Studies on Actinomycetes Isolated from Plant Leaves

—New plant growth inhibitors A-79197-2 and -3 from *Dactylosporangium aurantiacum* SANK 61299—

Masaaki Kizuka¹, Ryuzo Enokita¹, Kayoko Shibata¹, Yoshihiro Okamoto²,
Yoshiko Inoue² and Takao Okazaki³

¹Lead Discovery Research Laboratories, Sankyo Co., Ltd., 33, Miyukigaoka, Tsukuba-shi, Ibaraki 305-0841, Japan

²Agroscience Research Laboratories, Sankyo Co., Ltd., 1041 Yasu, Yasu-cho, Yasu-gun, Shiga 520-2342, Japan

³Research Information Department, Sankyo Co., Ltd., 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan

(Received Apr. 23, 2002 / Accepted May 28, 2002)

New plant growth inhibitors, substances A-79197-2 and -3, were found to be produced by strain SANK 61299 isolated from fresh plant leaves. Strain SANK 61299 was identified as *Dactylosporangium aurantiacum*. Substances A-79197-2 and -3 were determined to be di- and tri- saccharides of streptol, an active ingredient, respectively. Streptol related compounds have been found as components of plants.

We have isolated actinomycetes from fresh plant leaves using newly devised methods and studied their flora in search of their secondary metabolites. As a result of a comparative study on leaf and soil isolates, non-*Streptomyces* strains were isolated at a higher frequency than *Streptomyces* strains. In particular, the isolation frequency of the genus *Microbispora* spp. was remarkably high.¹⁾ These leaf isolates were subjected to many kinds of screening and a few new bioactive compounds were found. We reported that two strains of isolates produced new herbicidal substances, herbicidin H by *Streptomyces* sp. SANK 63997 and γ -glutamylmethionine sulfoximine by *Microbispora* sp. SANK 62597.²⁾ In this paper, we report new plant growth inhibitors A-79197-2 and -3 and their producing strain SANK 61299.

The producing strain, SANK 61299, was isolated from a leaf of *Cucubalus* sp. collected at Futtsu in Chiba prefecture. The strain was characterized by its finger-shaped claviform sporangia on short sporangiophores of the substrate myceria (Fig. 1). Each sporangium contained a single row of two to five zoospores with

polar flagella (Fig. 2). The cultural characteristics are shown in Table 1. The color of the substrate myceria was pale brown, pale yellow orange, or light brownish gray. No soluble pigments were produced on any media. The physiological characteristics are shown in Table 2. Whole-cell hydrolysates contained *meso*-diaminopimelic acid as a cell wall diamino acid, and arabinose and xylose as whole-cell sugars. The major menaquinone was MK-9(H₈). The phospholipid pattern was type PII. The G+C content of DNA was 71 mol%. These characteristics suggested that strain SANK 61299 belonged to genus *Dactylosporangium*. In order to investigate phylogenetic relationships, the 16S rDNA of strain SANK 61299 was sequenced and the 16S rDNA homology to known species of this genus was evaluated. As a result, strain SANK 61299 showed 99.6% homology to *D. aurantiacum* IFO 12592^T (Fig. 3). DNA-DNA hybridization was performed between the two strains. The DNA relatedness was 82%. Based on these findings, this strain was identified as *D. aurantiacum* SANK 61299.

Fig. 1. Scanning electron micrograph of the sporangium of strain SANK 61299 on PCA agar at 28°C for 21 days.

Fig. 2. Transmission electron micrograph of a zoospore of strain SANK 61299.

Table 1. Morphological characteristics of strain SANK 61299.

Medium	SANK 61299
Yeast extract-malt extract agar (ISP-2)	G* ¹ : Abundant, flat, pale brown (7.5YR 7/4) * ² R: Yellowish brown (10YR 7/6) SP: Pale yellow orange (10YR 8/3) SG: None
Oatmeal agar (ISP-3)	G: Good, flat, pale brown (7.5YR 7/4) R: Pale brown (7.5YR 7/4) SP: None SG: Poor
Inorganic salts-starch agar (ISP-4)	G: Good, flat, pale brown (7.5YR 7/4) R: Pale brown (7.5YR 7/6) SP: None SG: Good
Glycerol-asparagine agar (ISP-5)	G: Good, flat, pale brown (7.5YR 7/4) R: Pale brown (7.5YR 7/4) SP: None SG: Poor
Peptone-yeast extract-iron agar (ISP-6)	G: Moderate, flat, pale yellow orange (10YR 8/3) R: Pale brown (7.5YR 7/4) SP: None SG: None
Potato extract-carrot extract agar	G: Moderate, flat, pale brown (2.5Y 8/2) R: Pale brown (2.5Y 8/2) SP: None SG: Good

*¹ G: Growth, R: Reverse, SP: Soluble pigment, SG: Sporangium*² Color code according to the munthell system

Table 2. Physiological characteristics of strain SANK 61299.

Hydrolysis of starch	+
Liquefaction of gelatin	NG
Reduction of nitrate	+
Coagulation of milk	+
Peptonization of milk	+
Production of melanoid pigment	—
Decomposition of Casein	+
Tyrosine	—
Xanthine	—
Temperature range for growth*	15–39°C
Optimum temperature for growth*	17–33°C
Sodium chloride tolerance*	2%
Utilization of D-Glucose	+
L-Arabinose	+
D-Xylose	+
Inositol	—
D-Mannitol	+
D-Fructose	+
L-Rhamnose	+
Sucrose	+
Raffinose	±

+: Positive utilization, ±: Weakly positive utilization,

—: Negative utilization, NG: No growth

*: Yeast extract-malt extract agar (ISP-2)

Streptol and two new plant growth inhibitors A-79197-2, -3 (Fig. 4), which inhibited the germination of *Brassica rapa*, were isolated from the culture broth of strain SANK 61299. The compounds were determined to be di- and tri-saccharides consisting of streptol and glucose based on their physico-chemical analysis. The minimum concentrations of these compounds to inhibit the germination of *Brassica rapa* were 8, 18 and 50 ppm, respectively. Although the herbicidal activity of the di- and tri-saccharides was almost nonexistent, streptol itself had weak herbicidal activities. The streptol moiety, therefore, seemed to be important for herbicidal activity.

There is evidence that streptol³⁾ was obtained from *Streptomyces* sp. and its stereoisomers were obtained from *Streptomyces* sp. and a fungus (*Curvularia* sp.). Other reports indicate that streptol related compounds have been found as components of plants. The compounds are as follows: (1*S*,2*R*,5*S*,6*R*)-benzoic acid 3-benzoxymethyl-2,5,6-trihydroxy-cyclohex-3-enyl ester (piperenol A)⁴⁾ and (1*S*,2*R*,5*S*,6*R*)-benzoic acid 3-benzoxymethyl-5-acetoxy-2,6-dihydroxy-cyclohex-3-enyl ester (acetyl piperenol A) from *Piper clarkii* and *Piper cubeb*, (1*S*,2*R*,5*S*,6*R*)-benzoic acid 3-benzoxymethyl-2,5,6-triacetoxy-cyclohex-3-enyl ester (piperenol A triacetate)⁵⁾ and (3*S*,4*R*,5*S*,6*R*)-benzoic acid 3,4,5,6-tetraacetoxy-cyclohex-1-enylmethyl ester (piperenol C)⁵⁾ from *Piper cubeb* and benzoic acid 2,3-diacetoxy-4,5-dihydroxy-7-oxa-bicyclo[4,1,0]hept-1-ylmethyl ester from *Piper wightii*. It is interesting to note that a compound closely similar to plant components was produced by an actinomycete strain isolated from fresh plant leaves and showed herbicidal activity.

Fig. 3. Phylogenetic tree of SANK 61299 and *Dactylosporarium* species derived from 16S rRNA gene sequence data using neighbour-joining method. *Streptomyces ambofaciens* ATCC 23877(M27245) was used as root organism. The number on the branches are confidence limits (expressed as percentages) estimated from a bootstrap analysis with 1000 replicates.

REFERENCES

- 1) Okazaki, T., K. Takahashi, M. Kizuka & R. Enokita: Studies on actinomycetes isolated from plant leaves. Annu. Rep. Sankyo Res. Lab. 47, 97–106, 1995
- 2) Kizuka, M., R. Enokita, K. Takahashi, Y. Okamoto, T. Otsuka, Y. Shigematsu, Y. Inoue & T. Okazaki: Studies on actinomycetes isolated from plant leaves. Actinomycetol. 12, 89–91, 1998
- 3) Isogai A., S. Sakuda, J. Nakayama, S. Watanabe and A. Suzuki: Isolation and structural elucidation of a new cyclitol derivative, streptol, as a plant growth regulator. Agric. Biol. Chem. 51, 2277–2279, 1987
- 4) Taneja, S. C., S. K. Koul, P. Pushpangadan, K. L. Dhar, W. M. Daniewski & W. Schilf: Oxygenated cyclohexanes from *Piper* species. Phytochemistry 30, 871–874, 1991
- 5) Koul, J. L., S. K. Koul, S. C. Taneja & K. L. Dhar: Oxygenated cyclohexanes from *Piper cubeb*. Phytochemistry 41, 1097–1099, 1996

Fig. 4. Structure of A-79197-2, -3 and streptol.