学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称： 応用生物科学専攻　　氏名： Dongyeop Kim

学 位 論 文 題 名

Studies on the effects of plant secondary metabolites on bacterial developments for xenobitotic biodegradation, biofilm formation and production of biocontrol agent
（細菌による生体異物分解，バイオフィルム形成，ならびに他者制御物質生産に対する植物二次代謝産物の効果に関する研究）

1. The cometabolic effect of pyrogallol-type plant polyphenols on the degradation of *N*-heterocyclic aromatic compounds by *Burkholderia unamae* CK43B

Indole, a xenobiotic and a typical *N*-heterocyclic aromatic compound (NHAC), is distributed widely in nature.[1)] In the course of the thesis research, it was found that pyrogallol-type plant polyphenols such as tannic acid, gallic acid, pyrogallol, and (–)-epigallocatechin stimulate degradation of indole by *Burkholderia unamae* CK43B via pyrrole ring cleavage and decarboxylation-coupled oxidative deamination to produce catechol, under shake-culture conditions (Scheme 1). This cometabolic trait of *B. unamae* CK43B is closely related to its habitat. These bacterial cells adapt to environmental conditions rich in plant polyphenols and poor in nitrogen, because addition of pyrogallol-type polyphenols to the medium results in chemical stress-adapted bacteria, such as *B. unamae* CK43B, to degrade exogenous indole. Some betaproteobacteria possessing NHAC-degrading properties that are responsive to polyphenols may play an important role in nitrogen fixation in the polyphenol-rich rhizosphere. Thus, this kind of rhizospheric bacteria that are associated with polyphenol-rich tanniferous plants may be used for a new phytobioremediation technology for soils polluted with NHACs.

2. Biofilm formation by indole-degrading *B. unamae* CK43B and indole-producing *E. coli* K-12 mediated by indole and plant polyphenols

In microbes, indole and its derivatives often act as cell signaling molecules for biofilm formation, which is just one of their diverse biological roles.[2)] *B. unamae* CK43B degrades indole to 3-hydroxyindoxyl in static culture. Because indole signaling is especially active in multispecies communities,[3)] we investigated the effect of exogenous indole on biofilm formation in a monoculture of *B. unamae* CK43B and in its coculture with indole-producing *Escherichia coli* K-12. An excessive amount of exogenous indole (1.7 mM) did not induce biofilm formation by *B. unamae* CK43B because of the toxicity of 3-hydroxyindoxyl, to which indole is converted by cytochrome C. Gallic acid (1.0 mM) added as a polyphenolic supplement inhibited the degradation of indole by static-cultured *B. unamae* CK43B, and the unmetabolized indole induced production of extracellular polymeric substances (EPS) by the bacterium. Furthermore, the addition of gallic acid to the coculture system of *B. unamae* CK43B and *E. coli* K-12 led to the formation of mixed cell aggregates, i.e., to a biofilm state. These results indicate that indole facilitates intergenus communication between indole-producing gammaproteobacteria and some indole-degrading betaproteobacteria, particularly in a gallic-acid-rich environment.

For *E. coli* K-12, extracellular signaling molecules such as autoinducer 2 (AI-2) and indole play a crucial role in biofilm formation.[3,4)] Similarly, the biofilm formability in the coculture system of *E. coli* with *B. unamae* CK43B strongly correlates with the concentration of exogenous indole. LuxS is an enzyme that participates in the biosynthesis of AI-2. *LuxS*$^{+}$ *E. coli* cocultured with *B. unamae* CK43B showed indole-dependent biofilm formation in response to the exposure to gallic acid. Thus, we used a *LuxS*-null mutant of *E. coli* (*LuxS*$^{-}$), which cannot produce AI-2, to investigate the effects of exogenous indole on biofilm formation in the coculture with *B. unamae* CK43B, without the influence of AI-2. The *LuxS*$^{-}$ *E. coli* did not respond to indole, regardless of the presence or absence of gallic acid. Nevertheless, the coculture of *LuxS*$^{-}$ *E. coli* with *B. unamae* formed harmonious mixed cell aggregates, as demonstrated by fluorescence in situ hybridization (Figure 1). We concluded that indole

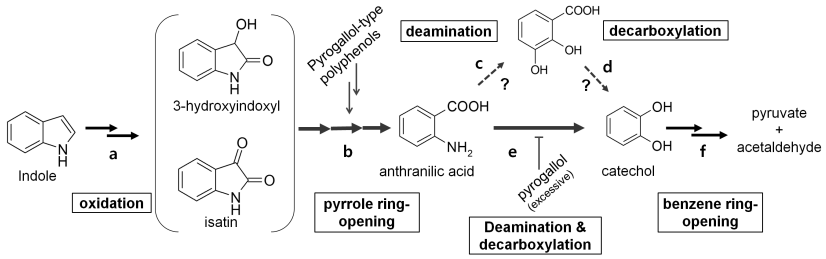
is the main extracellular signaling molecule for *LuxS*$^{-}$ *E. coli* and an important signaling molecule for the formation of biofilm by *LuxS*$^{+}$ *E. coli* in coculture with *B. unamae* CK43B.

3. Characterization of an active chemical substance in okara that accelerates production of antifungal cyclic peptides, including itulin A, by *Bacillus amyloliquefaciens*

Biological control by using a natural antagonistic microorganism has emerged as a promising alternative to chemical pesticides, just like the strategy for bioremediation of xenobiotics such as NHAC-type pesticides. Okara, a byproduct of soybean curd production, consists of carbohydrates, peptides, and indigestible fiber. We extracted and fractionated okara and tested whether it promotes the production of antifungal cyclic lipopeptides in *Bacillus amyloliquefaciens*. Using fractionated okara constituents, we showed that the culture fluid of *B. amyloliquefaciens* that was cometabolized with okara actively inhibited the conidial germination of the fungus *Fusarium oxysporum*. The active molecule, an arabinogalactan-like polar substance with size over 700 Da, acted as a stimulator leading to enhanced bacterial cell growth and production of antifungal cyclic lipopeptides, along with production of EPS and sporulation.

4. Conclusion

These studies show that some secondary metabolites of plant-produced polyphenols and oligosaccharides can participate in bacterial cell functions such as biodegradation, biofilm formation, EPS production, and/or cell differentiation, and these biologically active substances can alter metabolic pathways. These studies can contribute to the development of new technologies for the regulation of bacterial cell differentiation and biofunctionalities, including induction of biofilm formation.



Scheme 1. Postulated indole degradation pathway by *B. unamae* strain CK43B under exposure of pyrogallol-type polyphenolic additives.

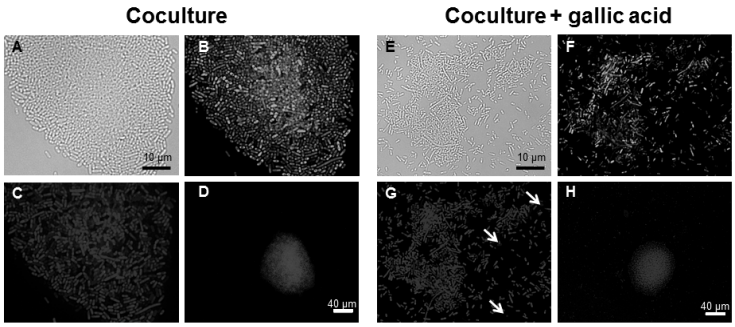


Figure 1. FISH on cell aggregates cocultured LuxS^{-} *E. coli* DH5α with *B. unamae* CK43B with or without gallic acid.

References

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