日植病報 62 : 559-565 (1996)
Ann. Phytopathol. Soc. Jpn. 62 : 559-565 (1996)

Biological Control of Cyclamen Gray Mould (*Botrytis cinerea*) by *Serratia marcescens* B2*

Hiroyuki IYOZUMI**,§, Tomoyuki KOMAGATA***, Kazuyuki HIRAYAE†, Kenichi TSUCHIYA††, Tadaaki HIBI††† and Katsumi AKUTSU**

Abstract

Cyclamen petals were treated with a highly chitinolytic bacterium, *Serratia marcescens* isolate B2, and 24 hr later, were challenge-inoculated with *Botrytis cinerea* conidia. The bacterium suppressed fungal disease incidence by *ca.* 60% on attached petals of cyclamen in the greenhouse. The efficacy of isolate B2 against gray mould caused by *B. cinerea*, which was resistant to benzimidazole and dicarboximide fungicides, was nearly equal to that of 200 ppm of iprodione, a dicarboximide fungicide. Although isolate B2 failed to survive on petals and leaves more than two weeks after the initial application, populations on leaf discs placed on the soil near the base of the cyclamen plant, where gray mould regularly developed, increased 10-fold during this period. Cyclamen leaf discs were challenge-inoculated with *B. cinerea* conidia either 1 hr, 3 days or 7 days after isolate B2 application, then placed near the base of the plant in the greenhouse. Fungal sporulation on the discs was suppressed by more than 85%. *S. marcescens* B2 was significantly more effective than iprodione treatment against fungal sporulation on the discs.

(Received May 15, 1996 ; Accepted September 11, 1996)

Key words : biocontrol, *Botrytis cinerea*, cyclamen, *Serratia marcescens*, chitinolytic bacterium.

INTRODUCTION

Botrytis cinerea Persoon ex Fries, the causal agent of gray mould, can infect an extremely wide range of crop plants, such as field-grown crops like grapes, and greenhouse-grown vegetables, flowers and fruits, during production or post-harvest during storage and transport[14]. Since disease control using resistant cultivars and so on is difficulty under these conditions, the control of gray mould in many crops has been based on frequent fungicide applications. With the introduction of systemic fungicides, effective control was possible for a short period. After a few years of using these fungicides, however, control became less effective with the development of fungicide-resistant isolates. In the 1970s, resistance of *B. cinerea* populations to benzimidazoles was widespread. During the early 1980s, resistance to dicarboximide fungicides was also reported in some greenhouses. In recent years resistance to dicarboximides has become common[6]. Likewise, resistance has developed to the mixture of diethofencarb with carbendazim used for gray mould control[15]. Since resistance to currently available fungicides is common in greenhouses, alternative methods to suppress *B. cinerea* are of great importance to commercial growers.

Biocontrol of *Botrytis* spp. has been attempted mainly with fungal agents, such as hyperparasitic *Trichoderma* spp.[4,7,9,10], *Gliocladium* spp.[20,22], and saprophytic yeast[5]. When using *T. harzianum* to control gray mould of grapes in the field, all the data suggested that a conidial concentration in the order of 10^8/ml was needed for adequate control[4,9]. Below this concentration, a rapid decline in efficacy was observed ; no particular improvement seemed to be achieved using a higher inoculum density. Under commercial greenhouse conditions, however, *T. harzianum* survived at an effective population on cucumber plants for one month, proving to be a reliable biocontrol agent of gray mould[7].

We previously reported that a highly chitinolytic

* This work was supported in part by the grant in aid for scientific research from the Ministry of Education, Science and Culture of Japan (06660046).
** Faculty of Agriculture, Ibaraki University, Ami-machi, Inashiki-gun, Ibaraki 300-03, Japan 茨城大学農学部
*** Ibaraki Horticultural Research Institute, Minori-machi, Ibaraki 319-02, Japan 茨城県園芸研究所
† Kyushu National Agricultural Experiment Station, Nishigoshi, Kikuchi-gun, Kumamoto 861-11, Japan 九州農業試験場
†† National Institute of Agrobiological Resources, Tsukuba 305, Japan 農業生物資源研究所
††† Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan 東京大学農学部
§ Present address : Shizuoka Agricultural Experiment Station, Iwata-gun, Shizuoka 678-1, Japan 静岡県農業試験場

bacterium, *Serratia marcescens* B2, suppressed the growth of *Botrytis* spp. *in vitro*, as well as controlling chocolate spot disease of broad bean caused by *B. fabae* in a growth chamber. It controlled growth and infection of the fungicide-resistant isolates as effectively as that of the fungicide-sensitive isolates, both *in vitro* and *in vivo*[2,13,26]. *S. marcescens* is a ubiquitous organism in water, soil and food[8]. The isolate B2 was isolated from tomato phylloplane in the greenhouse[2], suggesting that this bacterium could survive on the phylloplane under humid conditions, which are conducive to gray mould development[12,17].

This study was initiated to control cyclamen gray mould with *S. marcescens* B2 under greenhouse conditions. Gray mould in cyclamen plants develops throughout the cultivation period, particularly on petals in the winter or just before sale. Because chemical control is limited during this period to avoid polluting the petals with fungicides, safer control methods are urgently needed for commercial growers. The present work is aimed at establishing an efficient biocontrol strategy for gray mould and evaluating integrated disease control which incorporates the use of *S. marcescens* B2.

MATERIALS AND METHODS

Inoculum production. *S. marcescens* B2 (Plate I-①) was routinely cultured on chitin-amended lima bean agar medium (LBCA) at 28°C[2]. For applications, a 2-to 3-day-old bacterial mass was collected by centrifugation at 10,000 rpm for 10 min and diluted with sterile water to OD_{555} 1.0 (*ca.* 1×10^9 cfu/ml).

B. cinerea IH36 was isolated from diseased cyclamen plants treated with fungicides in Ibaraki Horticultural Research Institute greenhouses (Minori-machi, Ibaraki). From *in vitro* tests, the *B. cinerea* isolate showed resistance to benzimidazole (MIC of benomyl>10 ppm) and dicarboximide (MIC of iprodione, procymidone and vinclozolin>10 ppm) fungicides. To produce inoculum, the fungus was grown on potato sucrose agar (PSA) at 20°C for five days, and incubated under BLB (Black light blue lamp, Toshiba) for 2 days. Conidia were suspended in sterile distilled water and adjusted to *ca.* 5.0×10^5 conidia/ml.

Plant materials. Cyclamen plants (*Cyclamen persicum*, Pastel strain cv. John Strauss) were grown for 10 to 12 months in soil (Metro-Mix 360, Grace Sierra) in pots (diameter of 15 cm and high of 10 cm) under greenhouse conditions. Before blossoming, plants were cultivated under a fungicide control regimen.

Biocontrol test on cyclamen petals. *S. marcescens* B2 (10 ml of *ca.* 1×10^9 cfu/ml per plant) was sprayed on cyclamen petals. The plants were then kept at *ca.* 18°C for 1 hr, before spraying with *B. cinerea* (10 ml of *ca.* 5×10^5 conidia/ml per plant). After fungal inoculation, the plants were placed in humid conditions (R.H.>95%) for 24 hr, then kept in the greenhouse at 15-20°C for. 6 days before measuring the diameter of the lesions on plant tissue. Disease incidence was evaluated according to a damage index (Plate I-③). Disease incidence rate (%) was calculated by the following formula : disease incidence rate (%)$=\{(0\times n_0+1\times n_1+2\times n_2+3\times n_3+4\times n_4+5\times n_5)/5\times(n_0+n_1+n_2+n_3+n_4+n_5)\}\times100$ ($n_{0\sim5}$: number of index 0～5 petals).

The effectiveness of *S. marcescens* B2 application was compared to that obtained with iprodione (0.2 mg/ml), isolate B2 (*ca.* 1×10^9 cfu/ml) combined with iprodione (0.2 mg/ml), or a sterile water control (six replicates/each treatment).

Light microscopy. Petals were collected from cyclamen plants from *S. marcescens* B2 alone, iprodione alone, the mixture of isolate B2 and iprodione, and sterile water control, 48 hr after inoculation. One square cm^2 pieces of the petals were fixed and decolorized in FAA (formalin : ethanol : acetic acid=1 : 1 : 1) solution. After staining with a 0.5% methyl blue solution, the petal pieces were observed under light microscope (12 pieces per treatment).

Population dynamics of S. marcescens B2 on cyclamen plants. Cyclamen plant and leaf discs (diameter of 12 mm), which were placed on the soil near the base of the plants, were sprayed with a suspension of isolate B2, *ca.* 1 ml per disc (*ca.* 1×10^9 cfu/ml), and then kept in the greenhouse at 15-20°C. At 1 hr, 3 days, 7 days and 14 days after the application, five discs (diameter of 12) were cut from petals, outer and inner leaves using a cork-borer. In addition, five leaf discs were excised near the base of the plant, transferred to 25 ml bottles, and washed in 10 ml sterile water for 5 min. Dilutions of the aliquot were cultured on LBCA plate for 24 hr. Colonies with a reddish pigment and producing a chitinolytic halo (Plate I-①) on the plates were counted to estimate the *S. marcescens* B2 population.

Effects of S. marcescens B2 against sporulation of B. cinerea. Cyclamen leaf discs (12 mm diameter) were sprayed with a suspension of *S. marcescens* B2 (*ca.* 1×10^9 cfu/ml). Nine discs were placed on the soil near the base of each cyclamen plant grown in pots under greenhouse conditions. These discs were challenge-inoculated with a suspension of *B. cinerea* (*ca.* 5×10^5 conidia/ml), 1 hr, 3 days and 7 days after application. At 11 days after inoculation, the nine discs were washed with 10 ml sterile water in 25 ml universal bottles. After 5 min, the number of *B. cinerea* conidia in the aliquot was determined with a haemacytometer. The effect of isolate B2 application on *B. cinerea* sporulation was compared with that of iprodione alone (0.2 mg/ml), a mixture of isolate B2+iprodione or sterile water control (three replicates/each treatment).

Data analysis. Results of biocontrol tests were statistically analysed using Tukey's[25] method (at $P=0.05$).

RESULTS

Suppressive effect of S. marcescens B2 against gray mould on cyclamen petals

Under greenhouse conditions, gray mould incidence was reduced to *ca.* 20% on cyclamen petals treated with *S. marcescens* B2 for up to five days after inoculation, compared to a 56% incidence of gray mould on the controls inoculated with only the pathogen (Fig. 1 and Plate I-②). *S. marcescens* was as effective as iprodione (0.2 mg/ml) in limiting lesion development on petals inoculated with the dicarboximide-resistant *B. cinerea* (Fig. 1). In this test, the efficacy of isolate B2 was not enhanced in the presence of iprodione (Fig. 1). Nor did *S. marcescens* B2 cause any significant damage to cyclamen petals during the tests.

Light microscopy showed that conidial germination of *B. cinerea* was inhibited markedly on the petals treated with isolate B2, but that penetration was not inhibited (Table 1). The conidia became swollen and sometimes burst within 48 hr after inoculation of the petals treated with the bacterium.

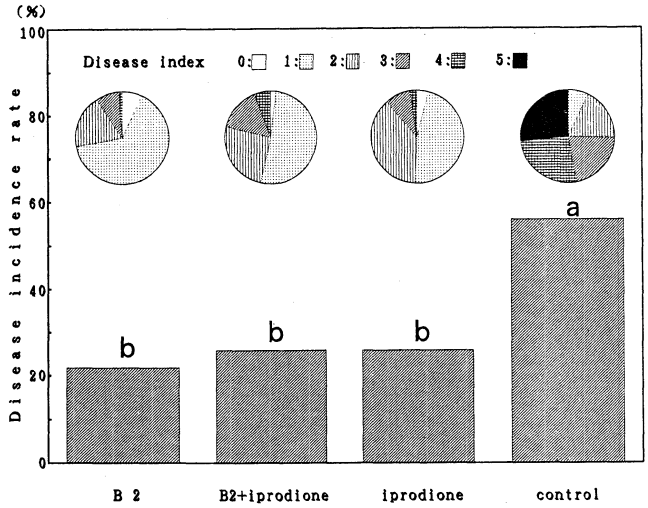


Fig. 1. Effects of *Serratia marcescens* B2 (*ca.* 10 ml per plant of 1×10^9 cfu/ml), iprodione (10 ml per plant of 0.2 mg/ml) or *S. marcescens* B2 (*ca.* 5 ml per plant of 2×10^9 cfu/ml) plus iprodione (5 ml per plant of 0.4 mg/ml) on lesion development by *Botrytis cinerea* on cyclamen petals in the greenhouse. Different letters indicate statistical significance at the 5% level by Tukey's method.

Survival of S. marcescens B2 on cyclamen plants

The level of the *S. marcescens* B2 population was examined on each treated organ of the cyclamen plants. On the petals and both the outer and inner leaves, the population of isolate B2 was reduced to *ca.* $1/10^4$ for two weeks under greenhouse conditions (Fig. 2). However, the population level of isolate B2 on leaf discs placed on

Table 1. Infection behavior of *Botrytis cinerea* conidia on cyclamen petals treated with *Serratia marcescens* isolate B2[a)]

Treatment	Germination (%)[b)]	Penetration (%)[c)]
Isolate B2	15.6 [x]	30.6 [z]
Control	39.2 [y]	28.2 [z]

a) Cyclamen petals were treated with *S. marcescens* B2 (*ca.* 10 ml per plant of 1×10^9 cfu/ml) or distilled water control, then challenge-inoculated with conidial suspension *B. cinerea* (*ca.* 10 ml per plant of 5×10^5 cfu/ml) after 1 hr. Samples were collected 48 hr after inoculation, and observed under a light microscope.

b) Ratio of germinated conidia to total conidia (*ca.* 150 conidia/piece). Different letters indicate statistical significance at the 5% level by Tukey's method.

c) Ratio of infection hyphae-forming conidia to germinated conidia.

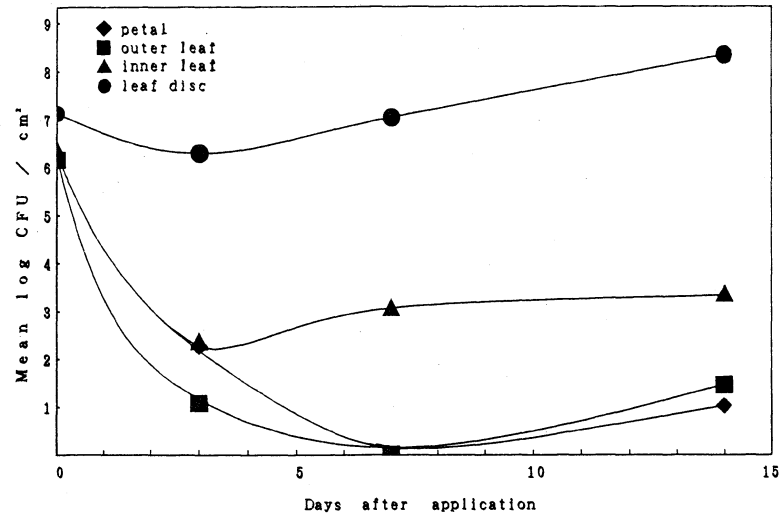


Fig. 2. Population dynamics of *Serratia marcescens* B2 on petals, inner or outer leaves or leaf discs placed on the soil near the base of cyclamen plants in the greenhouse. Samples were collected 1 hr, 3 days, 7 days and 14 days after application, and the density per cm^2 of the each sample was calculated.

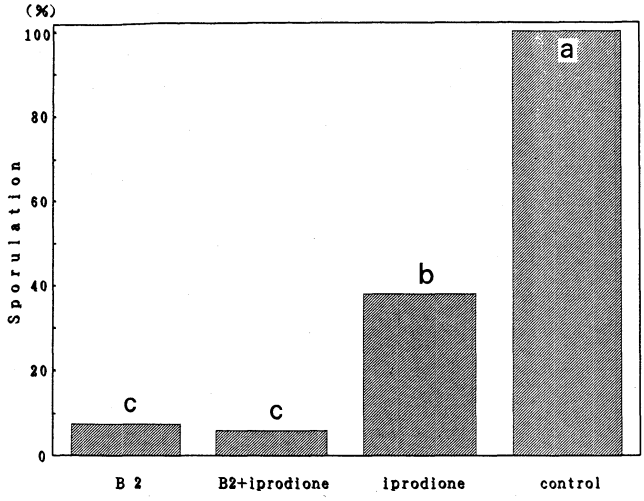


Fig. 3. Effects of *Serratia marcescens* B2 (*ca.* 1×10^9 cfu/ml), iprodione or isolate B2 plus iprodione against sporulation of *Botrytis cinerea* on the leaf discs placed on the soil near the base of cyclamen plant in the greenhouse. The discs were challenge-inoculated with the conidial suspension (*ca.* 5×10^5 conidia/ml) 1 hr after isolate B2 application and collected 11 days after inoculation and the conidial concentration determined. Different letters indicate statistical significance at the 5% level by Tukey's method.

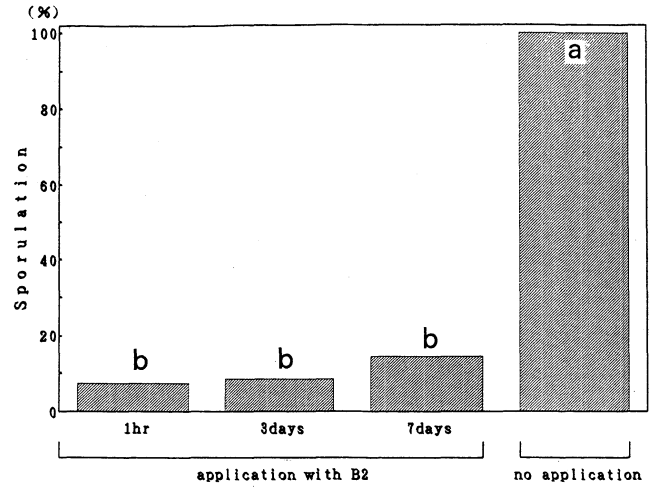


Fig. 4. Duration of inhibition of *Botrytis cinerea* sporulation by *Serratia marcescens* B2 (*ca.* 1×10^9 cfu/ml) on leaf discs placed near the base of cyclamen plants in the greenhouse. The discs were challenge-inoculated with *B. cinerea* conidial suspension (*ca.* 5×10^5 conidia/ml) 1 hr, 3 days and 7 days after isolate B2 application, collected 11 days after inoculation and the conidial concentration formed on the discs determined. Different letters indicate statistical significance at the 5% level by Tukey's method.

the soil near the base of plants remained at *ca.* 10^8 cfu/cm^2 for two weeks, a more than 10-fold increase over the density (cfu/cm^2) present on the disc at application (Fig. 2). Survival of *S. marcescens* B2 was ensured at the base of cyclamen plants in an area of high humidity, where gray mould frequently develops. *S. marcescens* B2 and other chitinolytic organisms were not detected on the untreated control plants.

Suppressive effects of S. marcescens B2 against sporulation of B. cinerea

B. cinerea sporulated heavily by 11 days after inoculation on untreated cyclamen leaf discs placed on the soil near the base of the plants (Plate I-④). Fungal sporulation on discs treated with isolate B2 and B2 plus iprodione was reduced to *ca.* 8% and on those treated with iprodione alone to 38% in comparison to the untreated discs (Fig. 3). Although the suppressive effect of isolate B2 on *B. cinerea* sporulation declined slowly after application, the sporulation was inhibited by *ca.* 85% on discs treated with isolate B2 7 days before the challenge-inoculation compared with that on untreated discs (Fig. 4).

DISCUSSION

S. marcescens B2 inhibited the growth of *B. cinerea* isolated from diseased cyclamen plants and showing *in vitro* resistance to benzimidazole and dicarboximide fungicides. Isolate B2 treatments markedly suppressed both lesion formation on the petals and sporulation on leaf discs placed on the soil near the base of the plants in the greenhouse.

Gray mould develops particularly on the petals in winter or just before the sale, when the use of polluting chemicals must be avoided. Since *S. marcescens* B2 did not pollute cyclamen petals in this experiment, this bacterium could be applied repeatedly on the petals to suppress more effectively petal infection by *B. cinerea* during anthesis. *S. marcescens* B2, therefore, may prove to be a useful microbial pesticide for control to cyclamen gray mould during anthesis.

Although *S. marcescens* B2 failed to survive on dry and aerial parts of cyclamen plants, this bacterium established stable populations near the base of cyclamen plants in areas prone to high humidity. On cyclamen plants inoculated with *B. cinerea* prior to anthesis, the disease developed mainly on senescing leaves and petioles near the base of the plant, but not on the topmost plant organs. The fungus sporulated primarily on the dead, fallen tissues near the base of the plant; these propagules caused secondary infections on petals during anthesis. In this experiment, population survival of *S. marcescens* B2 was ensured near the base of cyclamen plants, where the gray mould often developed extensively and the fungus formed numerous propagules. Because this bacterial treatment suppressed sporulation of a dicarboximide fungicide-resistant *B. cinerea* isolate better than iprodione, *S. marcescens* B2 may have potential as a biocontrol agent against cyclamen gray mould under greenhouse conditions.

S. marcescens B2 efficiently inhibited sporulation of *B. cinerea* on leaf discs near the base of cyclamen plants,

where the B2 population was maintained at a level of more than 10^8 cfu/cm^2. The survival of *S. marcescens* B2 at more than 10^8 cfu/cm^2 could, therefore, effectively control sporulation of *B. cinerea* and halt the secondary dispersal to other plant organs and plants. As shown in the cases using *T. harzianum* and *Candida* sp. to control gray mould of other crops, antagonistic populations in the order of 10^8 cfu/cm^2 have been reported to be the most suitable for biopreparations[4,9,18].

Against the fungicide-resistant isolate of *B. cinerea* alone, isolate B2 showed nearly equal efficacy to that of 200 ppm of iprodione in the control to gray mould on cyclamen petals. Mixing isolate B2 with iprodione did not increase control of the gray mould compared with isolate B2 alone. However, the efficacy of *S. marcescens* B2 was lower than that provided by iprodione against cucumber gray mould with the fungicide-sensitive isolate of *B. cinerea* (data not shown) and chocolate spot disease of broad bean by the fungicide-sensitive isolate of *B. fabae*[13]. Similar results have been reported for biocontrol of gray mould with other antagonists, *e.g. Trichoderma* spp. or *Glicocladium* spp. on strawberry and cucumber[3]. Under commercial greenhouse conditions with both fungicide-resistant and -sensitive populations of *B. cinerea*[1], therefore, the combination of isolate B2 with various fungicides may be expected to be highly effective against gray mould.

S. marcescens B2 causes well-developed, clear zones around its colonies on chitin plates, indicating its ability to produce chitinolytic enzyme(s). When conidia of *B. cinerea* were placed on the clear zone, germination was completely inhibited[2]. The conidia became swollen, with the appearance of spheroplasts. The tips of germ tubes and hyphae also swelled markedly. Likewise, on cyclamen petals treated with *S. marcescens* B2, conidial germination of *B. cinerea* was inhibited markedly. Swelling of the conidia within 48 hr was observed frequently. Conidial germination was similarly suppressed on cyclamen leaf discs treated with isolate B2 (data not shown). Although other antibiotic substance(s) are known to be produced by *S. marcescens*[19], the above data strongly suggest that chitinolytic activity of the bacterium was primarily responsible for inhibiting conidial germination of *B. cinerea*, due to the degradation of the fungal cell walls and interference with cell wall synthesis.

To enhance the intrinsic properties of the antagonists and to assist in its survival on the phyllosphere, adjuvants have been added to biocontrol agents, *e.g. Trichoderma* spp.[10,18]. Tronsmo[24] found that *Trichoderma* was more efficient against gray mould on apple and strawberry blosomes, if the conidial suspension contained soluble cellulose. McLaughlin *et al.*[18] also noted that the addition of salt solutions, such as $CaCl_2$, improved the efficacy of *Candida* sp. against gray mould on apples in storage. These reports suggest that the antagonistic activity of *S. marcescens* B2 can also be enhanced with adjuvants or nutritional substances.

To improve the efficacy of *S. marcescens* B2 against cyclamen gray mould, we are investigating nutritional effects of chitin and other compounds on the antagonistic activity and survival of isolate B2 on cyclamen plants under greenhouse conditions. Studies on the efficacy of isolate B2 on other diseases cyclamen plants will be carried out at the same time.

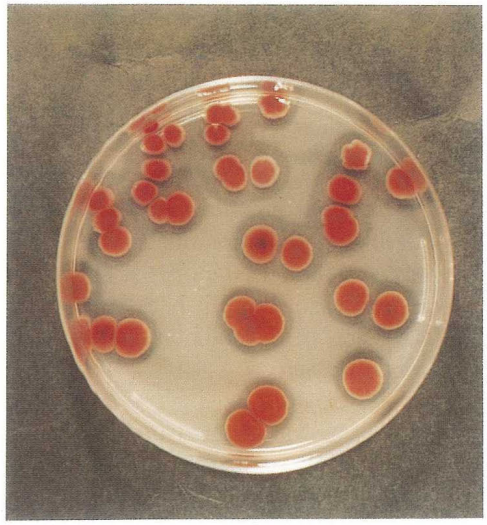
Some strains of *S. marcescens* which form white colonies are known to be opportunistic wound pathogens of vertebrate animals including mammals[11,16,21,23]. Even though the *S. marcescens* isolate used in this study forms red colonies, the potential hazard of this bacterium must be determined before using it as a biocontrol agent of fungal diseases of cyclamen plants.

The authors gratefully acknowledge Emeritus Prof. Dr. S. Okuyama of Faculty of Agriculture, Ibaraki University, for his helpful comments. We also gratefully thank Dr. I. Havukkala of the STAFF Institute, Rice Genome Research Program, Tsukuba, Japan for his English corrections.

Literature cited

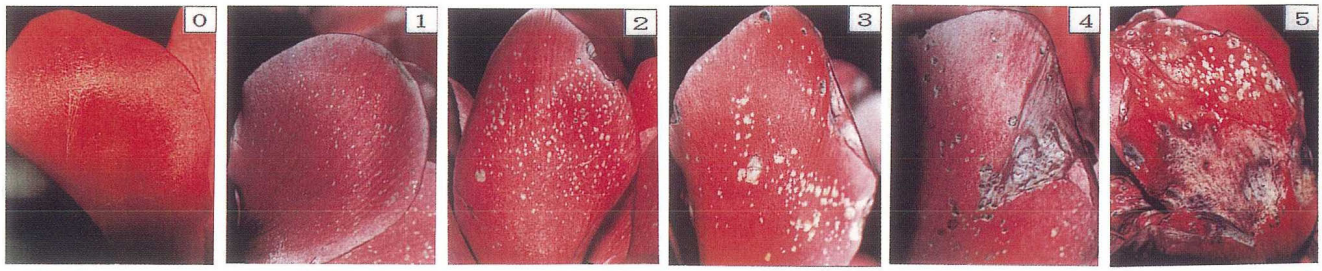
1. Akutsu, K. (1990). Occurrence mechanisms of fungicide-resistance in gray mould. Plant Prot. 44 : 398-401.
2. Akutsu, K., Hirata, H., Yamamoto, M., Hirayae, K., Okuyama, S. and Hibi, T. (1993). Growth inhibition of *Botrytis* spp. by *Serratia marcescens* B2 isolated from tomato phylloplane. Ann. Phytopathol. Soc. Jpn. 59 : 18-25.
3. Dubos, B. (1992). *In* Recent Advances in *Botrytis* Research (Verhoeff, K. *et al.* eds.), Pudoc Scientific Publishers, Wageningen, pp. 169-178.
4. Dubos, B., Roudet, J. and Bulit, J. (1983). Influence de la temperature sur lesaptitudes antagonists des *Trichoderma* a l'egard du *Botrytis cinerea* Pers. Les antagonisms microbiens. Colloq. l'INRA 18 : 81-90.
5. Elad, Y., Kohl, J. and Fokkema, N.J. (1994). Control of infection and sporulation of *Botrytis cinerea* on bean and tomato by saprophytic yeasts. Phytopathology 84 : 1193-1200.
6. Elad, Y., Shtienberg, D., Yunis, H. and Mahrer, Y. (1992). *In* Recent Advances in *Botrytis* Research (Verhoeff, K. *et al.* eds.), Pudoc Scientific Publishers, Wageningen, pp. 147-158.
7. Elad, Y. and Zimand, G. (1991). Experience integrated chemical-biological control of grey mould (*Botrytis cinerea*). WPRS Bull. 14 :195-199.
8. Grimont, P.A.D. and Grimont, F. (1981). *In* The Prokaryotes, Vol. II. (Starn, M.P. *et al.* eds.), Springer-Verlag, Berlin, pp. 1187-1203.
9. Gullino, M.L., Mezzalama, M. and Garibaldi, A. (1986). Biological and integrated control of grey mould : results and perspectives. Quad. Sc. Spec. Vitic. Ecol. 9 : 299-308.
10. Gullino, M.L., Aloi, C. and Garibaldi, A. (1989). Evaluation of the influence of different temperatures, relative humidities and nutritional supports on the antagonistic activity of *Trichoderma* spp. against grey mould of grape. Proceedings of the EC Experts Group, 100-104.

Plate I

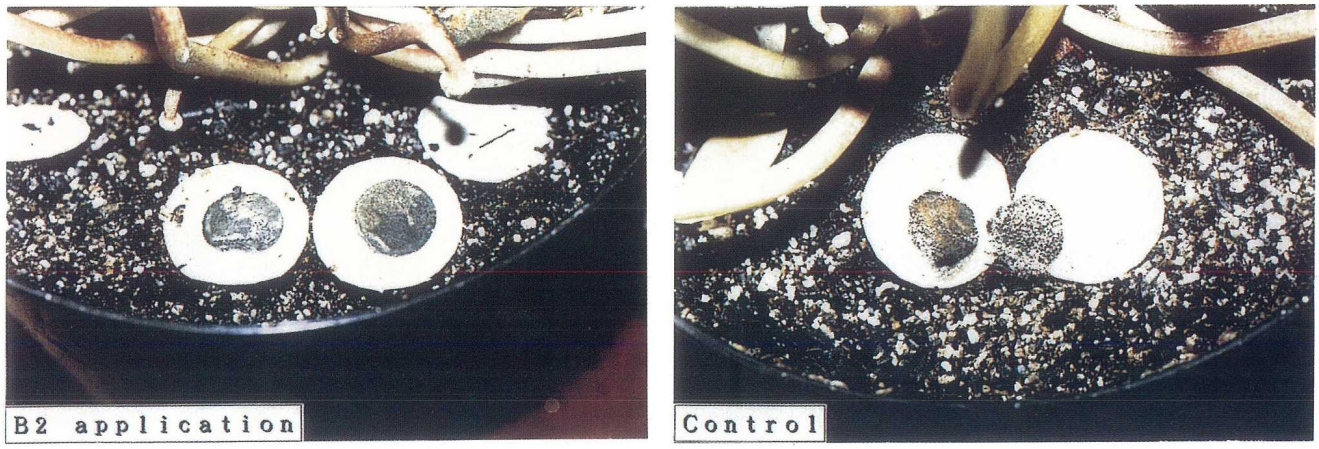


①

②



③



④

11. Heimpel, A.M. (1971). *In* Microbial Control of Insects and Mites. (Burges, H.D. and Hussey, N.W. eds.), Academic Press, New York and London, pp. 469-490.
12. Huber, L. and Gillespie, T.J. (1992). Modeling leaf wetness in relation to plant disease epidemiology. Annu. Rev. Phytopathol. 30 : 553-577.
13. Iyozumi, H., Akutsu, K., Hirayae, K., Tsuchiya, K., Hibi, T. and Okuyama, S. (1993). Biological control of broad bean chocolate spot disease by *Serratia marcescens* B2. Ann. Phytopathol. Soc. Jpn. 59 : 723 (Abstr. in Japanese).
14. Jarvis, W.R. (1980). *In* The Biology of *Botrytis*. (Coley-Smith, J.R. *et al*. eds.), Academic Press, London, New York, Toronto, Sydney, San Francisco, pp. 219-250.
15. Katan, R., Elay, Y. and Yunis, H. (1989). Resistance to diethofencarb (NPC) in benomyl-resistant field isolates of *Botrytis cinerea*. Plant Pathol. 38 : 86-92.
16. Bryant, M.P. (1984). *In* Bergey's Manual of Systematic Bacteriology. Vol. I (Kreig, N.R. *et al*. eds.), The Williams & Wilkins Company, Baltimore, pp. 477-484.
17. Kressies, A. (1993). Influence of environmental condition on dispersal of *Botrytis cinerea* conidia on postharvest infection of gerbera flowers grown under glass. Plant Pathol. 42 : 754-762.
18. McLaughlin, R.H.J., Wisnicwski, M.E., Wilson, C.L. and Chalutz, E. (1990). Effect of inoculum concentration and salt solutions on biological control of postharvest disease of apple with *Candida* sp. Phytopathology 80 : 456-461.
19. Okamoto, H., Sato, M., Sato, Z. and Isaka, M. (1991). *In* Plant Growth-Promoting Rhizobacteria Progress and Prospects (Keel, C. *et al*. eds.). WPRS Bull., Zurich. pp. 122-126.
20. Peng, G. and Sutton, J.C. (1993). Evaluation of microorganisms for biological control of *Botrytis cinerea* in strawberry. Can. J. Plant Pathol. 13 : 247-257.
21. Saik, J.E., Lacey, L.A. and Lacey, C.M. (1990). *In* Safety of Microbial Insecticides. (Laird, M. *et al*. eds.), CRC Press, Boca Raton, pp. 115-132.
22. Sutton, J.C. and Peng, G. (1993). Biological control of *Botrytis cinerea* in strawberry. Phytopathology 83 : 615-621.
23. Tanada, Y. and Kaya, H.K. (1993). Insect Pathology. Academic Press, San Diego and London.
24. Tronsmo, A. (1986). *Trichoderma* used as a biocontrol agent against *Botrytis cinerea* rots of strawberry and apple. Meld. Nor. Landbrukshogsk. 65 : 1-22.
25. Tukey, J.W. (1984). Exploratory data analysis. Reading, MA, Addison-Wesley.
26. Yamamoto, M., Iyozumi, H., Akutsu, K., Hirayae, K., Hibi, T. and Okuyama, S. (1992). Suppressive effects by *Serratia marcescens* B2 against *Botrytis fabae*-induced chocolate spot disease of broad bean. Ann. Phytopathol. Soc. Jpn. 58 : 582-583 (Abstr. in Japanese).

和 文 摘 要

伊代住浩幸・駒形智幸・平八重一之・土屋健一・日比忠明・阿久津克己：*Serratia marcescens* B2 によるシクラメン灰色かび病のバイオコントロール

キチナーゼ活性細菌 *Serratia marcescens* B2 株を用いて *Botrytis cinerea* によるシクラメン灰色かび病に対する防除効果を温室栽培条件下で検討した。B2 株懸濁液（*ca.* 1×10^9 cfu/ml）をポット栽培シクラメン（パステル系ヨハンシュトラウス）に噴霧処理した後に *B. cinerea* IH36（ベンズイミダゾールおよびジカルボキシイミド系薬剤耐性菌）の分生胞子懸濁液（*ca.* 1×10^5 個/ml）を接種し，花弁における発病程度を調べた。B2 株処理区の発病指数は無処理区に対して約 60%低く，その効果はイプロジオン 200 ppm と同程度であった。次に，B2 株のシクラメンにおける定着性を調べた結果，B2 株密度は花弁および葉身では著しく低下したが，葉柄基部や地際付近の土壌に置床した葉片では増加を示し，定着が示唆された。B2 株を前処理した葉片（径：12 mm）に *B. cinerea* 胞子を接種してシクラメン地際付近の土壌に置床し，これらの葉片における胞子形成を調べた。その結果，B2 株処理葉片において 85%以上の顕著な胞子形成阻害が認められた。

Explanation of plate

Plate I

1. Colonies of *Serratia marcescens* B2 on lima bean agar plate containing chitin. The bacterium produced a reddish pigment and formed clear zones around the colonies.
2. Gray mould on petals of cyclamen plants. Disease severity on petals (left) treated with *Serratia marcescens* B2 1 hr before inoculation with *Botrytis cinerea* conidia was reduced markedly compared with that on control petals (right) treated with distilled water before inoculation.
3. Disease index of gray mould on cyclamen petals. Scale of lesion diameter : 0, no lesion ; 1, $\Phi<1$ mm ; 2, 1 mm$\leqq\Phi<$ 3 mm ; 3, 3 mm$\leqq\Phi<$5 mm ; 4, $\Phi\geqq$5 mm ; 5, whole petal destroyed.
4. Sporulation of *Botrytis cinerea* on leaf discs placed on the soil near the base of cyclamen plants. The number of *B. cinerea* conidia on discs (left) treated with *Serratia marcescens* B2 was greatly reduced compared with that on discs (right) treated with distilled water before the inoculation.