

Pseudomonas avenae

Mannsによるタンパク性抗糸状菌物質の生成

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Production of Proteinous Anti-Fungal Substance(s) by *Pseudomonas avenae* Manns

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Key words: antagonism, proteinous anti-fungal substance(s), *Pseudomonas avenae* Manns, *Pyricularia oryzae* Cavara.

It has been well-documented that non-phytopathogenic and phytopathogenic bacteria are antagonistic to plant parasitic bacteria and fungi¹⁻⁸), and some of them have been utilized for biological control^{1,8}). Previously, the authors reported the production of antibiotic substance(s) against phytopathogenic bacteria by some pseudomonads⁷). We also found the antibiotic activity of these pseudomonads to the rice blast fungus, *Pyricularia oryzae* Cavara.

Pyricularia oryzae Cavara isolate Ken 53-33 was pre-cultured on potato sucrose agar for one week at 27 C. Mycelial disk was cut with cork borer (6 mm in diameter) and transferred on the center of the potato semi-synthetic (PS) agar plate [potato (300 g) decoction 1,000 ml, 0.5 g Ca(NO₃)₂·4H₂O, 2.0 g K₂HPO₄·12 H₂O, 5.0 g peptone, 15.0 g sucrose, 18.0 g agar, pH 7.0], which is suitable for cultivation of bacteria and fungi. After 24 hr incubation at 27 C, each isolate of *Pseudomonas avenae* Manns, *Ps. cepacia* Palleroni *et* Holmes, *Ps. glumae* Kurita *et* Tabei and *Ps. gladioli* pv. *gladioli* Severini was confronted (2.5 cm aparted from the center of the disk of indicator, *Pyricularia oryzae*) and incubated at 27 C for 6 days. The anti-fungal activity was estimated by the distance (D) from the center of bacterial colony (antagonist) to the margin of the inhibition zone and exhibited as follows: - not inhibited, + slightly inhibited but the distance (D) was 6 mm or less ($D \leq 6$ mm), ++ inhibited ($6 < D \leq 12.5$ mm), +++ highly inhibited ($D > 12.5$ mm).

Most isolates of *Ps. avenae*, *Ps. cepacia* and *Ps. gladioli* pv. *gladioli* showed an antagonistic activity to *P. oryzae* except for *Ps. glumae*. Particularly, the activity of *Ps. avenae* was obvious among all bacteria tested (Table 1). This bacterium was also antagonistic to other isolates of *P. oryzae* and various species of *Pyricularia* in the same degree, and a lower degree against other fungi such as *Fusarium oxysporum* f.sp. *melonis* and *Alternaria bataticola* (Table 2).

To isolate the antibiotic substance(s) excreted in culture medium, preliminary experiments were conducted. *Pseudomonas avenae* isolate H8201⁹) was shake-(140 strokes/min with 6 cm amplitude) and still-cultured in PS liquid medium in Sakaguchi flask (500 ml) at 30 C for 10 days. Then, the culture medium was centrifuged at 10,000 rpm for 20 min and membrane

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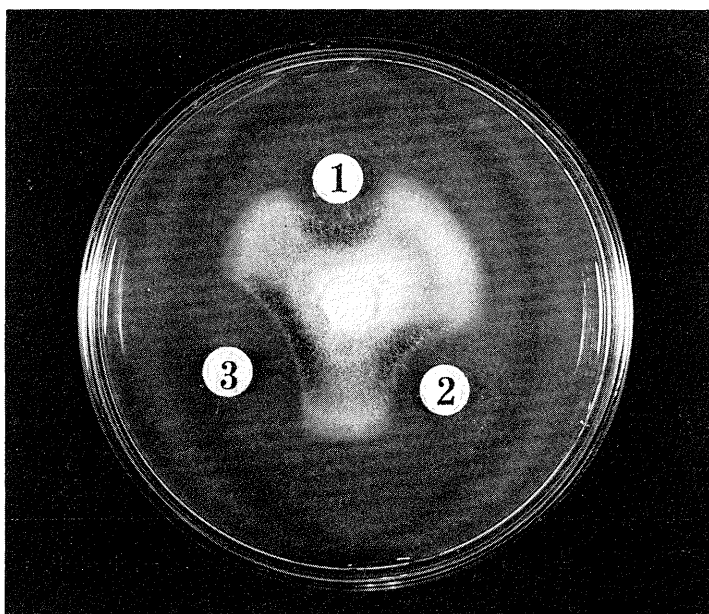


Fig. 1. The inhibition of mycelial growth of *Pyricularia oryzae* Cav. by the culture filtrate of *Pseudomonas avenae* Manns. 1: +, 2: ++, 3: +++.

Table 1. Anti-fungal activity of the pseudomonads against *Pyricularia oryzae*

Producer	Isolate	Activity
<i>Ps. glumae</i>	N7501	—
	N7503	—
	N7505	—
	YN7810	—
	8020	—
<i>Ps. avenae</i>	H8201	+++
	H8203	++
	H8205	++
	H8206	+++
	H8210	+++
<i>Ps. cepacia</i>	342-43	+
	342-44	+
	342-45	+
	342-46	+
	356-3	—
<i>Ps. gladioli</i> pv. <i>gladioli</i>	251-14	+
	251-15	++
	251-16	++
	251-20	++
	NIAES1064	+
	NIAES1065	++
	E-14	+

Note: Each producer was confronted to *Pyricularia oryzae* on the potato semi-synthetic medium.
—, +, ++, +++ ; See the text.

Table 2. Anti-fungal activity of *Pseudomonas avenae* against various phytopathogenic fungi

Indicator	Isolate	Activity
<i>Alternaria bataticola</i>	A4-1-2	+
<i>Fusarium oxysporum</i> f.sp. <i>melonis</i>	F1-8-1	++
<i>F. roseum</i>	78-13	—
<i>F. solani</i> f. sp. <i>pisi</i>	Ito-01	—
<i>Helminthosporium setariae</i>	IF6635	—
<i>Pestalotia funerea</i>	Ku-7	—
<i>Pyricularia oryzae</i>	Ken53-33	+++
<i>P. oryzae</i>	TH67-22	+++ a)
<i>P. oryzae</i>	Naga69-150	+++ a)
<i>P. panici</i>	STPM1-2-1	+++ a)
<i>P. setariae</i>	STSII-1-1	+++ a)
<i>P. grisea</i>	OM-1	+++ a)
<i>Rhizoctonia solani</i>	31	—
<i>Sclerotium hydrophilum</i>	NIAS-Ku-01	—
<i>S. delphinii</i>	S2-1-2	—

Note: *Pseudomonas avenae* Manns isolate H8201 was used as a producer of anti-fungal substance.

a) Tested for culture filtrate by using the paper-disk method. —, +, ++, +++; See the text.

filtered (0.45 μ m). Paper disk (8 mm in diameter, 1.5 mm in thickness, Toyo Co.) was dipped in the filtrate and placed on PS agar medium confronting to the indicator (Fig. 1). Wide inhibition zone appeared around paper disk suggesting production of the anti-fungal substance(s) by the bacterium. The substance(s) was predominantly produced under shake-culture condition. In this case, the bacterial population reached maximum by 24 hr after inoculation and rapidly decreased afterwards. The accumulation of the substance(s) increased correlatively with the death of the bacteria.

The activity of the substance(s) was lost by heat-treatment at 100 C for 10 min and protease K treatment (100 μ g/ml, Sigma Co.). The substance(s) was not dialyzable, not transferable to organic solvents such as chloroform, petroleum ether, diethyl ether, ethylacetate and *n*-butanol and was precipitated by addition of excess organic solvents. These results clearly indicate that the anti-fungal substance(s) is proteinous. Recently, Azegami *et al.*¹⁰⁾ reported the production of tropolone, an anti-fungal substance³⁾, by *Ps. plantarii*. No tropolone production, however, was observed in the case of *Ps. avenae*, the relative of *Ps. plantarii*.

和 文 摘 要

國武幸子・松山宣明・脇本 哲：*Pseudomonas avenae* Manns によるタンパク性抗糸状菌物質の生成

Pseudomonas 属植物病原細菌のなかには、*Pyricularia oryzae* (イネいもち病菌) に対して抗菌性を示すものがあり、とくに *Pseudomonas avenae* (イネ褐条病菌) は平板培地上において顕著な抗菌活性を示した。本菌は各種 *Pyricularia* 属菌のほか、その程度はやや弱いものの *Fusarium oxysporum* f.sp. *melonis* や *Alternaria bataticola* に対しても抗菌性を示した。*Pseudomonas avenae* の生成する抗いもち病菌物質は、PS 培地中で振とう培養することにより多量に生産された。本物質は透析されず、100 C、10 分間の熱処理および protease K 処理により容易に失活することから、タンパク性の物質と考えられる。本抗菌物質の精製については現在検討中である。

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