

ORIGINAL ARTICLE

Antifungal activities of bacteria producing bioactive compounds isolated from rice phyllosphere against *Pyricularia oryzae*

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Abstract

Rice blast is the main disease of rice plants in Indonesia and several countries worldwide. Controlling this disease using chemical fungicides has harmful effects on the environment. Therefore, we need biocontrol agents which are more environmentally friendly such as rice phyllosphere bacteria. This study aimed to explore bacteria producing bioactive compounds from the rice phyllosphere environment to control blast disease. A total of 88 isolates were successfully isolated from rice leaves in Sukabumi, Situgede, and Jasinga (West Java, Indonesia). From them, we obtained 22 bacteria isolates with antifungal activity against *Pyricularia oryzae* *in vitro* assay. In addition, seven non-pathogenic bacteria were obtained from further screening in hypersensitivity, hemolysis and pathogenicity assays, namely STGG 3, STGG 7, STGG 8, STGG 14, SKBV 1, STGV 8, and SKBG 78. To show their antifungal activity, we tested crude extracts of these seven isolates and the results revealed that all the crude extracts can inhibit the growth of *P. oryzae*. Based on a genetic approach, isolates STGG 3, STGG 7, and STGG 14 were found to have both nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) genes, while isolate SKBV 1 only had the NRPS gene. The NRPS and PKS genes from potential isolates were similar to NRPS and PKS genes of *Bacillus* sp. in different strains. Furthermore, molecular identification based on the 16S rRNA gene revealed that the seven potential isolates belong to three genera, i.e. *Bacillus* (STGG 3, STGG 7, STGG 8, STGG 14, SKBV 1), *Enterobacter* (STGV 8) and *Brachy bacterium* (SKBG 78). We suggest that the seven isolates found in this study have potency and could be recommended as biocontrol agents of blast disease caused by *P. oryzae*.

Keywords: bioactive compounds, blast disease, NRPS, phyllosphere, PKS, *Pyricularia oryzae*

Introduction

Pyricularia oryzae (anamorph: *Magnaphorte oryzae*) is one of the most destructive pathogens in rice fields, causing blast disease that attacks rice plants in vegetative and generative growth phases. Rice blast disease attacked 1.285 million ha or 12% of all rice fields in Indonesia (Kharisma *et al.* 2013) and can cause yield losses of up to 90% on susceptible rice and 40–70% in India, the Philippines, and Nigeria (Sukanya *et al.* 2011). In a conducive environment, leaf blast can quickly

develop and cause the death of rice plants, while neck blast causes crop failure and disease spreading by rice seeds. Several methods have been applied to manage blast disease including fungicide application, plant rotation, and resistant cultivar planting. Among them, the use of fungicides is the most effective and popular way to control blast disease. However, chemical residue from fungicides is harmful to the environment, human health and agricultural products. Therefore, eco-

friendly blast disease management is continuously being developed. The use of biocontrol agents (bacteria, fungi, actinomycetes, etc.) isolated from the environment has become the most effective method for plant disease control.

The phyllosphere or phylloplane is defined as the plant surface environment above the ground, either stems or leaves. The phyllosphere environment is dominantly occupied by bacteria that are living as commensal bacteria. The amount of bacteria that can be obtained from one gram of plant material ranges from 10^5 to 10^7 cells (Yadav *et al.* 2010). They play an important role in combating plant disease and increasing the growth of host plants through diverse mechanisms (Rastogi *et al.* 2013). From previous studies, Khrisanti *et al.* (2015) and Harsonowati *et al.* (2017) successfully isolated *Bacillus* sp. and actinomycetes from the rice phyllosphere environment which can inhibit *Xanthomonas oryzae* pv. *oryzae* and *P. oryzae*, respectively. Therefore, rice phyllosphere bacteria could be developed as biocontrol agents of plant pathogens.

The ability of bacteria to suppress plant pathogens is due to their production of bioactive compounds, siderophore and antibiotics (Junaid *et al.* 2013). Generally, bioactive compounds derived from bacteria are synthesized by nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) enzymes. Both enzymes encoded by NRPS and PKS genes consist of a conserved domain, an adenylation (A) domain for NRPS and a ketosynthase (KS) domain for PKS (Amoutzias *et al.* 2008). These genes could be amplified for screening of potential bacteria producing bioactive compounds. This study aimed to isolate rice phyllosphere bacteria producing bioactive compounds with antifungal activity against *Pyricularia oryzae*, the causal agent of blast disease on rice.

Materials and Methods

Isolation and purification of rice phyllosphere bacteria

Rice plants without blast symptoms were collected from Situgede, Jasinga, and Sukabumi (West Java, Indonesia). Rice leaves in vegetative and generative growth phases were taken from the plants. Phyllosphere bacteria were isolated following serial dilution (Yadav *et al.* 2010). Ten grams of rice leaves from the three regions were each cut into small pieces and mixed with 90 ml saline buffer solution (0.85% NaCl) prior to shaking at 150 rpm at room temperature (27°C) for 1 h. The serial dilution was conducted in saline buffer solution and 100 µl mixed solution from 10^{-3} to 10^{-6} serial dilution were aseptically plated on Luria-Bertani

(LB) agar medium (1% NaCl, 1% tryptone, 0.5% yeast extract, 2% agar in 1 l distilled water). Then, the plates were incubated for 3 days at room temperature. Each bacterial colony with different morphological characters that grew on the medium was purified in the same medium to obtain pure colonies.

Antifungal assay

The antifungal assay was conducted following the method described by Kandel *et al.* (2017) with modification in the distances between bacterial agents and the edge of Petri dishes, which was 2 cm. *Pyricularia oryzae* race 173 was inoculated in potato dextrose agar (PDA) medium 4 cm from the petri dish edge. The pathogenic fungi were first incubated at room temperature for 5 days prior to inoculation by phyllosphere bacteria. Observation of inhibition growth was carried out after 10 days of incubation and the percentage of inhibition of radial growth (PIRG) was calculated according to length differences of normal (R1) and inhibited (R2) hyphal growth by using the following formula:

$$PIRG = \frac{R1 - R2}{R2} \times 100 [\%].$$

Hemolysis, hypersensitivity and pathogenicity assays

Selected phyllosphere bacteria were streaked in blood agar medium (5% blood sheep; 2.5% NaCl; 1.5% agar in 1 l distilled water). Hemolysis response was observed after 2 days of incubation. A positive response was indicated by a clear zone around the bacterial colonies (Bernal *et al.* 2015). For hypersensitivity reaction and pathogenicity assays, each bacterial isolate was cultured in LB broth medium for 24 h (cell concentration was approximately $\pm 10^6$ CFU · ml⁻¹). One ml of each bacterial culture was injected into tobacco leaves using a needleless syringe (Sundh *et al.* 2011) and inoculated into one-month-old rice leaves var. Ciherang following leaf clipping. Observations were conducted 2 days after leaf injection and 14 days after rice plant inoculation. Isolates causing chlorosis on tobacco leaves or having a necrotic effect on rice leaves indicated that the bacterial isolates were pathogens and were not used for further analysis. *Xanthomonas oryzae* pv. *oryzae* and *Bacillus cereus* INT C1 were used as positive and negative controls, respectively.

Molecular identification

Identification of seven selected phyllosphere bacteria with antifungal activity was based on 16S rRNA

sequence. The bacterial genome was extracted following the procedure described by Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech Ltd). DNA concentration and purity were measured using Nanodrop 1000 (Thermo Scientific, Wilmington, DE, USA). The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using primers of 63F (5'-CAG GCC TAA CAC ATG CAA-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3') (Fredriksson *et al.* 2013) with an amplicon target of ~1,300 bp. The PCR cycle involved pre-denaturation (94°C, 4 min), followed with 30 cycles of denaturation (94°C, 30 sec), annealing (55°C, 30 sec), elongation (72°C, 1 min) and post-elongation (72°C, 10 min). The PCR mixture contained 25 µl Go Taq Green Master Mix 1x (Promega®, USA), 0.5 µl (10 pmol · µl⁻¹) of each primer, 1 µl DNA template (~ 100 ng · µl⁻¹) and adjusted with 23 µl nuclease free water. The PCR products were visualized using agarose gel electrophoresis of 1% (w/v). The sequences of 16S rRNA genes from seven bacterial isolates were compared to sequences in the DNA database in the National Centre for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) using the BLASTN program. The neighbor-joining analysis in MEGA 7.0 software was performed to construct the phylogenetic tree using the Tamura-Nei model. In addition, the stability of the phylogenetic tree was assessed with one thousand bootstrap replicates.

Extraction of crude bioactive compounds

Crude bioactive compounds of phyllosphere bacteria were extracted following the method described by Islam *et al.* (2012) with modification in the volume of ethyl acetate as solvent. Each phyllosphere bacteria was cultured in 1 l of LB broth medium by shaking at 150 rpm for 3 days at room temperature. The bacterial culture was mixed with 1 l of ethyl acetate and shaken vigorously for 20 min. To obtain the crude extract of bacteria, the organic layer was collected and evaporated to dryness in a vacuum evaporator at 40°C. Then, the bacterial crude extracts were stored at 5°C until used for the next analysis.

Antifungal assay of crude bioactive compounds

The crude extract was dissolved in ethyl acetate solvent to make 50,000 ppm of concentration and filtered using 0.22 µm syringe filter. The antifungal assay was conducted following the poison food technique described by Islam *et al.* (2012) with modification in the amount of PDA medium that was used in this assay. PDA medium containing 1% (v/v) crude extract was poured into petri dishes until solidified, and agar block

P. oryzae race 173 (4 mm in diameter) was inoculated to the center of a Petri dish prior to incubation at room temperature. The PDA medium with ethyl acetate and without crude extracts was used as negative control. Each treatment was made in three replications. Inhibition growth was observed 10 days after inoculation and the antifungal activity percentage was calculated according to the fungal diameter of the control (*Dc*) and treatment (*Dt*) following the formula:

$$\text{Antifungal activity} = \frac{Dc - Dt}{Dc} \times 100 [\%].$$

NRPS and PKS gene amplification

The adenylation (A) domain from the NRPS gene and the ketoacyl synthase (KS) domain from the PKS-I gene of the bacterial genomes were amplified by PCR using specific primers. Primers of MTF (5'-CCN CGD ATY TTN ACY TG-3') and MTR (5'-GCN GGY GGY GCN TAY GTN CC-3') were used to amplify the A domain of NRPS (Tambadou *et al.* 2014), while KS2F (5'-GCS ATG GAY CCS CAR CAR CGS VT-3') and KSR5 (5'-GTS CCS GTS CCR TGS SCY TCS AC-3') were used to amplify the KS domain of PKS (Zhang *et al.* 2009). The PCR mixture contained 25 µl Go Taq Green Master Mix 1x (Promega® USA), 1 µl of each primer (10 pmol · µl⁻¹), 1 µl DNA template (100 ng · µl⁻¹) and were adjusted with 22 µl nuclease free water to reach a total volume of 50 µl. The PCR reactions consisted of pre-denaturation (94°C, 5 min); 30 cycles of denaturation (94°C, 45 sec), annealing (58°C and 50°C, 45 sec), elongation (72°C, 1 min) and post-elongation (72°C, 7 min). PCR products were subsequently assessed by agarose gel electrophoresis of 1% (w/v) to visualize ~800 and ~1,000 bp size amplicon of A domain and KS domain, respectively. The PCR products were subsequently sequenced and the nucleotide sequences were compared to the protein database in NCBI (<http://www.ncbi.nlm.nih.gov>) using the BLASTX program. A phylogenetic relationship was built using the neighbor-joining method with Tamura-Nei parameter model in MEGA 7.0 software. The 1000x bootstrap value was performed to analyze the reliability of the phylogenetic tree.

Results

Isolated rice phyllosphere bacteria with antifungal activity

A total of 88 rice phyllosphere bacteria was successfully isolated from the leaves of rice var. Cihorang in Sukabumi, Situgede, and Jasinga, West Java, Indonesia.

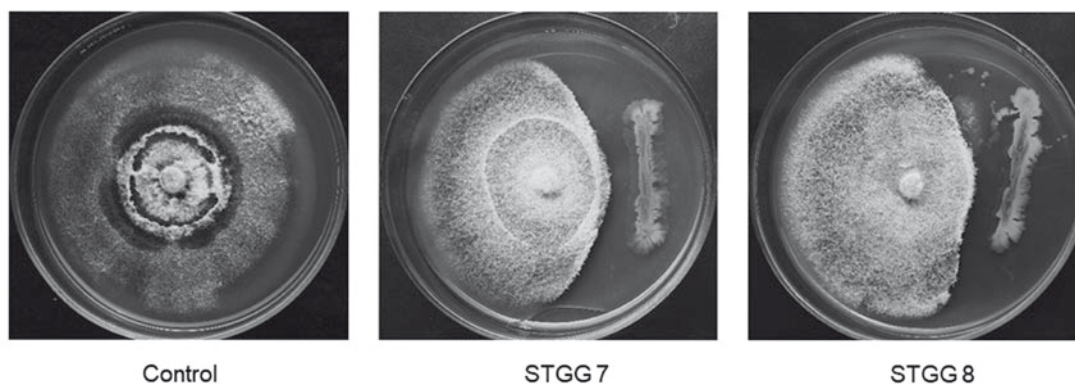


Fig. 1. Selected phyllosphere bacteria with antifungal activity are indicated by the formation of a clear zone

From the initial screening, 22 isolates were found to have antifungal activity against *P. oryzae* race 173, as indicated by the formation of a clear zone between the isolate and fungal pathogen (Fig. 1). Furthermore, from 22 isolates, we obtained seven non-pathogenic isolates from further screening in hemolysis, hypersensitivity and pathogenicity assays. The range of PIRG values from these seven bacteria isolates was 25.45% to 61.5% (Table 1). The highest PIRG value was shown by isolate STGG 14, while the lowest antifungal activity was shown by isolate STGV 8.

Amplification of 16S rRNA gene and sequence analysis

The 16S rRNA genes were successfully amplified from seven rice phyllosphere bacterial genomes. Sequence analysis of the DNA using the BLASTN program in NCBI revealed that the five bacterial isolates were homologous with *Bacillus* (isolates SKBV 1, STGG 3, STGG 7, STGG 8 and STGG 14), while two isolates were homologous with *Enterobacter* (isolate STGV 8) and *Brachybacterium* (isolate SKBG 78), respectively (Table 1). The phylogenetic tree of these seven bacterial

isolates that were constructed using the neighbor-joining method is shown in Figure 2.

Antifungal activity of crude bioactive compounds

Crude extracts of bioactive compounds were obtained from seven bacterial isolates and tested against *P. oryzae* race 173 *in vitro*. The crude extracts from all isolates were found to have antifungal activity which is characterized by inhibition of mycelial growth of treated fungi. Furthermore, the mycelial of treated fungi also had morphology that was different than the negative control (Fig. 3). The antifungal activities of crude bioactive compounds were very diverse between all isolates and ranged from 5.94% to 33.79%. The highest and the lowest antifungal activities were shown by isolate STGG 14 and isolate SKBG 78, respectively. This result is in line with the antifungal activity of the bacterial culture *in vitro* analysis, where isolate STGG 14 also showed the highest activity. A comparison of antifungal activities between bacterial culture and crude extracts is shown in Figure 4.

Table 1. Percentage inhibition, molecular identification, and the presence of PKS and NRPS genes of seven bacterial isolates isolated from rice phyllosphere

Bacterial isolates	PIRG [%] ^a	Closest bacteria strain (Accession number)	Identity [%]	E-value	Presence genes: NRPS/PKS
STGV 8	25.45 ± 1.44	<i>Enterobacter cloacae</i> strain Mc8 (MG238590.1)	99	0.0	-/-
SKBG 78	31.19 ± 1.64	<i>Brachybacterium paraconglomeratum</i> strain LMG 19861 (NR025502.1)	99	0.0	-/-
STGG 3	53.75 ± 1.77	<i>Bacillus</i> sp. strain WC5 (JN975953.1)	99	0.0	+/+
SKBV 1	56.25 ± 1.77	<i>Bacillus subtilis</i> strain NC1B 3610 isolate P5 (KY886246.1)	99	0.0	+/-
STGG 8	58.47 ± 2.91	<i>Bacillus amyloliquifaciens</i> strain P2 (MF358481.1)	91	0.0	-/-
STGG 7	60.74 ± 2.5	<i>Bacillus subtilis</i> strain A2R6 (KC634262.1)	92	0.0	+/+
STGG 14	61.5 ± 1.41	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> strain 168 isolate CESI5 (KY886250.1)	98	0.0	+/+

PKS – polyketide synthase; NRPS – nonribosomal peptide synthase; PIRG – percentage of inhibition of radical growth
^avalue means ±SD, NRPS/PKS: +/- (presence/absence)

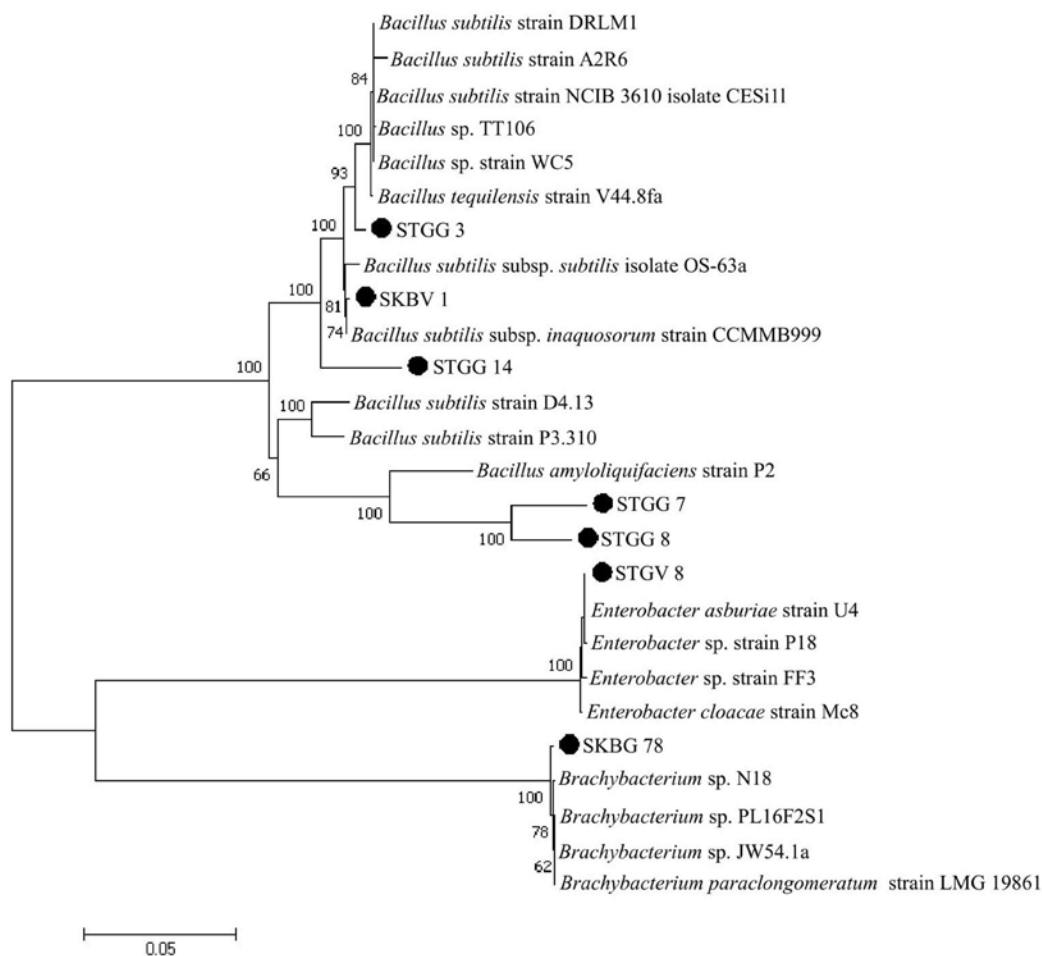


Fig. 2. Phylogenetic tree presenting the relationship of seven rice phyllosphere bacteria based on 16S rRNA gene using the neighbor-joining method. The percentage of homology is shown by bootstrap testing (1,000 replicates). Black circles indicate phyllosphere bacteria in this study

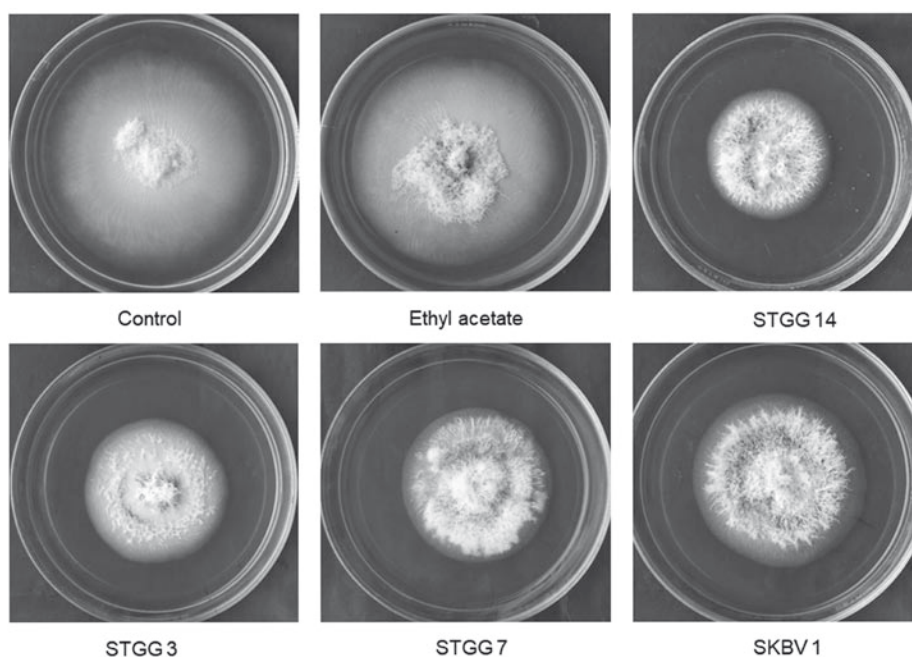


Fig. 3. Antifungal activity of crude bioactive compound extracts from bacterial isolates against *Pyricularia oryzae* with the poisoned food technique

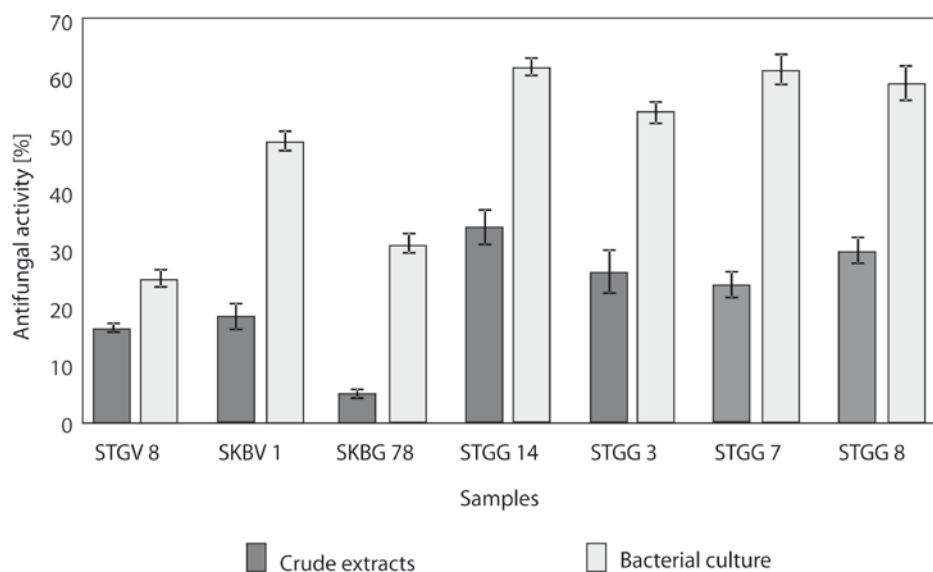


Fig. 4. Comparison of antifungal activity between bacterial culture and crude extracts against *Pyricularia oryzae* in vitro assay

NRPS and PKS gene sequence analysis

Among seven bacterial isolates, we identified three isolates having both NRPS and PKS genes, yet one isolate only had the NRPS gene and the other three isolates did not have both genes. As shown in Table 2, the NRPS and PKS genes identified from three isolates (STGG 3, STGG 7 and STGG 14) were homologous with NRPS and PKS from genera of *Bacillus* in different species and strains. In addition, the NRPS gene from isolate SKBV was also a homologous with *Bacillus* species. Furthermore, the results of sequence analysis using BLASTX program is presented in Table 2.

Discussion

In this study, healthy rice plants of the Cihurang variety without blast symptoms were taken from rice fields

that were infected by blast disease. Rice plant fitness is affected by phyllosphere microbe communities that are predominant with commensal bacteria. We estimated that there are several beneficial bacteria playing important roles in the health of rice plants without any blast disease infection. A total of 88 isolates were successfully isolated from rice phyllosphere environments in Sukabumi, Jasinga, and Situgede, West Java, Indonesia and they were tested for their antifungal activity against *P. oryzae* race 173. From the initial screening, we obtained 22 isolates with antifungal activity against *P. oryzae* in vitro, and further screening in hypersensitivity, hemolysis, and pathogenicity assays showed that seven isolates were non-pathogenic bacteria. Fungal growth inhibition was characterized by the presence of a clear zone between *P. oryzae* and phyllosphere bacteria (Fig. 1), that might be caused by water-soluble antifungal metabolites produced by rice phyllosphere bacteria. There are several mechanisms by which bacteria inhibit other microbes, e.g. competition for nutrients,

Table 2. Bioinformatics analysis of NRPS and PKS genes from rice phyllosphere bacteria using the BLASTX program

Bacterial isolates	Similarity	Identity [%]	E-value	Accession number
STGG 7	Poliketide synthase [<i>Bacillus</i> sp.]	97	1e-122	WP095713871.1
STGG 3	Poliketide synthase [<i>Bacillus subtilis</i>]	99	2e-141	WP095317817.1
STGG 14	Poliketide synthase [<i>B. subtilis</i>]	98	1e-141	WP080010800.1
STGG 7	Non-ribosomal peptide synthetase [<i>Bacillus</i> sp. MSP13]	94	3e-136	WP039075880.1
SKBV 1	Non-ribosomal peptide synthetase [<i>B. subtilis</i>]	96	1e-179	WP029317185.1
STGG 14	Non-ribosomal peptide synthetase [<i>Bacillus</i> sp. CC120222-01]	99	1E-178	WP085186744.1
STGG 3	Non-ribosomal peptide synthetase [<i>Bacillus</i> sp. GL120224-02]	99	0.0	WP097080590.1

production of siderophores, antibiotics, enzymes, and volatile compounds (Junaid *et al.* 2013). As such, further analysis is required to determine the antifungal mechanism of rice phyllosphere bacteria against *P. oryzae*.

Seven non-pathogenic bacterial isolates were obtained from vegetative and generative rice plants in Sukabumi and Situgede. The highest mycelial inhibition (61.5%) was shown by isolate STGG 14 from a generative rice plant in Situgede, while the lowest (25.45%) was shown by isolate STGV 8 from a vegetative rice plant in Situgede (Table 1). The difference in inhibition percentages between the isolates indicates different mechanisms against *P. oryzae in vitro*. This finding confirmed that the rice phyllosphere environment is inhabited by commensal bacteria with potency in managing plant pathogens. Furthermore, according to the identification analysis, the isolates belong to three genera, i.e. *Bacillus*, *Enterobacter* and *Brachybacterium*. Isolates STGG 7, STGG 14 and SKBV 1 are closely related with *B. subtilis*, STGG 3 with *Bacillus sp.* strain WC5, and STGG 8 with *B. amyloliquifaciens* strain P2 (Table 1, Fig. 2). *Bacillus* species have become the target of particular research because of their characteristics e.g. they are environmentally friendly, are easily found in various habitats and are able to survive under several natural conditions by developing endospores. This *Bacillus* species can also synthesize several bioactive compounds for agricultural application (Mora *et al.* 2011). Rungjindamai (2016) successfully isolated *Bacillus* species from the phyllosphere of mangoes with antifungal activity against *Colletotrichum gleosporioides in vitro* assay. This *Bacillus* could produce bioactive compounds to impede the spore germination of *C. gleosporioides* in artificially wounded mangoes. In addition, Sha *et al.* (2016) revealed the interaction between *B. subtilis* SYX04 and SYX20 and *Magnaporthe oryzae* P131 in controlling blast disease, where these two bacteria can suppress mycelial growth, conidial germination, germ tube formation and appressorium formation of *M. oryzae* P131. Commercially, *B. amyloliquifaciens* FZB42 is also known to be used as a bio-control agent especially efficient against fungal and bacterial pathogens on tomato, cucumber, cotton, tobacco, and lettuce (Chowdhury *et al.* 2015).

On the other hand, potential isolate STGV 8 was closely related with *Enterobacter cloacae* strain Mc8, and SKBG 78 with *Brachybacterium paraconglomeratum* strain LMG 19861. Several strains of *E. cloacae* were reported to be capable of colonizing and promoting plant growth in diverse crops such as soybean, rice, corn, cucumber, and ginger. It is also reported to have antifungal activity against *Phytophthora ultimum*, *Fusarium moniliforme* and *F. oxysporum* (Liu *et al.* 2013). In addition, *Brachybacterium* species also play an important

role in agriculture by producing ACC-deaminase enzyme related to ethylene production in crop plants and promoting plant growth (Gontia *et al.* 2011).

To confirm the antifungal activity, we extracted crude bioactive compounds from the seven rice phyllosphere bacteria. Seven isolates were cultured in LB broth medium, serving as a source of nutrients, and bioactive compounds were extracted after 3 days of incubation when the metabolites had accumulated. Ethyl acetate, as a semi-polar solvent, is used to bind more diverse types of bioactive compounds between polar and non-polar solvents. The antifungal activity from crude extract was tested against *P. oryzae in vitro*. Using poisoned food technique, each crude extract was dissolved in ethyl acetate to obtain 50,000 ppm concentration, but only 100 μ l crude extract was mixed with a tested medium to obtain 500 ppm concentration. All crude extracts were found to have antifungal activity against *P. oryzae* with different inhibition percentages (Fig. 3). The crude extract of isolate STGG 14 showed the highest antifungal activity (33.79%), while SKBG 78 showed the lowest activity (5.94%). Mycelial growth inhibition by crude extract was lower than by bacterial culture (Fig. 4). This may be because the bacteria possibly secrete several antifungal compounds, not only secondary metabolites but also hydrolytic enzymes and volatile organic compounds that inhibit mycelial growth. In addition, it is necessary to purify bioactive compound extracts to obtain pure antifungal compounds in order to increase antifungal activity of the extracts. These results also indicate that application of phyllosphere bacteria or their formulations directly to rice fields might be more effective than bioactive compound extracts themselves. The bacteria will survive and colonize in the phyllosphere environment, so rice plant fitness can be maintained over a long time.

The bioactive compounds from the crude extract might be encoded by non-ribosomal and polyketides genes. Therefore, NRPS and PKS genes from non-pathogenic phyllosphere bacteria were analyzed to show that they can produce antifungal bioactive compounds. The NRPS and PKS are multienzymatic, multimodular megasynthases of oligopeptides and polyketides secondary metabolites that contain catalytic modules for single rounds of chain elongation and intermediate product modification (Amoutzias *et al.* 2008). We amplified A domain from NRPS for activation of amino acids and hydroxy acids and transferred them to a peptidyl carrier domain and a KS domain from PKS-I for decarboxylative condensation of the core domains of PKS modules (Fisch 2013). Three isolates were found to have both A domain and KS domain, i.e. isolates STGG 3, STGG 7 and STGG 14 while 1 isolate (SKBV 1) only has KS domain. Sequence analysis of NRPS and PKS-I genes revealed that these amplified genes are similar to

polyketide synthase and non-ribosomal peptide synthetase of the genus *Bacillus* (Table 2). This finding is appropriate with molecular identification results since those isolates are also indeed closely related with *Bacillus*. Several strains from this genus are known to produce a wide variety of biocontrol metabolites, including ribosomally synthesized antimicrobial peptides (bacteriocins) as well as non-ribosomally synthesized peptides (NRPs) and polyketides (PKs) (Zhao and Kuipers 2016). NRPS, PKS or hybrid NRPS/PKS genes from *Bacillus* strains usually encode lipopeptide compounds mainly known for their antifungal properties such as iturins, fengycins and bacillomycin (Walia and Cameotra 2015). Lou et al. (2014) confirmed the contribution of lipopeptides bacillomycin L and surfactin, produced by *Bacillus subtilis* 916, in controlling sheath blight in rice plants caused by *Rhizoctonia solani*.

In this study, a total of seven rice phyllosphere bacteria and their crude extracts had antifungal activity against *P. oryzae* *in vitro*. This finding confirms that the rice phyllosphere environment is inhabited by beneficial microbes which can potentially be developed as biological control agents. However, greenhouse and field experiments need to be carried out in order to examine the effectiveness of isolated rice phyllosphere bacteria in plants. In addition, it is also important to identify and purify the bioactive compounds from these phyllosphere bacteria.

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