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Inhibition of the mycelial growth of cucurbit disease pathogens by *Streptomyces* sp. STS1 strain

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Abstract Cucurbits are used in diets worldwide. However, they are subject to damage that can further lead to various diseases. Here, we report that the STS1 strain, from Matsue city in Japan, can inhibit the mycelial growth of pathogenic fungi infecting cucurbits (*Colletotrichum orbiculare, Corynespora cassiicola, Monosporascus cannonballus, and Stagonosporopsis cucurbitacearum*). STS1 strain can also be grown in the presence of fungicides. These results indicate that the STS1 strain might be able to control diseases caused by pathogenic fungi in cucurbits.

Keywords : *Streptomyces*, Cucurbits, Plant pathogenic fungi, Biological control

Introduction

The cucurbit plant family, which includes cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin, and squash (*Cucurbita* sp.) are taxonomically divided into 118–122 genera, including 940–980 species (Khalaf and Raizada, 2018). Cucurbits are used in diets worldwide. However, the cucurbit plant is subject to damage that can further lead to several diseases. The control strategies applied for cucumber diseases mainly involve those that use chemical fungicides. Over time, this leads to the development of resistance towards some chemicals. (FRAC, 2018; Ishii *et al*., 2001; Lebeda *et al*., 2010; McGrath *et al*., 2017; Miyamoto *et al*., 2010). However, the development of resistance to microbial fungicides has not yet been reported. Therefore, the development of new microbial fungicides is required to control cucurbit diseases. In a previous study, we suggested that microorganisms (STS1: *Streptomyces blastmyceticus*) isolated from Matsue city (Shimane prefecture) can control cucumber anthracnose caused by *Colletotrichum orbiculare* (Ueno *et al*., 2012). However, the inhibitory effects of the STS1 strain against other pathogenic fungi of cucurbits have not yet been clearly elucidated.

In this study, we examined the STS1 strain as a potential biological control agent against pathogenic fungi infecting cucurbits.

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Materials and Methods

Cultivation of the bacterial and plant pathogen

The STS1 strain was grown on Luria-Bertani (LB) medium (10 g bactotryptone, 10 g NaCl, and 5 g yeast extract dissolved in a final volume of 1 L distilled water) and was used to individually inoculate test tubes containing 20 mL LB medium. The liquid cultures were incubated at 25 ± 2℃ with constant shaking on a rotary shaker (130 rpm).

The plant pathogens (*Colletotrichum orbiculare, Corynespora cassiicola, Monosporascus cannonballus* and *Stagonosporopsis cucurbitacearum*) were grown on potato sucrose agar (PSA; 200 g potato, 20 g sucrose, 20 g agar, 1 L distilled water) medium at 25 ± 2℃ for 10–14 days.

Dual culture assay

The antagonistic activity of STS1 strain against the pathogenic fungi (*C. orbiculare, C. cassiicola, M. cannonballus, S. cucurbitacearum*) was studied using the dual culture assay employing potato sucrose agar (PSA) medium as described previously (Lemtukei *et al*. 2016,

2017). Mycelial plugs of plant pathogenic fungi and paper discs (8 mm) for antibiotic tests were placed on PSA plates, 4.5 cm apart. Subsequently, the paper disc was inoculated with culture (30μL) from the isolate. LB liquid medium was used for control treatments. All petri dishes were incubated at 25±2℃ for 14 days, and the mycelial area (cm^2) was measured using the LIA 32 software (http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index-e.html).

Fungicide sensitivity test

To determine the fungicide sensitivity of the STS1 strain, Benlate (Benomile: Kumiai Chemical Industry Co. Ltd.), Kantasudoraifuroaburu (Boscalid: Nippon Soda Co., Ltd.), Ososaido (Captan: Hokko Chemical Industry Co., Ltd.), Polyoxin AL (Polyoxin: Kumiai Chemical Industry Co. Ltd.), and Rizolex (Tolclofosmethyl: Hokko Chemical Industry Co., Ltd.) were used in this experiment. Each fungicide was diluted (500-fold) in liquid LB media and mixed thoroughly before being dispensed on labeled 9-cm Petri plates. Culture suspensions of the STS1 strain (50μL) were spread on LB media in the presence of fungicides, and the plates were incubated at 25±2℃. After 48 h, the growth of the STS1 strain was observed. LB medium without the addition of fungicide was used as a control.

Statistical analysis

Data are presented in terms of the mean ± standard deviation values. Statistically significant differences were determined using the t -test ($P < 0.05$).

Results and Discussion

The inhibitory activity of the STS1 strain towards the mycelia growth of pathogenic fungi of cucurbits was evaluated using a dual culture method employing PSA. The STS1 strain inhibited mycelial growth of the pathogenic fungi (*C. orbiculare, C. cassiicola, M. cannonballus, S. cucurbitacearum*) to a greater extent than that observed in the control plates (Fig. 1A).

Mycelial growth of *C. orbiculare, C. cassiicola, M. cannonballus, and S. cucurbitacearum* was inhibited by the STS1 strain (mycelial area, 17.2±0.2 cm^2, 16.0±0.2 cm^2, 23.2±0.4 cm^2, 21.8±0.3 cm^2) (Fig. 1B). In the control plates, the mycelial area of *C. orbiculare, C. cassiicola, M. cannonballus,* and *S. cucurbitacearum* was 25.0±2.7 cm^2, 25.0±1.7 cm^2, 31.5±0.7 cm^2, and 28.7 ±4.2 cm^2, respectively (Fig. 1B). This result suggested that the STS1 strain can inhibit both soil-borne and aboveground diseases caused by pathogenic fungi.

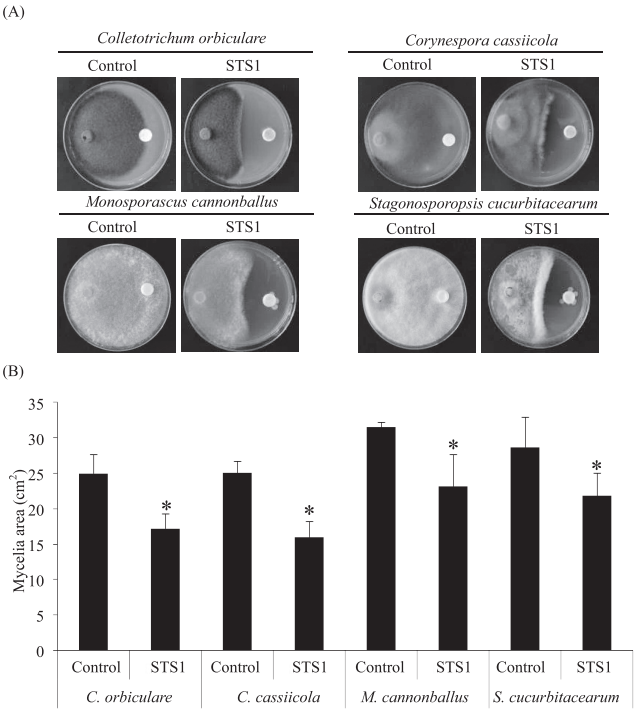

Fig. 1 Antagonistic activity of the STS1 strain to the mycelia growth of pathogenic fungi of cucurbits observed by dual culture assay on potato sucrose agar plate (A) and the mycelial area of pathogenic fungi of cucurbits without or with the STS1 strain (B). The experiments were repeated three times. Bar: represents±SD. Asterisk indicate the significant difference compared with the result of the control (t-test, $P < 0.05$).

Furthermore, we determined the agrochemical sensitivity of the STS1 strain. The growth of STS1 was observed in the presence of Benlate, Kantasudoraifuroaburu, Ososaido, Polyoxin AL, and Rizolex on LB medium (Table 1). It is known that these agrochemicals are used against cucurbit diseases. This indicates that these agrochemicals can be used for the control of cucurbit diseases along with the STS1 strain.

Table 1 Effect of agrochemical on growth of the STS1 strain

Isolate	Fungicides	
STS1	Benlate (Benomile)	+
	Kantasudoraifuroaburu (Boscalid)	++
	Ososaido (Captan)	+
	Polyoxin AL (Polyozin)	++
	Rizolex (Tolclofosmethyl)	+

-: no growth, +: scanty growth, ++: maximum growth

Further studies are required to investigate the suppression effect of cucurbit diseases by the STS1 strain in field conditions.

These results suggest that the STS1 strain might be useful in controlling diseases caused by pathogenic fungi in cucurbits.

References

Fungicide resistance action committee (FRAC). (2018) Online. http://www. frac.info/publications/downloads.

Ishii, H., Fraaije, B. A., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T. and Hollomon, D. W. (2001) Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. Phytopathology, **91**: 1166-1171.

Khalaf, E. M. and Raizada, M. N. (2018) Bacterial seed endophytes of domesticated cucurbits antagonize fungal and oomycete pathogens including powdery mildew. Frontiers in Microbiology, **9**: 42. doi:10.3389/fmicb.2018.00042. eCollection 2018.

Lebeda, A., McGrath, M. T. and Sedláková, B. (2010) Fungicide resistance in cucurbit powdery mildew fungi. Fungicides, 221-246.

Lemtukei, D., Tamura, T., Nguyen, Q. T., Kihara, J., and Ueno, M. (2016) Antagonistic potential of isolated microorganisms from soil in Shimane prefecture against rice blast disease cause by *Magnaporthe oryzae*. Bulletin of the Faculty of Life and Environmental Science, Shimane University, **21**: 9-12.

Lemtukei, D., Tamura, T., Nguyen, Q. T., and Ueno, M. (2017) Inhibitory activity of *Burkholderia* sp. isolated from soil in Gotsu city, Shimane, against *Magnaporthe oryzae*. Advances in Microbiology, **7**: 137-148.

McGrath, M.T. and Wyenandt, C. A. (2017) First detection of boscalid resistance in *Podosphaera xanthii* in the United States with failure to control cucurbit powdery mildew in New York and New Jersey in 2009. Plant Health Progress, Online publication. doi: 10.1094/PHP-03-17-0014-BR.

Miyamoto, T., Ishii, H. and Tomita, Y. (2010) Occurrence of boscalid resistance in cucumber powdery mildew in Japan and molecular characterization of the iron-sulfur protein of succinate dehydrogenase of the causal fungus. Journal of General Plant Pathology, **76**: 261-267.

Ueno, M., Suzuki, Y., Kumura, Y., Ueda, K., Nguyen Q. T., Kihara, J., Arase, S. and Oshima A. (2012) Isolation of *Streptomyces* strain STS1 that inhibits the growth of *Colletotrichum orbicula*. Journal of Japanese Society of Agricultural Technology Management, **18**: 191-195.