ISME

BRIEF COMMUNICATION

Specific inactivation of an antifungal bacterial siderophore by a fungal plant pathogen

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Abstract

Bacteria and fungi secrete many natural products that inhibit each other's growth and development. The dynamic changes in secreted metabolites that occur during interactions between bacteria and fungi are complicated. Pyochelin is a siderophore produced by many *Pseudomonas* and *Burkholderia* species that induces systemic resistance in plants and has been identified as an antifungal agent. Through imaging mass spectrometry and metabolomics analysis, we found that *Phellinus noxius*, a plant pathogen, can modify pyochelin and *ent*-pyochelin to an esterification product, resulting in reduced iron-chelation and loss of antifungal activity. We also observed that dehydroergosterol peroxide, the fungal metabolite, is only accumulated in the presence of pyochelin produced through bacteria–fungi interactions. For the first time, we show the fungal transformation of pyochelin in the microbial interaction. Our findings highlight the importance of understanding the dynamic changes of metabolites in microbial interactions and their influences on microbial communities.

Microorganisms use various strategies to establish themselves within an ecological niche while facing keen competition in the environment. Natural products such as antibiotics, quorum sensing molecules, and siderophores are crucial in microbial interactions [1–3]. Certain microorganisms are equipped with uptake systems that enable them to acquire siderophores, even by those that may not produce them [4]. For example, pyochelin is a siderophore produced by many *Pseudomonas* and *Burkholderia* strains. Such bacterial strains are commonly found in soils, as endophytes, and from the rhizosphere where they may inhibit plant pathogens [5, 6].

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Burkholderia cenocepacia 869T2 was isolated as an endophyte and showed beneficial abilities to control banana Fusarium wilt [7]. It harbors many biosynthetic gene clusters of secondary metabolites, such as pyochelin, pyrrolnitrin, and pyrroloquinoline quinone [8]. Recently, we found that this strain could temporarily inhibit the growth of *P. noxius*, a fungal pathogen of brown root rot disease, which is prevalent in tropical and subtropical regions and has a wide host range covering over 200 plant species [9]. However, in the competition between fungi and bacteria, *P. noxius* can resist this inhibition and overwhelm bacterial colonies after 1–2 weeks under dual-culture conditions (Fig. S1). These results imply that fungi might have resistance responses and undergo metabolic changes in bacteria–fungi interactions [10]. Here we unveiled metabolic changes in the competitive interaction between *B. cenocepacia* 869T2 and *P. noxius* 2252 using the matrix-assisted laser desorption ionization-time of flight imaging mass spectrometry (MALDI-TOF IMS) [11, 12].

We specifically monitored the metabolites in the inhibition region of *B. cenocepacia* 869T2 and *P. noxius* 2252 dual-culture using MALDI-TOF IMS. Several induced or enzymatically modified metabolites were detected, including *m/z* 275, 362, 383, and 427 (Fig. 1A). In particular, pyochelin (*m/z* 325), surrounding the *B. cenocepacia* 869T2 colony, showed asymmetric distribution in dual-culture samples. Near the *P. noxius* 2252 mycelia, a new metabolite

Fig. 1 Metabolic changes in the bacteria–fungi interaction.
A Spatial distribution of selected mass signals (*m/z*) in MALDI-TOF IMS analysis of *Phellinus noxius* 2252 (Pn2252) dual-cultured with *Burkholderia cenocepacia* 869T2 (869T2) and a *pchF*-null mutant strain (Δ *pchF*). **B** Molecular networking analysis of pyochelin and analogs from the dual-culture sample. The red node is pyochelin, and the green node is pyochelin-GA. The structures of pyochelin, pyochelin-GA, and dehydroergosterol peroxide (DHEP), together with their mass signals in MALDI-TOF IMS, are shown. **C** Iron-chelating abilities of pyochelin and pyochelin-GA were evaluated by Chrome Azurol S liquid assay using different concentrations (2.5, 1.25, 0.63, 0.31, and 0.16 mM, *n* = 3). Proportions of siderophore units are shown in Fig. S14. **D** Fungal transformation of pyochelin and *ent*-pyochelin by treating *P. noxius* 2252 with ethyl acetate crude extracts of *B. cenocepacia* 869T2, *Pseudomonas aeruginosa* PAO1, and *P. protegens* Pf-5 for 8 h. LC-MS was used to monitor the signals of pyochelin (red), *ent*-pyochelin (blue), and transformation product 383 (black).
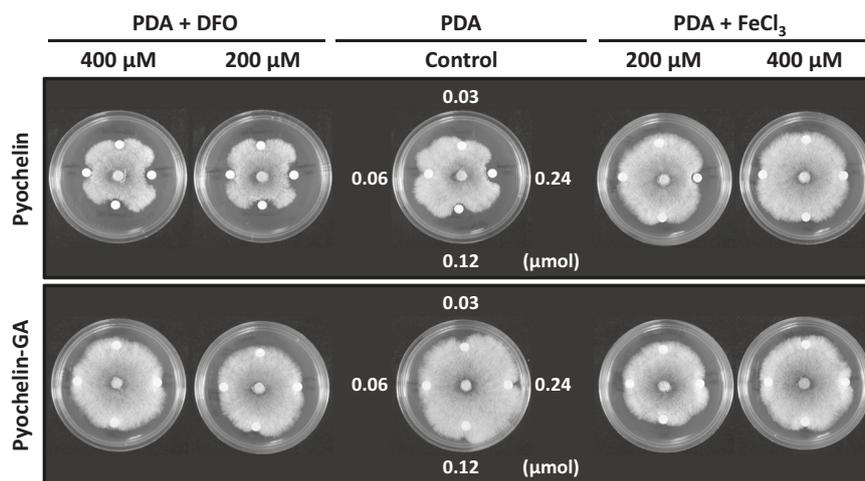
Fig. 2 Pyochelin inhibition of mycelial growth of *Phellinus noxius* 2252 is inversely associated with iron concentration. Pyochelin-GA did not have an inhibition effect on *P. noxius* 2252. Potato dextrose agar (PDA) with deferoxamine (DFO; 200 and 400 μM) was used to mimic iron-deficiency conditions. Iron-rich conditions was prepared by adding FeCl₃ (200 and 400 μM) in PDA. *P. noxius* 2252 was treated with 0.03, 0.06, 0.12, and 0.24 μmol of pyochelin or pyochelin-GA at 30 °C for 24 h. The antifungal assay was performed in two biological replicates.

with m/z 383 was detected with a complementary distribution to pyochelin (Fig. 1A). In LC-MS/MS-based molecular networking analysis [13], we found that this new metabolite structure is an esterification product of pyochelin and glycolic acid, which we named pyochelin-GA (Fig. 1B). We then constructed a *pchF*-null mutant strain, Δ*pchF*, which cannot produce pyochelin, and then dual cultured it with *P. noxius*. Pyochelin and pyochelin-GA were not observed in the MALDI-TOF IMS and LC-MS analysis of dual-culture samples (Fig. 1A and Fig. S2). We further inoculated *P. noxius* 2252 with pyochelin-GA-free extract harvested from *B. cenocepacia* 869T2 single culture, and the complementary distribution of pyochelin and pyochelin-GA was observed by MALDI-TOF IMS again (Fig. S3). These results demonstrated that pyochelin-GA was transformed from pyochelin by *P. noxius* 2252, rather than produced by *B. cenocepacia* 869T2 under dual-culture conditions.

The chemical structure of pyochelin-GA was further confirmed via total synthesis, NMR, and LC-MS/MS analysis (Supplementary Material and Methods, and Figs. S4–7). The purified pyochelin and pyochelin-GA were also evaluated for their iron-chelating ability. Chrome Azurol S assay indicated that pyochelin had the dose-dependent iron-chelating ability, but pyochelin-GA had lower iron-binding efficiency (Fig. 1C, Fig. S8). Pyochelin chelates iron in the extracellular medium and transports it into cells via the specific outer membrane transporter FptA. The X-ray structure of FptA-pyochelin-Fe indicated that the terminal carboxylic acid of pyochelin plays an essential role in the iron uptake ability [14, 15]. Our docking analysis suggested that the glycolic ester moiety of pyochelin-GA would affect the binding pocket shape of FptA and result in different binding properties compared to FptA-pyochelin (Fig. S9).

Pyochelin and *ent*-pyochelin are produced independently by different biosynthetic gene clusters in *Pseudomonas* species [16]. To determine whether *P. noxius* 2252 can transform both enantiomers via this esterification process, we treated *P. noxius* 2252 with the extracts of pyochelin producers (*P. aeruginosa* PAO1 and *B. cenocepacia* 869T2) and an *ent*-pyochelin producer (*P. protegens* Pf-5). After 8 h of treatment, both pyochelin and *ent*-pyochelin were converted to pyochelin-GA (or *ent*-pyochelin-GA) (Fig. 1D), demonstrating this is a non-stereospecific transformation.

To better understand the iron-chelating ability of pyochelin, we used pyochelin and pyochelin-GA to treat *P. noxius* 2252 under iron-deficiency conditions, by adding the iron chelator deferoxamine, and iron-rich conditions by adding FeCl₃ (Fig. 2). Pyochelin-GA did not affect the growth of *P. noxius* 2252 under all conditions. However, *P. noxius* 2252 was more sensitive to pyochelin in iron-deficient conditions and more resistant to pyochelin in iron-rich conditions, demonstrating that iron availability directly affected the tolerance of *P. noxius* 2252 to pyochelin. A similar phenomenon was reported previously for *Aspergillus fumigatus* [17].

Using MALDI-TOF IMS analysis of the dual-culture of *B. cenocepacia* 869T2 and *P. noxius* 2252, we observed that several metabolites (e.g., m/z 275, 362, and 427) were only observed in the boundary of fungal mycelia (Fig. 1A). Although those metabolites were not detected in the dual-culture of Δ*pchF* and *P. noxius* 2252 (Fig. 1A), they were present when we treated *P. noxius* 2252 with pyochelin (Fig. S10). We identified the metabolite associated with m/z 427 as dehydroergosterol peroxide (DHEP) (Fig. S11), which was initially oxidized from ergosterol and dehydroergosterol [18]. Pyochelin can enhance intercellular reactive oxygen species (ROS) and ultimately disrupts membrane integrity,

leading to cell death [17, 19, 20]. To clarify whether ROS induced the accumulation of DHEP, we treated *P. noxius* 2252 with pyochelin, pyochelin-GA, and 2,2′-bipyridyl (an iron chelator). Pyochelin and 2,2′-bipyridyl showed antifungal effects on *P. noxius* 2252 and induced ROS production (Fig. S12). However, the accumulation of DHEP in *P. noxius* 2252 was only associated with pyochelin treatment (Fig. S13). The induction of ROS in *P. noxius* 2252 by pyochelin and pyochelin-GA was not significantly different (Fig. S14). Therefore, we predict that pyochelin-induced accumulation of DHEP in *P. noxius* 2252 is independent of ROS production and iron-deficiency.

Overall, we demonstrate that pyochelin transformation by fungi, in the interaction between pyochelin-producing bacteria and the plant pathogen *P. noxius* transforms pyochelin and *ent*-pyochelin into pyochelin-GA (and *ent*-pyochelin-GA). This product no longer functions as an iron chelator and no longer shows antifungal activity. The production of a fungal metabolite, dehydroergosterol peroxide, was induced explicitly by pyochelin through an unknown mechanism. These results highlight the importance of monitoring dynamic changes of metabolites in situ to better understand the functions and influences of metabolites on microbial community interactions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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