Rice Bran Amendment Suppresses Potato Common Scab by Increasing Antagonistic Bacterial Community Levels in the Rhizosphere

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ABSTRACT

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Potato common scab (PCS), caused by pathogenic *Streptomyces* spp., is a serious disease in potato production worldwide. Cultural practices, such as optimizing the soil pH and irrigation, are recommended but it is often difficult to establish stable disease reductions using these methods. Traditionally, local farmers in southwest Japan have amended soils with rice bran (RB) to suppress PCS. However, the scientific mechanism underlying disease suppression by RB has not been elucidated. The present study showed that RB amendment reduced PCS by repressing the path-

ogenic *Streptomyces* population in young tubers. Amplicon sequencing analyses of 16S ribosomal RNA genes from the rhizosphere microbiome revealed that RB amendment dramatically changed bacterial composition and led to an increase in the relative abundance of gram-positive bacteria such as *Streptomyces* spp., and this was negatively correlated with PCS disease severity. Most actinomycete isolates derived from the RB-amended soil showed antagonistic activity against pathogenic *Streptomyces scabiei* and *S. turgidiscabies* on R2A medium. Some of the *Streptomyces* isolates suppressed PCS when they were inoculated onto potato plants in a field experiment. These results suggest that RB amendment increases the levels of antagonistic bacteria against PCS pathogens in the potato rhizosphere.

Additional keywords: rhizosphere bacterial community.

Potato common scab (PCS), caused by soil- and seedborne pathogenic *Streptomyces* spp., occurs throughout potato-growing areas in Japan and around the world. PCS infection damages the skins of tubers and greatly reduces their market value, making it one of the most economically limiting factors affecting potato production (Dees and Wanner 2012; Lazarovits et al. 2007; Liu et al. 1995). In Japan, at least three pathogenic *Streptomyces* spp. are known to cause PCS: *Streptomyces scabiei*, *S. turgidiscabies*, and *S. acidiscabies* (Kobayashi et al. 2012; Tashiro et al. 2012; Tomihama et al. 2016). Of these species, both *S. scabiei* (Lambert and Loria 1989) and *S. turgidiscabies* (Miyajima et al. 1998) are thought to be common pathogens in most Japanese potato cultivation areas (Nishi et al. 2015; Tashiro et al. 2012).

There has been an increasing interest in the utilization of cultural practices as alternatives to pesticides for controlling soilborne diseases (Katan 2000). To date, several cultural practices for controlling PCS have been developed, including optimizing soil pH (Lambert and Manzer 1991; Lacey and Wilson 2001), irrigation during tuber formation (Adams and Lapwood 1978), green manuring (Wiggins and Kinkel 2005), crop rotation (Hooker 1981), and organic amendment (Lazarovits et al. 2001). *S. scabiei* is able to grow between pH 5 and 8 (Hiltunen et al. 2009; Tashiro et al. 2012). Therefore, reducing the soil pH to below 5.2 has been recognized as one of the most efficient

ways of suppressing PCS (Lacey and Wilson 2001; Lazarovits et al. 2007). However, regulation of soil pH is labor intensive and expensive due to soil buffering effects. Irrigation, particularly during the 6 weeks following tuber initiation, has also been shown to reduce PCS (Lapwood et al. 1973) and is a common control measure adopted by growers. However, some studies have suggested that scab levels increased after irrigation (Haynes et al. 2010), and it is thought that the relationship between soil moisture and PCS is more complex than previously thought (Larkin et al. 2011). Hence, an alternative, reliable approach for suppressing PCS is needed.

Organic amendments are known to considerably influence soil microorganism community structures (Bernard et al. 2012; Cretoiu et al. 2013), and organic amendment is generally thought to affect the severity of soilborne diseases by changing the diversity and function of rhizosphere microbial communities (Singhai et al. 2011). Organic amendment reportedly enriches some bacterial groups that are antagonistic toward PCS pathogens, especially some *Streptomyces* populations (Bernard et al. 2012; Kinkel et al. 2012; Wiggins and Kinkel 2005). Noticeably, application of these antagonistic microbes, including species of *Streptomyces* (Hiltunen et al. 2009; Kobayashi et al. 2012; Liu et al. 1995), *Bacillus* (Han et al. 2005), and *Pseudomonas* (Singhai et al. 2011), has been shown to reduce the incidence of PCS under field conditions. In recent studies, inoculation with antagonists has been employed in conjunction with the application of organic amendments to improve PCS reduction efficiency (Kinkel et al. 2012; Larkin et al. 2011; Singhai et al. 2011).

In southwest Japan, mainly Kagoshima Prefecture, which is one of the largest cultivation areas for both rice and potato plants in Japan, amending soils with rice bran (RB), a byproduct of rice milling, has empirically been used to control PCS by local farmers. RB is thought to be primarily consumed by a wide range of

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microorganisms such as fungi and bacteria; therefore, RB amendment would be expected to considerably alter the microbial composition of a soil (Kanaiso and Yonemoto 2003; Yamagata and Ae 1999). However, the impact of RB amendment on a soil microbial community structure has not yet been assessed, and the scientific mechanism underlying disease suppression by RB amendment has not been elucidated.

In the present study, we first examined the effects of soil RB amendment on the population levels of *Streptomyces* spp. that cause PCS in the young tubers at the early tuber formation stage by quantitative polymerase chain reaction (qPCR) using *txtAB* genes, which are required for the production of the essential pathogenicity factor thaxtomin A (Loria et al. 2006; Nishi et al. 2015). Then, the bacterial community composition under RB amendment was characterized by amplicon sequencing of the 16S ribosomal RNA (rRNA) gene in order to survey the bacterial groups that responded to RB amendment. To clarify the functionality of the soil microbial community under RB amendment, actinomycetes were isolated from a soil amended with RB, and their antagonistic activities against PCS pathogens were examined under various culture and field conditions.

MATERIALS AND METHODS

Bacterial strains and culture media. Three strains of PCS pathogens (*S. scabiei* strain S58, *S. turgidiscabies* strain T45, and *S. acidiscabies* a10) were isolated from diseased tubers in Kagoshima Prefecture, Japan, and used in this study (Nishi et al. 2015; Tomihama et al. 2016). Fresh RB was obtained from a local rice milling company in Kagoshima Prefecture. The chemical properties of the RB used in this study were analyzed by the Japan Food Research Laboratories (Tokyo) and a typical data set is shown in Supplementary Table S1. All chemicals were purchased from Nacalai Tesque (Kyoto, Japan) or Wako Chemicals (Osaka, Japan). The R2A, starch agar, and vermiculite-starch media, which were used for antimicrobial assays and for preparing a spore inoculum source, were prepared as described previously (Collins and Willoughby 1962; Matsumoto 1979; Nishi et al. 2015). The media pH were adjusted with 0.1 to 1 M NaOH or HCl (unless otherwise stated in the text) using a pH spear tip electrode (F-21; Horiba Co., Kyoto, Japan) before autoclaving.

Field experimental design for assessing the disease suppression effect of RB amendment. Field experiments investigating the disease suppression effect of RB amendment on PCS were conducted in an experimental field at the Kagoshima Institute for Agricultural Development (31°28'56''N, 130°20'34''E, 25 m above sea level) in Kagoshima Prefecture, Japan, during the fall 2013 and 2014. The soil at the experimental site was classified as a volcanic ash soil (Andosol) with an initial pH (H₂O) of 5.86 in 2013 and 5.84 in 2014 (Supplementary Table S2). The field inoculum levels had been maintained since 1980 by artificial inoculation through the incorporation of tubers mainly infected by *S. scabiei* and *S. turgidiscabies* (Nishi et al. 2015).

In 2013, three different management approaches were included as factors: amendment, soil pH, and fertilizer. The amendment factor consisted of either the presence (RB amendment) or absence of RB amendment (nonamendment). RB was applied at a rate of 3 t/ha in the furrow when the seed tubers were planted. The pH factor consisted of a soil pH of 5.8 (control, original soil with no pH adjustment) or 4.8. To adjust the soil pH from 5.8 to 4.8, commercial-grade ferrous sulfate (Ferro-sand; Ishihara BioScience, Tokyo) was applied to the soil at a rate of 5 t/ha 12 days before planting. Finally, two types of commercial fertilizers (conventional or slow-release nitrogen fertilizer) were applied at a rate of 1 t/ha (N-P-K at 140:140:140 kg/ha) at the time of planting. The research plots were established in a completely randomized design with two replication blocks for each treatment. There were 16 plots in total (two amendment levels × two pH levels × two fertilizer levels × two blocks) and the individual plots were 10 m long by 1 m wide. Potato seed tubers

(‘Nishiyutaka’) treated with the fungicide fluazinam (Frownicide; Ishihara BioScience) were manually planted 20 cm apart (50 seed potatoes per plot) on 18 September 2013. No pesticide was applied over the growing season and the weeds were controlled manually.

In 2014, three different management approaches were included as factors: amendment, fertilizer, and calcium application. In this survey, diseased seed tubers (disease severity index score was 1 for these tubers, which had been collected during the previous growing season) were used to ensure high inoculum intensity during the experiment. The RB and fertilizer treatments were the same as described for the field experiment in 2013. The calcium factor consisted of either the presence or absence of a gypsum application, which was applied at a rate of 1 t/ha at the time of planting. The research plots had a completely randomized design with two replication blocks for each treatment. There were 16 plots in total (two amendment levels × two fertilizer levels × two calcium levels × two blocks) and the individual plots were 8 m long by 1 m wide. The potato seed tubers (‘Nishiyutaka’) were manually planted 20 cm apart (40 potatoes per plot) on 10 September 2014. No pesticide was applied over the growing season and manual weed control was carried out.

The experiments, which included the amendment factor, were repeated twice in 2015. The effects of lowering the soil pH (2013) and the gypsum application rate (2014) were not examined in the repeated experiments (Supplementary Table S3). Therefore, we have not mentioned the effect of these factors on disease incident in the main text.

Measurement of disease and yield parameters. Disease severity and the proportion of diseased tubers were measured at harvest (2 December 2013 and 29 November 2014). Individual tubers were scored in order to calculate the mean disease severity index at the plot level using the following formula: $\Sigma (\text{disease index score} \times \text{number of tubers}) / (5 \times \text{total number of tubers}) \times 100$. The disease index score (0 to 5) is the percentage of the surface area covered by scab lesions, where 0 = no lesion and 0.5 = up to 1, 1 = 1 to 3, 2 = 3 to 13, 3 = 13 to 25, 4 = 25 to 50, and 5 = over 50% covered. The total number and weight of tubers and the average weight of tubers per plant were recorded for each treatment plot. These data were then used to calculate the yield parameters.

Soil sampling, enumeration of microbial populations, and isolation of actinomycetes. In 2013, bulk soil samples for microbial enumeration and isolation were collected at three growth stages: the preplanting stage (16 September, 10 days after soil pH adjustment and 2 days before planting), the early tuber formation stage (18 October, approximately 4 weeks after planting), and the harvesting stage (22 December). Three soil cores (5 cm in diameter and 15 cm in depth) were taken from a position that was equidistant between plants in each plot and were pooled to form a composite sample for each plot. At the preplanting soil stage prior to the applications of RB and fertilizers, three soil cores were collected from each block. After sieving (2-mm mesh), each composite soil sample was divided into two subsamples for microbial enumeration and soil chemical analysis. After air drying, the soil samples were ground in a mortar and pestle and their general chemical characteristics, including soil pH, were analyzed by Katakura Coop Agri Co., Ltd. (Tsuchiura, Japan).

Enumeration of total culturable bacteria and actinomycetes in the bulk soil was carried out using a plate counting method. Each soil subsample (10 g) was added to 40 ml of sterile water and gently shaken at 200 rpm for 1 h after ultrasonic treatment (SUW150; Sanyo, Tokyo) for 5 min. Each soil suspension was serially diluted and 100 µl of the diluent was spread onto two plates of R2A medium (pH 7.0). The plates were incubated at 25°C for 5 days in the dark. Then, the number of culturable actinomycetes in the bulk soil was measured by counting the number of actinomycete colonies (CFU/g of soil) on a plate based on their colony morphology. Subsequently, 96 isolates derived from the actinomycete colonies were collected from the bulk soils with and without RB amendment at the early

tuber formation stage (18 October) (96 isolates were collected: 24 colonies × [nonamendment + conventional fertilizer] × two blocks and 24 colonies × [RB amendment + conventional fertilizer] × two blocks).

Bulk soil samples for culturable actinomycete enumeration in 2014 were collected only at the early tuber formation stage (6 October). Two soil cores were taken from a position that was equidistant between two plants in each plot and were pooled to form a composite sample representing each plot. The number of total culturable actinomycetes in each composite sample was assessed using the plate counting method described above. In addition, 240 bacterial colonies (including actinomycetes) were collected from the fertilized bulk soil with and without RB amendment at the early tuber formation stage (240 isolates in total: 60 colonies × [nonamendment + conventional fertilizer + no gypsum application] × two blocks and 60 colonies × [RB amendment + conventional fertilizer + no gypsum application] × two blocks).

In both 2013 and 2014, the colonies were randomly isolated and purified twice by single-colony isolation, and a piece of each colony (mycelial mat) was stored in 10% glycerol stock at −30°C for further investigation.

Soil and tuber DNA extraction. Following the method used in the field experiment, rhizosphere soil samples for amplicon sequencing were taken from the soil adhering to the roots of three potato plants in each of eight selected plots on 6 November 2013 at the early tuber formation stage (24 samples in total: two amendment levels × two fertilizer levels × intact soil at pH 5.8 × two blocks × three potato plant replicates). The potato roots were gently shaken to remove loosely adhered soil, and the rhizosphere soil was collected by vigorously shaking the roots in a polyvinyl propylene bag. DNA was extracted from 500 mg of the rhizosphere soil sample using a FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH).

In the 2014 field experiment, rhizosphere soil samples and young tuber samples (approximately 5 to 10 mm in diameter) were taken in each plot on 6 October from two potato plants at the early tuber formation stage for amplicon sequencing and qPCR (32 samples in total: two amendment levels × two fertilizer levels × two calcium levels × two blocks × two potato plant replicates). The rhizosphere soil samples were collected as described above, and DNA was extracted from 500 mg of rhizosphere soil or 100 mg of young tuber surface using an ISOIL kit (Nippon Gene, Tokyo) and a Quantum Prep Kit (Bio-Rad Laboratories, Hercules, CA), respectively (Tanaka and Ikeda 2002).

Following the extraction, the quality and quantity of the extracted DNA samples were assessed by agarose gel electrophoresis and spectrophotometry (ND-1000; NanoDrop Technology, Wilmington, DE).

Quantification of the pathogenic *Streptomyces* spp. Quantification of the pathogenic *Streptomyces* spp. in the 2014 potato tuber DNA samples was carried out by qPCR using the *txtAB* operon, as previously reported (Nishi et al. 2015; Qu et al. 2008). Briefly, primer pairs StrepF (5'-GCAGGACGCTCACCAGG TAGT-3') and StrepR (5'-ACTTCGACACCGTTGTCCTCAA-3') were used in an SYBR Green real-time PCR assay. Real-time PCR amplifications were performed in 25-μl reaction mixtures containing 1× DimerEraser master mix (TaKaRa Co., Ltd., Kusatsu, Shiga, Japan) and 0.3 μM each primer. Tuber DNA extract (3 μl) was used as the DNA template per reaction. Real-time PCR amplifications were carried out on an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA), with an initial activation step of 95°C for 10 s followed by 45 cycles of 95°C for 5 s, 55°C for 10 s, and 72°C for 35 s. Serial dilutions of genomic DNA from pathogenic *S. scabiei* S58 were used as the standard. Quantifications of the pathogenic *Streptomyces* spp. in young tubers were repeated twice in 2015 and gave similar results (Supplementary File S1; Supplementary Table S4).

16S rRNA gene amplicon sequencing, sequence editing, and community analyses. In 2013, amplicon sequencing and amplification of the V4 region of the 16S rRNA gene using primers

515F and 806R (Caporaso et al. 2012) were performed using the paired-end Illumina MiSeq at FASMAC Co., Ltd. (Atugi, Japan). Sequence data editing was carried out as described in a previous study (Narihiro et al. 2014). Briefly, raw 16S rRNA gene sequences were processed using QIIME 1.8.0 (Caporaso et al. 2010). Low-quality sequences shorter than 300 bp, with an average quality score lower than 25 or with ambiguous bases, or mismatching primer sequences were eliminated from downstream analyses. The filtered and denoised sequence data were grouped into operational taxonomic units (OTU) by using the `pick_denovo_otus` command with default parameters (furthest neighbor). The sequences with ≥97% identities were grouped into one OTU by the UCLUST algorithm (Edgar 2010), and then the representative sequences of each OTU were aligned using PyNAST (Narihiro et al. 2014). The taxonomic assignment of each OTU was performed by an RDP classifier trained using a dataset from Greengenes v.13.8 at a minimum confidence level of 0.8 (Wang and Qian 2009). The sequence data were compared with the 16S rRNA genes in the RDP release 10 database using the BLAST+ program (Cole et al. 2009). After taxonomic assignment, α diversity indices (Chao1 and Shannon) for the 16S rRNA genes libraries and the weighted UniFrac distances were calculated and used for principal coordinates analysis (PCoA) by the QIIME pipeline (Caporaso et al. 2010). The α diversity and relative abundance were calculated from subsampled data (36,463 reads per sample), such that all samples had an equal sampling effort. An average relative abundance and diversity index was calculated for the three potato replicates in each plot. We used a two-way analysis of variance (ANOVA) to fit the linear models in JMP ver. 12 (SAS Institute, Tokyo). This included both amendment and fertilizer factors as fixed effects and the block factor as a random effect.

Amplification of the V4 region of the 16S rRNA gene using primers 515F and 806R (Caporaso et al. 2012) and amplicon sequencing and editing in 2014 were performed using the Roche GS junior at the RIKEN BioResource Center (Tsukuba, Japan) and followed Roche technical bulletins 2009-005 (April 2009) and 2011-007 (January 2012). The sequence data were denoised and the low-quality sequences, pyrosequencing errors, and chimeras were removed. Then, the sequences were clustered into OTU at 97% identity using QIIME 1.8.0, as described above. The α diversity and relative abundance were calculated from subsampled data (1,983 reads per sample), such that all samples represented an equal sampling effort. For relative abundance and diversity statistical analyses, the average relative abundance and diversity index of two potato replicates in each plot were taken as a single plot-level mean. We used a three-way ANOVA to fit the models in JMP ver. 12 (SAS Institute). This included amendment, fertilizer, and calcium factors as fixed effects and the block factor as a random effect.

Assessment of the antagonistic potentials of actinomycete isolates against PCS pathogens. To assess the antagonistic activity of the 96 actinomycetes isolated in 2013 against PCS pathogens, two strains of pathogenic *Streptomyces* spp. (*S. scabiei* S58 and *S. turgidiscabies* T45) and the 96 actinomycete isolates were grown on starch agar plates (pH 7.0) at 25°C for 10 days in the dark. Spore suspensions were then made by scraping the mycelium with a sterile loop and mixing the loop with 1 ml of sterile water to detach the spores from the mycelium. Spore suspension at approximately 10⁵ CFU/ml was prepared for each strain and isolate. To assess the antagonistic activity of actinomycete isolates under two different pH conditions (pH 7.0 and 5.0), a 500-μl spore suspension of a pathogenic *Streptomyces* bacterium was spread on an R2A plate that was adjusted to either pH 7.0 or 5.0 to act as an indicator. Then, a 10-μl spore suspension of an actinomycete isolate was dropped onto a plate (4 drops/plate). The inoculated R2A plates were incubated at 25°C in the dark for 3 days (pH 7.0) or 6 days (pH 5.0). Antagonistic activity (including competitive and antibiotic interactions) was evaluated by the presence of a clear zone around an actinomycete isolate. The proportion of the actinomycete isolates producing a clear zone of inhibition was calculated for the total

number of isolates derived from the bulk soil with or without RB amendment. The radius of each clear zone was used as an indicator of the growth inhibitory activity. The experiments were repeated twice and gave similar results.

Phylogenetic distribution analyses of actinomycete isolates by 16S rRNA gene sequences. The genomic DNA of the isolates and pathogenic *Streptomyces* spp. was extracted from a piece of colony grown on an R2A plate at 25°C in the dark for 5 days using a PrepMan Ultra Reagent (Applied Biosystems, Warrington, UK). Amplification of the 16S rRNA gene was performed using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3'), as previously described (Ikeda et al. 2011), and sequencing analyses were performed at TaKaRa Co., Ltd. In total, 140 actinomycete sequences (73 sequences in 2013 and 67 sequences in 2014) and sequences from three strains of pathogenic *Streptomyces* spp. were obtained. These sequences were assigned by MOTHUR into OTU using a cutoff of 0.01 (≥99% identity) for the distance matrix, as described previously (Ikeda et al. 2011). BLASTN was used to classify the OTU and to identify their closest relatives in the GenBank database. During the phylogenetic analyses, their sequences were aligned by CLUSTALW, and the neighbor-joining method was used to build a phylogenetic tree. The PHYLIP format tree output was obtained and 1,000 bootstrap trials were carried out. The trees were constructed using MEGA6 software (Tamura et al. 2013). Statistical differences in the number of isolates belonging to each OTU were evaluated between the nonamendment and RB-amendment treatments for 2013 and 2014 by using the compare algorithm in RDP. The *P* value was estimated from the *Z* critical value (Wang et al. 2007).

PCS suppression after inoculation with *Streptomyces* isolates under field conditions. A field experiment to assess the ability of *Streptomyces* isolates to suppress PCS was carried out in 2014 using “the bottomless frame method” (Sakamoto et al. 2011). Two different management approaches were included as factors: biocontrol treatments and RB amendment. The biocontrol treatment factor consisted of a pathogen inoculation (*S. scabiei* S58) without a *Streptomyces* isolate (positive control) and a dual inoculation of a pathogen and one of four *Streptomyces* isolates. The four candidate biocontrol agents isolated from RB-amended soil (ANA105, ANA109, ANA1007, and ANA1906) were selected after phylogenetic analyses of *Streptomyces* isolates for representing a wide phylogenetic diversity range. The four *Streptomyces* isolates and the pathogenic *S. scabiei* S58 were cultured on 1 kg of vermiculite-starch medium at 25°C for 20 days. Inoculum at approximately 10⁷ CFU/g was prepared for each isolate and pathogen. A proportion of each inoculum source (20 g for strain S58 and 80 g for each *Streptomyces* isolate) was mixed, and this mixture was gently blended with 1 kg of soil and poured into a bottomless frame (32 cm

in diameter and 30 cm in depth; 0.08 m²) in a field. The amendment factor consisted of nonamendment and RB amendment. The RB was applied at a rate of 24 g/frame (equivalent to 3 t/ha). The biocontrol and amendment treatments were applied just before planting (15 October 2014). In total, 40 framed, 0.08-m² plots (two amendment levels × five biocontrol levels × four replicates) were established with a completely randomized design. One healthy potato seed tuber (Nishiyutaka) per frame was manually planted in the frame soil on 15 October 2014. No pesticide was applied over the growing season, and manual weed control was carried out. Disease severity and the proportion of diseased tubers were measured at the harvesting stage (24 December 2014), as described above. The experiments, which included the soil amendment factor, were repeated in spring 2015 and gave similar results.

Statistics. Experimental designs fitting linear model analysis (one-way, two-way, and three-way ANOVA), a linear regression analyses, Spearman's rank correlation, stepwise regression of factors, and calculation of false discovery rate (FDR) *P* values (Benjamini and Hochberg 1995) were performed using JMP ver. 12.0 (SAS Institute). For statistical comparisons, we conducted a fitting linear models analysis that included all factors as the main effects; however, the interactions between factors were not included in the analysis. Disease severity data were arcsine transformed, and the actinomycete population and the pathogenic *Streptomyces* data were log transformed. For the responses that were shown to be significantly different by the models, a Student's *t* test or a Wilcoxon signed test was performed to compare between levels at $\alpha = 0.05$.

Nucleotide sequence accession numbers. Sequence data from amplicon sequencing in 2013 were made publicly available at the DNA Data Bank of Japan (DDBJ) Sequence Read Archive under accession number DRA003981, BioProject (PRJDB3924), and BioSample (SAMD00031517-SAMD00031540). Sequence data from amplicon sequencing in 2014 were made publicly available at the DDBJ Sequence Read Archive under accession number DRA004010, BioProject (PRJDB3924), and BioSample (SAMD00041214-SAMD00041245). The sequences of the actinomycete isolates used in phylogenetic analyses have been deposited in DDBJ under accession numbers LC061295–LC061437.

RESULTS

RB amendment reduced the occurrence of common scab. The suppressive effect of RB amendment against PCS was confirmed in field experiments conducted over two consecutive years (2013 and 2014) (Table 1). In 2013, the RB amendment significantly reduced PCS disease severity compared with the nonamendment control (Table 1). Similarly, in 2014, RB amendment again significantly reduced PCS (Table 1). The fertilizer factor had

TABLE 1. Effect of rice bran amendment on the severity of potato common scab, the actinomycete population, and the amount of pathogenic *Streptomyces* bacteria in young tubers after the field experiments in 2013 and 2014^w

Year	Factor ^x	Level	Disease severity ^y	Actinomycetes (log CFU/g of soil) ^y	Pathogenic <i>Streptomyces</i> (log fg/g of tuber) ^z
2013	Amendment	Nonamendment	12.4 ± 7.3 a	5.9 ± 0.2 b	NT
		Rice bran amendment	4.1 ± 3.2 b	7.2 ± 0.1 a	NT
		<i>P</i> value	0.011	<0.001	
2014	Amendment	Nonamendment	28.8 ± 9.0 a	6.7 ± 0.2 b	5.1 ± 0.5 a
		Rice bran amendment	15.9 ± 7.2 b	7.8 ± 0.3 a	4.4 ± 0.8 b
		<i>P</i> value	<0.001	<0.001	0.002

^w Lowercase letters represent significant differences within columns at $\alpha = 0.05$ and NT indicates not tested.

^x Factors other than amendment (fertilization in 2013 and 2014, pH treatment in 2013, and gypsum application in 2014) are shown in Supplementary Table S3.

^y Disease severity at harvesting and the actinomycete population at the early tuber formation stage are shown. Values are the least square means (± standard deviation) for each level by three-way analysis of variance (ANOVA) in 2013 (*n* = 8) and 2014 (*n* = 8). Student's *t* test was used for further comparison when the ANOVA showed significant differences between levels (*P* < 0.05). Disease severity and actinomycete population data were arcsine and log transformed, respectively.

^z Amount of pathogenic *Streptomyces* bacteria (log transformed) in young tubers at the early tuber formation stage was examined by qPCR analysis and the values are the least square means (± standard deviation) for each level by three-way ANOVA in 2014 (*n* = 8). A Wilcoxon signed test was used for further comparison when the ANOVA showed significant differences between levels (*P* < 0.05).

no effect on the incidence of PCS in both years. In 2014, the amounts of pathogenic *Streptomyces* bacteria in the young tubers were reduced by the RB amendment compared with the nonamendment control (Table 1). The declines in disease severity and the pathogenic *Streptomyces* population in young tubers after RB amendment were also confirmed in 2015.

The correlation analyses indicated that, in 2014, there was a significant positive correlation between the amount of pathogenic *Streptomyces* bacteria in young tubers and disease severity but a negative correlation between actinomycete population and disease severity (Supplementary Fig. S2A and B). In addition, stepwise screening of factors that correlated with disease severity in the experiment conducted in 2014 indicated that disease severity was significantly estimated by two factors: the amount of pathogenic *Streptomyces* bacteria in the tuber and the actinomycete population (Supplementary Fig. S2C).

Impact of RB amendment on microbial composition in the rhizosphere. Bacterial community analyses of the potato rhizosphere by amplicon sequencing in 2013 revealed that the RB amendment significantly increased the relative abundance of gram-positive bacteria. The α diversity analysis indicated that the number of OTU and the diversity indexes, such as the Chao1 and Shannon indexes, were significantly reduced by RB amendment in both 2013 and 2014 (Supplementary Table S5). Other factors such as fertilization had no effect on the α diversity of the rhizosphere bacterial community. Weighted UniFrac PCoA analysis showed that nonamended rhizosphere soil samples formed a tight cluster (Fig. 1A). They were distinctly separated from RB-amended samples along PC1 (64.6%), and variability was high among the RB-amended samples in 2013 (Fig. 1A).

Analysis of relative abundances in 2013 indicated that, at the phylum level, the most abundant phyla in the nonamendment soil were *Proteobacteria*, followed by *Acidobacteria*, *Actinobacteria*, and *Firmicutes* (Table 2). These four phyla accounted for 73.1% of the total reads (Table 2). RB amendment exclusively increased the relative abundance of *Actinobacteria* but decreased the relative abundance of *Acidobacteria* compared with the nonamendment soil (Table 2). At the order level, the relative abundances for *Actinomycetales* and *Saprospirales* were significantly increased by RB amendment (Table 2). In contrast, *Acidobacteriales*, *Gaiellales*, and *Solibacteriales* were significantly reduced by RB amendment in 2013 (Table 2). Accordingly, at the genus level, the

relative abundances of genera *Bacillus*, *Streptomyces*, *Chitinophaga*, and *Actinomadura* significantly increased, whereas unclassified *Koribacteraceae* and unclassified *Gaiellaceae* were significantly reduced after RB amendment in 2013 (Table 3). There was a power law relationship between the magnitude of increase by RB amendment (ratio of RB amendment to nonamendment relative to abundance at the genus level) and the number of genera at each magnitude (Supplementary Fig. S3A). With the exception of amendment, other factors such as fertilization had no effect on the rhizosphere bacterial community (Supplementary Tables S6 to S8). A similar trend for rhizosphere bacterial community shifts after RB amendment was also confirmed in 2014 (Supplementary Tables S9 and S10; Supplementary Figs. S3B and S4).

The Spearman's rank correlation analyses indicated that the relative abundance of *Streptomyces* spp. was most negatively correlated with disease severity in 2014 (Table 4). In addition, OTU 1 (closest relative species: *S. avellaneus*, 100% identity) and OTU 2 (closest relative species: *S. diastaticus*, 100% identity), which were the two most abundant *Streptomyces* OTU out of the 296 identified in 2013, had significantly increased following RB amendment (Fig. 1B). However, not all *Streptomyces* OTU were significantly changed by RB amendment (Fig. 1B), which suggested that RB amendment differentially affected the populations of certain groups of *Streptomyces* spp. in the potato rhizosphere.

Antagonistic activities of actinomycetes against pathogenic *Streptomyces* spp. The increase in soil actinomycetes, including the genus *Streptomyces*, in the bulk soil amended with RB was also confirmed by colony counting. In the 2013 experiment, the actinomycete population significantly increased after RB amendment by up to 10^7 CFU/g of soil at the early tuber formation stage (Table 1), and this was maintained at a similar level until the harvesting stage (data not shown). A similar result was obtained in the 2014 experiment (Table 1). However, fertilization had no effect on the cultural population of soil actinomycetes.

The PCS pathogens belong to the genus *Streptomyces*. Therefore, we isolated and characterized the soil actinomycetes, including *Streptomyces* spp., from nonamended soil and from RB-amended soil. More than 80% of the actinomycete isolates in 2013 produced an inhibitory zone on the R2A medium plate against both *S. scabiei* S58 and *S. turgidiscabies* T45 (Fig. 2A). The average diameter of the inhibitory zone on the pH 5.0 medium was significantly greater than on the pH 7.0 medium (Fig. 2A and B). The diameters of the

Fig. 1. Bacterial composition changes in the potato rhizosphere caused by rice bran (RB) amendment in 2013. **A**, Principal coordinates analysis of bacterial communities in the potato rhizosphere. Bacterial communities in potato rhizospheres were analyzed based on weighted UniFrac distance matrixes in 2013. Solid squares and open squares indicate samples from nonamended and RB-amended rhizospheres, respectively. **B**, Changes in the relative abundances of *Streptomyces* operational taxonomic units (OTU) (dominant 10 of 296 OTU belong to *Streptomyces* spp.). Relative abundance of OTU 1 and 2 were significantly increased by RB amendment. Not all OTU belonging to *Streptomyces* spp. responded in a similar way to RB amendment. When OTU were significantly different according to the analysis of variance (false discovery rate [FDR] $P < 0.05$), a Wilcoxon signed test was performed to compare the nonamendment and RB-amendment treatments. Asterisks represent significant differences for each OTU at $\alpha = 0.05$. Columns and error bars indicate the least square means and standard deviation ($n = 4$), respectively.

inhibitory zones of isolates from the nonamended soil were slightly but significantly greater than those from the RB-amended soil. However, there was no significant difference among the average diameters of the inhibitory zones against *S. scabiei* S58 and *S. turgidiscabies* T45 (Fig. 2B). These data clearly suggest that most of the indigenous actinomycetes have antagonistic activity against *S. scabiei* S58 and *S. turgidiscabies* T45, especially under low pH conditions. More importantly, RB amendment increased the populations of the indigenous actinomycetes that were antagonistic to PCS pathogens.

Phylogenetic distribution analyses of actinomycete isolates. The 16S rRNA gene sequences from a total of 140 actinomycete isolates were assigned to 39 OTU with $\geq 99\%$ identity (Fig. 3). Most of the OTU were classified into the genus *Streptomyces* (92.2%), and cluster analyses identified two abundant OTU (OTU ANA109 and OTU ANA105) (Fig. 3). The cluster analyses revealed that RB amendment increased the number of isolates belonging to OTU ANA109 (closest relative species: *S. glaucescens*, 99% identity) and OTU ANA105 (closest relative species: *S. roseochromogenus*, 99% identity) but decreased the number of isolates in OTU ANA2110 (closest relative species: *S. flavidovirens*, 99% identity) (Fig. 3).

Suppression of PCS after inoculation with antagonistic *Streptomyces* isolates under field conditions. The RB amendment significantly reduced the incidence of PCS compared with the nonamendment control (two-way ANOVA, $P < 0.001$) (Fig. 4). Under the nonamendment condition, inoculation with isolates ANA1906 or ANA109 significantly reduced disease severity compared with the noninoculated control (Fig. 4). Furthermore, inoculation with isolate ANA105 or ANA1906 significantly reduced

disease severity after RB amendment compared with the non-inoculated control (Fig. 4).

DISCUSSION

There have been previous reports on the suppressive effects of organic amendments on PCS (Bonanomi et al. 2010; Larkin 2008; Lazarovits 2001; Wiggins and Kinkel 2005). However, such effects

TABLE 3. Relative abundance of genera responding to rice bran (RB) amendment of the potato rhizosphere after the field experiment in 2013

Genus ^y	Relative abundance (%) ^x		R/N ^z	FDR <i>P</i> value
	Nonamendment	RB amendment		
<i>Bacillus</i>	5.34 ± 0.49 b	12.48 ± 3.26 a	2.34	0.026
<i>Uc Koribacteraceae</i>	3.84 ± 0.15 a	2.46 ± 0.23 b	0.64	<0.001
<i>Burkholderia</i>	3.60 ± 1.25	2.54 ± 0.49	0.71	0.210
<i>Uc Gaiellaceae</i>	3.45 ± 0.37 a	2.40 ± 0.19 b	0.70	0.007
<i>Streptomyces</i>	1.65 ± 0.18 b	3.58 ± 0.52 a	2.17	0.008
<i>Uc Gemmatimonadetes</i>	2.81 ± 0.48	2.16 ± 0.47	0.77	0.283
<i>Uc Chitinophagaceae</i>	2.13 ± 0.12	2.63 ± 0.39	1.23	0.102
<i>Rhodoplane</i>	2.64 ± 0.29 a	1.73 ± 0.15 b	0.66	0.002
<i>Uc iii1-15</i>	2.18 ± 0.29 a	1.64 ± 0.09 b	0.75	0.017
<i>Uc Myxococcales</i>	1.60 ± 0.08	1.68 ± 0.11	1.05	0.414
<i>Uc Bradyrhizobiaceae</i>	1.63 ± 0.13	1.47 ± 0.36	0.90	0.380
<i>Kaistobacter</i>	1.57 ± 0.25	1.48 ± 0.15	0.94	0.393
<i>Paenibacillus</i>	1.49 ± 0.09	1.37 ± 0.19	0.92	0.668
<i>Uc Rhodospirillaceae</i>	1.57 ± 0.22	1.27 ± 0.04	0.81	0.177
<i>Rhodanobacter</i>	1.03 ± 0.06	1.75 ± 1.42	1.70	0.351
<i>Ca. Koribacter</i>	1.64 ± 0.20 a	0.98 ± 0.19 b	0.60	0.031
<i>Uc Sinobacteraceae</i>	1.44 ± 0.21	1.14 ± 0.10	0.79	0.177
<i>Uc Xanthomonadaceae</i>	0.84 ± 0.16	1.40 ± 0.28	1.67	0.021
<i>Uc Solibacterales</i>	1.31 ± 0.13 a	0.92 ± 0.16 b	0.70	0.018
<i>Uc Gemmatimonadetes</i>	1.06 ± 0.44	1.17 ± 0.34	1.10	0.997
<i>Uc Gemmatimonadetes</i>	1.43 ± 0.27 a	0.74 ± 0.14 b	0.52	0.015
<i>Chitinophaga</i>	0.34 ± 0.14 b	1.80 ± 0.52 a	5.29	0.018
<i>Uc Micrococcaceae</i>	0.87 ± 0.06	1.19 ± 0.43	1.37	0.221
<i>Uc Syntrophobacteraceae</i>	1.27 ± 0.13 a	0.79 ± 0.11 b	0.62	0.021
<i>Uc MND1</i>	1.20 ± 0.09 a	0.83 ± 0.02 b	0.69	0.005
<i>Actinomadura</i>	0.16 ± 0.05 b	1.69 ± 0.34 a	10.56	0.002
<i>Nitrospira</i>	1.06 ± 0.22	0.62 ± 0.08	0.58	0.057

^x Values are the least square means (± standard deviation) for each level of the amendment factor obtained by two-way analysis of variance (ANOVA) in 2013 ($n = 4$). A Wilcoxon signed test was performed to compare relative abundance between nonamendment and RB amendment when the ANOVA showed significant differences between taxa (FDR $P < 0.05$). Letters represent significant differences at $\alpha = 0.05$. Relative abundances of genera responding to fertilization are shown in Supplementary Table S7.

^y In all, 27 dominant genera are shown. Uc = unclassified. Unclassified taxa were based on the highest taxonomic level of resolution.

^z Ratio of RB amendment to nonamendment relative abundance.

TABLE 4. Dominant 10 taxa that showed negative correlations for disease severity or the amount of pathogenic *Streptomyces* bacteria in young tubers after the field experiment in 2014

Genus ^y	Disease severity		Pathogenic <i>Streptomyces</i> bacteria	
	ρ^z	<i>P</i> value	ρ^z	<i>P</i> value
<i>Bacillus</i>	-0.552	0.026	-0.526	0.036
<i>Uc Chitinophagaceae</i>	-0.511	0.042	-0.111	0.680
<i>Uc Xanthomonadaceae</i>	-0.764	<0.001	-0.447	0.082
<i>Rhodanobacter</i>	-0.720	0.001	-0.526	0.036
<i>Chitinophaga</i>	-0.729	0.001	-0.447	0.082
<i>Burkholderia</i>	-0.820	<0.001	-0.679	0.003
<i>Streptomyces</i>	-0.902	<0.001	-0.620	0.010
<i>Uc Sphingobacteriales</i>	-0.673	0.004	-0.155	0.564
<i>Shingomonas</i>	-0.708	0.002	-0.435	0.032
<i>Uc Coxiellaceae</i>	-0.752	<0.001	-0.247	0.356

^y Uc = unclassified. Taxa were based on the highest taxonomic level of resolution.

^z Spearman's rank correlation value.

TABLE 2. Relative abundance of phyla or orders responding to rice bran amendment of the potato rhizosphere after the field experiment in 2013

Taxon ^x	Relative abundance (%) ^w		R/N ^y	FDR ^z <i>P</i> value
	Nonamendment	RB amendment		
Phylum				
<i>Proteobacteria</i>	33.22 ± 0.80	30.49 ± 2.25	0.92	0.411
<i>Acidobacteria</i>	15.68 ± 0.40 a	10.47 ± 0.43 b	0.67	<0.001
<i>Actinobacteria</i>	13.66 ± 0.10 b	20.18 ± 0.21 a	1.48	0.004
<i>Firmicutes</i>	10.54 ± 0.89	16.33 ± 3.73	1.55	0.086
<i>Chloroflexi</i>	6.83 ± 0.29 a	4.99 ± 0.57 b	0.73	0.008
<i>Gemmatimonadetes</i>	6.59 ± 0.79	5.13 ± 1.13	0.78	0.299
<i>Bacteroidetes</i>	3.66 ± 0.30	5.11 ± 0.95	1.40	0.063
<i>Verrucomicrobia</i>	2.58 ± 0.36	1.83 ± 0.32	0.71	0.121
<i>Planctomycetes</i>	2.05 ± 0.07	1.57 ± 0.23	0.77	0.042
<i>Nitrospira</i>	1.86 ± 0.37	1.18 ± 0.15	0.63	0.077
<i>Armatimonadetes</i>	0.79 ± 0.09 a	0.48 ± 0.01 b	0.61	0.004
WS3	0.70 ± 0.12 a	0.51 ± 0.06 b	0.73	0.017
Order				
<i>Bacillales</i>	9.32 ± 0.92	15.66 ± 3.77	1.68	0.070
<i>Rhizobiales</i>	7.32 ± 0.62	7.80 ± 1.50	1.07	0.227
<i>Actinomycetales</i>	7.12 ± 0.62 b	15.20 ± 1.86 a	2.13	<0.001
<i>Acidobacteriales</i>	6.08 ± 0.44 a	3.87 ± 0.19 b	0.64	<0.001
<i>Burkholderiales</i>	5.83 ± 1.19	4.88 ± 0.50	0.84	0.505
<i>Gaiellales</i>	3.81 ± 0.46 a	2.69 ± 0.25 b	0.71	0.012
<i>Xanthomonadales</i>	3.73 ± 0.24	5.19 ± 0.14	1.39	0.181
<i>Saprospirales</i>	3.10 ± 0.25 b	4.94 ± 0.84 a	1.59	0.044
<i>Myxococcales</i>	2.65 ± 0.15	2.54 ± 0.10	0.96	0.681
<i>Solibacterales</i>	2.42 ± 0.21 a	1.76 ± 0.20 b	0.73	0.004
<i>Sphingomonadales</i>	2.13 ± 0.30	2.03 ± 0.21	0.95	0.502
<i>iii1-15</i>	2.34 ± 0.30 a	1.76 ± 0.11 b	0.75	0.018
<i>Rhodospirillales</i>	1.84 ± 0.25	1.74 ± 0.16	0.95	0.521
<i>Nitrospirales</i>	1.86 ± 0.37	1.18 ± 0.15	0.63	0.077

^w Values are the least square means (± standard deviation) for each level of the amendment factor obtained by two-way analysis of variance (ANOVA) in 2013 ($n = 4$). A Wilcoxon signed test was used to compare nonamendment and rice bran (RB) amendment data when the ANOVA showed significant differences between taxa (FDR $P < 0.05$). Letters represent significant differences at $\alpha = 0.05$. Relative abundances of phyla or orders responding to fertilization is shown in Supplementary Table S6.

^x Dominant 12 phyla and 14 orders are shown.

^y Ratio of RB amendment to nonamendment relative abundance.

^z False discovery rate.

Fig. 2. Antagonistic activity of actinomycete isolates against pathogenic *Streptomyces* spp. **A**, Clear zones produced by antagonistic actinomycete isolates against the indicator bacterium (*Streptomyces scabiei* S58) on R2A medium plates at pH 7.0 and 5.0 (left and right plates, respectively). Spore suspensions of isolates ANA105, ANA109, ANA1007, and ANA1906 were dropped on the top, left, bottom, and right positions of each plate, respectively. Note that the clear zones on R2A pH 5.0 were larger than those on pH 7.0. **B**, Effect of medium pH on the antagonistic activity of actinomycete isolates against *S. scabiei* S58 and *S. turgidiscabies* T45 as evaluated by the radius of the clear zone on R2A medium at pH 7.0 and 5.0. The diameter of the inhibitory zone on the pH 5.0 medium was significantly greater than that on the pH 7.0 medium (three-way analysis of variance, $P < 0.001$). Inhibitory zones of isolates from the nonamendment soil were slightly but significantly greater than for the rice bran (RB)-amendment soil ($P = 0.005$) but there was no significant difference in the inhibitory zone diameters between *S. scabiei* S58 and *S. turgidiscabies* T45 ($P = 0.972$). Columns and error bars indicate means and standard deviation ($n = 48$), respectively.

	OTUs	Number of isolates				Closest known species	Accession No.	Identity (%)
		2013		2014				
		NA	RA	NA	RA			
	ANA1011	0	3	0	0	<i>Streptomyces roseochromogenus</i>	AB184512	99
	ANA2801	0	2	0	0	<i>Streptomyces roseochromogenus</i>	AB184512	98
	ANA1004	0	1	0	0	<i>Streptomyces roseochromogenus</i>	AB184512	99
	ANA105	0	7*	4	14	<i>Streptomyces roseochromogenus</i>	AB184512	99
	ANA1007	0	2	0	1	<i>Streptomyces jiujiangensis</i>	NR125706	100
	D330	1	0	1	1	<i>Streptomyces durhamensis</i>	NR112399	99
	ANA1904	1	1	0	0	<i>Streptomyces recifensis</i>	EU216596	98
	D1433	2	1	1	0	<i>Streptomyces recifensis</i>	EU216596	99
	ANA2104	1	0	0	0	<i>Streptomyces polychromogenes</i>	KF032545	100
	ANA3005	2	1	0	0	<i>Streptomyces shenzhenensis</i>	AB999927	98
	ANA1906	4	1	0	1	<i>Streptomyces rishiriensis</i>	KF317972	99
	D1406	0	0	1	0	<i>Streptomyces pseudovenezuelae</i>	KJ573013	99
	D104	0	0	1	0	<i>Streptomyces galbus</i>	KP233822	99
	D323	2	0	0	1	<i>Streptomyces chrestomyceticus</i>	NR115395	99
	ANA2110	4	0*	1	1	<i>Streptomyces flavidovirens</i>	FN649466	99
	<i>Streptomyces scabiei</i> S58							
	<i>Streptomyces turgidiscabies</i> T45							
	<i>Streptomyces acidiscabies</i> a10							
	D405	0	0	0	1	<i>Streptomyces asterosporus</i>	NR041193	98
	ANA305	1	0	0	0	<i>Streptomyces diastaticus</i>	KJ019008	100
	D309	0	0	0	1	<i>Streptomyces cinerochromogenes</i>	AB968639	100
	D334	0	0	0	1	<i>Streptomyces anandii</i>	KF595303	99
	ANA3003	1	0	0	0	<i>Streptomyces ollivaceus</i>	KP128878	99
	D401	0	0	0	1	<i>Streptomyces fumanus</i>	NR041101	99
	ANA2112	1	0	0	0	<i>Streptomyces glaucescens</i>	EU593712	98
	ANA109	3	8	2	15*	<i>Streptomyces glaucescens</i>	CP009438	99
	ANA1208	1	0	0	0	<i>Streptomyces phaeochromogenes</i>	EU554479	99
	ANA308	1	0	0	0	<i>Streptomyces purpeofuscus</i>	JK707864	99
	D1608	0	0	0	3	<i>Streptomyces purpeofuscus</i>	JN553847	99
	D1632	0	1	0	1	<i>Streptacidiphilus luteoalbus</i>	AY530190	99
	ANA302	1	0	0	0	<i>Kitasatospora arboriphila</i>	NR042904	100
	ANA1205	1	0	0	0	<i>Streptomyces xanthocidicus</i>	KM067120	99
	D340	2	7	4	4	<i>Streptomyces avellaneus</i>	FJ481075	100
	D320	2	1	0	2	<i>Streptomyces herbaricolor</i>	NR112506	99
	D1604	0	0	0	1	<i>Kitasatospora atroaurantiaca</i>	NR043493	99
	D1328	0	1	0	1	<i>Kitasatospora cystarginea</i>	NR112446	99
	D217	0	0	1	0	<i>Kitasatospora cystarginea</i>	NR112446	99
	ANA2811	0	1	0	0	<i>Streptomyces aomiensis</i>	NR112998	98
	D417	0	1	0	1	<i>Streptomyces aomiensis</i>	NR112998	99
	ANA1210	1	0	0	0	<i>Terrabacter tumescens</i>	FN386765	100
	ANA110	0	1	0	0	<i>Actinomodura oligospora</i>	AJ293709	99
	ANA303	1	0	0	0	<i>Nocardia niigatensis</i>	NR117402	99
	<i>Pseudomonas aeruginosa</i> PAO1							

Fig. 3. Phylogenetic distribution of actinomycete isolates derived from nonamended (NA) and rice bran amended (RA) soils in the 2013 and 2014 experiments. The 16S rRNA gene sequences in 140 actinomycete isolates (73 isolates in 2013 and 67 isolates in 2014) and three pathogenic *Streptomyces* spp. used in this study were determined, and they were assigned to 39 operational taxonomic units (OTU) by clustering with $\geq 99\%$ identity. The dendrogram indicates the phylogenetic relationships among the representative sequences of OTU. *Pseudomonas aeruginosa* PAO1 was used as an outgroup. Asterisks indicate a significant difference ($P < 0.05$) in the number of isolates for each OTU between the nonamended and RB-amended treatments using the compare algorithm in RDP (Wang et al. 2007). Strains used in the field inoculation test are indicated in bold.

are often inconsistent among studies and their modes of action are not fully understood. The important role that soil microbes play in suppressing PCS has been generally accepted. This has been confirmed by some studies on naturally occurring common-scab-suppressive soils (Rosenzweig et al. 2012; Sagova-Mareckova et al. 2015).

The natural soil suppression of PCS is associated with low copy numbers of *txtAB*, which is essential for the pathogenicity of PCS pathogens (Loria et al. 2006), in either the bulk soil or the rhizosphere (Sagova-Mareckova et al. 2015). The present study quantified the amount of *txtAB* in the tuber at the early tuber formation stage, which is a critical period for pathogen infection (Khatri et al. 2010, 2011). The results showed that RB amendment reduced PCS by repressing the pathogen population in the young tuber. Regression analyses of the results in 2014 showed that the amount of pathogenic *Streptomyces* bacteria in the young tubers at the early tuber formation stage was significantly correlated with disease severity. This fundamental correlation between disease severity and the densities of pathogenic *Streptomyces* bacteria in the tubers was probably identified for the first time because recently established methods for estimating the pathogen populations were used (Qu et al. 2008). Our results suggest that RB amendment represses the growth of pathogens on the surface of young tubers. Furthermore, stepwise regression analyses revealed that disease severity was significantly explained by two factors: the amount of pathogenic *Streptomyces* bacteria in the young tuber and the population level of actinomycetes in the rhizosphere soil at the early tuber formation stage. Our data indicated that these two factors greatly affected the occurrence of PCS in RB-amended soil.

In the present study, molecular ecological analyses revealed that RB amendment decreased the diversity of the rhizosphere bacterial community but increased the relative abundance of certain groups of gram-positive bacterial populations, such as *Streptomyces* spp. Although the relative abundance of *Streptomyces* bacteria significantly increased after RB amendment at the genus level, not all *Streptomyces* spp. increased after RB amendment. More importantly, RB amendment increased the population of antagonistic *Streptomyces* spp. against PCS pathogens, suggesting that these

actinomycetes could potentially suppress PCS in the field. Thus, all data in the present study indicate that RB amendment exclusively increases certain groups of antagonistic *Streptomyces* spp. but suppresses the populations of pathogenic *Streptomyces* bacteria in the potato rhizosphere.

It has been reported that high population densities of antagonistic *Streptomyces* bacteria are associated with naturally occurring disease-suppressive soils (Kinkel et al. 2012) and PCS-resistant potato plant lines (Wanner 2007). It has also been demonstrated that the inoculation of seed tubers with antagonistic *Streptomyces* spp. can suppress PCS under field conditions (Kobayashi et al. 2012; Liu et al. 1995). The densities and the species compositions of antagonistic *Streptomyces* populations in a field soil would vary considerably depending on geological location, plant genetic diversities, soil nutritional levels, and so on (Kinkel et al. 2012; Otto-Hanson et al. 2013; Schlatter et al. 2013; Wiggins and Kinkel 2005). However, the present study clearly showed that RB amendment could be intentionally used to manipulate the microbial community toward increasing antagonism against PCS.

As shown in a number of previous studies, antagonistic microorganisms that were selected based on their antagonism under culture conditions often fail to control plant diseases in the field (Kinkel et al. 2012). However, the present study showed that the applications of antagonistic *Streptomyces* spp. could suppress PCS in the field. Among them, strain ANA 1906 had the greatest biocontrol stability under both nonamendment and RB-amendment conditions. Interestingly, the 16S rRNA gene sequence for strain ANA 1906 has 99% identity to *Streptomyces* strain WoRs-501, which has been also reported as an effective biocontrol agent for PCS (Kobayashi et al. 2015). Noticeably, the application of antagonistic *Streptomyces* sp. strain ANA105, whose population tends to increase after RB amendment, suppressed PCS only under RB amendment. As previously reported for the suppression of soilborne fungal diseases through an increase in actinobacteria by other organic amendments, such as chitin and seed meal (Cohen et al. 2005; Cretoiu et al. 2013), the present study also suggest that an increase of antagonistic *Streptomyces* population in a soil is directly linked to the suppression of PCS by RB. Interestingly, nitric oxide production by *Streptomyces* bacteria in *Brassica napus* seed meal amendments has been postulated to have a role in the induction of plant systemic resistance (Cohen et al. 2005). Therefore, RB may also act in the same way for decreasing the pathogenic *Streptomyces* population in young tubers by inducing plant systemic resistance.

The two most abundant *Streptomyces* spp. identified by the amplicon sequencing analysis in the RB-amended rhizosphere were different from the results obtained by culture-dependent isolation. It is well known that culture-dependent methods are biased because a microorganism can only be cultivated after its physiological niche has been identified (Ward et al. 1990). DNA extraction methods are also known as culture-independent methods (Feinstein et al. 2009). The differences in the abundances we observed might reflect the growth efficiency of actinomycetes on the R2A medium used for isolation or the soil sampling method (bulk or rhizosphere soil). However, the results from both the culture-dependent and -independent analyses suggested that disease suppression by the RB amendment could be partially explained by increases in the indigenous antagonistic *Streptomyces* populations in the potato rhizosphere.

There were power law relationships between the magnitude of increase by RB amendment and the number of genera at each magnitude in both 2013 and 2014. This indicated that only a small proportion of the rhizosphere bacterial community could be significantly altered by RB amendment. Power law relationships between the magnitude and frequency of an incident have been observed in various self-organized critical phenomena such as earthquakes (Bak and Tang 1989) and financial market fluctuations (Gabaix et al. 2003). Recently, it has also been postulated

Fig. 4. Disease suppression effect after inoculations with *Streptomyces* isolates under field conditions. Four *Streptomyces* isolates (ANA105, ANA109, ANA1007, and ANA1906) derived from rice bran (RB)-amended soil were selected. Columns and error bars indicate the means and standard deviation ($n = 4$), respectively, and an asterisk on a column indicates a significant difference in disease severity (arcsine transformed) between the noninoculated control and those samples inoculated with an antagonistic strain under each amended condition when analyzed by the Wilcoxon signed rank test at $\alpha = 0.5$.

that soil-microbe complexes are self-organizing, and interactions and feedbacks between soil mineral constituents, organic matter, and microbial activity create and stabilize soil structure (Young and Crawford 2004). The bacterial community power law relationships we observed in the RB-amended rhizosphere might identify some of the relationships between the soil structure of volcanic ash soils and soil microorganisms after amendment with RB. It needs to be confirmed whether the microbial community shift and the power law relationships observed in the volcanic ash soil in our experimental field are applicable to other soil types such as red-yellow soil.

Several gram-positive bacteria other than *Streptomyces* spp. such as *Bacillus* and *Chitinophaga* spp. also increased after RB amendment and were negatively correlated with PCS disease severity as *Streptomyces* bacteria. However, their potential for disease suppression in the field was not investigated in the present study. The RB amendment increased the rhizosphere fungal population and affected community structure (T. Tomihama and S. Ikeda, unpublished data), which suggests that RB may also suppress PCS by changing the antagonistic fungal population. The possible contribution of other antagonistic microorganism groups responding to RB amendment should be investigated in the future.

Soil pH, which is one of the factors that influences the occurrence of PCS, probably affects disease suppression by RB because soil actinomycete antagonism against *S. scabiei* and *S. turgidiscabies* was greater at pH 5.0 than at 7.0. The application of calcium is also known to influence the incidence of PCS, presumably by promoting the growth of *S. scabiei* (Goto 1985; Lazarovits et al. 2007; Natsume et al. 2001). However, no definitive conclusions could be made in the present study due to the inappropriate experimental designs (small sample size and nonrepeated experiments). Therefore, the effects of soil pH and calcium application on PCS should be reassessed in a future study.

RB amendment has been reported to suppress the occurrence of sweet potato (*Ipomoea batatas* (L.) Lam.) soil rot disease caused by *S. ipomoea* (Kanaiso and Yonemoto 2003) and to have nematocidal activity against root-knot nematodes (Taba et al. 2008). These studies indicate that RB amendment could potentially contribute to sustainable agriculture in countries where rice is widely grown as a main crop.

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