Bull. Fac. Life Env. Sci. Shimane Univ., 21 : 9 − 12, September 30, 2016

Antagonistic potential of isolated microorganisms from soil in Shimane prefecture against rice blast disease cause by *Magnaporthe oryzae*

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Abstract Rice blast disease caused by *Magnaporthe oryzae* is an economically important fungal disease leading to extensive losses in many regions globally. In this study, microorganisms were isolated from soil in Gotsu City (Shimane prefecture) and screened for their inhibitory potential against *M. oryzae* using a dual culture assay. Based on this, 165 isolates were obtained from four locations. Forty-two of these isolates inhibited mycelial growth of *M. oryzae* relative to growth of the control. Furthermore, 11 of them inhibited *M. oryzae* mycelial growth by more than 50% relative to that of the control. These results strongly suggest that microorganisms isolated from the soil of Gotsu City could yield candidate control agents for plant diseases such as rice blast.

Keywords : *Magnaporthe oryzae,* Culture filtrate, Microorganisms, Antifungal compound, Biological contro

Introduction

Rice is known to be attacked by many pests and diseases; this results in huge annual losses worldwide. Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world (Strange and Scott 2005). Among fungal diseases, rice blast caused by *Magnaporthe oryzae* is of significant economic importance.

Rice blast disease is devastating especially when rice is irrigated or receiving high amounts of rainfall and high levels of nitrogen fertilizer. Epidemics of rice blast have led to yield losses ranging from 50 to 90% (Agrios 2004) in different parts of the world. Control strategies applied against rice blast disease mainly involve the use of chemical fungicides. However, the development of resistance to these chemicals has been reported in instances of extensive use. In fact, resistance against kasugamycin and organophosphorus thiolate fungicides (Ishii 2006) were observed in the field where they were intensively used. These chemical fungicides could also affect non target organisms and have negative environmental impact (Aktar et al. 2009). Therefore, the search for alternative methods of plant disease control, including the utilization of soil microorganisms could significantly contribute to reducing chemical fungicides use.

The biological control activity of bacteria is exerted either directly through antagonism of pathogen development, or indirectly by eliciting a plant-mediated resistance response. Mechanisms responsible for antagonistic activity include inhibition of pathogen growth, competition for colonization sites, nutrients and minerals, parasitism, and mycophagy (Elshafie et al. 2012). It is a common strategy for bacterial antagonists to inhibit plant pathogens by excretion of antimicrobial metabolites (AMMs) (Compant et al. 2005). It is well known that AMMs are antibiotics, toxins, and bio-surfactants.

In this study, soil microorganisms were isolated and screened for their inhibitory potential against *M. oryzae* by the dual culture assay.

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Materials and Methods

Isolation of microorganisms from soil samples

Microorgonisms were isolated from soil samples (GT1, GT2, GT3, and GT4) using humic acid-vitamin (HV) agar medium as described previously (Hayakawa and Nomura 1987). Soil samples previously collected from Gotsu City (Shimane prefecture) and maintained at 4°C were used in this experiment to obtain isolates. Each soil sample was suspended in a micro tube containing 1 ml distilled water (DW). Each sample (20μL) was spread on HV agar media with 10mg/ml cycloheximide and 10μg/ml nalidixic acid. All petri plates were incubated for 4 days at 25℃. A single colony of each isolate was grown for 4 days at 25℃ on nutrient broth glucose agar (NGA) medium (4 g nutrient broth, 2g glucose, and 2g agar). Distinct colonies on NGA medium were inoculated into test tubes containing 3ml PC-I medium (10g starch, 10g polypeptone, 10g molasses, and 10g meat extract in 1000ml deionized water, pH 7.2) and incubated on a rotary shaker for 7 days at 25℃. The cultures were used for a dual culture assays. In addition, isolated Microorganisms were suspended in 15–20% glycerol solution and stored at −80℃ until use.

Dual culture assay

The antagonistic potential of isolated microorganisms against *M. oryzae* was investigated by the dual culture method using potato sucrose agar (PSA). Mycelial plugs (6mm) of *M. oryzae* and paper discs (8mm) for antibiotic tests were placed on PSA plates 4.5 cm apart. Subsequently, the paper disc was inoculated with culture (20μl) of isolated microorganisms. Sterile DW and PC-I liquid media were used for control treatments. The experiment was replicated three times. All petri dishes were incubated at 25℃ for 14 days and mycelial area (mm^2) of *M. oryzae* was measured using LIA 32 software.

Statistical analysis

Data are presented as the mean ± standard deviation. Statistically significant differences were determined by a t-test ($P < 0.05$) using the Statistical Package for the Social Sciences (IBM SPSS version 22.0).

Results and Discussion

In this study, preliminary experiments were carried out to evaluate the inhibitory potential of soil microbial isolates against rice blast fungus using dual culture. Isolates (165) were obtained from the soil in Gotsu City (four locations). Forty-two of the 165 isolated microorganisms inhibited mycelial growth of *M. oryzae* relative to growth of the control (Fig. 1). Ten isolates from the GT1 soil samples, ten from GT2, eight from GT3, and fourteen from GT4 significantly inhibited mycelial growth of *M. oryzae* on PSA petri plates. Furthermore, 11 (GT1005, GT1007, GT1014, GT1022, GT2002, GT2027, GT4013, GT4025 GT4027, GT4028, and GT4041) of the 165 isolated microorganisms inhibited *M. oryzae* mycelial growth by more than 50% compared to growth of the control (PC-I) (Fig. 1). Soil microorganisms of the genus *Pseudomonas* have been extensively used for biological control against many soil-borne plant pathogens (Aghighi et al. 2004); *Pseudomonas fluorescens* F113 was shown to control the potato rot pathogen *Erwinia carotovora* subsp. *atroseptica* (Cronin et al. 1997). Application of non-pathogenic strains of *Streptomyces* to control potato scab caused by *Streptomyces scabies* has been reported (Meng et al. 2013). In addition, studies of the antifungal potential of *Streptomyces sindenensis* against rice blast disease (*M. oryzae*) indicated significant inhibition both in dual cultures and in vivo (Zarandi et al. 2009). *Streptomyces globisporus* JK-1 inhibited mycelial growth of various plant pathogens including *M. oryzae* in vitro, and its culture filtrates were effective in control of rice blast in a greenhouse environment (Li et al. 2011).

Further studies are needed to investigate the activity of antifungal compound (s) from the isolated microorganisms in plants. Also, the application of culture or culture filtrate of these microorganisms for rice blast disease control will require further investigation. In conclusion, the present study indicates that some isolated microorganisms have inhibitory activity against mycelial growth of *M. oryzae*. Therefore, these results strongly suggest that isolated microorganisms from the soil of Gotsu City could yield candidate control agents for plant diseases such as rice blast.

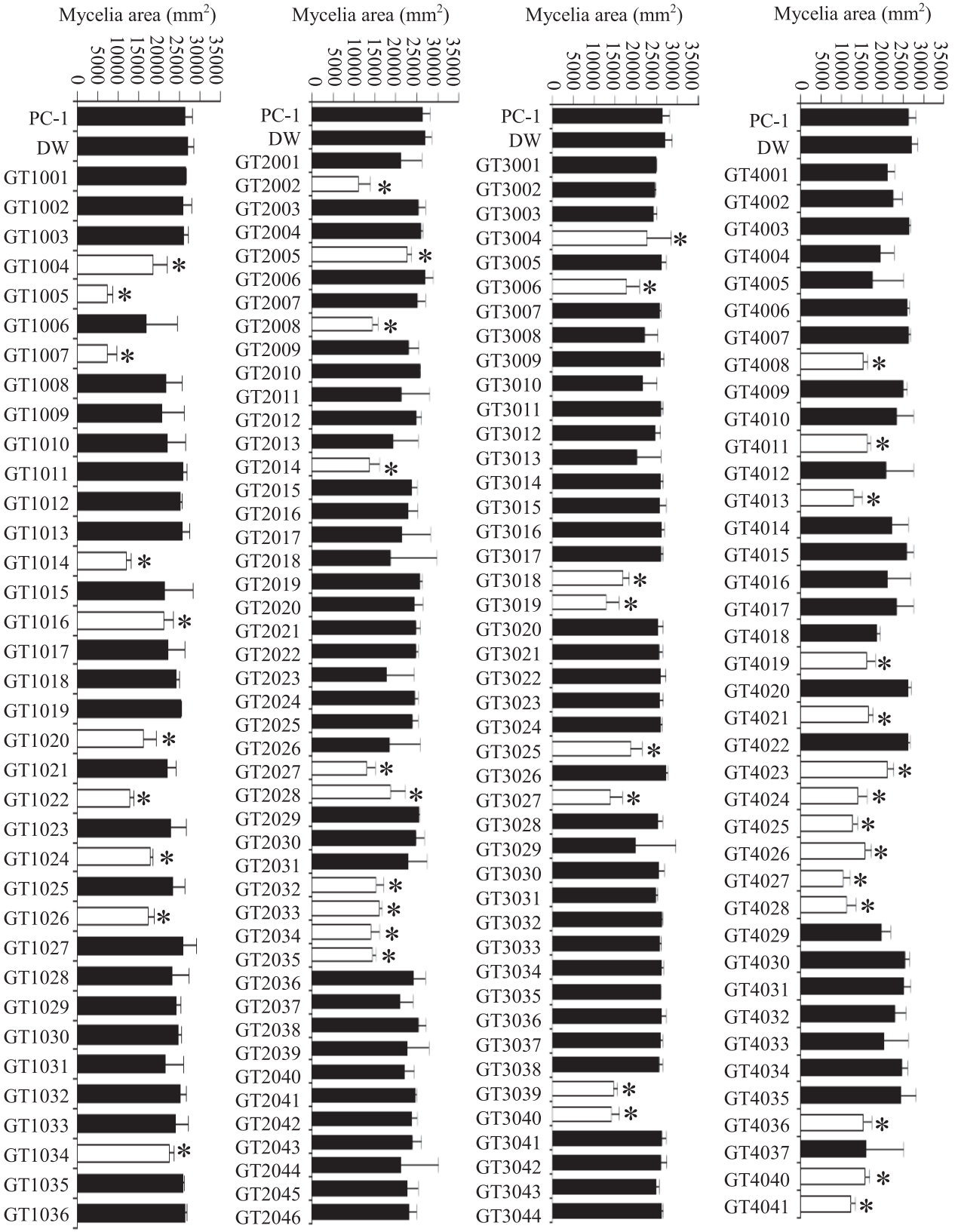

Fig. 1 Antagonistic potential of isolated microorganisms from soil in Gotsu city (Shimane prefecture) to the growth of *Magnaporth oryzae* observed by dual culture on potato sucrose agar plate. GT×× is sample code. Bar: represents ± SD. Asterisk indicate the significant difference compared with the result of the control (PC-1) (t-test, $P < 0.05$).

Acknowledgment（s）

This investigation was supported by the African Business Education Initiative for Youth Program of the Japan International Cooperation Agency

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