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Induction of systemic resistance in Chinese cabbage against black rot by plant growth-promoting rhizobacteria

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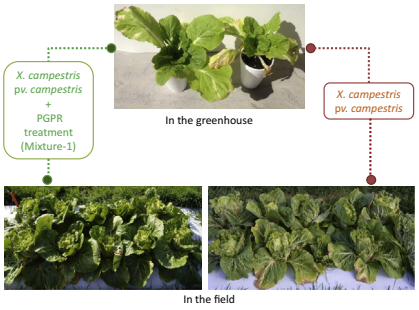
HIGHLIGHTS

- Plant growth-promoting rhizobacteria induced systemic resistance to black rot.
- Two mixtures of strains were formed for biocontrol of black rot.
- The marketable yield was increased by individual strains and mixtures of strains.

GRAPHICAL ABSTRACT



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ABSTRACT

Black rot, caused by *Xanthomonas campestris* pv. *campestris*, is the most important and potentially destructive disease of cabbage. The objectives of this study were to select plant growth-promoting rhizobacteria (PGPR) strains and to form strain mixtures with the capacity to elicit induced systemic resistance or to increase plant growth in Chinese cabbage. In preliminary screening, 10 of 12 tested individual PGPR strains (AP136, AP188, AP209, AP213, AP217, AP218, AP219, AP282, AP295, and AP305) reduced the number of foliar lesions, and 2 PGPR strains (AP7 and AP18) increased all tested parameters of plant growth promotion, including shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and plant diameter. In advanced tests, four individual stains (AP136, AP209, AP282 and AP305) were combined into mixture-1. Mixture-2 contained all strains in mixture-1 plus three additional stains (AP7, AP18 and AP218). Both mixtures and three individual strains (AP136, AP209 and AP305) significantly reduced the number of black rot lesions, and mixture-2 increased shoot dry weight and root dry weight in greenhouse tests. In a field test, all the tested treatments significantly reduced disease incidence on whole plants at three weeks after transplanting and reduced head disease severity at harvest time. All treatments also increased marketable yield compared to the nonbacterized control. These results demonstrated that specific individual PGPR strains and strain mixtures induced systemic resistance to black rot in the greenhouse and field.

1. Introduction

Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), is the most important and potentially destructive disease of cabbage (Williams, 1980; Vicente and Holub, 2013). Black rot may arise from systemic infection (infected seeds) and from secondary spread. Infected seed is a source of secondary infections (Akhtar, 1989) if the bacteria are exuded from the hydathodes, which are natural openings on the leaf edge that connect to the xylem. Splashing rain or sprinkler irrigation can spread the pathogen from the source plant to hydathodes of neighboring plants (Hugouvieux et al., 1998).

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Tactics for management of black rot include using hot water treatment (Nega et al., 2003), certified disease-free transplants and seeds, resistant cultivars, biological control, and chemical control (Mew and Natural, 1993). Biological control can reduce pesticide usage, making it an attractive alternative management option for crop protection (Waard et al., 1993; Chandler et al., 2011). The use of plant growth-promoting rhizobacteria (PGPR) as biopesticides is reported to be an effective way to reduce the use of agrichemicals (Banerjee et al., 2005). PGPR are beneficial bacteria that influence the growth (Khalid et al., 2004), yield (Mia et al., 2010), and nutrient uptake of the plant (Liu et al., 2013). Some PGPR strains also provide biological control of plant disease (Chithrashree et al., 2011; Beneduzi et al., 2012). The two main genera of PGPR strains include asporogenous fluorescent *Pseudomonas* spp. and sporogenous *Bacillus* spp. (Piggot and Hilbert, 2004; Figueiredo et al., 2011). Although the preponderance of PGPR studies have been with fluorescent *Pseudomonas* spp., most commercially available PGPR are bacilli (Sivasakthi et al., 2014). This is because *Bacillus* spp. can form dormant endospores by the process of sporulation, and these spores are tolerant to heat, desiccation, UV irradiation and organic solvents (Nicholson, 2002).

PGPR exhibit two major mechanisms of biological control: including antagonism (Beneduzi et al., 2012), which is a direct mechanism, and induced systemic resistance (Kloepper et al., 2004), which is an indirect mechanism. Biological control of black rot by antagonistic bacteria has been demonstrated experimentally with PGPR on crucifers (Wulff et al., 2002; Massomo et al., 2004; Monteiro et al., 2005; Mishra and Arora, 2012b). Compared to antagonism, with ISR, the physiological (Benhamou et al., 1996) and metabolic response (Ongena et al., 2000) of the host plant is altered, leading to an enhanced synthesis of plant defense chemicals to challenge the pathogen. Some PGPR strains have induced systemic resistance against multiple plant diseases (Kloepper et al., 1997; Ramamoorthy et al., 2001). In addition to having the capacity for biocontrol, PGPR have been reported to enhance plant growth directly by a wide variety of mechanisms, including biological nitrogen fixation (Bhattacharjee et al., 2008), solubilization of mineral phosphate (Yazdani et al., 2009), secretion of plant hormones (Idris et al., 2007), and siderophore production (Sharma and Johri, 2003).

Although the beneficial effects of PGPR on plants usually are separated into two categories: biological control (Beneduzi et al., 2012) and growth promotion (Vessey, 2003), there is a close relationship between them. A single PGPR strain can exhibit both of these effects through multiple mechanisms (Wahyudi and Astuti, 2011). In search of efficient PGPR strains, multiple traits related to plant growth promotion and biocontrol activity were tested together during the screening process, and selected strains showed multiple functions related to crop production (Ahmad et al., 2008; Praveen Kumar et al., 2014).

Currently there is very limited knowledge available regarding the biological suppression of black rot in cabbage by induced systemic resistance. Objectives of this study were to 1) screen individual PGPR strains *in vitro* for multiple traits reported to be related to growth promotion and induction of systemic resistance to black rot *in planta*, and 2) form mixtures of PGPR strains based on results from objective 1 and evaluate them in the greenhouse and field.

2. Materials and methods

2.1. PGPR strains and inoculum preparation

Twelve PGPR strains (*Bacillus velezensis* AP136, AP188, AP213, AP218, AP219, AP295, and AP305; *Bacillus safensis* AP7; *Bacillus altitudinis* AP18; *Bacillus mojavensis* AP209; *Fictibacillus solisalsi* AP217; *Lysinibacillus macrolides* AP282) from the culture collection of Auburn University were used in the study. The bacteria were maintained in tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA), supplemented with 20% glycerol at −80 °C. For *in vitro* tests, inoculum of PGPR was grown on tryptic soy agar (TSA) at 28 °C for 48 h. For *in planta* tests, theses strains were used as spore preparations (Zhang et al., 2010).

2.2. Xanthomonas campestris pv. campestris and inoculum preparation

X. campestris pv. *campestris* strain OHS-001B-92 was provided by J. Olive, Ornamental Horticulture Research Center, Mobile, Alabama, and stored under the conditions described above. For experimental use, Xcc was grown on Yeast Dextrose Calcium Carbonate Agar plate (YDC) at 28 °C for 72 h (Schaad and Alvarez, 1993). A single colony was incubated in 25 ml TSB with continuous shaking (150 rpm) at 28 °C for 48 h. Bacterial cultures were centrifuged at 3500 rpm for 15 min. Pellets were resuspended in sterilized water, and the concentration was adjusted to 10^8 CFU/ml for challenge inoculation.

2.3. Preliminary screening

Four traits reported to be related to plant growth promotion were tested *in vitro*: nitrogen fixation, phosphate solubilization, siderophore production, and indole-3-acetic acid (IAA) production. Presumptive nitrogen fixation was qualitatively evaluated by growing the PGPR in the nitrogen-free semisolid medium (JNFb) as described by Olivares et al. (1996). Phosphate solubilizing capacity was qualitatively evaluated by the plate assay using National Botanical Research Institute's phosphate growth medium (NBRIP) which contained calcium phosphate as the inorganic source of phosphate (Nautiyal, 1999). Siderophore production was qualitatively evaluated by Chrome Azurol S medium (Alexander and Zuberer, 1991). IAA production was assayed by the quantitative analysis using ferric chloride-perchloric acid reagent ($FeCl_3$-$HClO_4$) (Gordon and Weber, 1951). Each of these tests was conducted three times.

The induction of systemic resistance to black rot was tested *in planta*. Kaboko hybrid organic Chinese cabbage seeds (Park Seed, Hodges, SC 29653) were planted in germination trays containing 25 cm^2 holes. One ml of PGPR spore suspension (10^7 CFU/ml) was applied to each seed prior to covering with commercial potting substrate (Sunshine mix, Sun Gro Horticulture, Agawam, MA 01001). Seeds were placed in a temperature controlled greenhouse at the Plant Science Research Center at Auburn University. Ambient air temperature in the greenhouse was maintained at 25 °C day/21 °C night throughout the year. Fourteen days after seeding, cabbage seedlings were transplanted into 10 cm diameter round pots. Each pot was drenched with 50 ml of PGPR spore suspension (10^6 CFU/ml) at transplanting time. Freshly prepared suspensions of Xcc were sprayed onto the leaves two weeks after transplanting. Pathogen-challenged plants were placed into a dark dew chamber (100% humidity) for two days at 24 °C, and then moved to the greenhouse. Pots were watered daily as needed. Fourteen days after pathogen challenge, total lesion number was recorded for each plant. Plants were harvested at the same time and the following plant parameters were measured: shoot fresh weight, shoot dry weight (oven dry at 90 °C), root fresh weight, root dry weight, and plant diameter. The experiment included 13 treatments (12 single PGPR strains and a non-bacterized but pathogen-challenged disease control) arranged in a randomized complete block design (RCBD) with 8 replications, with a single cabbage per pot.