Screening of Novel Bioactive Compounds from Plant-Associated Actinomycetes

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INTRODUCTION

Although soil-derived microorganisms have been intensively screened as a source of therapeutically important molecules over a half century1, the frequency of discovering structurally new compounds is apparently decreasing these years. This trend seems to imply that the easily accessible microorganisms in soil had been exhausted and there is a need to seek unutilized microorganisms from unexplored sources. Since the role of natural products in the drug discovery is still large, new approaches such as the utilization of eDNA2, combinatorial biosynthesis3 and screening of microorganisms from extreme environments4 are investigated to discover novel chemical structures.

It is likely that the diversity of secondary metabolites relies more or less on the isolation source, namely, the habitat of the producers. As for the interrelationship between plants and microorganisms, a couple of evidences have been provided suggesting the exchange or the transfer of biosynthetic gene cluster beyond the kingdom. Taxol, an antitumor diterpene, was first isolated from Pacific yew, Taxus brevifolia. Recently, the production of taxol was identified in the culture broth of a fungus Taxomyces andreanae which is an endophyte of Pacific yew5. Another example is the isolation of maytansine from a Celastraceae plant, Maytenus ovatus, and ansamitocin from Actinosynnema sp6. Maytansine and ansamitocin are comprised of an ansamycin backbone and a fatty acid side chain, and the structure of their ansamycin backbone is identical. These findings suggest that endophytes possibly possess metabolic genes different from the microorganisms in other habitat.

We have identified several new bioactive compounds from actinomycetes isolated from live plants (Fig. 1). Two new novobiocin analogs7 were produced by Streptomyces from Aucuba japonica, and cedarmycins8 by Streptomyces from Cryptomeria japonica as antimicrobial metabolites. Fistupyrone9 is a metabolite of Streptomyces from Allium fistulosum which inhibits the infection of Alternaria brassicicola to Brassica plant. Furthermore, in the culture broth of S. hygroscopicus from Pteridium aquilinum were found phytanic acids that induce the formation of adventitious roots in hypocotyl of kidney beans with the effectiveness equivalent to that of indoleacetic acid, a plant hormone, at 1 nM10.

6-Prenylindole11 and clethramycin12 are antifungal metabolites from Streptomyces sp. Anicemycin is a potent antitumor antibiotic produced by S. thermoviolaceus isolated from a leaf of Aucuba japonica. These findings indicate that plant is a potential isolation source of strains producing new bioactive molecules. In this article, our recent results on the screening of novel bioactive compounds from plant-associated actinomycetes are described.

1. Isolation of actinomycetes from live plants

In 1978, Hasegawa et al first reported the isolation of an actinomycete which was neither symbiotic nor pathogenic but associated host-specifically with plant and the identification of a new genus Actinosynnema13. Later, Okazaki et al investigated the plants inhabiting in seashores and proved the existence of endophytic actinomycetes in leaves by showing the scanning electron micrographs of aerial hypha and spore chains growing in plants14. These studies prompted us to investigate thoroughly the distribution of actinomycetes in herbaceous and arbor plants along with their potency of producing new bioactive compounds15.

Plant samples were collected in Toyama and Miyagi prefectures, Japan. In order to compare the distribution of actinomycetes in leaves, stems and roots of a whole plant, we chose healthy seedlings with no apparent physical or physiological damages. Samples were surface-sterilized, and incubated on an agar plate at 32°C for a month. The numbers of isolates were determined by counting the colonies different from each other by macroscopic observation of morphology on a Bn-2 agar slant. From 24 species of herbaceous and arbor plants, 398 actinomycete strains were isolated. Actinomycetes were isolated from all plants used in this study, regardless of herbaceous or arbor, or wild or agricultural species, suggesting their wide distribution in association with plant in natural environment. These strains were used for the screening of bioactive compounds.

2. Fistupyrone, an inhibitor of spore germination of Alternaria brassicicola

Alternaria brassicicola is the cause of black leaf spot, a major disease of cultivated Brassica plants. In the screening of the inhibitor of infection by A. brassicicola to Chinese cabbage, we identified fistupyrone in the fermentation broth
of *Streptomyces* sp. TP-A0569 which was isolated from a leaf of spring onion, *Allium fistulosum*. Although fistupyrone does not show the *in vitro* antifungal activity against *A. brassicicola*, it completely inhibits the infection of *A. brassicicola* by pretreating the seedlings with 100 ppm of the compound. Further analysis revealed that fistupyrone does not give any effect on the growing hyphae but specifically suppresses the spore germination at 0.1 ppm16). In addition, fistupyrone does not show any activity against *A. alternata*, a pathogen of apple and pear trees.

3. Pteridic acid, an auxin-like plant growth promoter
   In the screening of plant growth regulators, pteridic acids A and B were found in the culture broth of *S. hygroscopicus* TP-A0451 isolated from a stem of bracken, *Pteridium aquilinum*. Pteridic acids A and B are stereoisomers regarding to the spiro carbon. Pteridic acids are probably biosynthesized in the biosynthetic pathway similar to that for azalomycin B because the absolute configurations of hydroxyl and methyl groups in pteridic acid and azalomycin B are identical. Pteridic acids inhibit the rice germination at 100 ppm, but very surprisingly, pteridic acid A promotes the root elongation at 20 ppm. Furthermore, pteridic acid A induces the adventitious root formation of the kidney bean hypocotyl at 1 nM as effectively as indoleacetic acid, a native plant growth hormone. There is no report on the microbial secondary metabolites that show plant growth promotion at such an extremely low concentration except for plant hormones.

4. Clethramycin, an inhibitor of pollen tube growth
   In pollen tube growth, actin/myosin cytoskeleton plays an important role in the transport of the vesicles containing precursors for cell wall biosynthesis from the sites of their synthesis to the growing pollen tube tip. This process is inhibited by cytochalasin or latrunculin B, an inhibitor of actin polymerization, and therefore pollen tube growth is also inhibited. An inhibitor of cytoskeletal function is expected to be a tool to probe the cell function and further to be a lead for therapeutic agents. Clethramycin is an inhibitor of pollen tube growth produced by *S. hygroscopicus* TP-A0326. It shows antifungal activity against *Candida albicans* and *Cryptococcus neoformans* with the MIC of 1 μg/ml. Although the relationship between pollen tube growth and the antifungal action is obscure, the cell wall biosynthesis is the key event in both processes.

5. Cedarmycin, an antifungal butyrolactone
   Cedarmycins A and B were isolated from the culture broth of *Streptomyces* sp. TP-A0456 which was isolated from a twig of cedar, *Cryptomeria japonica*. Cedarmycin is an ester of a butyrolactone and a fatty acid. The structure reminds us of *A*-factor and related microbial hormones in *Streptomyces*. Biosynthetically, cedarmycin is presumably related to these butyrolactone hormones and supposed to be derived from a coupling of two C-3 intermediates that derived from the EMP pathway. Cedarmycin A shows *in vitro* antifungal activity against *Candida glabrata* with the MIC of 0.4 μg/ml.

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**Fig. 1.** Secondary metabolites isolated from plant-associated actinomycetes.
6. Other novel metabolites from plant-associated actinomycetes

7'-Demethylvobioicin and 5''-demethylvobioicin were isolated from Streptomyces sp. TP-A0556. This strain produces novobiocin as a major metabolite. Isolation yield of the demethyl analogs was 1–3% of that of novobiocin. Antimicrobial activity of the demethylnovobiocins was weaker than that of novobiocin. The production of the most bioactive congener, novobiocin, is highest among the three congeners produced by strain TP-A0556. It is likely that the production of antibiotics has the inevitability for the producer.

6-Prenylindole was isolated from the culture broth of Streptomyces sp. TP-A0595. This simple molecule shows a significant antifungal activity against plant pathogens, A. brassicicola and Fusarium oxysporum. 6-Prenylindole was first reported as a component of the liverwort (Hepaticae). Therefore, this is an additional example of the isolation of the same compound from plant and microorganism. After the isolation of 6-prenylindole from strain TP-A0595, we identified this compound is often produced by Streptomyces including the pyridic acid-producing strain.

Ancicemycin is a novel cytotoxic substance produced by S. thermoviolaceus TP-A0648. It is a new analog of spicamycin and septacidin. Ancicemycin possesses an unsaturated fatty acid side chain while the fatty acid part of spicamycin and septacidin is saturated. The absolute configuration of anicemycin is not yet determined. Ancicemycin shows the cytotoxic activity against tumor cell lines with the IC₅₀ of less than 1 nM. Although this class of compounds has an outstanding cytotoxicity, anicemycin is the third example of isolation.

CONCLUDING REMARKS

What we expect natural products is their structure diversity that cannot be created by chemical synthesis or rational design. Although it is not so simple to rationally assess the potency of natural products in drug discovery, lactacycin derivatives, geldanamycin analogs and epothilone are the hopeful examples of microbial secondary metabolites that are currently investigated for therapeutic usages. In addition, natural products have driven the development of basic research over the chemistry and biology. I believe that the impact given by natural products will not be changed in future.

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