



Formulations of *Bacillus subtilis* BY-2 suppress *Sclerotinia sclerotiorum* on oilseed rape in the field



Xiaojia Hu^a, Daniel P. Roberts^{b,*}, Lihua Xie^a, Jude E. Maul^b, Changbing Yu^a, Yinshui Li^a, Mulan Jiang^a, Xiangsheng Liao^a, Zhi Che^a, Xing Liao^{a,*}

^a Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, People's Republic of China

^b Sustainable Agricultural Systems Laboratory, USDA-Agricultural Research Service, Beltsville, MD 20705-2350, USA

HIGHLIGHTS

- *Bacillus subtilis* BY-2 controlled *Sclerotinia sclerotiorum* on oilseed rape in field trials.
- Control with *B. subtilis* BY-2 was similar to the standard chemical control used in China.
- *B. subtilis* BY-2 responded to oilseed rape root exudate and was detected within root tissue.

GRAPHICAL ABSTRACT



BY-2 + oilseed rape seed → healthy plant



Untreated oilseed rape seed → diseased plant

ARTICLE INFO

Article history:

Received 27 September 2013

Accepted 9 December 2013

Available online 14 December 2013

Keywords:

Bacillus subtilis

Biological control

Colonization

Formulation

Oilseed rape

Sclerotinia sclerotiorum

Spray

ABSTRACT

We are developing a collection of *Bacillus* strains, isolated from different environments, for use in controlling *Sclerotinia sclerotiorum* on oilseed rape in China and elsewhere. Strain BY-2, isolated from internal tissues of an oilseed rape root, was demonstrated to be *Bacillus subtilis* based on biochemical and morphological characteristics and on 16S RNA gene sequence. Photographic evidence from gnotobiotic studies using the *lacZ*-tagged strain BY-2(pUC18) confirmed that this strain was capable of colonizing internal root tissues. Strain BY-2 did not effectively colonize the ectorhizosphere or the surface of the stems or leaves of oilseed rape when applied in pellet or wrap seed treatment formulations. Populations of BY-2 dropped from 10^8 CFU seed⁻¹ to 10^4 CFU g root⁻¹ and $\leq 10^2$ CFU g stem⁻¹ or leaf⁻¹ after 60 days. Strain BY-2 was applied as a pellet seed treatment formulation alone, as a spray at flowering alone, and as the pellet seed treatment formulation combined with the spray application in two field trials at the Wuxue location conducted in two consecutive years. These three treatments containing BY-2 provided disease control (disease incidence) and mean seed yield that was similar to the chemical control treatment and significantly greater than the pellet without bacteria and non-treated control treatments. All three of these BY-2 treatments were similar to each other with regard to these two metrics and to treatments containing *B. subtilis* Tu-100, a genetically distinct strain previously shown to be effective against this disease. In two additional field trials, conducted in consecutive years at the Wuhan location, strain BY-2 applied as a wrap seed treatment formulation alone, as a spray at flowering alone, and as the wrap seed treatment combined with the spray application provided disease control (disease incidence) and mean seed yield that was similar to the chemical control treatment. These three BY-2 treatments also were significantly greater than the non-treated control treatments and compared favorably to treatments containing Tu-100. There was no evidence of BY-2 promoting growth of oilseed rape when applied in the pellet or wrap seed treatment formulations in field trials conducted at the Wuxue or at the Wuhan loca-

* Corresponding authors. Address: Sustainable Agricultural Systems Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, United States Department of Agriculture – Agricultural Research Service, Beltsville, MD 20705-2350, USA. Fax: +1 301 504 6491 (D.P. Roberts). Dr. Liao works for the Key Laboratory and Genetic Improvement of Oils Crops, Oil Crops Research Institute, Wuhan. Fax: +86 27 86816451 (X. Liao).

E-mail addresses: dan.roberts@ars.usda.gov (D.P. Roberts), liaofox@oilcrops.cn (X. Liao).

tions. We now have three *Bacillus* strains (*B. subtilis* strains BY-2 and Tu-100, *B. megaterium* A6) that control *S. sclerotiorum* on oilseed rape in the field that can be tested in strain combinations for enhanced disease control. We also have multiple methods for application of *Bacillus* strains as both seed treatment and foliar applications were effective.

Published by Elsevier Inc.

1. Introduction

Sclerotinia stem rot of oilseed rape, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is an important disease in the People's Republic of China and other regions of the world (Purdy, 1979; Boland and Hall, 1994; Lu, 2003; Zhao and Meng, 2003). *Sclerotinia sclerotiorum* overwinters as sclerotia in soil and generally infects plants as mycelia originating from these sclerotia or as airborne ascospores that directly penetrate host leaf or stem tissue (Abawi and Grogan, 1979; Lu, 2003). Crop rotation to nonsusceptible hosts and the application of fungicides are the typical methods for controlling diseases caused by *S. sclerotiorum* (Yu and Zhou, 1994b; Lu, 2003). Crop rotation as a disease control tactic can be ineffective due to the wide host range of this pathogen and the long-term persistence of sclerotial survival structures in soil (Nelson, 1998). Fungicides have limitations as they can be expensive and hazardous, and there are concerns regarding the development of fungicide resistance in populations of *S. sclerotiorum* (Gossen et al., 2001). Also, traditional oilseed rape breeding programs for disease resistance have been hampered by a limited gene pool (Lu, 2003). Alternative disease management strategies for this pathogen on oilseed rape are clearly needed.

Biological control of *S. sclerotiorum* has received considerable attention from scientists as an alternative disease management strategy due to its potential to provide safe and environmentally compatible disease control (Hu et al., 2011). However, acceptance of commercial biological control products by growers using conventional crop production systems has been slow due, in part, to inconsistent performance by biological control agents (Pierson and Weller, 1994; Fravel, 2005; Glare et al., 2012). Disease control efficacy and consistency of performance with regard to soilborne pathogens can be strongly impacted by abiotic (e.g. mineral concentrations, pH, oxygen tensions) and biotic factors (genetic structure of populations of target and nontarget pathogens, soil microbial community) that vary in the soil, and by crop production methods that influence the plant and soil environment (Handelsman and Stabb, 1996; Compant et al., 2005; Roberts and Kobayashi, 2011). One approach to overcoming inconsistent performance by microbial biological control agents is the integration of multiple microbes into individual biological control formulations (Lemanceau and Alabouvette, 1991; Pierson and Weller, 1994; Raupach and Kloepper, 1998). A second approach uses multiple methods of applying microbial biological control agents (Wilson, 1996, 1997; Ji et al., 2006). With regard to *S. sclerotiorum* on oilseed rape, applications of microbial biological control agents as a seed treatment and as a foliar spray at flowering may improve disease control as this pathogen can infect the host near the soil line and in the foliar canopy.

Strains from the genus *Bacillus*, including those from *Bacillus subtilis* (Ehrenberg), are ideal candidates for biological control strategies because they have properties consistent with commercial development of microbes for use in sustainable agriculture (Jacobsen et al., 2004). Most notably, they produce a number of antibiotics (Stein, 2005; Ongena and Jacques, 2007) and have

been demonstrated to have a durable shelf-life due to their ability to form endospores (e.g. Hu et al., 2011, 2013a). Our long-term goal is to develop commercially viable products based on combinations of *Bacillus* and other isolates for use in sustainable production strategies for oilseed rape in The People's Republic of China and elsewhere. Although commercial products for control of *Sclerotinia* exist (de Vrije et al., 2001) they are not used in China, possibly due to monetary exchange rates (Hu et al., 2005). We have isolated two *Bacillus* strains, *B. subtilis* Tu-100 and *B. megaterium* A6, that provide control of *S. sclerotiorum* on oilseed rape in the field when applied as seed treatment formulations (Hu et al., 2011, 2013a). *Bacillus megaterium* (de Bary) A6 has also been shown to promote growth of oilseed rape; most likely through phosphate solubilization (Hu et al., 2013b). The major objective of this study was to determine the ability of an additional *Bacillus* isolate, strain BY-2, to control *S. sclerotiorum* on oilseed rape in the field using seed treatment and foliar application methods.

2. Materials and methods

2.1. Bacterial and fungal isolates

Bacillus subtilis BY-2 was isolated from internal tissues of an oilseed rape root (*Brassica napus* L. cv. Zhongshuang 4) from a research plot at the Oil Crops Research Institute, Wuhan. *Bacillus subtilis* Tu-100 was isolated from the rhizosphere of oilseed rape in Jingzhou County, Wuhan Province, and shown to control *S. sclerotiorum* disease on oilseed rape (Hu et al., 2005; Hu et al., 2011). Strain BY-2 was routinely cultured in Luria–Bertani (LB) (Miller, 1972) broth supplemented with streptomycin (50 µg mL⁻¹) and rifampicin (50 µg mL⁻¹) while strain Tu-100 was routinely cultured in LB broth supplemented with kanamycin (30 µg mL⁻¹) and rifampicin (50 µg mL⁻¹). Strain BY-2 and Tu-100 were naturally resistant to these antibiotics at these concentrations. Strain BY-2(pUC18) was constructed by introduction of plasmid pUC18 (Promega Corp., Wuhan, People's Republic of China), containing *lacZ*, into isolate BY-2 by electroporation (Chassy et al., 1988). Strain BY-2(pUC18) was similar to strain BY-2 in growth rate, colony morphology, *in vitro* inhibition of *S. sclerotiorum* Ss-1, and inhibition of isolate Ss-1 in detached-leaf assays (data not shown). Strain BY-2(pUC18) was cultured on LB broth or agar plus ampicillin (50 µg mL⁻¹), streptomycin (50 µg mL⁻¹), and rifampicin (50 µg mL⁻¹). All bacterial strains were stored long-term in 20% glycerol at –70 °C. *Sclerotinia sclerotiorum* Ss-1 was isolated from a sclerotium formed on oilseed rape (Hu et al., 2005). Isolate Ss-1 was maintained on potato dextrose agar (PDA) at 20–22 °C. All microorganisms were obtained from the culture collection of the Plant Protection Laboratory, Oil Crops Research Institute, Wuhan, People's Republic of China. Strains BY-2 and Tu-100 are also held in the Agricultural Culture Collection of China (Beijing, China) as ACCC 06137 and ACCC 05846, respectively, and *S. sclerotiorum* Ss-1 is held as ACCC 36169.

Download English Version:

<https://daneshyari.com/en/article/4503934>

Download Persian Version:

<https://daneshyari.com/article/4503934>

[Daneshyari.com](https://daneshyari.com)