Production of Phytotoxins, Concanamycins A and B by *Streptomyces* spp. Causing Potato Scab

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Key words : *Streptomyces* sp., thaxtomin, concanamycin, potato scab.

Potato scab is an important disease caused by the Actinomycetes. Only a few reports have been published on the phytotoxin produced by the causal organism of common scab. Sakai *et al.* reported that *Streptomyces* spp. associated with scab of potato tuber and sugar beet produce a phytotoxic compound that inhibits the root growth of rice seedlings, but its structure was not identified¹⁰. Recently, thaxtomins A and B were reported by King *et al.* to be vivotoxins^{6,7}.

Antibiotic production by Actinomycetes is closely related to such morphological differentiation as aerial mycelium formation and sporulation. Repeated transplantation of *S. griseus* caused a marked decrease in streptomycin production and the appearance of nonsporulating colonies⁹. Mutants of *S. lactamudurans* which failed to produce aerial mycelium also failed to synthesize the antibiotic, cephamycin C³. We therefore have studied the relation between phytotoxin production and aerial mycelium formation in the causal organism of common scab.

During our investigation, we found that *Streptomyces* sp. IFO 13767 produces phytotoxins other than thaxtomins and identified them as concanamycins A and B. We here report the isolation and identification of these concanamycins as well as their production and that of thaxtomin A in *Streptomyces* spp. IFO 13767 and IFO 13768 isolated from potato scab.

Procedures for the production and purification of the phytotoxins were as follows: IFO 13767 or IFO 13768 was inoculated to a 500-ml shaking flask containing 100 ml of oatmeal broth medium¹¹, after which the flask was incubated at 28°C for 3 days on a reciprocal shaker (150 rpm). Portions of 0.5 ml of this culture were inoculated to petri dishes (9 cm diameter) containing 10 ml of oatmeal agar medium, and the dishes incubated at 28°C for 14 days. Both strains formed a few aerial mycelia, and no difference in growth was found between the strains.

The cultured material was macerated with acetone, and the acetone extract evaporated to an aqueous residue, which then was extracted with CHCl₃ at pH 9. The CHCl₃ extract was partitioned between 90% aq. MeOH and *n*-hexane, and the aq. MeOH layer evaporated to

dryness. The extract was applied to a silica gel column which was flushed first with CHCl₃, 10% MeOH-CHCl₃ then with 20% MeOH-CHCl₃. In the above purification procedures, authentic thaxtomin A was recovered in the 10% MeOH-CHCl₃ fraction.

The phytotoxic activity of the fractions in each isolation step was assayed using rice seedlings as reported by Sakai *et al.*¹⁰ A sample equivalent to 10 ml of cultured material was used for the bioassay. In both strains, phytotoxic activity was found only in the 10% MeOH-CHCl₃ fraction from the silica gel chromatography.

Thaxtomin production in the two strains was investigated by HPLC (Develosil ODS-5, 30% aq. CH₃CN, UV 220 nm, Rt 12.5 min). IFO 13768 produced thaxtomin A, which was confirmed by UV spectrum and phytotoxic activity of the peak fraction, whereas IFO 13767 did not produce any detectable amount of it (Table 1). This result suggests that IFO 13767 produces a phytotoxin other than thaxtomin A.

The phytotoxins produced by IFO 13767 were eluted from an ODS-HPLC column with 60% aq. CH₃CN (Fig. 2). The peak 1 toxin showed a quasi molecular ion at m/z 874 (M+Na)⁺ on FAB-MS, the molecular formula of which was determined to be C₄₅H₇₃NO₁₄ based on results of high resolution FAB-MS (m/z 874.4962, calcd. 874.4929). The UV spectrum of peak 1 showed maxima at $\lambda_{\max}$ 245 (ϵ 34,000) and 285 (16,100). These data were identical with those of concanamycin B⁵. Comparison of the retention time in ODS-HPLC and of the ¹H-NMR spectrum of the peak 1 toxin with those of an authentic sample confirmed that the peak 1 toxin was concanamycin B. The peak 2 toxin showed a quasi molecular ion at m/z 888 (M+Na)⁺ on FAB-MS that was 14 mass units larger than that of concanamycin B. As it had the same UV spectrum ($\lambda_{\max}$ 245 (ϵ 31,700), 285 (15,400)) as concanamycin B, it was identified as concanamycin A⁵.

The production of thaxtomin A and concanamycins A and B by the two *Streptomyces* strains is shown in Table 1. It is noteworthy that IFO 13767 which induce common scab did not produce thaxtomin A. We also identified these concanamycins in the extract of IFO 13767 cultured in one of the media (soluble starch-polypeptone)

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Fig. 1. Structures of thaxtomin A and concanamycins A and B.

Fig. 2. HPLC purification (upper) and root growth inhibitory activity (lower) of the phytotoxins produced by *Streptomyces* sp. IFO 13767.Table 1. Production of thaxtomin A and concanamycins A and B by two *Streptomyces* strains

Strain	Production ($\mu\text{g/l}$ medium)		
	Thaxtomin A	Concanamycin A	Concanamycin B
IFO 13767	nd*	380	110
IFO 13768	241	92	75

* nd: not detected (less than 1 $\mu\text{g/l}$).

reported by Sakai *et al.*¹⁰⁾ (concanamycin A: 900 $\mu\text{g/l}$, concanamycin B: 5000 $\mu\text{g/l}$).

The IC_{50} of concanamycin B for the root growth of rice seedlings was 0.80 μM , which was comparable to that of thaxtomin A. The IC_{50} of concanamycin A was 2.0 μM .

Concanamycins originally were isolated from *S. diastatochromogenes* as the inhibitors of the proliferation of mouse splenic lymphocytes and the growth of several fungi and yeasts⁵⁾. Recently they have been shown to

specifically inhibit vacuolar H^+ -ATPase (V-ATPase)²⁾. Inhibition of root growth by concanamycins is presumed to be due to a decrease in vacuole function caused by the inhibition of V-ATPase.

Potato scab has several classifications that are based on the appearance of symptoms; *e.g.* common, deep, tumulus and superficial (russet) scabs¹¹⁾. *Streptomyces* sp. IFO 13767 was isolated from infected potato with the tumulus type symptom and IFO 13768 from that with the superficial type symptom⁴⁾. As thaxtomin A induces symptoms typical of the common scab disease⁸⁾, it is of interest whether concanamycins show other types of characteristic symptoms. The possibility that concanamycins induce a different type of symptoms in cooperation with thaxtomin A also needs to be examined. The relationship between the symptoms induced by *Streptomyces* sp. strains and the production of thaxtomins and concanamycins is now being investigated.

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Literature cited

1. Babcock, M.J., Eckwall, E.C. and Schottel, J.L. (1993). Production and regulation of potato-scab-inducing phytotoxins by *Streptomyces scabies*. J. Gen. Microbiol. 139: 1579-1586.
2. Dröse, S., Bindseil, K.U., Bowman, E.J., Siebers, A., Zeeck, A. and Altendorf, K. (1993). Inhibitory effect of modified bafilomycins and concanamycins on P- and V-type adenosinetriphosphatases. Biochemistry 32: 3902-3906.
3. Ginther, C.L. (1979). Sporulation and the production of serine protease and cephamycin C by *Streptomyces lactamdurans*. Antimicrob. Agents Chemother. 15: 522-526.
4. Kimura, S. (1976). Causal organism of a so-called Zohi-sho disease. Proc. Assoc. Pl. Prot. Kyushu 22: 51-54 (in Japanese).
5. Kinashi, H., Someno, K. and Sakaguchi, K. (1984). Isolation and characterization of concanamycins A, B and C. J. Antibiot. 37: 1333-1343.
6. King, R.R., Lawrence, C.H., Clark, M.C. and Calhoun, L.A. (1989). Isolation and characterization of phytotoxins associated with *Streptomyces scabies*. J.C.S., Chem. Commun. 849-850.
7. King, R.R., Lawrence, C.H. and Calhoun, L.A. (1992). Chemistry of phytotoxins associated with *Streptomyces scabies*, the causal organism of potato common scab. J. Agric. Food Chem. 40: 834-837.
8. Lawrence, C.H., Clark, M.C. and King, R.R. (1990). Induction of common scab symptoms in aseptically cultured potato tubers by the vivotoxin, thaxtomin. Phytopathology 80: 606-608.
9. Perlman, D., Greenfield, R.B., Jr. and O'Brien, E. (1954). Degeneration of a *Streptomyces griseus* mutant on repeated transfer. Appl. Microbiol. 2: 199-202.
10. Sakai, R., Kawamura, H., Mino, Y., Emami-Saravi, R. and Tanii, A. (1984). Toxin production by *Streptomyces* spp. associated with scab of potato tuber and sugar beet I. Effect of carbon and nitrogen sources. Ann. Phytopathol. Soc. Jpn. 50: 646-648.
11. Waksman, S.A. (1967). The Actinomycetes, Ronald Press, New York, pp. 232-237.

和 文 摘 要

夏目雅裕・龍 理恵・安部 浩：ジャガイモそうか病菌による植物毒素 concanamycin A と B の生産

ジャガイモそうか病菌の毒素生産と菌の形態分化との関係を解明する研究の過程で、IFO 13767 株が、これまでに本菌の生産する毒素として構造決定されている thaxtomin A 以外の毒素を生産していることを、イネ根の伸長阻害を指標とする生物検定により見いだした。この毒素は HPLC による精製で 2 種類の成分に分離され、concanamycin A および B と同定された。そこで IFO 13767 株と IFO 13768 株について毒素の生産性を調べた結果、IFO 13767 株は concanamycin A および B を生産したが thaxtomin A は生産せず、IFO 13768 株は concanamycin A および B と thaxtomin A を生産していた。上記 2 菌株は異なった形態の病巣から単離されていることから、concanamycins の生産性と病徴との関係に興味を持たれる。

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