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Inhibitory Activity of *Burkholderia* sp. Isolated from Soil in Gotsu City, Shimane, against *Magnaporthe oryzae*

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Abstract

An isolate GT4028 was obtained from soil samples collected from a field in Gotsu city (Kawahira), Shimane. The use of a culture suspension and culture filtrate of this isolate significantly suppressed the spore germination in *Magnaporthe oryzae*. The inhibitory activity of the culture filtrate was heat-stable. The formation of rice blast lesions by *M. oryzae* was significantly suppressed in the presence of the culture suspension of isolate GT4028. Furthermore, mycelial growth of some plant pathogenic fungi was inhibited by the isolate in a dual culture assay. Sequence analysis of 16S rDNA region of the isolate indicated that it shared similarities with species of the genus *Burkholderia*. Also, isolate GT4028 could be grown even in the presence of fungicides (Blastin, Kasugamycin, and Amistar) that act against *M. oryzae*. These results suggest that isolate GT4028 might be a potential control agent for plant protection against diseases, such as rice blast disease.

Keywords

Magnaporthe oryzae, Rice Blast, *Burkholderia* sp., Culture Filtrate

1. Introduction

The ascomycete fungus, *Magnaporthe oryzae*, the causative agent of rice blast disease, is one of the most important plant pathogenic fungi and is considered a threat to food security globally. This disease is responsible for annual losses of 10% to 30% of the harvest and rice is vulnerable to it, wherever it is grown [1] [2]. 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 were observed in the field

where they were intensively used [3]. However, development of resistance to microbial fungicides has not been reported. The inhibitory activity of microorganisms is exerted either directly through antagonism to pathogen development, or indirectly by eliciting a plant-mediated resistance response. Mechanisms responsible for inhibitory activity include inhibition of pathogen growth, competition for colonization sites, nutrients, and minerals, parasitism, and mycophagy [4]. It is well known that microorganisms have different physiological characteristics and produce different compounds even among members in the same species. Therefore, it is necessary to examine a collection with wide biological diversity to find new inhibitory compounds and microorganisms for plant diseases control. Shimane prefecture is geographically elongated from east to west and it has various characteristic diversities of climate in each region. Consequently, soil of Shimane prefecture is expected to have diverse microbial resources. However, little has been reported on the microbial communities found in Shimane, or on their utility in pathogen control for rice blast disease. In a previous study, we indicated that microorganisms isolated from the soil of Gotsu city (Shimane prefecture) inhibited the mycelial growth of *M. oryzae* [5]. In this paper, we report that culture suspension and culture filtrate of the isolate GT4028 from soil of Gotsu city can protect rice from rice blast fungus, *M. oryzae*.

2. Material and Methods

2.1. Bacteria, Fungus and Plant

The isolate GT4028 was obtained from soil samples collected from a field in Gotsu city (Kawahira), Shimane by the method described previously [5]. It was suspended in 15% - 20% glycerol solution and stored at −80˚C until use. A single colony of the isolate 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˚C - 27˚C for 7 days with constant shaking on a rotary shaker (130 rpm).

M. oryzae (strain Naga 69 - 150, race 007) was grown on rice bran agar medium (50 g rice bran, 20 g sucrose, and 20 g agar dissolved in a final volume of 1 L distilled water) at 25˚C - 27˚C for 10 days, washed with running water to remove aerial hyphae, and kept at 25˚C under near-ultraviolet radiation (FL20s BL-B; Panasonic, Osaka, Japan) for 4 days to induce sporulation. BL-B lamps, which emit wavelengths of 320 - 420 nm (mainly 360 nm), were used as the source of near-ultraviolet radiation to induce sporulation.

Seedlings of *Oryza sativa* L. 'Koshihikari' were used in this study. Seeds were soaked in water for 4 days, and the germinating seeds were sown in plastic pots. Seedlings were grown to the three-leaf stage under glasshouse conditions.

2.2. Inhibitory Activity of Culture and Culture Filtrate

M. oryzae spores (1×10^5 spores/mL) suspended in the culture suspension or culture filtrate of GT4028 were dropped on glass slides and kept in a moist

chamber at 25˚C - 27˚C. After 24-h incubation, the percentage of spore germination was determined by light microscopy. LB liquid medium was used as a control. Culture suspension of the isolate GT4028 was filtered through a 0.22-μm filter to obtain the culture filtrate.

2.3. Heat Stability Test

To evaluate heat stability, the culture filtrate of GT4028 was heated at 121˚C for 20 min. The treated and untreated culture filtrates were inoculated with a spore suspension (1×10^5 spores/mL) of *M. oryzae* on glass slides and kept in a moist chamber at 25˚C - 27˚C. After 24-h incubation, the percentage of germinating spores was determined by light microscopy.

2.4. Effect of Treatment with Culture Suspension on Development of Blast Lesion in Rice Leaves

The rice plant at three-leaf stage was sprayed with the culture suspension of isolate GT4028 (5 mL/pot). LB was sprayed as a control. The sprayed rice plants were maintained under natural conditions until the leaf dried, which were then inoculated with a spore suspension of *M. oryzae* (1×10^5 spores/mL, 2 mL/pot). The inoculated rice plants were kept in a moist chamber covered with a black polythene bag for 24-h and then maintained under natural light conditions. The experiments were performed in duplicates and repeated three times. The blast lesions formed were counted 7 days after the inoculation.

2.5. Fungicide Sensitivity Test

We determined the fungicide sensitivity of the isolate GT4028. Blastin (triazolobenzo-thiazole, Hokko Chemical Industry Co., LTD), Kasugamycin (hexopyranosyl antibiotic, Hokko Chemical Industry Co., LTD), and Amistar (azoxystrobin, Syngenta Japan K.K.) were used in this experiment. Each fungicide was diluted (1000-fold) in molten LB media and mixed thoroughly before dispensing to the respective, labeled 9-cm petri plates. The culture suspension of isolate GT4028 (50 μL) was spread on LB media in the presence of fungicides and then incubated at 25˚C - 27˚C. After 3 days, the growth of isolate GT4028 was observed. LB medium without fungicide addition was used as a control.

2.6. DNA Extraction, PCR Amplification, Sequencing, and Generation of Phylogenetic Tree

To identify the isolate GT4028, the sequence of the 16S rDNA was determined by PCR using the primers mentioned in **Table 1** [6] [7]. The bacterial genomic DNA was extracted from the bacteria colony by the method described previously [8], and was used as the PCR template. PCR amplification of the 16S rDNA region consisted of the following steps: an initial step of 30 s at 95˚C, followed by 30 cycles of denaturation at 95˚C for 30 s, annealing at 62˚C for 30 s, and elongation at 72˚C for 1.45 min, and a final extension step for 10 min at 72˚C. The

Table 1. The sequence of primers used for the isolates' sequence determination.

Primers	Sequence (5' → 3')
fD1	AGAGTTTGATCCTGGCTCAG
rP2	ACGGCTACCTTGTTACGACTT
EUB906F	AAACTCAAAGGAATTGRCGG
EUB532R	TTACCGCGGCKGCTGGCAC
1115R	GTTGCTCGCGTTGGGA
802R	TACCAGGGTATCTAATCC
926F	AAACTCAAAGGAATTGACGG
785F	GGATTAGATACCCTGGTAGTC
536R	GTATTACCGCGGCTGCTG

PCR-amplified fragments were purified by using HiYield Gel/PCR DNA fragment extraction kit (RBC Bioscience, Taipei, Taiwan). DNA sequencing was performed with a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA). The DNA sequence analysis was performed on an ABI PRIZM 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). The sequence homology was determined by using the BLAST suite of programs (DNA Data Bank, Japan). The phylogenetic tree was constructed using the neighbor-joining method [9].

2.7. Thin Layer Chromatography (TLC)-Profile of the Culture Filtrate of GT4028

Fifty microliter of the culture filtrate (5-fold) of the isolate GT4028 was spotted onto silica gel TLC plates (Silica gel 60, Merck KGaA, Darmstadt, Germany) and then developed using ethyl acetate and toluene (1:1, v/v) solvent system. After the development, inhibitory compounds were detected on the TLC plates by a growth inhibition assay for *M. oryzae* spores. Briefly, the TLC plates were sprayed with a concentrated spore suspension of *M. oryzae* in the presence of potato sucrose agar (PSA). The inoculated plates were kept in a moist chamber at 25˚C - 27˚C for 7 days.

2.8. Dual Culture Assay

The antagonistic potential of the isolate GT4028 against plant pathogenic fungus (*Alternaria alternata* Japanese pear pathotype, *Corynespora cassiicola*, *Colletotrichum orbiculare*, *Fusarium oxysporum* f. sp. *spinaciae*, and *Cochliobolus miyabeanus*) was investigated by a dual culture method using PSA. The plant pathogenic fungus was prepared as described by Nguyen *et al.* (2012) [10]. Mycelial plugs (6 mm) of plant pathogenic fungus and paper discs (8 mm) for antibiotic tests were placed on PSA plates, 4.5 cm a part. Subsequently, the paper disc was inoculated with culture (20 µL) from the isolate. LB liquid medium was used for control treatments. The experiment was replicated three times. All the petri dishes were incubated at 25˚C - 27˚C for 14 days and the mycelial area

(mm^2) of the plant pathogenic fungus was determined using LIA 32 software (http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index-e.html).

2.9. Data Analysis

Data have been shown in terms of the mean ± standard deviation values. Statistically significant differences were determined by *t*-test ($P < 0.05$) using Statistical Package for the Social Sciences (IBM SPSS version 22.0).

3. Results

3.1. Effect of Culture Suspension and Culture Filtrate of GT4028 on *M. oryzae* Spore Germination

To assess the inhibitory activity of the isolate GT4028, we investigated the effects of the culture suspension and culture filtrates of the isolate on the germination of *M. oryzae* spores. The percentage germination of *M. oryzae* spores in culture suspension and culture filtrate of the isolate was 4.7% ± 3.3% and 2.0% ± 2.9%, respectively (Figure 1). In contrast, in the control, the percentage of spore germination was 100% (Figure 1). In addition, we investigated the effect that heat treatment had on the ability of the culture filtrate of GT4028 to suppress spore germination. In the heat-treated LB medium (Control) and heat-treated culture filtrate (GT4028), the percentages of spore germination were 100% and 33.5% ± 12.5%, respectively (Figure 2).

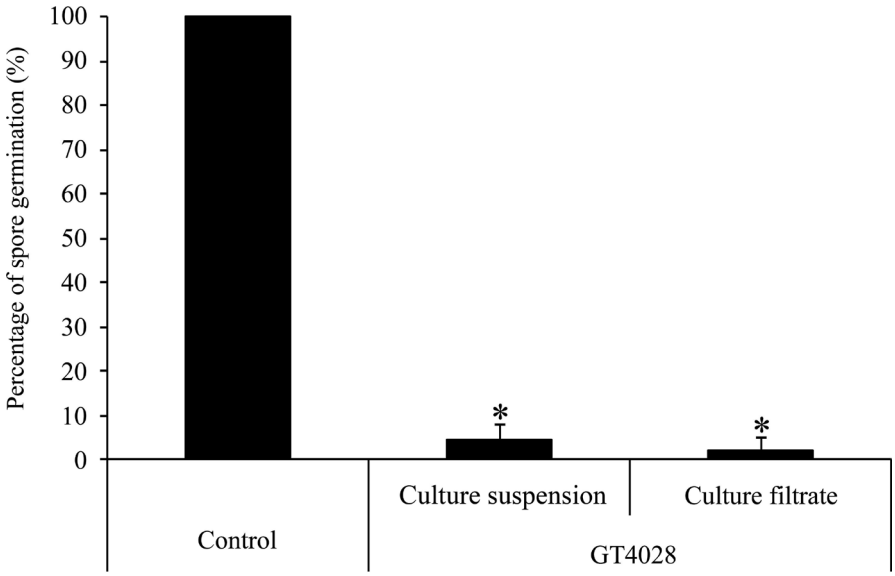

Figure 1. Effect of culture suspension and culture filtrates of isolate GT4028 on the germination of *Magnaporthe oryzae* spores. The spores of *M. oryzae* were suspended in the culture suspension and culture filtrates of isolate GT4028 and dropped on glass slides. After 24-h incubation in a moist chamber, the spore germination was observed by light microscopy. The rates of spore germination were calculated. The experiments were repeated thrice. Data are representative of the mean values of the results from three separate experiments. The bar at the top of each column represents the standard deviation of the mean. The asterisk indicates a significant difference compared to the result obtained in the control (*t*-test, $P < 0.05$).

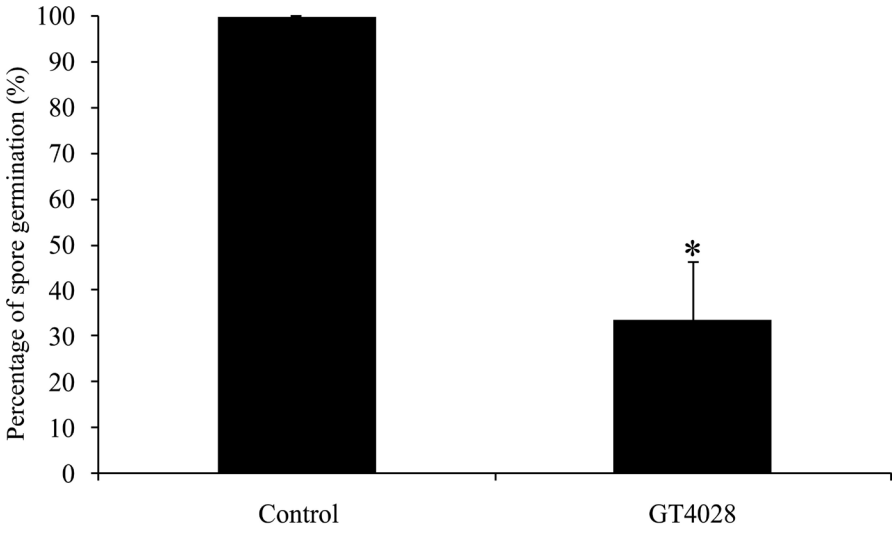

Figure 2. Suppression by heat-treated culture filtrates of isolate GT4028 on the germination of *Magnaporthe oryzae* spores. The spores of *M. oryzae* were suspended in the culture filtrates of isolate GT4028 and dropped on glass slides. After 24-h incubation in a moist chamber, the germination of spores was observed by light microscopy. The rates of spore germination were calculated. The experiments were repeated thrice. Data are representative of the mean values of the results from three separate experiments. The bar at the top of each column represents the standard deviation of the mean. The asterisk indicates a significant difference compared to the result obtained in the control (*t*-test, $P < 0.05$).

3.2. Suppression of Blast Lesion Formation by *M. oryzae* in Culture Suspension of GT4028 on Rice

To determine the inhibitory activity against *M. oryzae* in rice, the culture suspension-pretreated rice leaves (cultivar "Koshihikari") were inoculated with spores of *M. oryzae*. As a result, the blast lesion formation was significantly suppressed by culture suspension of the isolate, relative to that by the control (**Figure 3(a)**). In the controls, rice leaves sprayed with LB liquid medium showed the number of blast lesions to be 18.7 ± 4.7 (**Figure 3(b)**). The number of blast lesions in the treatment with culture suspension of isolate GT4028 was 9.2 ± 3.4.

3.3. Identification and Characterization of GT4028

To identify the isolate GT4028, we used specific primers to PCR amplify the 16S rDNA by using its genomic DNA. The phylogenetic analyses showed that the microorganism was most closely related to the type strain of genus *Burkholderia* (**Figure 4**) and therefore, isolate GT4028 was identified as a member of the genus *Burkholderia*. Morphological observation by the SEM revealed that GT4028 was rod-shaped (data not shown). In the present study, the growth of GT4028 was observed at 20˚C, 28˚C, and 38˚C, but not at 4˚C (**Table 2**). Furthermore, the growth of GT4028 was observed in the presence of fungicides against rice blast fungus, such as Blastin, Kasgamycin, and Amistar (**Table 2**). The inhibitory activity of isolate GT4028 against other plant pathogenic fungi was tested by

dual culture method. Isolate GT4028 inhibited the mycelial growth of *Alternaria alternata* Japanese pear pathotype, *Corynespora cassiicola*, *Colletotrichum orbiculare*, *Fusarium oxysporum* f. sp. *spinaciae*, and *Cochliobolus miyabeanus* by 40% to 60%, compared to the control (Table 3).

3.4. Detection of an Inhibitory Compound on the Thin Layer Chromatography Plate

The inhibitory compounds were detected on the TLC plates by a growth inhibition assay of *M. oryzae* spores. The growth inhibition zone was observed at origin (Rf 0.0) in the presence of culture filtrate of isolate GT4028 (Figure 5).

Figure 3. Suppressive effect of isolate GT4028 on the formation of blast lesions by *Magnaporthe oryzae* in rice plants. The rice plants were inoculated with *M. oryzae* in the presence of GT4028 culture suspension and were kept in a moist chamber for 24-h. After 7 days, blast lesion development (a) and number of blast lesions (b) was determined. Data are representative of the mean values of the results from three separate experiments. The bar at the top of each column represents the standard deviation of the mean. The asterisk indicates a significant difference compared to the result obtained in the control (*t*-test, $P < 0.05$).

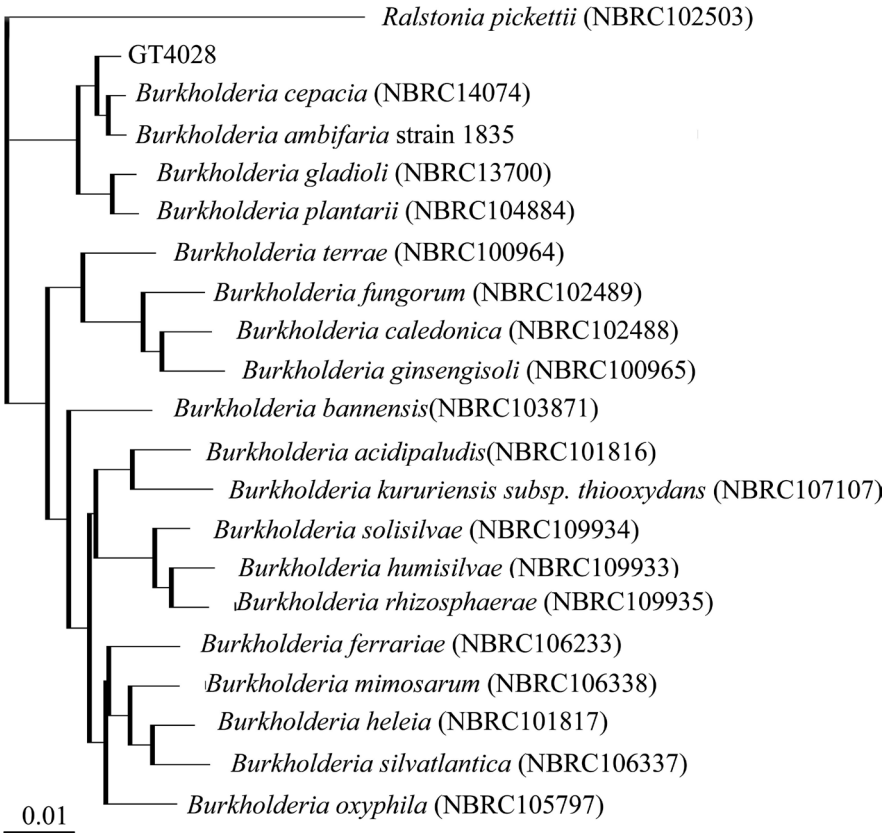

Figure 4. Phylogenetic tree constructed on the basis of 16S rDNA sequences of isolate GT4028. A bootstrap consensus neighbor-joining tree for the isolate was created based on the Kimura 2-Parameter distance matrix (1000 replicates). *Ralstonia pickettii* (NBRC102503) was used as an out-group. The scale bar represents 1% sequence dissimilarity.

Table 2. Effect of fungicides and temperature on growth of isolate GT4028 on fungicides-amended LB media and under different incubation temperatures.

Isolate	Temperature (˚C)				Fungicides			
	4	20	28	37	Control	Blastin	Kasgamycin	Amister
GT4028	-	+	++	+	++	++	++	++

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

Table 3. Effect of isolate GT4028 to the growth of plant pathogens observed by dual culture method.

Plant pathogens	Mycelia area (mm^2)	
	Control	Isolate GT4028
Alternaria alternata Japanese pear pathotype	276.8 ± 3.0	144.7 ± 26.4*
Corynespora cassiicola	286.6 ± 14.0	125.5 ± 26.3*
Colletotrichum orbiculare	278.5 ± 5.8	109.5 ± 49.9*
Fusarium oxysporum f. sp. *spinaciae*	274.0 ± 7.6	168.9 ± 13.2*
Cochliobolus miyabeanus	271.9 ± 8.7	124.9 ± 16.8*

Asterisk indicate the significant difference compared with the result of the control (*t*-test, $P < 0.05$).

Figure 5. Thin-layer chromatography (TLC)-profiles of the culture filtrate of isolate GT4028. The culture filtrate of isolate GT4028 was spotted onto a TLC plate. After development, the TLC plate was sprayed with a concentrated spore suspension of *Magnaporthe oryzae* in the presence of potato sucrose agar medium. The inoculated plate was kept in a moist chamber at 25˚C - 27˚C for 7 days.

4. Discussion

The application of fungicides is generally an effective control method for fungal pathogens of plants. However, extensive use of synthetic fungicides to control these pathogens has induced fungal resistance. The use of microorganisms as biological control agent for these pathogens has been reported. Recently, we constructed a library of microorganisms isolated from soil in Shimane. The isolate GT4028 is one of the microorganisms exhibiting a high inhibitory activity against the mycelial growth of *M. oryzae* [5]. In this study, it was observed that GT4028 had inhibitory activity against several plant pathogenic fungi, such as *A. alternata* Japanese pear pathotype, *C. cassiicola*, *C. orbiculare*, *F. oxysporum* f. sp. *spinaciae*, and *C. miyabeanus* as well as against *M. oryzae*. The phylogenetic analysis of isolate GT4028 revealed that the organism was most closely related to genus *Burkholderia*. The bacteria in the genus *Burkholderia* comprise more than 80 species that are inhabitants of diverse ecological niches and are nutritionally versatile. However, their use in experiments requires caution partly because of

the presence of opportunistic human pathogens in the population [11]. However, an increasing number of plant-associated strains and species of *Burkholderia* have been reported to confer resistance to plants against biotic and abiotic stresses; many species enhance the nitrogen fixing capabilities of the plants, as well [4]. Field trials have highlighted the ability of *Burkholderia cepacia* strains to colonize the rhizosphere of many crops, including, corn, rice pea, sunflower, and radish, leading to the enhanced growth of such plants and suppression of soil borne pathogens [12]. A study by Mao *et al.* (2006) demonstrated the *in vitro* inhibitory activity of purified compounds of *Burkholderia* sp MP-1 against a wide range of plant pathogenic fungi [13]. These compounds include phenylacetic acid, hydrocinnamic acid, 4-hydroxyphenylacetic acid, and 4-hydroxyphenylacetate methyl ester. The antifungal 2-hydroxymethyl-chroman-4-one was isolated from *Burkholderia* sp. CEB01056 [14] and was shown to be active against *Pythium ultimum*, *Phytophthora capsici*, and *Sclerotinia sclerotiorum.* Burkholderines isolated from *Burkholderia ambifaria* 2.2N were characterized as a complex heat stable cyclic lipopeptide capable of inhibiting a broad spectrum of fungal pathogens [15]. Similarly, occidiofungin is a compound purified from *Burkholderia contaminan*s MS14 that shows broad-spectrum activity against plant and animal fungal pathogens [16] [17]. Another strain known for its gibberellin-production and phosphate solubilization ability is *Burkholderia* sp KCTC 11096BP. Treatment of cucumber and Crown daisy plants with culture suspension of this strain promotes plant growth parameters [18]. In our study, plant growth promotion activity of the isolate GT4028 has not yet been investigated. The cultures and culture filtrates of *Burkholderia gladioli* pv *agaricicola* and *B. gladioli* have been shown to inhibit the growth of important soil borne plant pathogenic fungi as well as the postharvest fungal rot of apple, lemon, and orange [4] [19]. These strains showed protease, chitinase, and cellulase activities, which could have played important inhibitory roles against the development of fungal disease via cell wall-degradative effects of the extracellular enzymes. In our study, the inhibitory compounds against *M. oryzae* were detected at origin (Rf 0.0) on the TLC plates by a growth inhibition assay. Inhibitory compounds in the culture filtrate of isolate GT4028 were heat-stable and had molecular weight of 3000 or less. However, the activity of heat-treated culture filtrate was slightly decreased compared to that of non-heat-treated culture filtrate. These results suggested that isolate GT4028 might produce both heat-stable and heat-labile compounds, such as chitinase. In addition, the formation of blast lesions was significantly inhibited in the presence of the culture suspension of the isolate. Furthermore, isolate GT4028 was tolerant to Blastin/tricyclazole (triazolobenzo-thiazole), Kasugamycin (hexopyranosyl antibiotic), and Amistar (azoxystrobin) fungicides. However, the inhibitory compound that provided this protection has not yet been identified. Thus, further studies are required to identify the active compounds secreted in the culture of isolate GT4028.

The present study on isolate GT4028 might contribute to the development of a new fungicide and a biological fungicide.

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References

[1] Dean, R., Van Kan, J.A., Pretorius, Z.A., Hammond-Kosach, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J. and Foster, G.D. (2012) The Top 10 Fungal Pathogens in Molecular Plant Pathology. *Molecular Plant Pathology*, **13**, 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

[2] Liu, J., Wang, X., Mitchell, T., Hu, Y., Liu, X., Dai, L. and Wang, G.L. (2010) Recent Progress and Understanding of the Molecular Mechanisms of the Rice-*Magnaporthe oryzae* Interaction. *Molecular Plant Pathology*, **11**, 419-427. https://doi.org/10.1111/j.1364-3703.2009.00607.x

[3] Ishii, H. (2006) Impact of Fungicide Resistance in Plant Pathogens on Crop Disease Control and Agricultural Environment. *Japan Agricultural Research Quarterly*, **40**, 205-211. https://doi.org/10.6090/jarq.40.205

[4] Elshafie, H.S., Camele, I., Racioppi, R., Scrano, L., Iacobellis, N.S. and Bufo, S.A. (2012) *In Vitro* Antifungal Activity of *Burkholderia gladioli* pv. *agaricicola* against Some Phytopathogenic Fungi. *International Journal of Molecular Sciences*, **13**, 16291-16302. https://doi.org/10.3390/ijms131216291

[5] Lemtukei, D., Tamura, T., Nguyen, T.Q., 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.

[6] Huong, N.L., Itoh, K. and Suyama, K. (2007) Diversity of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T)-Degrading Bacteria in Vietnamese Soils. *Microbes and Environments*, **22**, 243-256. https://doi.org/10.1264/jsme2.22.243

[7] Matsui, T., Kato, K., Namihira, T., Shinzato, N. and Semba, H. (2009) Stereospecific Degradation of Phenylsuccinate by Actinomycetes. *Chemosphere*, **76**, 1278-1282. https://doi.org/10.1016/j.chemosphere.2009.06.021

[8] Suzuki, S., Taketani, H., Kusumoto, K. and Kashiwagi, Y. (2006) High-Throughput Genotyping of Filamentous Fungus *Aspergillus oryzae* Based on Colony Direct Polymerase Chain Reaction. *Journal of Bioscience and Bioengineering*, **102**, 572-574. https://doi.org/10.1263/jbb.102.572

[9] Saitou, N. and Nei, M. (1987) The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*, **4**, 406-425.

[10] Nguyen, Q.T., Ueda, K., Kihara, J., Arase, S. and Ueno, M. (2012) Effect of Culture Filtrates of *Trichoderma* sp. Isolated from Wild Mushrooms on the Infectious Behavior of Plant Pathogenic Fungi. *Bulletin of the Faculty of Life and Environmental Science*, *Shimane University*, **17**, 23-27.

[11] Coenye, T. and Vandamme, P. (2003) Diversity and Significance of *Burkholderia* species Occupying Diverse Ecological Niches. *Environmental Microbiology*, **5**, 719-729. https://doi.org/10.1046/j.1462-2920.2003.00471.x

[12] McLoughlin, T.J., Quinn, J.P., Bettermann, A. and Bookland, R. (1992) *Pseudomonas cepacia* Suppression of Sunflower Wilt Fungus and Role of Antifungal Compounds in Controlling the Disease. *Applied and Environmental Microbiology*, **58**, 1760-1763.

[13] Mao, S., Lee, S.J., Hwangbo, H., Kim, Y.W., Park, K.H., Cha, G.S., Park, R.D. and

Kim, K.Y. (2006) Isolation and Characterization of Antifungal Substances from *Burkholderia* sp. Culture Broth. *Current Microbiology*, **53**, 358-364. https://doi.org/10.1007/s00284-005-0333-2

[14] Kang, J.G., Shin, S.Y., Kim, M.J., Bajpai, V., Maheshwari, D.K. and Kang, S.C. (2004) Isolation and Anti-fungal Activities of 2-Hydroxymethyl-Chroman-4-One Produced by *Burkholderia* sp. MSSP. *The Journal of Antibiotics*, **57**, 726-731. https://doi.org/10.7164/antibiotics.57.726

[15] Tawfik, K.A., Jeffs, P., Bray, B., Dubay, G., Falkinham, J.O., Mostafa, M., Youssef, D., Khalifa, S. and Schmidt, E.W. (2010) Burkholdines 1097 and 1229, Potent Anti-fungal Peptides from *Burkholderia ambifaria* 2.2N. *Organic Letters*, **12**, 664-666. https://doi.org/10.1021/ol9029269

[16] Lin, Z., Falkinham, J.O., Tawfik, K.A., Jeffs, P., Bray, B., Dubay, G., Cox, J.E. and Schmidt, E.W. (2012) Burkholdines from *Burkholderia ambifaria*: Antifungal agents and Possible Virulence Factors. *Journal of Natural Products*, **75**, 1518-1523. https://doi.org/10.1021/np300108u

[17] Lu, S., Novak, J., Austin, F.W., Gu, G., Ellis, D., Kirk, M., Wilson-Stanford, S., Tonelli, M. and Smith, L. (2009) Occidiofungin, a Unique Antifungal Glycopeptide Produced by a Strain of *Burkholderia contaminans*. *Biochemistry*, **48**, 8312-8321. https://doi.org/10.1021/bi900814c

[18] Joo, G.J., Kang, S.M., Hamayun, M., Kim, S.K., Na, C.I., Shin, D.H. and Lee, I.J. (2009) *Burkholderia* sp. KCTC 11096BP as a Newly Isolated Gibberellin Producing Bacterium. *The Journal of Microbiology*, **47**, 167-171. https://doi.org/10.1007/s12275-008-0273-1

[19] Scuderi, G., Bonaccorsi, A., Panebianco, S., Vitale, A., Polizzi, G. and Cirvilleri, G. (2009) Some Strain of *Burkholderia gladioli* are Potential Candidates for Postharvest Biocontrol of Fungal Rots in Citrus and Apple Fruits. *Journal of Plant Pathology*, **91**, 207-213.