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Efficacy of the bacterium *Lysobacter enzymogenes* strain ch3B10 as a new biocontrol agent on the pathogenic fungi *Alternaria solani* and *Fusarium oxysporum* under laboratory conditions

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Abstract

Background: The plant pathogenic fungi, *Alternaria solani* and *Fusarium oxysporum*, are considered among the fungal pathogens which cause severe damages to tomato plants. The application of chemicals fungicides reduced fungal infection and entails great risks to human health and to the environment. Using biological control agents is considered one of the most effective techniques which suppress fungal pathogens and preserve the environment. The beneficial bacterium, *Lysobacter enzymogenes*, is applied as a biocontrol agent against different plant pathogens.

Results: The present work was carried out, under laboratory conditions, to investigate the efficacy of *L. enzymogenes* different concentrations of the strain ch3B10 on controlling the linear growth of *A. solani* and *F. oxysporum* compared to using the fungicide Benlate®. Treatment with Benlate® and the highest concentration (2×10^8 CFU/ml) of *L. enzymogenes* strain ch3B10 showed the highest inhibition of 70.6–94.0% on linear growth of all the tested fungi. Also, treatments with two concentrations (2×10^6 and 2×10^7 CFU/ml) of *L. enzymogenes* strain ch3B10 inhibited linear growth of all tested fungi by means of 47.1–69.7%. The low concentration of 2×10^3 CFU/ml of the strain ch3B10 resulted in the lowest linear growth inhibition 19.8–28.1% in all tested than the check treatment.

Conclusion: Further experiments under both greenhouse and field conditions are needed to approve the efficacy of the strain ch3B10 as an effective bioagent and ecologically safer approach than chemical treatments.

Keywords: Biological control, In vitro conditions, Lytic activity, Antagonistic fungi and parasitic fungi

Background

Tomato is a widespread crop cultivated all over the world. Tomato cultivation has been attacked by many pathogens including fungi, bacteria, viruses and nematodes (Jones et al. 2000). Tomato plants are affected by several or many fungal pathogens, *Alternaria solani* and *Fusarium oxysporum*, which caused diseases including early blight and *Fusarium* wilt, respectively. These diseases resulted in a severe yield loss worldwide and considered very difficult to be controlled (Dun-chun et al. 2016).

In spite of their negative influence on the environment and human health and its high cost, chemical fungicides have been used extensively for the management of many fungal diseases (Terna et al. 2016). Lately, demand is increasing for applying eco-friendly management methods (Odhiambo et al. 2017).

The potential of the genus *Lysobacter* as a biological control agent for controlling plant pathogenic diseases

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has been described since 1978 because of their antagonistic relationships with other microorganisms (Odhiambo 2021). *Lysobacter* from the family *Xanthomonadaceae* in *Gammaproteobacteria* is Gram-negative bacteria with gliding motility characterized by a high G + C content, and antimicrobial lytic activity against different microorganisms (Reichenbach 2006). Over 30 *Lysobacter* spp. have been identified and used as biocontrol agents against various plant pathogens, e.g. bacteria, fungi and nematodes (Singh et al. 2015). Recently, *Lysobacter* has been applied as a biological control agent against different fungal pathogens (Zhang et al. 2011).

The most important common features of all *Lysobacter* species are secreting different extracellular enzymes, including the production of chitinases, glucanases, proteases and lipases (Vasilyeva et al. 2014). Different studies have revealed that chitinases have the most important role in biocontrol (Ko et al. 2009).

Thus, the objective of this research was to evaluate the antagonistic effects of *L. enzymogenes* strain ch3B10 on *A. solani* and *F. oxysporum* under laboratory conditions.

Methods

Lysobacter enzymogenes strain ch3B10 isolation, identification and growth conditions.

Lysobacter enzymogenes strain ch3B10, a bioagent (Ba) isolated from mango soil samples collected from Jazan area southwest KSA (Alharbi 2017). Isolated strain was purified, identified and recorded in Gene bank under the accession No. LT601528. The strain ch3B10 was stored in sterilized 20% (vol/vol) glycerol at − 82 °C until use (Kobayashi et al. 2005).

In all experiments, the strain ch3B10 was cultured at 28 ± 2 °C with shaking overnight (200 rpm) on 10% tryptic soy agar medium. The bacterial suspension was centrifuged at 3000 g and concentrated up to 2×10^8 CFU/ml by measuring absorbance at optical density of 590 nm (OD_{590}) (Palumbo et al. 2003).

Source of pathogenic fungi

Two isolates of each of the pathogenic fungus, *Alternaria solani* (Sorauer) and *Fusarium oxysporum* (Synder & Hans), were obtained from the culture collection of the Biology Department, Science College, Jazan University KSA, which isolated from infected tomato plants.

All fungal isolates were cultured on Petri plates filled with a Czapek Dox agar (CDA) medium at 28 ± 2 °C. Pure cultures grown on CDA plates were saved for further work.

In vitro assay for *L. enzymogenes* strain ch3B10 and the fungicide Benlate® was developed on growth diameters of *A. solani* and *F. oxysporum* isolates.

Under laboratory conditions, two experiments were conducted to study the efficiency of the bioagent, *L. enzymogenes* strain ch3B10, using four concentrations of 2×10^3, 2×10^6, 2×10^7 and 2×10^8 colony-forming unit (CFU)/ml compared to one concentration 50 µg/ml of the fungicide Benlate®. The inhibition % of linear growth diameters of *A. solani* and *F. oxysporum* isolates was determined. Petri plates were filled with CDA medium (20 ml/each) and inoculated at the centre with 5-mm fungal disc from a week-old culture of each isolate of *A. solani* and *F. oxysporum* by using cork borer.

Data of fungal growth diameter inhibition percentage/each treatment were determined 3 and 5 days later. Two hundred and forty Petri plates (120 plates/each experiment) were used. Plates were incubated at 28 ± 2 °C. Treatments were replicated to 10 times. Ten plates of each fungal isolate untreated with the bioagent were used as a check treatment.

Statistical analysis

Data obtained were statistically analysed using ANOVA procedure (SAS 1997). Comparison among means was made via the least significant difference test (LSD) at ≤ 5% level of probability.

Results

Data presented in Tables 1 and 2 showed the effects of *L. enzymogenes* strain ch3B10 and the fungicide Benlate® on linear growth inhibition % of the tested fungi after 3 and 5 days of incubation. Treatments with the fungicide, Benlate® and the highest concentration of *L. enzymogenes* strain ch3B10 (2×10^8 CFU/ml) showed great inhibitions of 70.6–94.0% on linear growth of *A. solani* and *F. oxysporum* isolates followed by treatments of the two concentrations (2×10^6 and 2×10^7 CFU/ml) of *L. enzymogenes* which resulted in 47.1–69.7% inhibition on linear growth of all tested fungal isolates. Meanwhile, treatment with the lowest concentration (2×10^3 CFU/ml) of *L. enzymogenes* revealed that the lowest inhibition % on linear growth of *A. solani* and *F. oxysporum* ranged 17.9–30.3% than the linear growth of check treatment.

Discussion

The present data indicated that treatments with different tested concentrations of *L. enzymogenes* strain ch3B10 resulted in a significant inhibition on linear growth of all tested species of *F. oxysporum* and *A. solani* under laboratory conditions. Many investigations reported that all known strains of *L. enzymogenes* have been considered bioagents against several microorganisms through their ability of produced different extracellular degradation enzymes (Pidot et al. 2014).

Table 1 Effect of *Lysobacter enzymogenes* strain ch3B10 (Ba) and the fungicide Benlate® on the linear growth (cm) of two *Fusarium oxysporum* isolates after 3 and 5 days of incubation and inhibition % (*I*)

Treatments	Isolate 1				Isolate 2			
	3 days	*I***	5 days	*I*	3 days	*I*	5 days	*I*
Check*	3.4 a	0.0	9.9 a	0.0	3.5 a	0.0	10.0 a	0.0
Ba (CFU/ml)								
2×10^3	2.4 b	29.4	6.9 b	30.3	2.7 b	22.9	7.4 b	26.0
2×10^6	1.8 c	47.1	3.6 c	63.6	1.6 c	54.3	4.2 c	58.0
2×10^7	1.4 cd	58.8	3.0 c	69.7	1.3 cd	62.9	3.5 c	65.0
2×10^8	1.0 de	70.6	1.0 d	89.9	0.9 de	74.3	0.9 d	91.0
Benlate® 0.50 (µg/ml)	0.5 e	85.3	0.5 d	93.9	0.5 e	85.7	0.6 d	94.0

*Untreated plates. $I^{**} = 1 -$ [diameter of growth zone in the test plate/diameter of growth zone in the control (check) plate] $\times$ 100. Data are averages of 10 replicates. Values, within each column, followed by the same letter(s) are not significantly different at ($P \leq 0.05$)

Table 2 Effect of *Lysobacter enzymogenes* strain ch3B10 (Ba) and the fungicide Benlate® on the linear growth (cm) of two *Alternaria solani* isolates after 3 and 5 days of incubation and inhibition % (*I*)

Treatments	Isolate no. 1				Isolate no. 2			
	3 days	*I*	5 days	*I*	3 days	*I*	5 days	*I*
Check*	3.9 a	*0.0*	8.6 a	*0.0*	4.6 a	*0.0*	10.0 a	*0.0*
Ba (CFU/ml)								
2×10^3	3.2 b	17.9	6.9 b	19.8	3.4 b	26.1	7.4 b	26.0
2×10^6	2.0 c	48.7	4.0 c	53.5	2.1 c	54.3	4.6 c	54.0
2×10^7	1.3 d	66.7	3.3 c	61.6	2.2 c	52.2	3.1 c	69.0
2×10^8	1.0 de	74.4	0.8 d	90.7	1.2 d	73.9	1.0 d	90.0
Benlate® 0.50 (µg/ml)	0.6 e	84.6	0.6 d	93.0	0.6 d	87.0	0.6 d	94.0

Legend as in Table 1

The observations provide persuasive evidence that strain ch3B10 could have enzymatic activity like that produced by other *Lysobacter* strains against a variety of plant fungal pathogens. The present results are in harmony with those of Odhiambo et al. (2017).

Previous work indicated that culture filtrates of *L. enzymogenes* strains (3.1T8 and SB-K88) caused inhibition on fungal spore germination (Islam et al. 2005). Jochum et al. (2006) reported that *L. enzymogenes* strain C3 was very effective as a biological control agent against *Fusarium graminearum*.

Also, the studies of Postma et al. (2008) indicated the suppressive effect of several *Lysobacter* species on *Rhizoctonia solani.* Zhao et al. (2017) reported that *L. enzymogenes* strain OH11 could attach, penetrate and lyse the hyphae of *Aphanomyces cochlioides* and *Pythium aphanidermatum*.

Conclusions

Present observations made in this study refer to the efficacy of *L. enzymogenes* strain ch3B10 on inhibiting growth of *F. oxysporum* and *A. solani* under laboratory conditions. These inhibition activities may be attributed to the inhibition effect against mycelium growth or degradation of fungal structures. Insertion of strain ch3B10 as a biocontrol agent in integrated pest management systems for controlling plant pathogens needs further studies under greenhouse and field conditions.

Abbreviations

CFU: Colony-forming unit; I%: Inhibition %; CDA: Czapek Dox agar; Ba: *Lysobacter enzymogenes* Strain ch3B10 as a bioagent.

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