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Inhibitory potency of microbes isolated from soil in Shimane Prefecture against diseases in rice

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Abstract In this study, microorganisms were isolated from the soil in Ohda city (Shimane prefecture, Japan) and screened for their inhibitory activity against *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe oryzae*. Ninety–six microbial isolates were obtained from the soil samples. Three isolates (S1004, S1011, and S2001) inhibited the growth of *X. oryzae* pv. *oryzae* compared to the control. Mycelial growth of *M. oryzae* was inhibited by the isolate S2001, but not by the isolates S1004 and S1011. Furthermore, conidial germination in *M. oryzae* was inhibited by the culture filtrate of isolate S2001. This study showed that the isolate S2001 might be able to control the diseases caused by *M. oryzae* and *X. oryzae* pv. *oryzae*.

Keywords : Microorganisms, Rice, Rice bacterial leaf blight, Rice blast

Introduction

Rice blast (caused by *Magnaporthe oryzae*), bacterial leaf blight (caused by *Xanthomonas oryzae* pv. *oryzae*), and sheath blight (caused by *Thanatephorus cucumeris*) are the three major diseases of rice (Zou *et al.* 2000; Hu *et al.* 2008; Li *et al.* 1999). The use of resistant rice cultivars, fungicides, and bactericides plays an important role in the control of *M. oryzae* and *X. oryzae* pv. *oryzae*. However, the durability of genetic resistance in improved rice cultivars is often short–lived in the agricultural field (Ahn 1994). Additionally, chemical fungicides are required for controlling the rice blast disease; however, the development of resistance towards these chemicals has been reported in instances of extensive use (So *et al.* 2002; Yamaguchi *et al.* 2002). Furthermore, only a few bactericides have been developed for treating bacterial leaf blight caused by *X. oryzae* pv. *oryzae*. Therefore, a search for inhibitory compounds is necessary to develop novel bactericides and fungicides. Antimicrobial compounds of microbial and plant origin play an important role in the biological and chemical control of plant diseases (Fravel 1988; Shimizu *et al.* 2000; Uddin and Viji 2002; Chaijuckam and Davis 2010).

In this paper, we report the inhibition of *M. oryzae* and *X. oryzae* pv. *oryzae* by microorganisms isolated from the soil of Ohda city.

Materials and Methods

Cultivation of plant pathogen

M. oryzae (strain Naga 69–150, race 007) was grown on potato sucrose agar (PSA; 200 g potato, 20 g sucrose, 20 g agar, 1 L distilled water) medium at 26–28 ℃ for 10–14 days. For formation of conidia, the same strain was grown on rice bran agar medium (50 g rice bran, 20 g sucrose, 20 g agar, 1 L distilled water) at 26–28 ℃ for 14 days. Aerial hyphae were removed from the agar medium in plates containing sterile water, and the plates were maintained at 25–26℃ for 48 h under near–UV light. The synchronously formed conidia were used in the following experiments.

X. oryzae pv. *oryzae* (strain MAFF 210616) was grown on peptone sucrose agar (PeSA; 5 g peptone, 20 g sucrose, 20 g agar, 1 L distilled water, pH7) or PeS broth medium at 26–28℃ .

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Isolation of microorganisms from the soil samples

Microorganisms were isolated from the soil samples using humic acid–vitamin (HV) agar medium as described previously (Hayakawa and Nonomura 1987). The soil samples collected from Ohda city (Sanbe experimental forest of Shimane University, Japan) and maintained at 4 °C were used. The microorganisms, thus isolated, were stored as described previously (Lemtukei *et al.* 2017). The microbial isolates were subjected to disk diffusion method and dual culture assay. The medium containing the culture of the microbial isolates was filtered through a 0.22–μm filter to obtain the culture filtrate.

Disk diffusion method

The antibacterial activity of the isolated microorganisms against *X. oryzae* pv. *oryzae* was investigated by the disk diffusion method using PeSA medium. *X. oryzae* pv. *oryzae* was subcultured on PeSA and paper discs (8 mm), used for antibiotic testing, were placed on these petri dishes containing agar. Subsequently, the paper discs were inoculated with culture medium (50 μL) containing the isolates. All petri dishes were incubated at 26–28 ℃ for 7 days and the diameter of the zone of inhibition was measured using a scale.

Dual culture assay

The antagonistic activity of microbial isolates against *M. oryzae* was investigated by the dual culture assay using PSA as described previously (Lemtukei *et al.* 2016). All petri dishes were incubated at 26–28 ℃ for 10 days and the mycelial area (mm^2) was measured using the LIA 32 software.

Inhibition of infection by culture filtrates

M. oryzae conidia (1×10^5 conidia/mL) suspended in culture filtrates of the different microbial isolates or distilled water were placed onto glass slides and maintained in a moist chamber at 26–28℃. After 24 h incubation, the percentage of conidial germination was determined by light microscopy.

Statistical analysis

Data are reported as mean ± standard deviation (SD). Means labeled with different letters were significantly different from each other according to the Scheffe's test ($p<0.05$).

Results and Discussion

The inhibitory activity of microbial isolates from soil against bacterial leaf blight disease of rice was evaluated using disk diffusion method. Ninety–six microbial isolates were obtained from the soil in Ohda city (Sanbe experimental forest of Shimane University, Japan). Three isolates (S1004, S1011, and S2001) among the 96 microbial isolates, showed higher inhibition of *X. oryzae* pv. *oryzae* growth compared to inhibition of growth in the control plates (Fig. 1A). The diameter of the inhibition zone in the petri dish containing discs inoculated with isolates S1004, S1011, and S2001 was 2.5 ± 0.5, 3.9 ± 1.5, and 4.4 ± 0.8 cm, respectively (Fig. 1B). In contrast, inhibition zone was not observed in the control plates in the absence of isolated microorganisms (Fig. 1B).

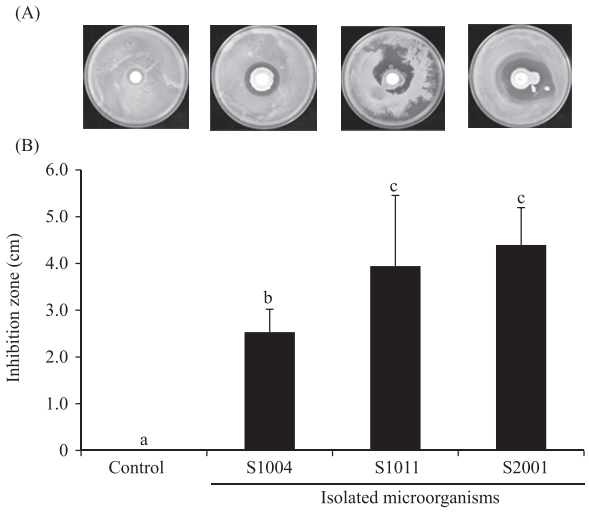

Fig. 1 Antibacterial activity of isolated microorganisms to the growth of *Xanthomonas oryzae* pv. *oryzae* observed by disk diffusion method on peptone sucrose agar plate (A) and the inhibition zone of *X. oryzae* pv. *oryzae* without or with isolated microorganisms (B). The experiments were repeated three times. Bar: represents ± SD. Mean values followed by the same letters are not significantly different at a 5% level according to a Scheffe's test.

Mycelial growth of *M. oryzae* was inhibited by isolate S2001 (mycelial area, 1575.8 ± 189.3 mm^2) (Fig. 2), but not by the isolates S1004 (mycelial area, 2139.4 ± 605.9 mm^2) and S1011 (mycelial area, 1951.8 ± 390.2 mm^2). In the control plates, the mycelial area of *M. oryzae* was

found to be 2266.5 ± 643 mm^2 (Fig. 2).

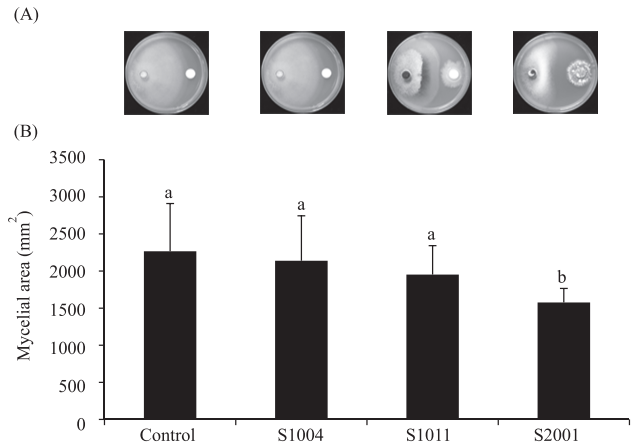

Fig. 2 Antagonistic activity of isolated microorganisms to the growth of *Magnaporthe oryzae* observed by dual culture assay on potato sucrose agar plate (A) and the mycelial area of *M. oryzae* without or with isolated microorganisms (B). The experiments were repeated three times. Bar: represents ± SD. Mean values followed by the same letters are not significantly different at a 5% level according to a Scheffe's test.

In order to understand the production of inhibitory compound (s) by isolates S1004, S1011, and S2001, we investigated the effects of the culture filtrates of different microbial isolates on the germination of *M. oryzae* conidia. Inhibition of conidial germination was observed in the culture filtrates of isolates S2001, but not in that of isolate S1004 and S1011 (Fig. 3A). The percentage inhibition of *M. oryzae* conidial germination by the culture filtrates of the isolates S1004, S1011, and S2001 was 100, 100, and 14.2 ± 6.4 % , respectively (Fig. 3B). In the control sample, the percentage of conidial germination was 100% (Fig. 3B).

These results indicated the production of inhibitory compound (s) by isolate S2001 against *M. oryzae* and *X. oryzae* pv. *oryzae*.

The biological control of plant diseases and promotion of plant growth by rhizobacteria is well known (Colo *et al.* 2014; Lugtenberg and Kamilova 2009: Yasmin *et al.* 2016).

Further studies are required to investigate the inhibitory activity of the suspension culture and culture filtrate of the isolate S2001 against bacterial leaf blight disease and rice blast disease of rice and their effects on the promotion of plant growth in plant. Thus, the identification of isolate S2001 was essential.

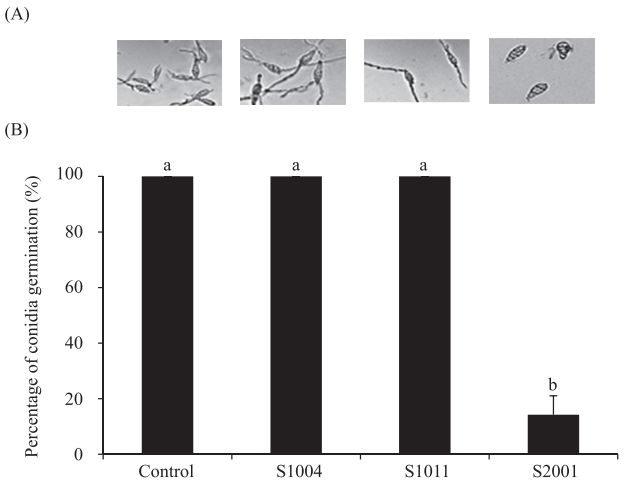

Fig. 3 Effect of culture filtrates of isolated microorganisms on the conidia germination of *Magnaporthe oryzae*. The conidia of *M. oryzae* were suspended in the culture filtrates of isolated microorganisms and dropped on glass slides. After 24h incubation in a moist chamber, the conidia germination was observed by light microscopy (A). The rates of conidia germination were calculated (B). The experiments were repeated three times. Bar: represents ± SD. Mean values followed by the same letters are not significantly different at a 5 % level according to a Scheffe's test.

These results suggest that the isolate S2001 might be useful in controlling the diseases caused by *X. oryzae* pv. *oryzae* and *M. oryzae* in rice.

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