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Bacillus Atrophaeus SYR 15b, a Promising Biocontrol Strain to Protect Barley Against Common Root Rot (*Cochliobolus sativus)*

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Abstract

Common root rot (CRR) is a devastating soil-borne fungal disease caused by *Cochliobolus sativus*. In recent years, some antifungal bacteria have been applied for biocontrol of pathogenic fungi. The present work was carried out to study the potential of *Bacillus atrophaeus SYR 15b* for the biocontrol of CRR by comparing plots with and without artificial inoculation under field experimental conditions. The universal susceptible barley genotype WI2291 was used, and a 0-5 scale based on the percentage of infected subcrown internodes (SCIs) surface was applied. The data showed that *B. atrophaeus SYR 15b* strain had a significant (P<0.001) antagonistic activity against the *C. sativus in vitro* (zone of inhibition was 67.3 mm), and in field where the percentage of infected SCIs averaged 26.66%, compared to the untreated controls (62.98%). Hence, the level of infection for *Bacillus* treatments was reduced by 60% compared to controls. In view of these, we can consider that *B. atrophaeus SYR 15b* strain is a promising natural biocontrol agent that could be used against CRR of barley.

Keywords: *Bacillus atrophaeus*, *Cochliobolus sativus*, common root rot, field test, antagonistic activity

1. Introduction

Common root rot (CRR), caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dast. [anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.], is an important disease of barley (*Hordeum vulgare* L.) found worldwide [3, 12]. It causes a brown to black discoloration of the subcrown internodes (SCIs) of barley (*Hordeum vulgare* L.), which is directly related to yield losses [6]. Barley susceptibility to CRR is commonly evaluated under field conditions by determining the visible disease symptoms as a percentage of SCIs [10].

Fungicides are currently the most widespread method to control CRR disease, but the long-term use of these chemicals are hazardous to humans and environment [4], therefore, alternative approaches that are potent and environmentally friendly require to be developed. Biocontrol is the most accepted option method for plant disease management, since it offers an effective and safe approach to avoid the drawbacks of fungicides [13, 17].

Among the bio-control bacteria, *Bacillus* has become the bacterium of choice for its flexibility and capacity to contain a big number of plant pathogens in different environments [12, 14].

During preliminary experiments, more than 525 bacilli were isolated from different regions of Syria. *B. atrophaeus SYR 15b* had a highest *in vitro* antagonism impact against various soil pathogens [7]. In the present work, the antagonistic activity of *B. atrophaeus SYR 15b* strain against *C. sativus* was evaluated under field control conditions.

2. Materials and Methods

2.1. Bacterial isolate

The *B. atrophaeus SYR 15b* strain was isolated from Syrian soil samples (AL-Jebsah E: 040º44º33.2 / N: 36º03º49.6) [1] and screened among *Bacillus* isolates on NB culture, the bacterial colonies were identified according to Wulff et al. [22]. A pure culture of *SYR 15b* strain was grown on NB and incubated for 24 h at 30 °C.

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2.2. Fungal isolate

The most virulent isolate (*Cs* 16) of *C. sativus* described by Arabi and Jawhar [2] was used in the experiments. The infected barley tissues with *Cs* 16 were cut into small pieces (10 mm long) and sterilized with 5% sodium hypochlorite for 5 min, then washed three times with sterile distilled water and transferred to Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI. USA) amended with 13 mg/l kanamycin sulphate, and incubated in the dark for 7 days at 20 ±1 ºC. The conidial suspension was adjusted to 5 X 10^5 condia/ml [2].

2.3. *In vitro* test

B. atrophaeus SYR 15b was streaked a few times until single colonies of a single type were observed on the NA plates. Then 5 mm diameter disc of *C. sativus* was cut from of an actively growing culture by a sterile cork borer and placed on the center of NA plates. Mycelial disc on Nutrient Agar (NA) medium without bacteria was used as control. Every elementary treatment was repeated five times. Mean diameter was measured after 4 days of incubation at 25°C. The inhibition of fungal growth was noted as described by Rabindran and Vidyasekaran [19].

2.4. Field test

Seeds of the universal susceptible barley cultivar WI2291 from Australia were inoculated with the *C. sativus Cs*16 isolate. Seed inoculation was performed according to the method described by van Leur [21], where, 30g barley seeds was immersed in a plastic Petri dish (12-cm diameter) containing 10g sterile neutralized peat, 40 ml spore suspension (5 × 10^5 condia/ml) and 8 drops of natural Arabic gum. The components were mixed thoroughly and then seeds were planted at 6 cm depth to promote long subcrown internodes [10] in with five replicate plots (1 m x1 m) separated with a 1-m wide borders. Each plot consisted of five rows, 20 cm apart and with 50 seeds per row. Based on laboratory preliminary tests on PDA media, bacteria and *CRR-free seeds were used* as controls.

2.5. CRR evaluation

Plants were examined 7 weeks post-inoculation by measuring the percentage of SCIs surface showing disease symptoms using a 0-5 scale, as described by Kokko *et al.* [10], where 0 (resistant); 1 = HT (highly tolerant):small light brown lesions covering 1-10% of the SCI; 2 = T(tolerant):light brown lesions covering 11-25% of the SCI; 3 = MS (moderately susceptible): light brown/black lesions covering 26-40% of the SCI; 4 = S (susceptible): black lesions covering 41-75% of the SCI; 5 = HS (highly susceptible): black lesions covering 76-100% of the SCI.

2.6. Statistical analysis

The means and standard deviations were determined and statistically analyzed using analysis of variance (ANOVA), and means with $p < 0.001$ were considered statistically significant [9].

3. Results and Discussion

In this present work, antagonistic potential of the *B. atrophaeus SYR 15b* strain against *C. sativus* fungus was noticed *in vitro* by forming inhibition zone on NA culture plate as shown in photo-plate comparing with the control (Fig. 1), and the average diameter for the zones of inhibition was 67.3 mm. This antagonism was checked directly on barley susceptible plants under field experiments by comparing plots with and without artificial inoculation.

CRR produced brown-dark lesions on SCIs, and these symptoms were severe on the susceptible cultivar WI2291, whilst no symptoms were observed in the control (Fig. 1). The results are in agreement with our previous observations under natural field conditions [2].

The data showed that *B. atrophaeus SYR 15b* strain had a significant (P<0.001) antagonistic activity against *C. sativus* under field conditions (Tables 1 and 2) where the percentage of infected SCIs averaged 26.66%, compared to the untreated controls (62.98%). Hence, the level of infection for *Bacillus* treatments was reduced by 60% compared to controls (Fig. 2).

Table 1. Analysis of variance for *B. atrophaeus SYR 15b* applications on barley CRR

Source	df	Mean Square	F-Value	P-Value
Treatment with bacteria	1	376.310	116.761	<.0001
Replicate	4			
Residual		132		

Table 2. Effect of *B. atrophaeus SYR 15b* applications on *C. sativus* of susceptible barley WI2291 expressed as percent of infected subcrown internode surface

Treatment	% SCI infected surface
Treated with bacteria only	0.00C
Treated with fungus only	62.98A
Treated with bacteria +fungus	26.66B

Means followed by different letters differ significantly at $P<0.001$ (ANOVA test)

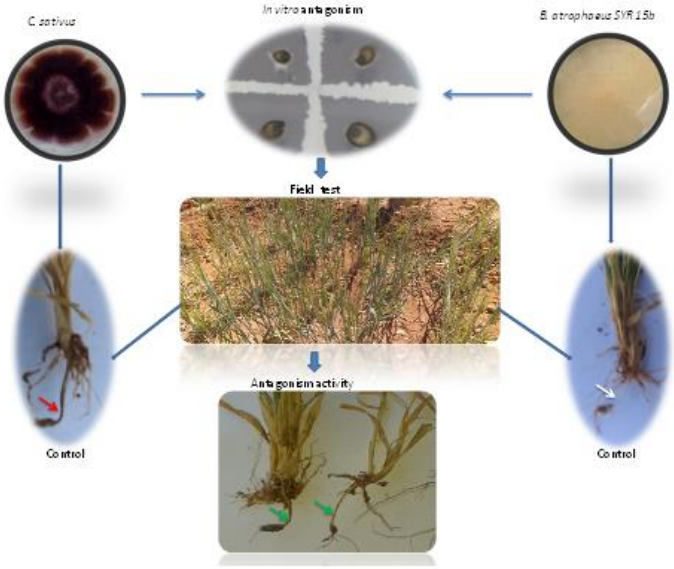

Figure 1. Scheme for *B. atrophaeus SY15b* antagonistic activity against *C. sativus* fungus *in vitro* and field tests.

B. atrophaeus has an ability to produce pigments in media containing organic nitrogen compounds, and it was earlier classified as *B. subtilis* var. *niger* [18]. Therefore, we identified *SYR15b* strain as *B. atrophaeus*. Moreover, 16S rRNA gene sequencing ratified the identification, that it was most closely related to *B. atrophaeus*, ATCC 49337 (96 % similarity), and it was deposited in GenBank under accession number MT159352 [8].

Our data demonstrated the field sensitivity of *C. sativus* to the antagonistic activity of *B. atrophaeus SYR 15b* strain, similar with those of antagonism against *Sclerotium rolfsii* [11] and *Verticillium dahlia* [16]. Miljaković, Dragana *et al.* [15] and Cao *et al.* [5] reported that *Bacillus* species have abilities to inhibit the growth of several phytopathogens fungi, which can be attributed to the production and secretion of antifungal compounds and antibiotics belonging to the family of iturins and subtilins that act on the fungi's cell wall. In addition, Rahman *et al.* [20] stated that the fungal mycelial malformation might be attributed to the antibiotic metabolites produced by the bacteria, which can penetrate and cause protoplasmic dissolution and disintegration. The highly antifungal effects on *C. sativus* in this work might be due to one or more antifungal compounds produced by this biocontrol agent.

4. Conclusion

In this work, *B. atrophaeus SYR 15b* strain was an effective biocontrol agent against *C. sativus* in barley plants under field conditions and reduced CRR by 60%, therefore, it has much potential for use as an economical and environmentally safe method to control this soil fungal pathogen.

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Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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