

学位論文要旨

ANTIFUNGAL AND BIOCONTROL ACTIVITIES OF BACTERIA ISOLATED FROM JAPANESE FROG SKIN AGAINST PLANT PATHOGENIC FUNGI

日本産カエル類から分離した細菌の植物病原菌に対する抗菌活性および生物防除活性に関する研究

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The bacterial communities on amphibians such as frogs play a pivotal role in host health. These communities of microbiota occupy the mucous layer forming a microbiological barrier for skin protection from invasive organisms such as fungi, bacteria, protozoa, and viruses by several mechanisms involved including competition of place or resources, production of antimicrobial compound production like volatile organic compounds and antimicrobial peptides. Frog-skin bacteria such as *Jatinobacterium lividum* and *Lysobacter gummomus* have been reported to have strong antifungal activity against the amphibian fungal pathogens. These bacteria having antifungal activity have the potential to be applied for other purposes, such as biocontrol agents for plant disease control. Concerns about chemical fungicides for controlling plant diseases are a social issue due to the burden on the environment and the emergence of resistant plant pathogenic fungi, and biological control is attracting attention as an alternative. Thus, employing some beneficial bacteria as environment-friendly “microbial fungicides” in agricultural fields could be applied for tackling plant diseases.

This study aimed to collect culturable bacteria from the skin of wild frogs sampled in Japan, and evaluate the antagonistic activity of the bacteria toward plant pathogenic fungi.

A collection of 106 bacterial isolates was obtained from three species of frogs, namely *Hyla japonica* (Japanese tree frog), *Pelophylax porosus porosus* (Tokyo daruma pond frog), and *Buergeria burgeri* (Kajika frog). Two most abundant phyla were detected. The predominant phylum from all three species of frogs was *Proteobacteria* which represented 79.3% of the total bacteria, followed by *Bacteroidetes* (15.1%). Bacteria in phyla *Firmicutes* and *Actinobacteria* were detected in a low abundance. In *H. japonica*, *P. p. porosus* and *B. burgeri*, the proportion of bacteria in the class *Gamma-proteobacteria* in the phylum *Proteobacteria* was 80.0, 62.5, and 60.0%, respectively. *Erwinia*, a genus in the phylum *Proteobacteria*, was dominant in *H. japonica* and *P. p. porosus* obtained from rice paddy fields, while *Cyrseobacterium* in the phylum *Bacteroidetes* and *Acinetobacter* in the phylum *Proteobacteria* were dominant in *B. burgeri* from a stream. These results suggested that host frog species and environment of habitat are significant factors influencing bacterial structure.

Using a dual-culture method on plate media three frog-skin bacteria, HJD52 and HJD92 from *H. japonica* and B341 from *B. buergeri* were selected based on their ability to significantly inhibit the growth of *Colletotrichum orbiculare*, the causal fungus of cucumber anthracnose disease. Among the 13 plant pathogenic fungi evaluated, the growth of 12 fungal species was significantly inhibited by HJD92 and B341. HJD57 inhibited the growth of five fungi. All three frog-skin bacteria strongly inhibited the mycelial growth of *Botrytis cinerea*, the gray mold pathogen, by 59.4, 55.0, and 63.1%, respectively. In contrast, none of the frog-skin isolates inhibited the mycelial growth of *Penicillium digitatum*, the green mold pathogen.

Furthermore, spray treatment with the suspensions (10^9 cfu/ml) of the three frog-skin bacteria effectively reduced the number of anthracnose lesions in greenhouse-grown, potted cucumber plants. Among the three frog-skin bacteria, B341 seemed to work the best in reducing symptoms. Moreover, pretreatment of tomato seedlings with isolates HJD57, HJD92 and B341 (10^9 cfu/ml) by soil drenching 1-week prior to inoculation with *Fusarium oxysporum* f. sp. *lycopersici* had significantly reduced symptoms of wilt disease. The three frog-skin bacteria also significantly reduced the severity of rice ‘bakanae’ symptoms, caused by *F. fujikuroi*, by submerging the infested rice seeds in their suspensions (10^9 cfu/ml), respectively. The three frog-skin bacterial isolates did not present negative influence on seed germination and plant growth of cucumber.

Based on the 16S rDNA sequence analysis and similarity search, isolates HJD57, HJD92 and B341 were identified as *Paenibacillus* sp., *Raoultella* sp. and *Citrobacter* sp., respectively.

Cell-free filtrates of isolates HJD57, HJD92 and B341 had strong antifungal activity against *C. orbiculare* mycelial growth which suggested that the possible mechanism of HJD57, HJD92 and B341 involved in the reduction of plant diseases was production of antifungal substances, respectively. The antifungal activity in cell-free filtrate did not lost after heat treatment.

In conclusion, *Paenibacillus* sp. HJD57, *Raoultella* sp. HJD92 and *Citrobacter* sp. B341 isolated from skins of two frog species, *H. japonica* and *B. buergeri*, are candidates for biocontrol agents of plant diseases caused by *C. orbiculare*, *F. oxysporum* f. sp. *lycopersici* and *F. fujikuroi*. This is the first report showing the potential of *Paenibacillus* sp., *Raoultella* sp. and *Citrobacter* sp. from frog skin to serve as potent biocontrol agents against plant diseases. The mode of action responsible for the control of plant diseases by these three frog-skin bacteria seemed to be antibiosis against pathogens by antifungal compounds produced by the bacteria, although further analyses are necessary to identify the compounds.