

Structure of Halo-toxin Produced by Phytopathogenic Bacterium,
Pseudomonas syringae pv. mori

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Halo-toxin was isolated from the incubated medium of pseudo-
monas syringae pv. mori which causes halo bright disease against
the leaves of mulberry trees. The structure of this compound was
revealed to be Pro-Phe-Pro-Gly-Pro-Ile by spectroscopic means and
amino acid sequence analysis.

Recent developments and improvements of agricultural techniques have caused
new plant diseases. The halo bright disease of mulberry trees is one of this kind
of diseases, being recorded in 1971 by one of the authors. The causal bacterium
was found to be pseudomonas syringae pv. mori from bacteriological and serological
view points.¹⁾ As for phytopathogenic Ps. syringae, it's known that there are
several kinds of pathover and each pathover produce each phytotoxin.²⁾ Thus, we
started out to search the phytopathogenic substances produced by the title
bacterium, as a part of our chemical studies on phytotoxins.

Pseudomonas syringae pv. mori isolated from infected mulberry leaves was
incubated in the King's B medium at 28 °C for 3 days. And incubated medium was
extracted with n-butanol and the organic layer was subjected to a combination of
chromatographies over Bondapak C-18(40-50% MeOH) and silica gel(CHCl₃:MeOH:H₂O=
8:2:0.1) to afford a homogeneous compound, designated as Halo-toxin.

Halo-toxin came as a pale yellow powder of $[\alpha]_D^{22} -114.0^\circ$ (c 0.4, MeOH) and
exhibited positive ninhydrin test. The ¹³C-NMR spectrum³⁾ showed that this compound
was composed from phenylalanine, glycine, isoleucine and proline, and furthermore
amino acid analysis⁴⁾ revealed the composed ratio of these amino acids as 1:1:1:3.
The molecular ion peak [M+H]⁺ at m/z 627 in the positive FAB-MS showed that neither
N- nor C-terminals of this peptide attach to other molecule. The result of amino
acid sequence analysis⁵⁾ confirmed the primary structure of this hexapeptide to be
Pro-Phe-Pro-Gly-Pro-Ile.

Although this hexapeptide had already been synthesized by Okai in the course
of their studies on bitterness of casein,⁶⁾ we synthesized the peptide having the
same sequence as shown in Fig.1 for the purpose of the identification. The
behavior on TLC, the mass spectrum, the value of $[\alpha]_D$ of Halo-toxin were well

coincided with those of the synthesized peptide.

Lastly, both the natural and synthesized peptides showed toxic activities against mulberry leaves at the concentration of 1γ .

L-Pro	L-Phe	L-Pro	L-Gly	L-Pro	L-Ile
		Boc-ONSu	TsOH·H		
		Et ₃ N / Dichloroethane	(100%)		
		Boc-ONSu			
		Et ₃ N / Dichloroethane	(100%)		
		Boc-ONSu			
		TFA			
Boc-ONSu	TFA·H			Boc-ONSu	TsOH·H
Et ₃ N / Dichloroethane				Et ₃ N / Dichloroethane	(100%)
(72%)				Boc-ONSu	
	NaOH (Dioxane/H ₂ O)				
	(43%)				TFA
				H	
			OH		
			WSC (CH ₂ Cl ₂)		
			(75%)		
		1) TFA			
		2) Pd-C / H ₂	(70%)		
H					OH

Fig.1. Synthesis of Halo-toxin.

We gratefully thank for the support from Ministry of Education, Sciences, and Culture, Japan (Grant No.633030013) and measurement of amino acid sequence analysis by Dr. Yoji Sengoku (Applied Biosystems Co.).

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- 3) The ¹³C-NMR(100 MHz) spectrum was measured in DMSO-d₆ at 90 °C and all signals were assigned as follows, by referring to the report of C.Grathwohl and K.Wüthrich [*J. Magn. Reson.*, **13**, 217 (1974)]. δ : 51.7(Phe, α -CH), 36.6(Phe, β -CH₂), 137.2(Phe, Ar-1), 128.1(Phe, Ar-2,6), 129.3(Phe, Ar-3,5), 126.4(Phe, Ar-4), 171.2(Phe, C=O), 41.4(Gly, α -CH₂), 167.1(Gly, C=O), 56.7(Ile, α -CH), 37.6(Ile, β -CH), 24.9(Ile, γ -CH₂), 15.6(Ile, γ -CH₃), 11.1(Ile, δ -CH₃), 169.9(Ile, C=O), 59.9, 59.7, 59.4(Pro, α -CH), 28.8, 28.6, 29.9(Pro, β -CH₂), 24.3, 24.2, 21.8(Pro, γ -CH₂), 46.7, 46.3(Pro, δ -CH₂), 172.6, 171.2(Pro, C=O).
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(Received January 23, 1989)