extracts of the combined culture and respective pure cultures were analyzed by LC-MS.

(2) Isolation of new metabolites from a combined culture of fungi

Two fungi species, *Phaeosphaeria oryzae* (13S070) and *Irpex lacteus* (13S052) isolated from plants collected in the Aso area, were cultured on malt agar plates in large scale and the MeOH extracts were purified to acquire new compounds.

Results

The combined culture of *Phaeosphaeria oryzae* (13S070) and *Irpex lacteus* (13S052) on malt agar plates produced a green pigment on the contact section (Figure 1). Analysis of the contact section MeOH extract by LC-MS indicated the presence of new metabolites generated by the combined culture, in addition to the pigment (Figure 2). Purification of the

combined culture in a large scale followed by spectroscopic analysis led to the identification of the pigment displayed in peak 1 (Figure 2) as hypoxyxylerone[1)] (Figure 3). LC-MS analysis showed the presence of several analogs of hypoxyxylerone. Neither *P. oryzae* nor *I. lacteus* produced hypoxyxylerone on malt agar medium, but *I. lacteus* produced it on rice medium, which clearly indicated that the hypoxyxylerone-producing fungus was *I. lacteus*. These results also revealed that the production of hypoxyxylerone by *I. lacteus* in malt agar medium was induced by the contact with *P. oryzae* or by compounds secreted from *P. oryzae*. The structure of the compound designated as peak 2 was studied by spectroscopy and found to be a new compound.[2)]

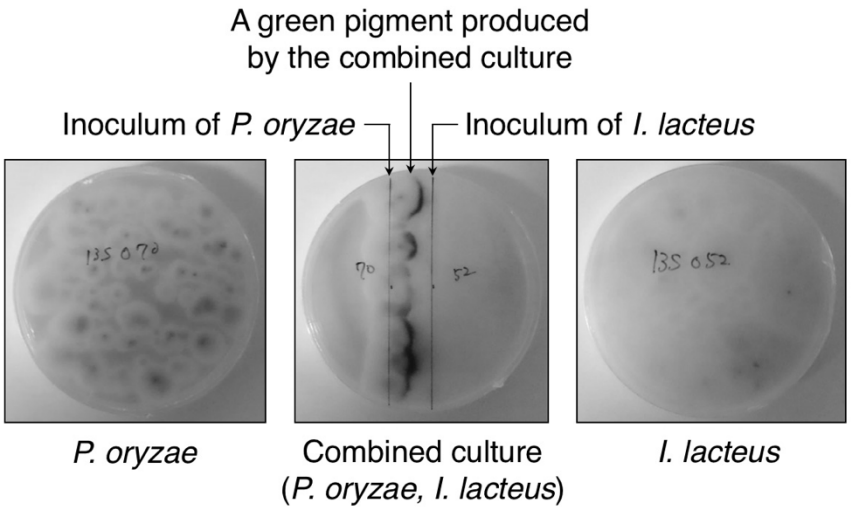

Figure 1. Cultures of *Phaeosphaeria oryzae* (13S070) (left) and *Irpex lacteus* (13S052) (right) and the combined culture (middle). In the combined culture, the two fungi cultures were inoculated 1 cm away from each other.

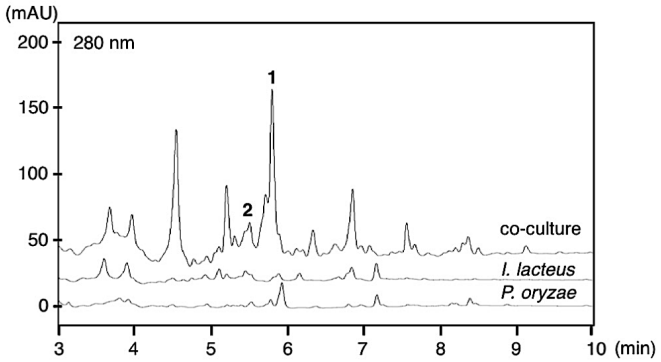

Figure 2. HPLC chromatogram of the culture extracts of *P. oryzae*, *I. lacteus*, and their combined culture detected at 280 nm.

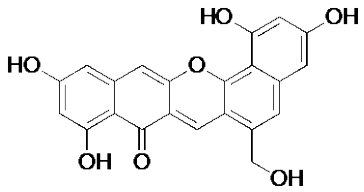

Figure 3. Structure of hypoxyxylerone.

Conclusion

Recently, pharmaceutical companies have shifted their target for drug development from infections to chronic diseases and have withdrawn from the search for new drugs from natural sources. As a result, the number of new anti-infective agents in the market has decreased. However, the development of anti-infective agents has been conducted vigorously all over the world, and in order to discover new anti-infective agents, the use of new resources and the activation of dormant genes are admittedly important. So far, activation of fungal genes by co-culturing them with bacteria such as actinomyces has been performed, but co-cultures of two fungi have been hardly conducted. In this study, we showed that the fungus *I. lacteus* produced a new compound in a combined culture with another fungus on solid medium. Production of new compounds from dormant genes may result in the development of new anti-infective agents, with potential applications on the fermentation and food industries as well.

References

1) Edwards, R. L., Fawcett, V., Maitland, D. J., Nettleton, R., Shields, L., and Whalley, A. J. S. (1991) Hypoxyxylerone. A novel green pigment from the fungus *Hypoxylon fragiforme* (Pers.: Fries) Kickx. *J. Chem. Soc., Chem. Commun*. 1009-1010.
2) Sadahiro, Y., Kato, H., Tsukamoto, S. (2018) A new metabolite produced by co-culture of fungi, *Phaeosphaeria oryzae* and *Irpex lacteus*. In preparation.