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Studies on screening of paddy-rhizospherous microbes against rice seedling blight disease and characterization of their physiological traits

1. Induction of conidiation of *Trichoderma virens* PS1-7 by means of an autoregulatory signal responsive to chemical stress caused by highly active iron chelators

Aiming to develop novel methods for biocontrol of this disease, we screened catechol-tolerant microorganisms by means of chemical-stress selection using catechol—a compound analogous to tropolone, and selected *Trichoderma virens* PS1-7. During the studies of the metabolic traits of *T. virens* PS1-7 exposed to catechol, we found a secondary metabolite that was released into the extracellular environment and that was accumulated selectively in the culture medium. This compound is induced by the chemical stress from catechol, and we isolated and identified it as carot-4-en-9,10-diol by spectroscopic analysis. The production of carot-4-en-9,10-diol, a metabolic response of *T. virens* PS1-7 to catechol, was enhanced in a dose-dependent manner from 0.1 to 1.0 mM. Similarly, *T. virens* PS1-7 also produced carot-4-en-9,10-diol in the range of 0.05 to 0.2 mM in response to tropolone. This chemical stress-responsive compound caused a morphological response in the form of conidiation in *T. virens* PS1-7 mycelia, suggesting that the secreted sesquiterpene diol is involved in the autoregulatory signaling system to induce conidiation of *T. virens* PS1-7. Carot-4-en-9,10-diol thus exhibits intracellular signaling properties linked with regulative conidiation of *T. virens*.

2. Repression of tropolone production by *T. virens* PS1-7, leading to induction of pseudobiofilm in *B. plantarii* by a disrupter of its cell-to-cell signaling system

To demonstrate an antagonistic action by *T. virens* PS1-7 on *B. plantarii* leading to repression of tropolone production in the coculture system, we conducted a bioassay-guided screening for active ingredients in a 3-day shake culture of *T. virens* PS1-7. Carot-4-en-9,10-diol was isolated and characterized as a component that repressed tropolone production of *B. plantarii* from 10 to 200 μM dose-dependently, and attenuated the virulence of *B. plantarii* against rice seedlings. Conversely, tropolone was shown to be an autoinducing signaling compound of *B. plantarii*. Besides, we observed a unique response of *B. plantarii* to carot-4-en-9,10-diol: biofilm formation in the static culture system. The initial stage in the biofilm formation of *B. plantarii* was actively induced by tropolone and carot-4-en-9,10-diol. Furthermore, the biofilm of *B. plantarii* induced by carot-4-en-9,10-diol at the late stage showed defects not only in the matrix structure but also in cell viability.

3. Conclusion

Characterization of the N-acyl homoserine lactone-type quorum-sensing regulatory system that target virulence-associated molecular systems of pathogens was newly found in this study.