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Unique ecosystems under plant-microbe interaction can produce new types of bioactive compounds.

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Introduction

Photosynthetic bacteria, algae, and plants are the key on earth through their unique position as primary producers of carbohydrate and oxygen molecules in animal kingdom. In this century, we have succeeded in establishing pure culture systems of microbes and plant / animal cells. Through the pure culture we have produced a number of useful products such as medicines, food, and biomaterials. However, in nature, organisms are not surviving independently and always co-existing with other living things under specific ecosystems. Recently, unique compounds with various biological activities have been isolated from field-grown marine animals as well as algae and microalgae. However, secondary metabolites found in the original sources are not always found in their pure cultures, which means that some metabolites are only produced under specific ecosystem.

In the case of plants, they are always exposed to microbes and react to a variety of environmental factors. In response to various biotic stimuli the metabolisms are activated and numerous metabolites are newly induced in the tissues. These metabolites as well as activated enzymes play an important role in the defense systems and such metabolites are only produced in abnormal physiological condition. Therefore, the modeling of natural ecosystems in a laboratory scale may bring us possible supply of new promising compounds for medicinal or industrial purposes.

In this seminar, the speaker will present a few examples performed in my lab. to produce new metabolites from the point of view aforementioned.

1. The mixed culture system for secondary metabolite production

In general, fungal pathogens in the field strictly select the host plants and have a narrow range of host-specificity. Antimicrobial compounds in incompatible races are often induced in the site of pathogenic attack.

It is generally accepted that plants contain antimicrobial components in the tissues in different levels, and the susceptibility of plants to pathogens are closely related to the quantity and quality of cell components as well as structural features of the tissue. However, dedifferentiated tissues (calli) no more sustain such physical and chemical barriers to pathogenic attack and the inoculated pathogens readily establish infection on the tissues.
As a model experiment, fungal pathogens were inoculated onto calli and dual cultures were performed. We chose several pathogens and plant calli, and set up the dual cultures with their arbitrary combinations. The dual culture was maintained for a certain period until each individual culture was well-grown. Among the several combinations, the methanolic extract from the dual culture consisting of a plant cell line, *Phytolacca americana* and a plant pathogen, *Botrytis fabae* exhibited a pronounced antifungal activity. Identification study enabled us to elucidate the chemical structure of the active principle (Fig.1). When the fungal mat of *B. fabae* was autoclaved and applied to the callus, no significant accumulation of Phytolaccoside B was seen, indicating that the viability of the fungus was essentially required for producing the antifungal compounds.

These findings suggest that the dual culture method we adopted is useful to produce unique metabolites in their structures and biological activities.

A number of random combinations with different pathogens and calli can be made in a simple procedure, and the dual cultures are easily established since calli no more sustain effective defense systems found in the mother plants.

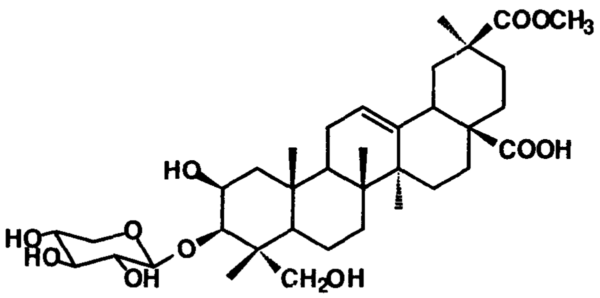


Fig. 1 Phytolaccoside B

2. Unique metabolites from field-grown cyanobacterium, *Nostoc commune.*

In recent years blue-green algae (cyanobacteria) have been recognized as a promising target in the search for new biologically active compounds such as antimicrobial compounds, enzyme inhibitors, cytotoxic compounds.

In a previous paper we have reported a novel antimitotic compound, nostodione A, from the field-grown terrestrial blue-green alga *Nostoc commune*. The following experiment indicates that the methanolic extract of this alga contains antifungal principle(s) as well as an additional antimitotic principle. A massive isolation work enabled us to give a novel antifungal lipopeptide named nostofungicidine and an antimitotic compound named nostolide A.

Field-grown *Nostoc commune* were collected at different places and the resulting samples were separately extracted with methanol and then biological activities were evaluated using our assay systems. Interestingly, the activities were quite different among the samples. Therefore, we tried to establish axenic culture of *Nostoc commune* and fortunately axenic *N. commune* was obtained. A large scale culture using the axenic strain was carried out in various media. However, the growth speed of axenic culture was slow, and any trace amount of the active metabolites was not found in the cultures. This strongly suggests that some environmental factors should take part in the growth and secondary metabolite production.

Therefore, we primarily focused on microbes residing in the jelly clumps of *Nostoc*. More than 100 different colonies were isolated from the samples of field grown *N. commune* and their metabolites were investigated. Addition of the isolated microbes to the axenic culture was found to promote the growth of the *Nostoc* culture. Among the Isolated microbes one strain belonging to *streptomyces* sp. was found to produce a known antibiotic, echinomycin.

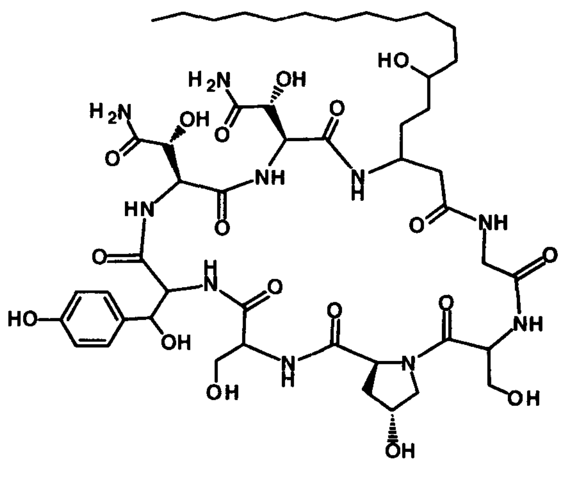


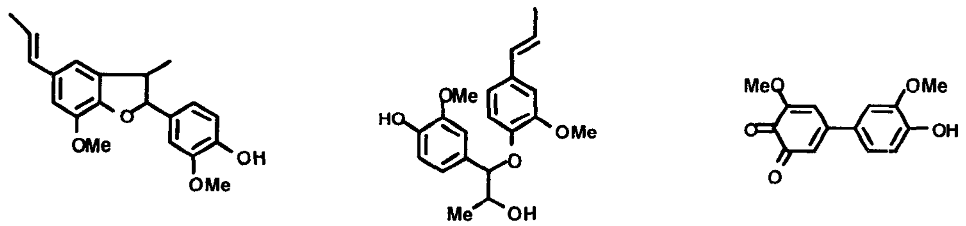
Fig. 2 Nostofungicidine

The predominant growth of this bacterium in the algal culture was found to assist the algal growth and interfere with the access of other microbes. Moreover, some methabolites which were not seen in the axenic culture were found in the co-culture. These findings suggest that the field-grown algae establish the own ecosystem suitable for their growth by means of co-existing with certain kind(s) of microbes.

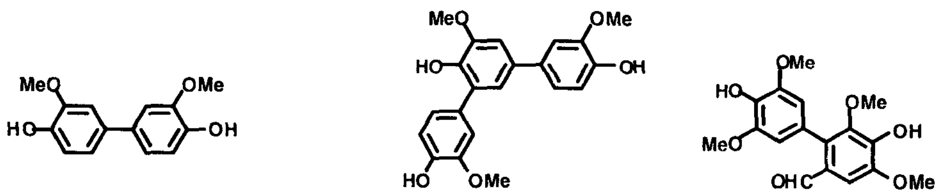
Close look at natural phenomena notices us numerous symbiotic relationships among living things and establishing precise model ecosystems operating in nature could offer incalculable value in the production of new bioactive compounds.

3. Usage of enzymes activated upon infection to produce bioactive compounds

On the way of evolution of plants, they have overcome the pathogenic attacks and survived. Plants have potency to resist to environmental stresses by setting up physiological and biological barriers.



MIC: 16 μg/ml MIC: 125 μg/ml MIC: 6.3 μg/ml



MIC: 25 μg/ml MIC: 100 μg/ml MIC: 500 μg/ml

Fig. 3 Several POD reaction products and their antimicrobial activities against *B.subtilis*

It has been known that various enzymes responsible for defense mechanisms are activated upon infection. Especially, peroxidase (POD) is one of the enzymes activated not only at the site of infection but also systemically.

We have proposed that POD, when activated by pathogenic challenge or abiotic stimulation, could convert common plant phenolics into bioactive compounds that interfere with pathogen and insect attacks. Plant PODs were examined to confirm this idea, and horse-radish POD was found to be an effective enzyme to convert simple phenolics into antimicrobial compounds. Model experiments with horse-radish and simple phenols indicated that isoeugenol and guaiacol can readily react and polymerize to yield polymeric products with antimicrobial activity. (Fig.3)

From these model reaction , a similar reaction is taking place in the plant tissues, prompted us to offer a useful reaction pot to afford new compounds with unknown biological potency.

In the practical use, POD products with a variety of bioactivities are promising substances and could be used as dyestuffs, timber preservatives, insecticides, deodorants and so on. Further efforts to investigate new POD products are now in progress using phenol-containing fractions from several plant species.

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