Antagonistic Action of Bacillus sp. AB89 to Phloeospora maculans, Causal Agent of Mulberry Leaf Spot

Kim, Kyung-Hee
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

Inoue, Shino
Fukuoka Agricultural Extension Center

Kawanami, Masakazu
Nissan Chemical Industries, Ltd.

Furuya, Naruto
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

他

https://doi.org/10.5109/24278

出版情報：九州大学大学院農学研究院紀要. 43 (3/4), pp.337-342, 1999-02. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **43** (3·4), 337–342 (1999)

Antagonistic Action of *Bacillus* sp. AB89 to *Phloeospora maculans*, Causal Agent of Mulberry Leaf Spot

Kyung-Hee Kim, Shino Inoue*[1], Masakazu Kawanami*[2], Naruto Furuya and Nobuaki Matsuyama

Laboratory of Plant Pathology, Faculty of Agriculture,
Kyushu University, Fukuoka 812 8581, Japan
(*Received October 28, 1998 and accepted November 6, 1998*)

The control of mulberry leaf spot caused by the infection of *Phloeospora maculans* (*Septogloeum mori*) has been important for sericulture in Goto islands. Since the chemical control of this leaf-spot disease was not available except for off-season, the possibility of other kind of control measure was tested. Phylloplane bacterium *Bacillus* sp. AB89 from a rice leaf was quite useful as an antagonist. Culture filtrate of the antagonistic bacterium inhibited spore germination and mycelial growth of leaf spot pathogen. Furthermore, the culture filtrate and living bacterial cells limited distinctly the development of lesions on mulberry leaves *in vivo*.

INTRODUCTION

Mulberry leaf spot by the infection of *Phloeospora maculans* was epidemic in China (1954, 1973～1978). In Japan this disease has been endemic and giving severe damage in Goto islands (Nagasaki prefecture) where sericulture is an important industry (Negi *et al.*, 1992; Baba *et al.*, 1995).

The use of agricultural chemicals, though they are effective to a causal pathogen, has been limited by the anxiety of effects on silkworm growth. Therefore the application of an antagonistic microorganism was intended. One of the authors, Matsuyama, isolated a phylloplane bacterium from a leaf of rice and named as AB89. This isolate showed wide antagonistic spectra to various phytopathogenic fungi and bacteria and was identified as *Bacillus* species based on the experimental results for numerous key characteristics in physiological, biochemical and serological tests (Inoue *et al.*, 1993). The authors estimated the ability of the isolate *Bacillus* sp. AB89 as a novel control measure against mulberry leaf spot disease. The results will be contributed in the present report.

MATERIALS AND METHODS

Plant used

Mulberry (*Morus alba* L. cv. Minamisakari) was raised in potted soil (Clay pot: 26.5 cm in inner diameter and 22 cm in depth) fertilized with the granulous compound fertilizer for mulberry tree (Toku-No. 10) in the green house.

---

*[1] Fukuoka Agricultural Extension Center, Fukuoka, Japan.
*[2] Nissan Chemical Industries, Ltd., Shiraoka, Japan.

Culture of inoculum

Phloeospora maculans (Bereng.) Allescher (*Septogloeum mori* Briosi *et* Cavara), the causal agent of the mulberry leaf spot was cultured on PSA medium [potato decoction (200 g) 1*l*, sucrose 15.0 g, agar 15.0 g]. Spores were obtained from the surface of PSA plates after culture for 10~15 days at 25 °C.

Culture of antagonistic bacterium

Bacillus sp. AB89 was shake-cultured (125 rpm) in potato semi-synthetic medium [PS medium: potato decoction (300 g) 1*l*, $Na_2HPO_4 \cdot 12H_2O$ 2 g, Ca $(NO_3)_2 \cdot 4H_2O$ 0.5 g, Peptone 5.0 g, Sucrose 15.0 g] at 30 °C for 72 hrs. The cultured fluid was centrifuged at 7,500 x *g* for 20 min. Bacterial pellet was washed twice with distilled water, adjusted to the concentration of $11 \sim 13 \times 10^8$cfu/ml and used for antagonist pretreatment. Supernatant after centrifugation was also used as a fluid of crude antibiotics.

Tests of antagonistic activity of *Bacillus* sp. AB89 *in vitro*

The culture filtrate was diluted 2, 4, 10 times with distilled water, membrane-filtered and tested for antifungal activity to germination of spores on glass slides in Petri-dish of RH 100% and mycelial growth of *P. maculans* by penicillin-cup method.

Evaluation of antagonist

Bacterial cells and culture filtrate of *Bacillus* sp. AB89 were sprayed on the leaves of potted mulberry trees and dried, respectively. Then the spore suspension ($11 \sim 27 \times 10^5$cfu/ml) of the pathogen of leaf spot, *P. maculans* was sprayed and inoculated trees were incubated at 25 °C for 24 hrs. Distilled water and not cultured fresh PS fluid were sprayed as the check.

Fifteen days after inoculation, the number of lesions formed on the 4 th leaf from the uppermost leaf was counted and expressed as lesions/cm^2. The leaf area was calculated by the automatic leaf-area meter (Hayashi denko Co.).

RESULTS AND DISCUSSION

The biological control with antagonistic *Bacillus* spp. has been well documented and some of the antagonists have been practically used for control of various foliate diseases (Fravel *et al.*, 1977; Baker *et al.*, 1983, 1985; Spurr *et al.*, 1985; Rytter *et al.*, 1989). The practical use of living cells of *Bacillus* sp. AB89 and antibiotic (s) which will be involved in the antagonism for control of the mulberry leaf spot disease was intended.

The antifungal activity of culture filtrate of *Bacillus* sp. AB89 against *P. maculans*, was presented in Table 1. Germination of spores from the lesions of diseased mulberry leaves and PSA medium was heavily inhibited. Rarely a few spores germinated but their growth was abnormal and ceased their elongation. As can be seen in Fig. 1, morphological abnormality such as swelling and granulation of the treated spore cells were observed.

The mycelial growth was also inhibited even when the culture filtrate was diluted 10 times. The hyphae of the surrounding region of the inhibition zone were morphologically abnormal and protrusion or swelling of hyphal cells was observed (Fig. 2). Such

Table 1. Inhibition of spore germination of *Phloeospora maculans* by culture filtrate of *Bacillus* sp. AB89 at various concentrations.

Concentration of culture filtrate				Distilled Water	Conidia from
Original	1/2	1/4	1/10		
0.00[1)]	0.00	0.00	0.00	88.6%	diseased leaves
0.00	0.01	0.01	0.01	76.6	PSA medium

1): Germination was counted at 24 th hr.

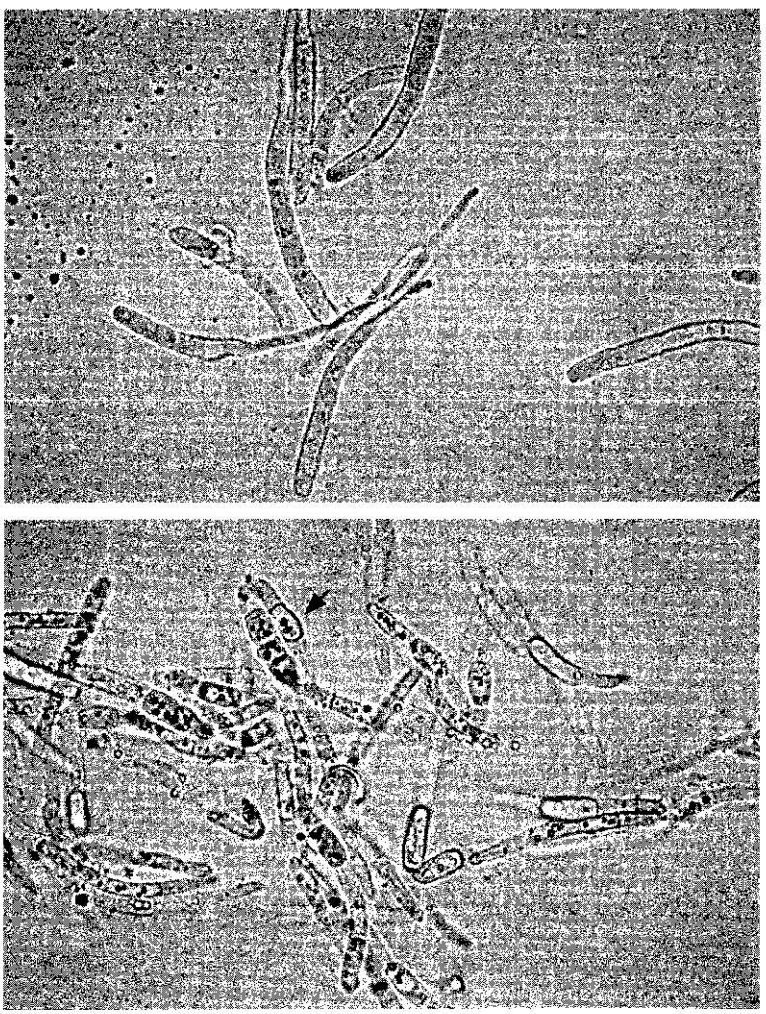

Fig. 1. Effect of antifungal substance (s) of *Bacillus* sp. AB89 on germination of *P. maculans* (Upper: Check, Lower: Treated with culture filtrate; Swelled and granulated cells)

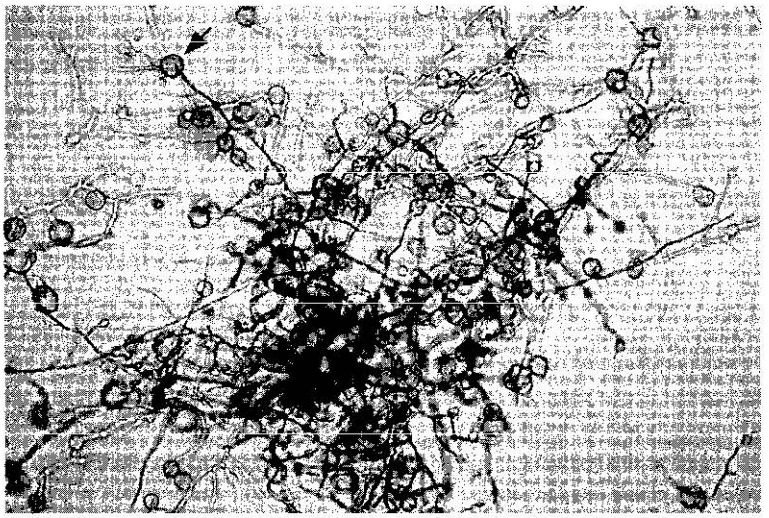

Fig. 2. Swelled hyphae of *P. maculans* at inhibition zone on the PSA plate

Table 2. Suppression of leaf-spot development by culture filtrate and bacterial cells of *Bacillus* sp. AB89.

Trial	Treated with[1]				Concentration of pathogen (*P. maculans*; cfu/ml)
	CF	BC	Check 1	Check 2	
1	0.00[2]b[3]	0.13b	0.39ab	0.78a	13×10^3
2	0.00b	0.02b	0.39a	0.49a	11×10^3
3	0.00b	0.12b	1.45a	1.24a	27×10^3
Mean	0.00	0.09	0.74	0.84	–

1) CF: Culture filtrate BC: Bacterial cells Check 1: Distilled water Check 2: Potato semi-synthetic medium

2) Number of lesions/cm^2 leaf area

3) Numbers followed by different letters differ significantly at P=0.05 level in Duncan's multiple range test.

phenomenon was sometimes observed at confronting fungus with antagonistic microorganisms and attributed as a result of the inhibition of chitin synthesis in fungal cell-wall (Ohta *et al.*, 1970; Kikutake *et al.*, 1991).

Mulberry leaves were pretreated with the culture filtrate or living cells of *Bacillus* sp. AB89 and challenge inoculated with *P. maculans*, the causal agent of leaf spot. As shown in Table 2 and Fig. 3, the infection was highly limited by the treatments. One of the authors tested the inhibitory effects of these antagonist and antibiotics on silkworm growth. No negative effects on the growth and behaviors of silkworm were observed (Kawanami *et al.*, 1997). These results will strongly indicate the usefulness of this isolate *Bacillus* sp. AB89 as a control measure.

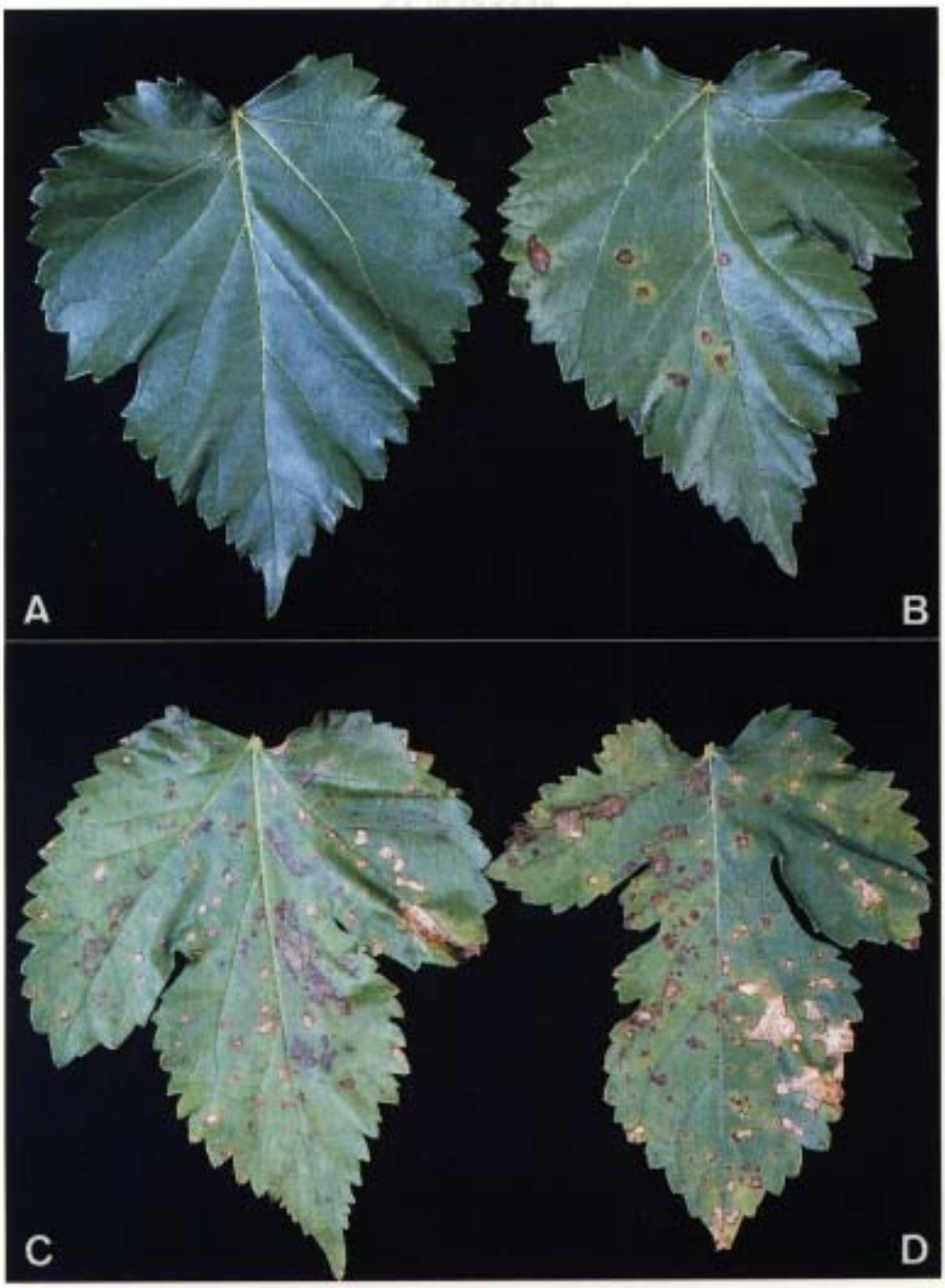

Fig. 3. Effect of *Bacillus* sp. AB89 on leaf-spot development caused by *P. macularis* [A: Culture filtrate (CF), B: Bacterial cells (BC), C: Distilled water (DW), D: Potato semi-synthetic medium(PS)]

REFERENCES

Baba, S., K. Matsuo, W. Negi, Y. Suga, T. Ogawa and M. Murayama 1995 Some ecological studies on leaf spot of mulberry. *Proc. Assoc. Pl. Prot. Kyushu*, **41**: 47–50 (in Japanese)

Baker, C. J., J. R. Stavely and N. Mock 1985 Biocontrol of bean rust by *Bacillus subtilis* under field conditions. *Plant Dis.*, **69**: 770–772

Baker, C. J., J. R. Stavely, C. A. Thomas, M. Sasser and J. S. MacFall 1983 Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. *Phytopathology*, **73**: 1148–1152

Fravel, D. R. and H. W. Spurr, Jr. 1977 Biocontrol of tobacco brown-spot disease by *Bacillus cereus* subsp. *mycoides* in a controlled enviroment. *Phytopathology*, **67**: 930–932

Inoue, S., K. H. Kim, M. Nishioka, N. Furuya, Y. Takanami and N. Matsuyama 1993 Production of antibiotic (s) by *Bacillus* sp. AB89. *Proc. Assoc. Pl. Prot. Kyushu*, **39**: 56–59 (in Japanese)

Kawanami, M., Y. Banno, K. H. Kim, S. Yamamoto, N. Furuya and N. Matsuyama 1997 On an antibiotic producing bacterium *Bacillus subtilis* AB89 II. Antifungal effect against *Phloeospora maculans* and characteristics of the antibiotics. *Ann. Phytopath. Soc. Jpn.*, **63**: 216 (abstract, in Japanese)

Kikutake, K., N. Matsuyama and S. Wakimoto 1991 Antagonistic activity of *Xanthomonads* against phytopathogenic fungi and isolation of antifungal substance. *Ann. Phytopath. Soc. Jpn.*, **57**: 61–64

Negi, W., K. Ide, T. Ota, N. Matsuyama and K. H. Kim 1992 Severe occurrence of mulberry leaf spot in Goto, Nagasaki Prefecture. *Proc. Assoc. Pl. Prot. Kyushu*, **38**: 52–54 (in Japanese)

Ohta, N., K. Kakiki and T. Misato 1970 Studies on the mode of action of Polyxin D part II. Effect of Polyxin D on the synthesis of fungal cell wall chitin. *Agr. Biol. Chem.*, **34(8)**: 1224–1234

Rytter, J. L., F. L. Lukezic, R. Craig, and G. W. Moorman 1989 Biological control of geranium rust by *Bacillus subtilis*. *Phytopathology*, **79**: 367–370

Spurr, H. W. Jr. and C. R. Knudsen 1985 Biological control of leaf diseases with bacteria. "Biological control on the phylloplane." (Winclels, C. E. and S. E. Lindow, eds.): 45–62