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Metabolites of Endophytic Actinomycetes Isolated from *Sophora flavescens*

Risa Takao[a], Ryuichiro Suzuki[a]*, Masashi Ueki[b], Yuki Sugiura[c] and Yoshiaki Shirataki[a]

[a] *Laboratory of Pharmacognosy and Natural Medicines, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan*

[b] *Nano Medical Engineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*

[c] *Department of Biochemistry, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*

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Endophytic actinomycetes were isolated from the root of *Sophora flavescens*. These isolated actinomycetes were identified by morphology characterization and analysis of partial 16S rDNA sequences. *Micromonospora* sp. and *Actinomadura* sp. isolated as endophytic actinomycetes were cultivated in liquid medium to analyze their metabolites. The results revealed that *Micromonospora* sp. and *Actinomadura* sp. produce k4610422 (**1**), and madurastatin A1 acetone adduct (**2**) and (–)-tetrangomycin (**3**), respectively.

Key words: *Sophora flavescens, Endophytic actinomycetes, metabolites*

Actinomycetes are Gram-positive bacteria known to constitute a large part of the rhizosphere microbiota. Some actinomycetes are found in plants as endophytic, and thought to influence plant growth and protect plant roots against pathogenic fungi.[1)] Furthermore, actinomycetes are well known as producers of various kinds of metabolites, such as antibiotics, anticancer and immunosuppression agents. Therefore, actinomycetes including endophytic have been said to be promising producers of medicinal sources for a long time. *Sophora flavescens* Aiton belongs to Leguminosae. The herbal drug SOPHORE RADIX, derived from the dry roots of *S. flavescens*, is a common traditional medicine used to combat gastric disturbance, and for its antifebrile, anodyne and anthelmintic activities.[2)] The major constituents of SOPHORAE RADIX are matrine alkaloids and a series of prenylated and lavandulated flavonoids.[3, 4)] On considering the quality control of traditional natural medicines, we should focus on their cultivated place, season, and endophytic microbes. However, no actinomycetes have been isolated from the root of *S. flavescens* so far.

Therefore, we describe the isolation of endophytic actinomycetes from the root of *S. flavescens* and their metabolites in this report. This is the first report on the isolation and identification of endophytic actinomycetes in *S. flavescens*. The studies focusing on the root of *S. flavescens* used as a traditional natural medicine and considering the quality control of natural medicines are few.

Five (M1-5) and eight (O1-8) actinomycetes strains were isolated from *S. flavescens* grown (*or* collected) at Moroyama, and Ogose, in Saitama Pref., respectively. These isolates were simply identified by determination of 16S rDNA sequences. Although the epidermis of the root was removed before being crushed into small pieces for the isolation of actinomycetes, we could not strictly distinguish if these isolates were endophytic or soil actinomycetes. However, some of isolates were found to be closely related to actinomycetes previously isolated in Leguminosae plants as endophytic. Though isolate M4 seemed to belong to genus *Micromonospora*, partial 16S rDNA of M4 was homologous to soil-isolated *Micromonospora* strains. Isolate M6 was also found to be related to *Micromonospora* strains which were isolated from Leguminosae plants.[5)] Thus it seemed that

we could successfully access endophytic actinomycetes of *S. flavescens*. On the other hand, O8 seemed to belong to genus *Actinomadura* according to the same method as described above. In this research, we have focused on isolates M4 and O8 strains and tried to determine their metabolites. Strain M4 was cultivated with 8 L of YMGS at 29 ˚C for 6 days in a 14 L jar fermenter. Strain O8 was cultivated with 7 L of YMG at 28 ˚C for 6 days and with 7.5 L of GLY at 28 ˚C for 10 days separately. The fermentation culture broth of M4 was extracted with acetone and filtered to remove the insoluble portion. The resultant filtrate was extracted with *n*-BuOH to give an *n*-BuOH soluble portion. After the evaporation of organic solvent with a rotary evaporator, the residue was loaded on Diaion HP-20 (H_2O→ 50% aqueous MeOH→ MeOH→ acetone). The methanolic elute was further purified with Sephadex LH-20, a silica gel open column and HPLC purification to afford k4610422 (**1**) (0.9 mg). The fermentation crude broth of O8 was partitioned with EtOAc to give a corresponding soluble portion. The EtOAc-soluble portion was purified by liquid chromatography to give madurastatin A1 acetone adduct (**2**) (19.7 mg) and (–)-tetrangomycin (**3**) (5.8 mg) (Fig. 1). The structures of these isolates were confirmed with spectroscopic data such as NMR and MS. Finally, comparisons of spectral data were conducted with literature values. The first of the three isolated compounds is diterpenoide k4610422, which acts as a 5α-reductase inhibitor.[6] The second compound is an acetone adduct of madurastatin A1. Madurastatin A1 is known as a siderophore and plays an important role in the uptake of Fe^{3+} from the surrounding environment.[7] The last is (–)-tetrangomycin, which is known as an anti-methicillin resistant *Staphylococcus aureus* agent, including antioxidant and anticancer agents.[8-10] Since endophytic actinomycetes potentially show ability to introduce biological metabolites, endophytic actinomycetes living in a medicinal plant may influence metabolite production of its medicinal plant. Considering the quality control of natural medicines, the existence of endophytic actinomycetes cannot be ignored any longer.

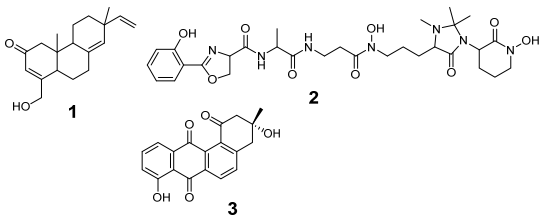

Fig. 1. Structures of Compound **1-3** from endophytic actinomycetes isolated from the root of *Sophora flavescens*.

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