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SUMMARY OF DOCTORAL THESIS

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Title: Bacterial–fungal interactions between *Paraburkholderia fungorum* GIB024 isolated from a *Rhizopogon roseolus* sporocarp and ectomycorrhizal fungi: Mycelial growth-promoting activity and fungal strain specificity
(ショウロ子実体から分離した*Paraburkholderia fungorum* GIB024細菌と外生菌根菌との相互作用：菌糸生育促進活性ときのこ菌株系統特異性)

Rhizopogon roseolus ("shouro" in Japanese) is an edible ectomycorrhizal (ECM) mushroom with high economic value. Previous study found that a sporocarp (fruiting body) of *R. roseolus* in a *Pinus thunbergii* (Japanese black pine) dominance forest harbored unique community of bacteria that might be committed in mutual bacterial-fungal interaction (BFI) toward its fungal host. One of the bacteria, *Paraburkholderia fungorum* strain GIB024, showed a mutual BFI by its ability to promote mycelial growth of *R. roseolus* trough in-vitro direct confrontation screening. However, the mycelial promoting mechanism of this bacterium was not revealed yet. Moreover, the literature studies showed that this bacterium was wide pervasive and co-occurance with various fungal host. In mutual BFI, the bacterium relies on fungal carbonaceous compounds as source of nutrients while extracellularly release mycelial growth-promotor, in the form of volatile or soluble compound. Therefore, the aim of this current study was to investigate the potential role of the extracellular mycelial growth-promoting metabolite, identified the possible fungal carbonaceous compounds during mutual BFI of *P. fungorum – R. roseolus* and specificity interaction of the bacterium with other ECM mushrooms.

First, I investigate the effect of potential metabolite(s) produced by *P. fungorum* GIB024. As comparison, I also selected other *R. roseolus* sporocarp bacterium from the same genera with GIB024, *P. caledonica* KN1. Direct confrontation assay at three different distances, a pour plate method that sampled bacterial spent broth either with and without agitation at 25 °C, and an indirect confrontation assay was carried out in order to assess the *R. roseolus* growth-promoting ability of *Paraburkholderia* spp. These assessments were carried out in a 1:5 diluted Melin-Norkran-modified medium with glucose (hs-dMMN) and without glucose (ls-dMMN). GIB024 promoted the growth of R. roseolus in ls-dMMN in short distance, whereas KN1 inhibited the growth of the fungus in that condition. In hs-dMMN, both bacteria have neutral or slightly promotion effect toward *R. roseolus*. I determined from the spent broth analysis that *Paraburkholderia* spp. that grew axenically under static conditions had a more pronounced mycelial growth-promoting effect on *R. roseolus* than under agitation conditions. I also found that high concentration of spent broth resulted in a decrease in mycelial growth-promoting ability. Volatile metabolite(s) produced by both bacteria did not promote the mycelial growth of *R. roseolus.* In conclusion, *Paraburkholderia* spp. exhibited a species- and nutrient (sugar)-dependent ability to promote the mycelial growth of *R. roseolus*, and the bacterial soluble metabolite(s) play a crucial role in their growth-promoting ability.

Then, I investigate whether specific carbon sources are responsible for bacterial stimulation of mycelial growth of *R. roseolus*, by *P. fungorum* GIB024. I designed a two-compartment media in a

non-separated Petri dish for a direct confrontation bioassay. The first compartment for growing the fungus was filled with 1:5 diluted MMN without glucose. The second compartment acted as the bacterial medium and was filled with various sole carbon sources, which were grouped into free sugars (glucose, fructose, trehalose, mannose, xylose, and arabinose), sugar alcohols (mannitol, glycerol, sorbitol), organic acids (malonic acid, maleic acid, citric acid, and oxalic acid), malt and yeast extract, soluble starch, chitin, and no addition of a carbon source. *R. roseolus* growth was stimulated when *P. fungorum* was grown in the organic acids rather than in other carbon source groups. Moreover, the bacterium grew better in organic acids than in free sugars or sugar alcohols. In the organic acid supplemented-medium, the bacterium increased the environmental pH and its sterilized suspension stimulated growth of *R. roseolus*. These results suggested a potential role of organic acids as a mediator during the mutual interaction between *P. fungorum* and *R. roseolus*.

Finally, I investigated the specificity among various ECM mushrooms regarding growth promotion by *P. fungorum* GIB024. In vitro agar-based plate approaches of confrontation assays as well as application of a sterilized bacterial suspension to ECM strains were employed. All assays were performed in 1:5 diluted Modified Melin-Norkrans (MMN) medium, with and without glucose. The bacterium significantly promoted the growth of *Suillus bovinus*, but not *R. roseolus*, in the medium without glucose. However, the opposite result was obtained in the medium supplemented with 1 g/L of glucose. I then extended the assay to focus on *Suillus* spp. in association with various plant hosts in medium without glucose. GIB024 significantly promoted *S. bovinus* growth in association with *P. thunbergii*, but not with *P. densiflora*. The bacterium had a neutral or inhibitory effect on the growth of other *Suillus* spp. associated with *P. thunbergii* as well as other *Pinaceae*. Furthermore, application of a sterilized bacterial suspension produced effects resembling those of the confrontation assay. These results suggest that combination of nutrient condition, ECM species, and plant host determines the mycelial growth-promoting specificity of ECM-associated bacteria.

The study indicated that there is a possible metabolite exchange between *P. fungorum* and *R. roseolus* modulating by fungal organic acids, and as an exchange, the bacterium produced soluble non-volatile mycelial growth-promoting metabolite(s) and suggested that *P. fungorum* GIB024 was a specific fungiphile bacterium, whose mycelial growth-promoting ability was specified against ECM fungal strains associated with *P. thunbergii*. The information will contribute to deepen understanding BFI in mushroom science and to promote application study in which GIB024 was effectively used in production of ectomycorrhizal pine trees and cultivation of shouro mushroom.