Studies on Actinomycetes Isolated from Plant Leaves

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Isolation method for the purpose of obtaining kinds of actinomycetes in plant leaves has been devised. All isolates were classified into genus based on their taxonomical characteristics. They included not only the genus *Streptomyces* but also the rare genera. In particular, strains belonging to the genus *Microbispora* represented more than 50% of the total share belonging to the rare isolates¹. Two strains of isolates produced new herbicidal antibiotics, herbicidin H produced by *Streptomyces* sp. SANK 63997 and γ -glutamylmethionine sulfoximine by *Microbispora* sp. SANK 62597.

Establishment of isolation method

The fresh leaves were put on the soft agar medium and incubated for colony formation at 28°C for 3 weeks. However, the leaf surfaces were frequently covered with rapidly growing fungi before the actinomycete colonies appeared, and they were often eaten by mites. Therefore, an isolation method was devised in order to prevent contamination of fungi or mite damage. The leaves were cut into several pieces, and after rinsing with sterile water, they soaked in 70% ethanol for 1 min. After rinsing with sterile water, they were soaked in 1% chlorinated lime for 3 min. and rinsed again with sterile water. After such treatment, the leaves were incubated on the soft agar medium at 28°C for several weeks.

Classification into genus of isolates

Two hundred and forty six strains of actinomycetes were isolated from various kinds of plant leaves. Ninety seven of these strains were identified as *Streptomyces* spp., 57 as *Microbispora* spp., 23 as *Nocardia* spp., 18 as *Micromonospora* spp., 4 as *Actinomadura-Microtetraspora* group, 2 as *Streptosporangium* spp., 1 as *Actinoplanes* sp., and 1 as *Thermomonospora* sp. Forty three of them were unclassified strains having meso-diaminopimelic acid in their cell walls. The strains belonging to the genus *Microbispora* represented more than 50 % of total isolates of rare genera of actinomycetes, although less than 1 per cent of its population exists in common soils. Therefore, *Microbispora* seemed to be a dominant genus in healthy fresh leaves.

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New antibiotic producers of isolates

Two strains of isolates produced new antibiotics having herbicidal activity.

1) Strain SANK 63997

The producing organism, strain SANK 63997, was isolated from a leaf of *Setaria viridis* var. *pachystachys*. The strain formed branched substrate mycelia and spiral spore chain with a smooth surface (Fig.1). The whole-cell hydrolysate of the strain contained LL-diaminopimelic acid. Among known species of *Streptomyces*, *Streptomyces saganonensis* most closely resembled strain SANK 63997. The strain exhibited DNA-DNA homologies greater than 82% with *S. saganonensis*²⁾. We found herbicidins A, B and new herbicidal antibiotics, designated as herbicidin H (Fig. 2) into the fermentation broth of the strain.

Fig.1. Scanning electron micrograph of spores of strain SANK 63997 on potato extract-carrot extract agar at 28°C for 14 days

2) Strain SANK 62597

The producing organism, strain SANK 62597, was isolated from a leaf of *Carex kobomugi*. The paired spores with smooth surface were produced on the aerial hyphae (Fig. 3). The major taxonomic characteristics of this strain were shown in Table 1. By comparison of the characteristics of strain SANK 62597, *Microbispora rosea* was selected as the most closely related species. The identification of this pro-

	R ₁	R ₂	R ₃
Herbicidin H	CH ₃	CO(CH ₂ OH)C=CHCH ₃	H
Herbicidin A	CH ₃	CO(CH ₂ OH)C=CHCH ₃	CH ₃
Herbicidin B	CH ₃	H	CH ₃

Fig.2. Structures of herbicidins A, B and H produced by strain SANK 63997

Fig.3. Scanning electron micrograph of spores of strain SANK 62597 on potato extract-carrot extract agar, at 28°C for 14 days)

Fig.4. Structure of γ glu-MSO produced by strain SANK 62597

Table 1. Major taxonomic characteristics of strain SANK 62597

Spore surface	Smooth	Utilization of	
Aerial mass color	Pink	D-Glucose	+
Growth at 25C/50C	+/-	L-Arabinose	+
Iodinin production	-	D-Xylose	+
Starch hydrolysis	-	Inositol	-
Nitrite from nitrate	+	D-Mannitol	-
Soluble pigments	-	D-Fructose	+
Melanoid pigment	-	L-Rhamnose	-
Cell wall type	III/B	Sucrose	+
Menaquinone	MK9(H ₄)	Raffinose	-

ducer is now under investigation. We found new herbicidal antibiotics, designated as γ -glutamyl-methionine sulfoximine (γ glu-MSO) (Fig.4) into the fermentation broth of the strain SANK 62597. γ Glu-MSO is very unique as metabolites of microorganisms and not express any herbicidal activity in vitro, while methionine sulfoximine (MSO), a known compound, show the strong activity³⁾. Therefore, it seems to speculate that γ glu-MSO is converted to MSO in vivo. MSO and the related compounds have been reported as the components of seed of plants as follows; methionine sulfoximine (MSO) from *Cnestis glabra*, γ -glutamylmethionine from *Allium cepa* and γ -glutamylmethionine sulfoxide from *Phaseolus radiatus*, respectively. It is very interesting that the compound closely similar to the plant

components was produced by the actinomycete strain isolated from plant leaves and showed herbicidal activity.

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