J Gen Plant Pathol (2004) 70:367–370
DOI 10.1007/s10327-004-0134-7

DISEASE CONTROL

Short communication

**Nobutaka Someya · Masami Nakajima
Hiroshi Hamamoto · Isamu Yamaguchi · Katsumi Akutsu**

Effects of light conditions on prodigiosin stability in the biocontrol bacterium *Serratia marcescens* strain B2

Received: December 4, 2003 / Accepted: February 17, 2004

Abstract *Serratia marcescens* strain B2 is a potential biocontrol agent that produces the antibiotic pigment prodigiosin. When this strain was incubated under white or blue light conditions ($<100\,\mu mol\,m^{-2}s^{-1}$), prodigiosin concentration in bacterial cells decreased, but growth did not. However, red and far-red light had no effect on prodigiosin concentration in bacterial cells. Purified prodigiosin was degraded under white or blue light conditions but was not degraded under red and far-red light. Because white and blue light appeared to affect the stability of prodigiosin itself, light conditions may affect the suppressive effects of the biocontrol agent *S. marcescens* strain B2.

Key words Antibiotic · Biological control · Light conditions · Prodigiosin biosynthesis · *Serratia marcescens*

Numerous bacteria produce antibiotics to ensure their own survival. Some of these antibiotics are effective inhibitors of phytopathogens and play important roles in the biological control of plant diseases. One such biocontrol agent, *Serratia marcescens*, effectively inhibits the growth of several phytopathogenic fungi and suppresses some crop diseases (Akutsu et al. 1993; Iyozumi et al. 1996; Okamoto et al. 1998a; Someya et al. 2000). *S. marcescens* produces antifungal factors including lytic enzymes, such as chitinases, and the antibiotic pigment prodigiosin (Okamoto et al. 1998b; Someya et al. 2001). Prodigiosin is a tripyrrole antibiotic (Bennett and Bentley 2000; Marks and Bogorad 1960) and has been shown to inhibit effectively the growth of phytopathogenic fungi (Okamoto et al. 1998b; Someya et al. 2001, 2003a, 2003b). This pigment is therefore one of the most important antifungal factors produced by *S. marcescens*.

Prodigiosin is a secondary metabolite; and numerous factors, including biotic and abiotic factors, have been reported to affect its production in *S. marcescens* (Anğ-Küçüker et al. 1999; Blessing and Süssmuth 1991; Cang et al. 2000; Dierstein et al. 1989; Qadri and Williams 1972; Silverman and Munoz 1973; Solé et al. 1994, 1997; Tsang and Feng 1983; Viñas et al. 1987). Similarly, light influences prodigiosin production by *S. marcescens*; Ryazantseva et al. (1995) reported that visible light induced phototranslation of prodigiosin and suggested that the pigmented bacterium uses prodigiosin to store visible light energy. However, a clear relation between light conditions and prodigiosin stability has not been demonstrated for biological control. Disease suppression is attributed to the ability of biocontrol agents to produce an antibiotic, either in the rhizosphere or on the plant surface; thus, the suppressive effects of a biocontrol agent are readily influenced by environmental factors. In the present article we report that light conditions can affect prodigiosin stability in the biocontrol agent *S. marcescens* strain B2.

Serratia marcescens strain B2 was isolated from the phylloplane of a tomato plant and stored at Ibaraki University, Ibaraki, Japan (Akutsu et al. 1993). For bacterial growth, Luria-Bertani (LB) medium (Sigma-Aldrich Japan, Tokyo, Japan) was used. A suspension of *S. marcescens* strain B2 cells (approximately $10^9\,cfu\,ml^{-1}$) was inoculated onto LB agar plates with a sterile toothpick. Plates were incubated at 25°C under continuous dark conditions or under white, blue, red, or far-red light. White light was provided by an FL20SW white fluorescent tube (Toshiba, Tokyo, Japan). Blue (470 nm), red (660 nm), and far-red (730 nm) light was provided by MIL-B18, MIL-R18, and MIL-IF18 light-emitting diodes, respectively (Sanyo Electric Biomedical, Osaka, Japan). The fluence rate of each light source was measured using a LI-250 quantum

N. Someya[1] · M. Nakajima · K. Akutsu (✉)
School of Agriculture, Ibaraki University, Ami-machi, Ibaraki 300-0393, Japan
Tel. +81-298-88-8561; Fax +81-298-88-8562
e-mail: kakutsu@mx.ibaraki.ac.jp

H. Hamamoto · I. Yamaguchi
RIKEN (The Institute of Physical and Chemical Research) Plant Science Center, Yokohama, Japan

Present address:
[1]RIKEN (The Institute of Physical and Chemical Research) Plant Science Center, Yokohama, Japan

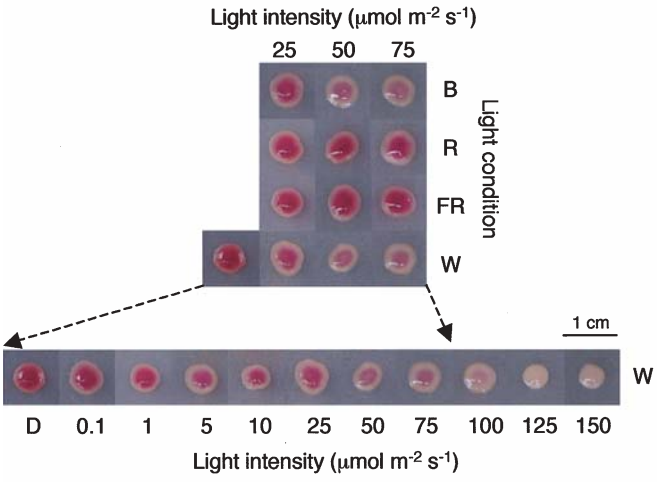

Fig. 1. Pigmentation of colonies of *Serratia marcescens* strain B2 on Luria-Bertani (LB) agar plates in the dark (*D*) or under blue (*B*), red (*R*), far-red (*FR*), or white (*W*) light. Each colony was incubated at 25°C for 72 h

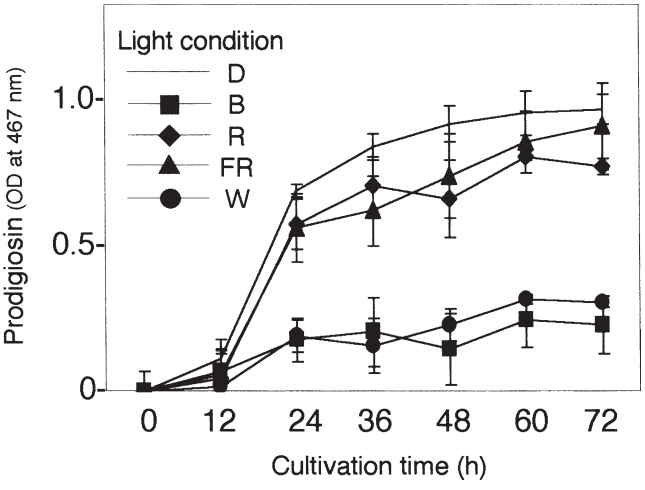

Fig. 2. Effect of light conditions on prodigiosin accumulation by *S. marcescens* strain B2. Bacteria were incubated on LB agar plates at 25°C in continuous dark (*D*) or under blue (*B*), red (*R*), far-red (*FR*), or white (*W*) light at 50 μmol $m^{-2} s^{-1}$. Prodigiosin was purified from 1 g of bacterial cells, and purified prodigiosin was dissolved in 100 ml of ethanol. Prodigiosin concentration was measured based on absorbance at 467 nm

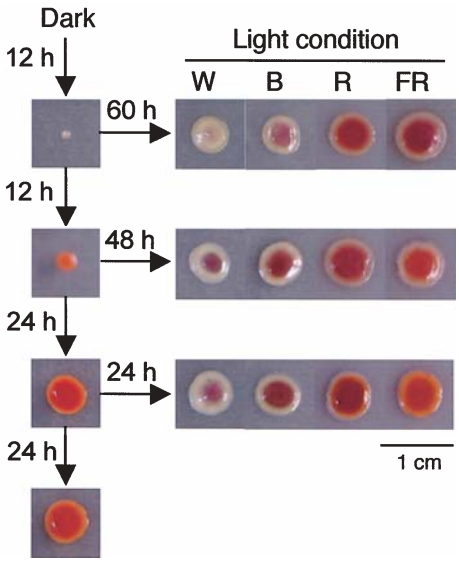

Fig. 3. Effect of light regimen on pigmentation of *S. marcescens* strain B2. Bacteria were incubated on LB agar plates at 25°C in the dark for 12, 24, or 48 h, then transferred to white (*W*), blue (*B*), red (*R*), or far-red (*FR*) light at 50 μmol $m^{-2} s^{-1}$

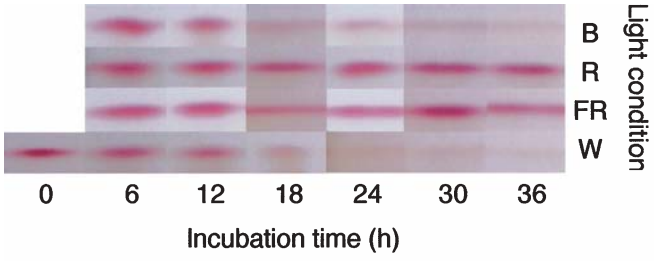

Fig. 4. Stability of prodigiosin under blue (*B*), red (*R*), far-red (*FR*), and white (*W*) light at 50 μmol $m^{-2} s^{-1}$. Purified prodigiosin was incubated at 25°C. After incubation, prodigiosin were refractionated using silica gel thin-layer chromatography. Bands indicate prodigiosin, with an R_f value in the range of 0.90 to 0.95

photometer (Li-Cor, Lincoln, NE, USA). The pigmentation of bacterial cells was observed after 72 h of incubation.

To examine the amount of prodigiosin, 1 g of bacterial cells, scraped from LB agar plates, was suspended in 9 ml of ethanol. Prodigiosin was then extracted from the cells by shaking this suspension for 1 h followed by centrifugation. The supernatant was then filtered through a 0.20-μm filter (Toyo Roshi, Tokyo, Japan) and concentrated under reduced pressure at room temperature in the dark. The ethanol extracts were then further extracted using chloroform. The chloroform extracts were fractionated using LK5 silica gel thin-layer chromatography (Whatman International, Maidstone, UK), and the pigment spots were scraped from the plates and extracted with ethanol. The red pigment having an *Rf* value in the range of 0.90 to 0.95 was defined as prodigiosin (Someya et al. 2001). Purified prodigiosin from 1 g of bacterial cells was then dissolved in 100 ml of ethanol. To determine prodigiosin concentration, the absorbance of each solution was measured at 467 nm using a spectrophotometer (Okamoto et al. 1998b; Someya et al. 2001). Each experiment was performed in triplicate.

To investigate the effects of light conditions on prodigiosin in the bacterial cells at a later stationary phase of bacterial growth, light conditions were changed during incubation. *S. marcescens* strain B2 was inoculated on LB agar

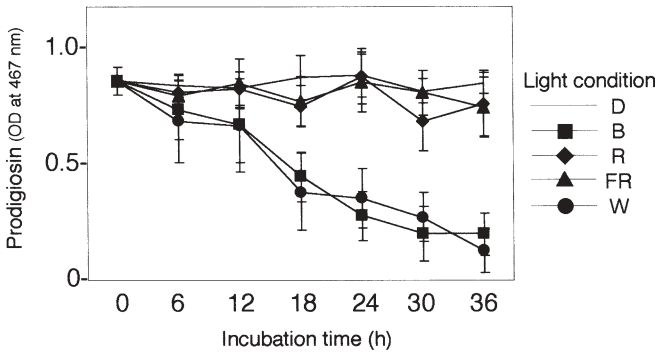

Fig. 5. Effect of light conditions on prodigiosin stability under dark (*D*), blue (*B*), red (*R*), far-red (*FR*), and white (*W*) light at 50 μmol $m^{-2}s^{-1}$. Purified prodigiosin from 1 g of bacterial cells was dissolved in 100 ml of ethanol. Prodigiosin solution was incubated at 25°C in the dark or under *B*, *R*, *FR*, or *W* light conditions. Prodigiosin concentration was measured based on absorbance at 467 nm

plates and was incubated at 25°C under dark conditions. After 12, 24, and 48 h of incubation, plates were transferred to white, blue, red, or far-red light conditions (50 μmol $m^{-2}s^{-1}$). The pigmentation of the bacterial cells was observed after 72 h of incubation.

Prodigiosin stability under various light conditions was investigated by purifying prodigiosin from bacterial cells incubated for 72 h under dark conditions. Purified prodigiosin from 1 g of bacterial cells was dissolved in 100 ml of ethanol, and 2 ml of this solution was poured into a petri dish (35 mm in diameter). Each petri dish was incubated at 25°C under dark conditions or under white, blue, red, or far-red light (50 μmol $m^{-2}s^{-1}$). The absorbance of prodigiosin was then measured at 467 nm using a spectrophotometer (Someya et al. 2001), and 100 μl of each solution was analyzed by silica gel thin-layer chromatography. Each experiment was performed in triplicate.

Growth of *S. marcescens* strain B2 was slightly inhibited under white light of 125 and 150 μmol $m^{-2}s^{-1}$ (Fig. 1). However, bacterial growth was not inhibited under blue, red, and far-red light conditions. Pigmentation of bacterial cells was inhibited by white light irradiation; and although the inhibitory effects were dependent on light intensity, pigmentation was inhibited even under weak white light (>0.1 μmol $m^{-2}s^{-1}$) conditions. Under white light of 125 and 150 μmol $m^{-2}s^{-1}$, bacterial cells were mostly whitish. Pigmentation of bacterial cells was not influenced by red or far-red light irradiation. However, blue light irradiation inhibited the pigmentation of bacterial cells in a manner similar to that seen with white light. Large amounts of prodigiosin accumulated in bacterial cells in the dark and under red or far-red light (Fig. 2). However, prodigiosin accumulation under white or blue light was lower than that in bacterial cells under red or far-red light.

Pigmentation of bacterial cells was initially observed after 18–24 h of incubation in the dark. White or blue light inhibited pigmentation of the bacterial cells at early stages of bacterial growth (Fig. 3). Red or far-red light had no effect on pigmentation. In addition, purified prodigiosin was degraded under white and blue light but was not degraded under red or far-red light (Figs. 4, 5). Almost all of the prodigiosin was degraded after 36 h of white or blue light irradiation at 50 μmol $m^{-2}s^{-1}$.

Antibiotic production is now recognized as an important mechanism in the function of biological control agents. For example, a prodigiosin-defective mutant of *S. marcescens* reportedly fails to suppress cucumber damping off (Okamoto et al. 1998a). Numerous factors, including biotic and abiotic factors, have been reported to affect prodigiosin production in *S. marcescens*. We also previously reported that some rice-isolated bacteria inhibited prodigiosin biosynthesis in *S. marcescens* without inhibiting growth (Someya et al. 2003a, 2003b). However, a clear relation between light conditions and prodigiosin stability had not been demonstrated. In the present study, prodigiosin in bacterial cells was strongly affected by white or blue light, and prodigiosin itself was degraded by white or blue light. These data suggest that prodigiosin, one of the important antifungal factors in *S. marcescens*, may be influenced by light conditions on the plant surface. We previously reported that *S. marcescens* strain B2 suppressed cyclamen gray mold, damping off, and fusarium wilt caused by *Botrytis cinerea*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *cyclaminis*, respectively (Iyozumi et al. 1996; Someya et al. 2000). The suppressive effects of *S. marcescens* strain B2 on aerial diseases was lower than that on soil diseases. We presume that this is due to antibiotic degradation by visible light, thus reducing the suppressive effects of *S. marcescens*. We are currently focusing on prodigiosin biosynthesis and degradation in *S. marcescens* strain B2 on plant surfaces under field conditions. The practical use of biocontrol agents under various climatic conditions requires further study in the field.

References

Akutsu K, Hirata A, Yamamoto M, Hirayae K, Okuyama S, Hibi T (1993) Growth inhibition of *Botrytis* spp. by *Serratia marcescens* B2 isolated from tomato phylloplane. Ann Phytopathol Soc Jpn 59: 18–25

Anğ-Küçüker M, Büyükbaba-Boral Ö, Tolun V, Törümküney D, Susever S, Anğ Ö (1999) Effect of some antibiotics on pigmentation in *Serratia marcescens*. Zentralbl Bakteriol Int J Med Microbiol Virol Parasitol Infect Dis 289:781–785

Bennett JW, Bentley R (2000) Seeing red: the story of prodigiosin. Adv Appl Microbiol 47:1–32

Blessing B, Süssmuth R (1991) Influence of combined chemicals on prodigiosin synthesis of *Serratia marcescens*. Environ Toxic Water 6:371–382

Cang S, Sanada M, Johdo O, Ohta S, Nagamatsu Y, Yoshimoto A (2000) High production of prodigiosin by *Serratia marcescens* grown on ethanol. Biotechnol Lett 22:1761–1765

Dierstein R, Kaiser I, Weckesser J (1989) Inhibition of prodigiosin formation in *Serratia marcescens* by extracts of toxic cyanobacteria. Syst Appl Microbiol 12:244–248

Iyozumi H, Komagata T, Hirayae K, Tsuchiya K, Hibi T, Akutsu K (1996) Biological control of cyclamen gray mould (*Botrytis cinerea*) by *Serratia marcescens* B2. Ann Phytopathol Soc Jpn 62:559–565

Marks GS, Bogorad L (1960) Studies on the biosynthesis of prodigiosin in *Serratia marcescens*. Proc Natl Acad Sci USA 46:25–28

Okamoto H, Sato M, Sato Z, Isaka M (1998a) Biocontrol of *Phytophthora capsici* by *Serratia marcescens* F-1-1 and analysis of biocontrol mechanisms using transposon-insertion mutants. Ann Phytopathol Soc Jpn 64:287–293

Okamoto H, Sato Z, Sato M, Koiso Y, Iwasaki S, Isaka M (1998b) Identification of antibiotic red pigments of *Serratia marcescens* F-1-1, a biocontrol agent of damping-off of cucumber, and antimicrobial activity against other plant pathogens. Ann Phytopathol Soc Jpn 64:294–298

Qadri SMH, Williams RP (1972) Induction of prodigiosin biosynthesis after shift-down in temperature of nonproliferating cells of *Serratia marcescens*. Appl Microbiol 23:704–709

Ryazantseva IN, Andreyeva IN, Klementyeva GS, Ogorodnikova TI, Petrov VY (1995) Pigment-dependent light influence on the energetics of *Serratia marcescens*. Thermochim Acta 251:63–67

Silverman MP, Munoz EF (1973) Effect of iron and salt on prodigiosin synthesis in *Serratia marcescens*. J Bacteriol 114:999–1006

Solé M, Rius N, Francia A, Lorén JG (1994) The effect of pH on prodigiosin production by non-proliferating cells of *Serratia marcescens*. Lett Appl Microbiol 19:341–344

Solé M, Francia A, Rius N, Lorén JG (1997) The role of pH in the "glucose effect" on prodigiosin production by non-proliferating cells of *Serratia marcescens*. Lett Appl Microbiol 25:81–84

Someya N, Kataoka N, Komagata T, Hirayae K, Hibi T, Akutsu K (2000) Biological control of cyclamen soilborne diseases by *Serratia marcescens* strain B2. Plant Dis 84:334–340

Someya N, Nakajima M, Hirayae K, Hibi T, Akutsu K (2001) Synergistic antifungal activity of chitinolytic enzymes and prodigiosin produced by biocontrol bacterium, *Serratia marcescens* strain B2 against gray mold pathogen, *Botrytis cinerea*. J Gen Plant Pathol 67:312–317

Someya N, Nakajima M, Watanabe K, Hibi T, Akutsu K (2003a) Influence of bacteria isolated from rice plants and rhizospheres on antibiotic production by the antagonistic bacterium *Serratia marcescens* strain B2. J Gen Plant Pathol 69:342–347

Someya N, Numata S, Nakajima M, Watanabe K, Hasebe A, Hamamoto H, Yamaguchi I, Akutsu K (2003b) Influence of environmental factors on the antagonism of a biocontrol agent and the genetic improvement for the development of the useful agents. In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW (eds) Proceedings of the sixth international workshop on plant growth-promoting rhizobacteria, Indian Institute of Spices Research, Calicut, India, pp 603–611

Tsang JC, Feng JS (1983) Effect of polymyxin B on the synthesis of prodigiosin and its precursors in *Serratia marcescens*. J Antibiot 36:1564–1566

Viñas M, El-Ebiary A, Guinea J, Loren JG (1987) Effect of gramicidin A and valinomycin on prodigiosin production by non-proliferating *Serratia marcescens*. J Antibiot 40:113–115