Tropolone as a Root Growth-Inhibitor Produced by a Plant Pathogenic *Pseudomonas* sp. Causing Seedling Blight of Rice

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Tropolone was isolated from the shake-cultured medium of a plant pathogenic *Pseudomonas* sp., and was found to act as a root growth-inhibitor. It can be used for the detection and the identification of the pathogen.

Recently, seedling blight of rice grown in nursery boxes has occurred annually in the northeastern part of Chiba prefecture, Japan. Root growth of diseased seedlings is seriously impaired and the seedlings easily tumble over, hence the symptoms are referred to as "korobi-nae (seedling tumbling)". When the growth of the affected seedlings exceeds several centimeters, chlorosis occurs at the basal parts of the leaves. Then, the seedlings wither and become brown, but never rot.

The Omigawa isolate of *Pseudomonas* sp. named after the town from which it was first isolated, was shown to be the causal agent of the disease, and was related to *P. gladioli* pv. *gladioli* and *P. glumae* based on bacteriological characteristics¹⁾. However, these three pseudomonads were different from one another pathologically, symptomatologically, serologically, and in some of their bacteriological characteristics. *P. gladioli* pv. *gladioli*, which is pathogenic to gladiolus, did not induce any disease to rice seedlings by seed soaking inoculation. Although the other two pseudomonads were pathogenic to rice seedlings, the symptoms were different, i. e. the seedlings infected with the Omigawa isolate withered, became brown, dried, and never rotted while those infected with *P. glumae* became brown and easily rotted. *P. glumae* is known to be serologically homogeneous and have a common antigen with *P. gladioli* pv. *gladioli*¹⁾. The Omigawa isolate reacted with neither antisera of *P. glumae* nor *P. gladioli* pv. *gladioli*.

Shake-cultured medium of the Omigawa isolate inhibited root growth of rice, and contained an inhibitory agent, which was purified. The bacterium was incubated at 28 C in the dark for 4 days in modified Ayers, Rupp and Johnson's medium (NH₄H₂PO₄ 1.0 g, KCl 0.2 g, MgSO₄·7H₂O 0.2g, distilled water 1 liter, pH 7.2~7.4) supplemented with 10 g glucose. The inhibition activity was recognized in the ethyl ether extract of the cultured medium (6 liter, pH 3.5~3.6) (Table 1). The solvent was evaporated and the residue

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1) Azegami, K., Nishiyama, K. and Watanabe, Y. (1983). Ann. Phytopath. Soc. Japan 49 : 411.

Table 1. Inhibition activity of crude ether extract from shake-cultured medium, sublimated crystal and authentic tropolone on the growth of rice

Substance	Length of seedling (mm)					Length of root (mm)				
Extract from shake-cultured medium inoculated with:	Concentration ^{a)}					Concentration ^{a)}				
	2	1	1/2	1/4	1/8	2	1	1/2	1/4	1/8
	Omigawa isolate AZ 8201	3.8 ^{b)}	4.5	9.6	29.8	40.0	0.0	0.0	0.9	0.8 15.0
	<i>P. glumae</i> AZ 8223	35.7	35.8	40.6	38.6	43.0	L ^{c)}	L	L	L L
none	44.5	49.0	51.7	51.1	45.1	L	L	L	L	L
Sublimated crystal (96 ppm)	3.3					0.0				
Authentic tropolone (87 ppm)	4.0					0.0				
Control (0.5% agar gel)	45.3					L				

a) Concentration 2, 1, 1/2, 1/4 and 1/8 indicates that 10 ml of 0.5% agar gel contained the extract from 20, 10, 5, 2.5, and 1.25 ml of the shake-cultured medium respectively. The medium was adjusted to pH 3.5~3.6 before extraction.

b) Means of 10 seedlings. Five rice seeds were sown on the agar gel in a small glass tube (2.6×6.0 cm). The length of the above-ground parts and the roots was measured 7 days after sowing and incubation at 30 C in the dark.

c) L: Longer than 20 mm.

was reextracted with acetone. After evaporation, the extract was fractionated by ethyl ether according to standard methods and the activity was detected in the acidic fraction. This fraction was applied on a silica gel column (1.5×25 cm) and the active material was eluted with acetone-benzene 1:1 (v/v). Colorless, transparent needle and prismatic crystals which showed the activity were sublimated at room temperature from the eluate. UV and MS analyses were performed on the sublimate, and the data suggested that the substance was tropolone^{2,3)}. The comparison of UV spectrum ($\lambda_{\max}$: 320, 353, 370 nm, in ethyl acetate), MS spectrum (m/e: 122 (M^+), 94), and the activity (Table 1) of the sublimate with those of authentic tropolone (Tokyo Chemical Industry Co., Ltd. EP grade) confirmed that the sublimate consisted of tropolone (2-hydroxy-2, 4, 6-cycloheptatriene-1-one).

Tropolone is a non-benzenoid aromatic compound with a seven-membered ring, and tropolone derivatives have already been isolated from the wood of several trees (hinokitiol, thujaplicins, nootkatin), *Penicillium* sp. (stipitatic acid), galls of *Quercus* sp. (purgogallin) and *Liliaceae* (colchicine). Tropolone is known to have an antibacterial activity⁴⁾. Lindberg *et al.*⁵⁾ were able to detect tropolone in the cultured medium of

2) Doering, W. von E. and Knox, L. H. (1951). J. Am. Chem. Soc. 73: 828-838. 3) Stenhagen, E., Abrahamsson, S. and McLafferty, F. W. eds. (1974): Registry of Mass Spectral Data, Vol. 1. John Wiley and Sons, New York. p. 190. 4) Trust, T. J. (1975). Antimicrob. Agents Chemother. 7: 500-506. 5) Lindberg, G. D., Larkin, J. M. and Whaley, H. A. (1980). J. Nat. Prod. 43: 592-594.

the *Pseudomonas* sp. which was found among the colonies of fungi isolated from Bermuda grass. Lindberg⁶⁾ reported that tropolone was also lethal to many fungi, although he did not mention the existence of the plant pathogenicity of the bacterial isolate, which was different from the Omigawa isolate in ten bacteriological characteristics.

Tropolone shows a strong UV absorption at 320 nm in ethyl acetate. When only a fragment of a diseased seedling which had been inoculated with a small amount of this bacterium was soaked in ethyl acetate in a quartz cell, the peak could be detected. Thus it is concluded that tropolone is produced by the bacterium in the medium as well as in rice plant, and is responsible for the symptoms of seedling tumbling without rotting. Tropolone was not detected in the shake-cultured media of the pathogenic *Pseudomonas* spp. (*P. gladioli* pv. *gladioli*, *P. glumae*, *P. avenae*, *P. solanacearum*) by UV analysis. Thus UV screening should be helpful in the detection and identification of the Omigawa isolate which is presently under way in the laboratory.

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和 文 摘 要

畔上耕児・西山幸司・渡辺康正・鈴木隆之・吉田 充・能勢和夫・戸田昭三：イネ苗立枯症の病原細菌が産生する発根抑制物質トロポロン

千葉県では数年来、箱育苗のイネ苗立枯症が発生している。罹病苗の根の生育はきわめて悪く、そのために育苗箱に播かれたイネもみは発芽しても倒れやすい。この症状を起こす *Pseudomonas* 属菌の培養ろ液からトロポロンが単離された。この物質は罹病イネ苗からも検出され、発根や根の生育、ひいては地上部の生育を抑制していることがわかった。*P. glumae* による苗腐敗症とは異なり、罹病苗は褐変しても腐敗しない。それはこの物質の抗菌作用によるものと考えられる。トロポロンは 320 nm に強い吸収ピークを持ち、罹病組織の一片を酢酸エチルなどの有機溶媒に浸漬するだけで抽出可能であり、また近縁の病原菌からは検出されないで、罹病組織より抽出した試料の紫外分光分析はこの病原菌の検出や同定に有効であると考えられる。

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6) Lindberg, G. D. (1981). Plant Disease 65 : 680-683.