

植物病原性 Pseudomonas 属細菌による抗細菌性物質の産生

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Production of Antibiotics by Plant Pathogenic *Pseudomonads*

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Abstract

Plant pathogenic pseudomonads such as *Pseudomonas glumae*, *Ps. gladioli* pv. *gladioli*, *Ps. cepacia* and *Ps. avenae* formed growth inhibition zone around their colonies on the lawn of other plant pathogenic bacteria, *Agrobacterium tumefaciens*, *Corynebacterium michiganense* pv. *michiganense*, *Erwinia carotovora* subsp. *carotovora*, *Ps. solanacearum*, *Ps. syringae* pv. *syringae*, *Xanthomonas campestris* pv. *citri* and *X. campestris* pv. *oryzae*, used as indicators. The activity spectra shown on the indicator bacteria were much varied depending upon the species and isolates of pseudomonads, suggesting that various antibiotic substances participated in the formation of inhibition zones.

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Key words : *Pseudomonas glumae*, *Pseudomonas gladioli* pv. *gladioli*, *Pseudomonas cepacia*, *Pseudomonas avenae*, antibacterial substances.

Introduction

Some saprophytic pseudomonads are known to be producers of antibiotics. Arima¹⁾ *et al.* reported that a strain of *Pseudomonas* produced a new antibiotic, pyrrolnitrin. Imanaka and his co-workers⁸⁾ identified the bacterium as *Pseudomonas pyrroctinia* and determined chemical structure of pyrrolnitrin. Howell and Stipanovic^{6,7)} also reported that an isolate of *Ps. fluorescens* obtained from rhizosphere of healthy cotton plants produced antibiotics, pyrrolnitrin and pyoluteorin. Lindberg *et al.*^{13,14)} found that an isolate of *Pseudomonas* sp. produced tropolone⁴⁾ which was bioactive to plants, fungi and bacteria. From the viewpoint of biological control of plant diseases, some strains of saprophytic or plant pathogenic pseudomonads were reported to be effective³⁾.

Some plant pathogenic pseudomonads also produce antibiotics. Sinden *et al.*¹⁶⁾ found that *Ps. syringae* produced syringomycin, which showed wide activity spectrum against various microorganisms as well as toxicity to plants. Hirayae and Wakimoto⁵⁾ made preliminary report that *Pseudomonas* sp. isolated from phylloplane of rice plant produced antibacterial substances effective to some plant pathogenic bacteria. They also made clear that the bacterium resembled to *Ps. gladioli* pv. *gladioli* as well as to *Ps. glumae* in bacteriological properties and pathogenicity to rice seedling and grains. Shirata *et al.*¹⁵⁾

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reported that many strains of *Ps. cichorii* produced antibacterial substances effective to homologous and heterologous plant pathogenic bacteria. Azegami *et al.*²⁾ recently reported that an isolate (Omigawa strain) of *Pseudomonas* sp., which resembled to *Ps. gladioli* pv. *gladioli* in bacteriological properties and pathogenicity to rice seedlings, produced tropolone. Bacteriocins¹¹⁾ were also reported by many workers to be produced by plant pathogenic bacteria including pseudomonads²¹⁾. Besides these reports, antagonistic effect of *Ps. cepacia* against the disease of onion seedlings caused by *Fusarium oxysporum* f. sp. *cepae* was reported by Kloepper *et al.*¹²⁾, and broad antibiotic spectrum to plant pathogens was clarified by Spurr and his colleagues¹⁷⁾. These reports are suggestive of the productivity of bioactive substances or antibiotics from many pseudomonads including plant pathogenic ones.

We have made an effort for several years to find some useful antibiotics and bacteriocins produced by plant pathogenic bacteria and saprophytic bacteria isolated from phylloplane. As a part of these serial work, the antibacterial activity or productivity of antibacterial substances from plant pathogenic pseudomonads was tested by using several plant pathogenic bacteria as indicators.

Materials and Methods

Bacteria used as producer. All of the isolates of *Ps. glumae* Kurita *et* Tabei, *Ps. gladioli* pv. *gladioli* Severini, *Ps. cepacia* Burkholder, and *Ps. avenae* Manns preserved in our laboratory were tested for their productivity of antibacterial substances. The origin of each isolate used in this experiment was shown in each Table.

Bacteria used as indicator. To detect the productivity of antibacterial substances from producers, seven species or pathovars of plant pathogenic bacteria, which are distantly related each other, were used as indicators (Table 1). These indicator bacteria were known to be distributed widely causing serious diseases in the world.

Culture media. To grow bacterial strains of both producers and indicators, PSA medium (potato semi-synthetic agar medium: decoction of potato (300 g/liter, 1 liter),

Table 1. Phytopathogenic bacteria used as indicator for testing productivity of antibacterial substances

Indicator bacteria	Code ^{a)}	Origin ^{b)}
<i>Agrobacterium tumefaciens</i> Ku7411	A. t	KU
<i>Corynebacterium michiganense</i> pv. <i>michiganense</i> N6601SR(3) ^{c)}	C. m	NIAES
<i>Erwinia carotovora</i> subsp. <i>carotovora</i> N7129	E. c	"
<i>Pseudomonas solanacearum</i> KT-2	P. sol	"
<i>Ps. syringae</i> pv. <i>syringae</i> I	P. syr	"
<i>Xanthomonas campestris</i> pv. <i>citri</i> N6113-1	X. c	"
<i>X. campestris</i> pv. <i>oryzae</i> T7174SR(12) ^{c)}	X. o	HAES

a) In the following Tables.

b) KU: Laboratory of Plant Pathology, Kyushu University.

NIAES: National Institute of Agro-Environmental Sciences.

HAES: Hokuriku Agricultural Experiment Station.

c) Streptomycin resistant mutant induced at KU.

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 2 g, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 0.5 g, peptone 5 g, sucrose 15 g, agar 15 g, pH 7.0) was used. For testing productivity of antibacterial substances, YPDA medium (yeast peptone dextrose agar medium: yeast extract 3 g, peptone 0.6 g, dextrose 3 g, agar 15 g (for bottom layer) or 5 g (for upper layer), distilled water 1 liter, pH 7.2) was used.

Detection of productivity of antibacterial substances. To detect the productivity of antibacterial substances from pseudomonads, the fresh (1-2 days) culture of the producer on the PSA slant medium was transferred to the center (or at 3 spots on each plate keeping distance from each other) of the plate of YPDA medium. The plate was then incubated at 30 C for 2 to 3 days. When the bacteria grew up to form colonies of several mm in diameter, the plate was turned upside down, a sheet of filter paper was placed in the lid, chloroform (0.5 ml) was dropped on the paper and kept the Petri dish at room temperature for 2 hr. After complete exclusion of the chloroform vapor, the suspension of indicator bacteria (conc.: ca. 10^8 cells/ml, 5 ml) mixed with YPDA medium (melted and kept at 50 C) was overlaid. The plate thus prepared was incubated at 30 C for 2 days. If the inhibition zone appeared, the width of it was measured to evaluate the activity or productivity of antibacterial substances (Fig. 1).

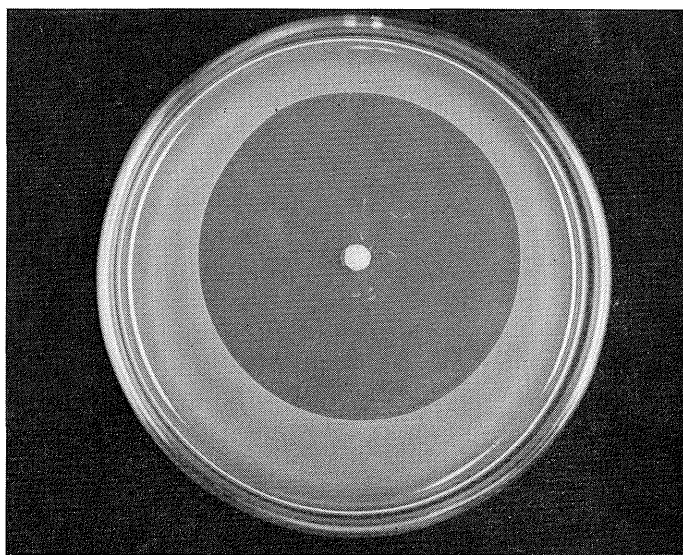


Fig. 1. Growth inhibition zone formed by *Ps. gladioli* pv. *gladioli* NIAES 1064 on the lawn of *C. michiganense* pv. *michiganense*.

Results

The majority of the isolates of *Ps. glumae* formed inhibition zone on some indicator bacteria as shown in Table 2, suggesting the productivity of antibacterial substance (s). The activity spectra, however, were greatly different depending upon isolates of producers. Among 47 isolates of *Ps. glumae*, 17 formed inhibition zone on the lawn of *Agrobacterium tumefaciens*, 22 on *Corynebacterium michiganense* pv. *michiganense*, 19 on *Erwinia carotovora* subsp. *carotovora*, and 26 on *Ps. solanacearum*, 28 on *Xanthomonas campestris*

pv. citri, and 27 on *X. campestris* pv. *oryzae*.

Among 9 isolates of *Ps. gladioli* pv. *gladioli* 3 isolates were active to *A. tumefaciens*, 4 to *C. michiganense* pv. *michiganense*, 7 to *E. carotovora* subsp. *carotovora*, all of the 9 to *Ps. solanacearum*, 7 to *X. campestris* pv. *citri*, and 8 to *X. campestris* pv. *oryzae*, as shown in Table 3. The activity spectra of these isolates also varied as in the case of *Ps. glumae*. The isolate NIAES1064 formed inhibition zone on the lawn of *A. tumefaciens* and *C. michiganense* pv. *michiganense* while did not on the lawn of *X. campestris* pv. *citri* and pv. *oryzae*, indicating great difference from the spectra shown by other isolates. The isolate 251-18 is also different from other isolates in the activity spectrum showing negative activity to *X. campestris* pv. *citri* and weak activity to *X. campestris* pv. *oryzae*.

In the case of *Ps. cepacia*, most isolates were strong in the activity to *C. michiganense* pv. *michiganense* but very weak or almost negative to *E. carotovora* subsp. *carotovora*. Some isolates such as 342-43, 342-44, 342-45, 342-46, 342-47 and 342-48 were highly active to *X. campestris* pv. *oryzae*. *Ps. cepacia* also showed wide variation in activity spectra depending upon isolates (Table 4), suggesting that various antibacterial substances were involved in the formation of inhibition zone as in the cases of *Ps. glumae* and *Ps. gladioli* pv. *gladioli*.

Table 2. Production of antibacterial substances by *Ps. glumae*

Isolate	Origin ^{a)}	Indicator							Pathogenicity ^{b)}
		A. t	C. m	E. c	P. sol	P. syr	X. c	X. o	
N7401	NIAES	—	—	—	—	†	—	—	—
N7501	"	—	—	‡	‡	+	‡	‡	+
N7502	"	—	+	—	—	†	—	—	—
N7503	"	—	+	—	†	+	(+)	(+)	—
N7504	"	—	—	—	—	+	—	—	—
N7505	"	—	+	—	—	†	—	+	—
YN7805	"	—	—	—	—	†	—	—	—
YN7810	"	—	—	—	—	†	—	—	—
YN7825	"	—	—	—	—	+	—	—	—
742	"	—	—	‡	‡	+	‡	‡	—
750	"	—	‡	‡	‡	(+)	‡	‡	—
752	"	—	†	‡	‡	(+)	‡	‡	—
805	"	—	‡	‡	‡	+	‡	‡	—
806	"	—	+	—	+	+	†	†	+
PI-22-1	"	—	‡	‡	‡	+	‡	‡	—
PI-22-2	"	—	—	—	—	(+)	—	(+)	+
PI-22-3	"	—	+	(+)	‡	(+)	‡	‡	—
PI-22-4	"	—	—	(+)	(+)	+	—	+	—
So 1	FARC	—	—	‡	‡	+	‡	‡	+
8001	KAES	—	‡	‡	‡	+	‡	‡	+
8012	"	—	—	—	—	+	—	(+)	+
8015	"	—	+	—	—	+	—	+	+
8017	"	†	+	†	‡	+	‡	‡	+
8020	"	†	+	†	‡	+	‡	‡	—

Table 2. (Continued)

Isolate	Origin ^{a)}	Indicator							Pathogenicity ^{b)}
		A. t	C. m	E. c	P. sol	P. syr	X. c	X. o	
8028	KAES	—	+	—	—	+	—	—	+
I	"	‡	—	+	‡	+	‡	‡	+
III	"	‡	—	‡	‡	+	‡	‡	+
2	"	(+)	—	‡	‡	+	‡	‡	+
Ku8101	KU	—	—	—	—	+	—	—	+
Ku8102	"	—	+	—	—	+	—	—	+
Ku8103	"	—	—	—	—	‡	—	—	+
Ku8104	"	—	+	—	‡	‡	‡	‡	+
Ku8105	"	—	+	—	—	‡	‡	‡	+
Ku8111	"	—	—	—	—	+	—	—	+
Ku8112	"	—	‡	‡	—	+	‡	(+)	+
Ku8113	"	—	‡	‡	—	(+)	‡	‡	+
Ku8114	"	—	—	—	‡	(+)	+	—	+
Ku8115	"	—	—	‡	‡	+	‡	‡	+
Ku8116	"	—	—	—	‡	+	—	—	+
Ku8117	"	—	—	—	+	+	+	‡	+
Ku8119	"	—	+	—	‡	‡	‡	‡	+
Ku8120	"	—	+	—	‡	‡	‡	‡	+
Ku8121	"	—	‡	‡	‡	‡	+	‡	+
Ku8122	"	+	—	—	‡	+	+	‡	+
Ku8123	"	‡	—	‡	—	+	‡	—	+
Ku8124	"	‡	—	‡	‡	+	‡	—	+

Activity: —not detected, (+) doubtful, +below 5mm, ‡ 5-10mm, ‡ above 10mm of the width of inhibition zone.

a) NIAES: National Institute of Agro-Environmental Sciences.

FARC: Fukuoka Agricultural Research Center.

KAES: Kyushu National Agricultural Experiment Station.

KU: Laboratory of Plant Pathology, Kyushu University.

b) Evaluated by inoculating each isolate to rice seedlings just after germination and to rice panicles at flowering stage.²²⁾

Table 3. Production of antibacterial substances by *Ps. gladioli* pv. *gladioli*

Isolate ^{a)}	Indicator						
	A. t	C. m	E. c	P. sol	P. syr	X. c	X. o
251-11	—	—	(+)	‡	+	‡	‡
251-14	+	+	‡	‡	+	‡	‡
251-15	+	+	‡	‡	+	‡	‡
251-16	—	+	‡	‡	+	‡	‡
251-17	—	(+)	‡	‡	+	+	‡
251-18	—	—	(+)	‡	+	(+)	+
251-19	—	—	‡	‡	+	‡	‡
251-20	—	+	‡	‡	+	‡	‡
NIAES1064	‡	‡	‡	‡	‡	(+)	(+)

a) Supplied from Dr. T. Uematsu, National Agricultural Research Center.

Table 4. Production of antibacterial substances by *Ps. cepacia*

Isolate ^{a)}	Indicator						
	A. t	C. m	E. c	P. sol	P. syr	X. c	X. o
342-1	+	++	(+)	—	++	—	(+)
342-2Y	—	++	(+)	—	++	—	(+)
342-4Y	+	+++	+	+	+	+	+
342-27	+	(+)	—	+	++	—	—
342-30	(+)	+	—	++	++	—	+
342-36	—	+	—	—	+	—	(+)
342-42	+	+	+	+	+	+	+
342-43	++	+++	—	++	++	++	+++
242-44	++	+++	—	++	+	++	+++
342-45	+	+++	—	+	++	+	+++
342-46	++	+++	—	++	+	+	+++
342-47	+	+++	—	++	++	+	+++
342-48	++	+++	—	+	(+)	(+)	+++
343-3	(+)	+	—	—	(+)	(+)	—
343-4	—	+	—	—	(+)	—	—
356-1	(+)	++	—	(+)	(+)	(+)	—
356-2	(+)	+++	+	+	—	+	+
356-3	(+)	+++	+	+	—	+	+
356-4	—	+	+	—	++	—	+
356-5	+	+++	+	+	+	+	+

a) Supplied from Dr. T. Uematsu, National Agricultural Research Center.

Table 5. Production of antibacterial substances by *Ps. avenae*

Isolate ^{a)}	Indicator						
	A. t	C. m	E. c	P. sol	P. syr	X. c	X. o
H8201	—	++	—	—	—	—	—
H8202	(+)	++	—	—	—	—	—
H8203	—	+	—	—	—	—	—
H8204	+	+	—	—	(+)	—	—
H8205	—	+	—	—	—	—	—
H8206	—	++	—	—	—	—	—
H8207	+	++	—	—	(+)	—	—
H8208	—	+	—	—	—	—	—
H8209	—	++	—	—	—	—	—
H8210	—	++	—	—	—	—	—

a) Supplied from Hokuriku Agricultural Experiment Station.

All isolates of *Ps. avenae* clearly showed antibacterial activity to *C. michiganense* pv. *michiganense*, but no or very weak activity to other indicators, as shown in Table 5.

Discussion

Plant pathogenic pseudomonads used in this experiment formed growth inhibition zone on the lawn of various plant pathogenic bacteria used as indicator, suggesting the productivity of some antibacterial substances. The activity spectrum and productivity were much varied depending upon species and isolates used.

As the causal agents for the formation of growth inhibition zone, antibiotics, bacteriocins, bacteriophages and bdellovibrios are well known. Besides these agents, siderophores^{12,18,19)} were also reported as an agent to form inhibition zone on some specific bacteria grown on the Fe^{3+} -unsupplemented medium.

Since the inhibition zones were formed on the indicator bacteria distantly related with the producers, the causal agent of inhibition zones observed in this experiment are considered to be neither bacteriocins nor bacteriophages. The causal agent is also not considered to be siderophores, because the inhibition zone appeared regardless of the concentrations of Fe^{3+} in the medium (unpublished data).

In the case of *Ps. glumae*, some isolates such as I, III, Ku8123, Ku8124 and 8020 showed high activity against *A. tumefaciens*. Among these isolates, some (I, III, and 8020) were also highly active to *E. carotovora* subsp. *carotovora*, *Ps. solanacearum*, *X. campestris* pv. *citri* and pv. *oryzae*, while others (Ku8123 and Ku8124) were inactive to *X. campestris* pv. *oryzae*. Ku8123 was inactive also to *Ps. solanacearum*. Some isolates such as Ku8112, Ku8121, 750, 752, 805, Pl-22-1 and 8001 formed wide inhibition zone on the lawn of *C. michiganense* pv. *michiganense*, but their activity spectra against other indicators showed much variation. Thus, the activity spectra of *Ps. glumae* were greatly different depending upon isolates, suggesting that several antibacterial substances participated in their activities.

The antibacterial spectra shown by *Ps. gladioli* pv. *gladioli* and *Ps. cepacia* were also complicated as in the case of *Ps. glumae*. While the activity spectra of *Ps. avenae* isolates were rather uniform, showing high activity only against *C. michiganense* pv. *michiganense*.

The antibacterial substances produced by plant pathogenic pseudomonads should be purified and chemical structures of these substances should also be made clear in future.

So far as *Ps. glumae* isolates are concerned the intensity or spectra of antibacterial activity had no correlation with the pathogenicity²²⁾ to rice plants or number and kinds of plasmids⁹⁾ found in each isolate.

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和 文 摘 要

脇本 哲・平八重一之・土屋健一・櫛間義幸・古屋成人・松山宣明：植物病原性 *Pseudomonas* 属細菌による抗細菌性物質の産生

Pseudomonas glumae, *Ps. gladioli* pv. *gladioli*, *Ps. cepacia* および *Ps. avenae* など、供試した植物病原 *Pseudomonas* 属細菌は、*Agrobacterium tumefaciens*, *Corynebacterium michiganense* pv. *michiganense*, *Erwinia carotovora* subsp. *carotovora*, *Ps. solanacearum*, *Ps. syringae* pv. *syringae*, *Xanthomonas campestris* pv. *citri*, および *X. campestris* pv. *oryzae* など、指示菌として供試した植物病原細菌の培養平板上に発育阻止帯を形成した。それらの活性スペクトラムは産生菌の種および分離株の違いによって著しく異なり、阻止帯形成には多種類の抗細菌性物質が関与しているものと推察された。