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Effect of light quality on *Bacillus amyloliquefaciens* JBC36 and its biocontrol efficacy

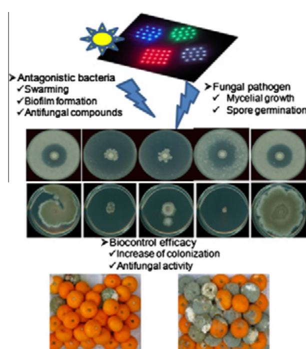
Sang-Mi Yu [a], Yong Hoon Lee [a,b,*]

[a] Division of Biotechnology, Chonbuk National University, 194-5 Ma-Dong, Iksan, Jeonbuk 570-752, Republic of Korea
[b] Advanced Institute of Environment and Bioscience, and Plant Medical Research Center, Chonbuk National University, Republic of Korea

HIGHLIGHTS

- Light quality affected growth, swarming motility, and biofilm formation.
- Light affected on production of secondary metabolites and antifungal activity.
- Red light increased the colonization on fruit surface.
- Red light increased biocontrol efficacy on green mold.

GRAPHICAL ABSTRACT



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ABSTRACT

Light is one of the most important environmental signals regulating physiological processes of many microorganisms. However, very few studies have been reported on the qualitative or quantitative effects of light on control of postharvest spoilage using antagonistic bacteria. In this study, we investigated the effects of white, red, green, and blue light at photon flux densities of 40, 240, and 360 μmol m^{-2} s^{-1} on *Bacillus amyloliquefaciens* JBC36 (JBC36), which has been reported as a promising candidate for biocontrol of green and blue mold on mandarin fruit. With the exception of blue light at 240 and 360 μmol m^{-2} s^{-1}, light generally stimulated growth of JBC36 compared to the controls grown in the dark. Red light increased swarming motility irrespective of intensity and significantly enhanced biofilm formation at 240 μmol m^{-2} s^{-1}. Production of antifungal metabolites and antifungal activity on *Penicillium digitatum* was also affected by light quality. Interestingly, antifungal activity was significantly increased when JBC36 and *P. digitatum* was co-incubated under red and green light at an intensity of 240 μmol m^{-2} s^{-1}. We also demonstrated that the quality of light resulted in changes in colonization of JBC36 on mandarin fruit and control of green mold. In particular, red light increased the population level on mandarin fruit and biocontrol efficacy against green mold. These results represent the first report on the effect of light quality on an antagonistic bacterium for the control of postharvest spoilage. We believe that an improved understanding of the JBC36 response to light quality may help in the development of strategies to increase biocontrol efficacy of postharvest spoilage.

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1. Introduction

Pathogenic fungi infecting plants and post-harvested agricultural products are the major threat to the production of economically valuable food and their storage. Application of huge amount of fungicides increases cost of food production and also leads to environmental hazards. Biocontrol using antagonistic

* Corresponding author at: Division of Biotechnology, Chonbuk National University, Republic of Korea. Fax: +82 63 850 0834.
E-mail address: yonghoonlee@jbnu.ac.kr (Y.H. Lee).

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microorganisms and their metabolic compounds to prevent post-harvest spoilage has emerged as a promising alternative to chemical pesticides (Alvindia and Natsuaki, 2009; Gálvez et al., 2010; Yu et al., 2010, 2012). Several strains of *Bacillus* genus are reported for their production of anti-fungal metabolites, which are potential biocontrol agents against wide range of fungal pathogens (Arguelles-Arias et al., 2009). Several studies reported that combination of chemical substances (such as glycolchitosan, essential oils, and various salts) with microbial agents enhance their antagonistic properties (Du Plooy et al., 2009; Hao et al., 2011; Smilanick et al., 2008; Torres et al., 2007). Visible light (Buchert et al., 2011) and blue light (Alfereza et al., 2012) were also employed to delay de-greening and control the post-harvest spoilage, respectively. Surprisingly, no reports were found to illustrate the combined effect of light and antagonistic bacterium. Hence, in the present study, we analyzed the effect of light quality on the antagonistic bacteria, *B. amyloliquefaciens* JBC36 (JBC36) and their effect on controlling post-harvest spoilage.

For most microorganisms light is one of the crucial environmental signals for regulating physiological processes (Mussi et al., 2010; Oberpichler et al., 2008; Tisch and Schmoll, 2010). These processes include stress responses, production of secondary metabolites, and multicellular behaviors, such as the formation of biofilm and swarming. Recently, Singh et al. (2009) reported that 33% of genes in *Synechocystis* species PCC6714 are regulated with response to changes in light quality.

Colonizing plant surfaces effectively and maintaining a mass of cells sufficiently have been demonstrated to be prerequisites for plant protective effects in antagonistic bacteria (Arguelles-Arias et al., 2009; Bais et al., 2004; Droby et al., 2009; Liu et al., 2010). These processes rely on surface motility and efficient biofilm formation, which are affected by light quality of the bacterial cell populations' environment. In a previous study, light was shown to repress the expression of flagella genes in *Agrobacterium tumefaciens*; alternatively, bacteria grown in the dark are more motile (Oberpichler et al., 2008). In the same study, reduced attachment of *A. tumefaciens* to tomato (*Solanum lycopersicum* L.) roots and decreased tumor formation in cucumber were also observed in the presence of light compared to those grown in darkness. Similarly, another study also demonstrated that *Escherichia coli* K12 showed a decreased biofilm formation and elevated motility when grown under blue light (van der Horst et al., 2007). In spite of the importance of light on ecological and metabolic processes of microorganisms, the effects of light on biocontrol agents have not been investigated in detail. Indeed, understanding the influence of light on biocontrol agents is essential for the design of new control strategies for postharvest spoilage.

Satsuma mandarin (mandarin; *Citrus unshiu* Marc.) fruits are usually stored in non-refrigerated commercial or farm warehouse, after their harvest. Green and blue molds caused by *P. digitatum* and *Penicillium italicum*, respectively, spoil 10–25% of mandarin fruits during storage in Korea. In a previous study, we reported the antagonistic bacterium JBC36 as a promising candidate for biocontrol of green and blue mold on mandarin fruit (Yu et al., 2012). Bacterial strain JBC36 suppressed the green and blue mold by producing antifungal metabolites and volatile organic compounds.

The objective of this study was to investigate the effect of light quality on the interaction between antagonist JBC36 and green mold on mandarin fruits. The influences of light wavelength and intensity on growth and production of antibiotics of JBC36 were primarily determined. Moreover, the effects of light quality on antifungal and biocontrol activity were also investigated. To the best of our knowledge, this is the first report on the effect of light quality on an antagonistic bacterium for the control of postharvest spoilage.

2. Materials and methods

2.1. Custom design of a LED panel

LED light source (embedded in plastic sheet of 8 cm diameter, 0.7 cm height) was custom designed at ODTech Co., Ltd. (Jeonju, South Korea) in such a way that each light source emits particular light wavelength with specific photosynthetically active photon flux densities (PPFDs). Distinctive LED light sources were used in this study to produce four different light wavelengths i.e. red (wavelength range from 630 to 655 nm with a typical light emission at 645 nm), green (wavelength range from 497 to 544 nm with a typical light emission at 524 nm), blue (wavelength range from 448 to 475 nm with a typical light emission at 458 nm) and white light. The LED light sources were connected to a circuit box that allowed to control of light intensity for each wavelength. As each light source was 8 cm in diameter, it can provide uniform light intensity from center to edges of Petri dish (9 cm diameter) and 100 ml conical flask

2.2. Effect of light on growth of bacterial strain JBC36

Bacterial strain JBC36 was previously selected as a biocontrol agent for the suppression of green and blue mold of mandarin fruits (Yu et al., 2012). The strain JBC36 was revived from glycerol stock (which was stored at −80 °C) on potato dextrose agar (PDA) plate. Single colony of JBC36 from PDA was inoculated into potato dextrose broth (PDB) and incubated at 25 °C and 180 rpm in darkness overnight. The overnight culture was centrifuged at 3000 rpm for 15 min to pellet down the bacterial cells and supernatant was discarded. The cell pellet was resuspended with fresh PDB in such a way that the final cell density was ca. 1×10^6 cfu/ml. Twenty milliliters aliquots of this culture were transferred to 100 ml conical flasks (Schott Duran, Germany). Individual culture flasks were kept on green, red, blue and white light sources; each with three different (40, 240 and 360) PPFDs. In order to avoid the effect of ambient light, the flasks were covered with aluminum foil. Control flasks were covered with aluminum foil without any light source. These flasks were incubated at 25 °C and 180 rpm in shaking incubator. The light sources did not affect the temperature of the growth media significantly (25.0 ± 1 °C). Growth of the bacterial strain was spectrophotometrically monitored by measuring optical density (OD) at 600 nm 48 h after incubation (HAI). Each treatment consisted of three replicates and was repeated three times.

2.3. Effect of light quality on swarming pattern and colony morphology of JBC36

Motility tests were performed as previously described (Kearns and Losick, 2003) with small modifications. Swarm agar plates (9 cm diameter) containing 20 ml of Luria Bertani (LB) media fortified with 0.7% agar were prepared fresh. Ten microliters of overnight grown (in LB media at dark) bacterial cells (1×10^6 cfu/ml) were transferred to the center of swarming plates. Light source was fixed immediately above the lid of the Petri dishes and the plates were incubated under white, red, green, and blue light sources at intensities of 40, 240 and 360 μmol m^{-2} s^{-1}. Control plates were incubated in the dark. Swarming motility was recorded by measuring colony diameter 12 HAI at 25 °C. This experiment was repeated three times with two replications.

2.4. Assay of biofilm formation

Biofilm formation was evaluated as described previously (O'Toole and Kolter, 1998) with small modifications. Briefly, strain

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