JARQ 51 (3), 251 – 257 (2017) http://www.jircas.affrc.go.jp

# Enhanced Suppressive Effect of Antagonistic *Streptomyces* sp. WoRs-501 on Potato Scab in Conjunction with Other Control Methods

**Yuki O. KOBAYASHI[1]*, Akira KOBAYASHI[1], Hiroshi SOEJIMA[2] and Shigehito TAKENAKA[3,4]**

[1] Division of Upland Farming Research, Kyushu Okinawa Agricultural Research Center, NARO (Miyakonojo, Miyazaki 885-0091, Japan)
[2] Technical Research Institute, Snow Brand Seed Co., Ltd. (Ebetsu, Hokkaido 069-0832, Japan)
[3] Memuro Research Station, Hokkaido Agricultural Research Center, NARO (Memuro, Hokkaido 082-0081, Japan)
[4] Western Region Agricultural Research Center, NARO (Fukuyama, Hiroshima 721-8514, Japan)

## Abstract

Potato scab is a serious disease affecting cultivated potato. Combinations of an inoculant application (*Streptomyces* sp. strain WoRs-501) and other control methods were tested for their ability to suppress potato common scab. The use of a moderately resistant variety of potato in combination with the soil application of WoRs-501 decreased an inoculant population of this strain required for suppressing scab disease as compared to a susceptible variety. When a susceptible variety of potato grown in paper pots was planted in a scab-infested field, the application of a small volume of WoRs-501 inoculum to soil in paper pots tended to increase the scab control effect. The incorporation of lopsided oat seedlings derived from seeds coated with a mycelial suspension of WoRs-501 into scab-infested soil as green manure prior to planting a susceptible variety of potato in pots tended to increase the disease-suppressive effect of lopsided oat, and would be a practical method of providing a significant disease-suppressive effect. Furthermore, when tubers of a susceptible potato variety coated with WoRs-501 suspension in 0.1% sodium carboxy methyl cellulose (CMC) were planted in pots, the disease incidence score was significantly reduced to almost the same level as that of NEBIJIN powder. The treatment of CMC alone at a concentration of 0.1% also tended to decrease the disease incidence score, suggesting that it could be a new candidate agent for practical disease control.

**Discipline:** Plant protection
**Additional key words:** biocontrol, lopsided oat, paper pot transplanting, potato tuber inoculation, sodium carboxy methyl cellulose

## Introduction

Common scab of potato (*Solanum tuberosum* L.) is prevalent in most potato-growing areas worldwide. The scab-like lesions that form on infected potato tubers significantly reduce their market quality. This disease is caused by a soil- and seed tuber-borne pathogen. Although several pathogenic *Streptomyces* species have been reported (Bukhalid et al. 2002, Goyer et al. 1996, Loria et al. 2006), common scab caused by *S. turgidiscabies* or *S. scabiei* is most prevalent in Hokkaido in northeastern Japan (Miyajima et al. 1998), and in Kagoshima and Nagasaki in southwestern Japan (Nishi et al. 2015), which are the main potato-growing regions of Japan.

Currently, several methods including the chemical treatment of seed potatoes (Souma et al. 2002), crop rotation (Loria 2001, Tanaka 2005), incorporation of green manure (Shiga & Suzuki 2005, Tanaka 2005, Sakuma et al. 2011), addition of organic materials (Nakagawa 2003), reduction of soil pH (Tanaka 2005), irrigation during tuber formation (Loria 2001, Mino & Nishiwaki 2001), and transplanting potato seedlings grown in paper pots (Naito 2001) are used to control potato common scab. But such methods fail to adequately control the disease. Cultivating varieties of potato that are resistant to common scab is the most practical, effective

*Corresponding author: e-mail oodaira@affrc.go.jp
Received 16 June 2016; accepted 14 November 2016.

method of controlling the disease (Kobayashi et al. 2002, Kobayashi 2005). However, few varieties of potato in Japan are scab resistant.

The biological control of plant diseases is generally considered to be more environmentally friendly than the use of chemical pesticides, and is expected to become a supplemental or alternative approach in the future. In a previous study, we isolated an actinomycete in the genus *Streptomyces*, strain WoRs-501, from the rhizosphere soil of lopsided oat cv. 'Hayoats' (*Avena strigosa* Schreb.), which had been cultivated as green manure in Hokkaido (Kobayashi et al. 2012). An inoculum of $2 \times 10^7$ colony-forming units (CFU) of WoRs-501 per g dry soil was sufficient to establish a population with a stable suppressive effect on potato scab disease caused by *S. turgidiscabies* and *S. scabiei* in a field pot trial (Kobayashi et al. 2015). In the field, mixing WoRs-501 inoculum (approx. $10^9$ CFU/g dry mass) at a ratio of 1:9 (v/v) with *S. turgidiscabies*-infested field soil in plots 30 cm in length × 30 cm in width × 15 cm in depth per plant (resulting in a final population of WoRs-501 in the area of approx. $10^7$ CFU/g dry soil) decreases the disease severity score by 26-54% compared to the untreated control. The WoRs-501 strain offers good potential as a biocontrol agent, although a 10% inoculum of the strain is difficult to apply to a large volume of field soil. Therefore, we tested treatments with lower populations of WoRs-501 in combination with other control methods for determining their ability to control potato scab.

## Materials and Methods

### 1. Transplanting potato seedlings in paper pots

The antagonistic *Streptomyces* sp. strain WoRs-501 inoculum was cultured in vermiculite-oatmeal mix (Liu et al. 1995). The WoRs-501 inoculum of $6.2 \times 10^8$ CFU/g dry mass was then mixed at a ratio of 1:1 (v/v) with field soil (volcanic ash soil), and with the mixture then being placed in paper pots (#91A-No.5, 5 cm in diameter × 7.5 cm in height; Nippon Beet Sugar Manufacturing, Tokyo, Japan). Field soil without WoRs-501 inoculum was placed in paper pots as a control. On May 28, 2007, seed tubers (cv. 'Irish Cobbler' or '*Danshaku-imo*' in Japanese) that had been soaked the preceding autumn for several seconds in two antibiotic solutions—40-fold diluted Agromycin-100 (active ingredients: oxytetracyclin and streptomycin; Pfizer, Tokyo, Japan) and 200-fold diluted Validacin solution 5 (active ingredient: validamycin; Sumitomo Chemical, Tokyo, Japan)—were planted in these treatment and control pots, as described by Kobayashi et al. (2005). The pots were kept in a greenhouse for one month, and growing potato plants in paper pots were then transplanted into a *S. turgidiscabies*-infested field in Memuro, Hokkaido. Seed tubers of the same cultivar pre-treated with antibiotics were also planted directly into the scab-infested field on May 25 as another control for customary planting practices. Potato plants were grown for four months as per standard management recommendations for potato.

### 2. Cultivation of scab-resistant potato variety

*S. turgidiscabies* strain 94-3 (Kobayashi et al. 2005) isolated in Hokkaido was used as the common scab pathogen. As described by Kobayashi et al. (2015), *S. turgidiscabies*-infested soil was adjusted and mixed thoroughly with WoRs-501 inoculum cultured in the vermiculite-oatmeal mix to obtain final populations of $2.0 \times 10^5$, $2.0 \times 10^6$ and $1.9 \times 10^7$ CFU/g dry soil for the *Streptomyces* WoRs-501 antagonistic strain, and $2.0 \times 10^4$ CFU/g dry soil for the *S. turgidiscabies* 94-3 pathogen. For the untreated control, vermiculite was added to the *S. turgidiscabies*-infested soil instead of the WoRs-501 strain inoculum. These soil mixtures were placed in nonwoven fabric pots (15 cm in diameter × 15 cm in height). The seed tubers of two potato varieties that differed in resistance to common scab and which had been treated with antibiotics as described above were then planted in the prepared pots (one tuber per pot). The pots with plants for this experiment were planted in a noninfested field in Memuro and grown for three months from June 2 to August 30, 2010. The scab resistance of both potato varieties—susceptible 'Irish Cobbler' and moderately resistant 'Snowden'—had previously been evaluated in a *S. turgidiscabies*-infested field in Memuro between 2004 and 2009.

### 3. Potato tuber inoculation

The WoRs-501 strain was cultured in tryptic soy broth (Difco, Detroit, MI, USA) at 25°C for three days at 120 rpm. The cultures were centrifuged and the pellets resuspended in 0.1% sodium carboxy methyl cellulose (CMC, Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) after being washed twice with sterile distilled water (SDW). The final population of the WoRs-501 suspension was $1.1 \times 10^7$ CFU/mL. Seed tubers (cv. 'Irish Cobbler') that had not been treated with antibiotics were soaked in the WoRs-501 suspension for 75 min and then planted in nonwoven fabric pots (one tuber per pot) containing *S. turgidiscabies*-infested soil ($2.0 \times 10^4$ CFU/g dry soil). Seed tubers soaked in 0.1% CMC alone were also planted in *S. turgidiscabies*-infested soil in the same manner as a control. As another control, 1.06 g of NEBIJIN powder (Mitsui Chemicals Agro, Inc., Tokyo, Japan), a commercially available agricultural chemical for potato scab control, was mixed with the *S. turgidiscabies*-infested soil, and seed tubers that had been treated with antibiotics were planted. These pots were planted in an noninfested field in Memuro and grown for three months from June 2 to August 30, 2010.

### 4. Treatment with green manure

*S. turgidiscabies* strain T45 isolated in Kagoshima (Nishi et al. 2015) was used as another common scab pathogen. The pathogen was cultured in vermiculite–oatmeal mix and adjusted to obtain a final population of $2.0 \times 10^4$ CFU/g dry soil. The inoculum was crushed with a mixer (Osterizer, John Oster Manufacturing Company, USA), and 2 g of inoculum was mixed thoroughly into each pot with 3 L of volcanic ash field soil that had been autoclaved twice at one-day intervals at 121°C for 60 min. Liquid inoculum of WoRs-501 suspended in SDW ($2.2 \times 10^5$ CFU/mL) was adjusted as previously described. Seeds of lopsided oat (cv. 'Hayoats', Snow Brand Seed Co., Ltd., Hokkaido, Japan), which had been treated with fungicides such as tebuconazole and metalaxyl, were soaked in the WoRs-501 inoculum for 10 min, then air-dried for 5 h. On May 22, 2013, the seeds treated with WoRs-501 or untreated seeds were planted in nonwoven fabric pots (13 seeds per pot) containing *S. turgidiscabies*-infested soil. For an untreated control, no lopsided oat seed was planted. For another control, 50 µL of the WoRs-501 suspension, which was approximately the same amount of the strain required for coating one seed, was dropped in each seeding hole without seeds. These pots were planted in a field in Miyakonojyo, Miyazaki, located in southwestern Japan. Eight weeks later on July 17, the growing oat plants were cut with scissors at a height of 0.5-1 cm and incorporated into soil in the pots. After three weeks (on August 6), seed tubers (cv. 'Nishiyutaka'—a leading scab-susceptible variety in southwest Japan) that had been treated with an unspecified mixture of antibiotics before commercial sale were planted in all of the pots (one tuber per pot), and growing potatoes were harvested on November 4.

### 5. Evaluation of disease incidence

After the tubers were harvested, the percentage of tubers with symptoms for each plant was calculated. As for the trial conducted in the field, the percentage of tubers with symptoms for each set of five consecutive plants per row was calculated. The percentage scores were normalized and the variances homogenized using arcsine transformation. The disease-suppressive effect of each treatment was evaluated using a one-way analysis of variance and Tukey's multiple comparison using Stat Flex software (version 6.0, Artech, Osaka, Japan).

## Results

When potatoes (cv. 'Irish Cobbler') grown in paper pots were transplanted in a field infested with *S. turgidiscabies*, the disease incidence score was significantly reduced by 41% compared to the customary practice control (Fig. 1). When WoRs-501 ($6.2 \times 10^8$ CFU/g dry mass) was applied to the paper pots, the disease incidence score was further reduced by 52% compared to the customary practice control, but the difference in disease incidence between treatments of transplanting in paper pots with or without the WoRs-501 strain was not statistically significant.

When scab-susceptible variety 'Irish Cobbler' was planted in nonwoven fabric pots containing *S. turgidiscabies*-infested soil ($2.0 \times 10^4$ CFU/g dry soil), the inoculation of WoRs-501 to soil ($1.9 \times 10^7$ CFU/g dry soil) in the pots significantly reduced the disease incidence score by 83% compared to the untreated control (Fig. 2). In contrast, the inoculation of WoRs-501 at lower populations ($2.0 \times 10^5$ and $2.0 \times 10^6$ CFU/g dry soil) reduced the disease incidence score by 20% and 16%, respectively, compared to the untreated control,

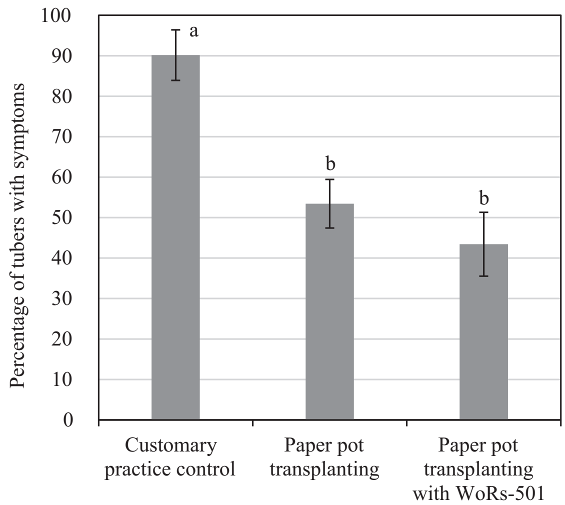


**Fig. 1. Suppressive effect of *Streptomyces* sp. strain WoRs-501 on incidence of potato scab combined with transplanting seedlings in paper pots.**
Potato seedlings (cv. 'Irish Cobbler') grown in paper pots containing field soil or field soil treated with WoRs-501 ($6.2 \times 10^8$ CFU/g dry mass) were transplanted into a field infested with *S. turgidiscabies. Vertical bars* indicate standard errors of the mean of three (customary practice) or four (paper pot transplanting) replicate plots consisting of five plants per replicate. *Different letters* indicate statistical significance according to Tukey's HSD test ($P = 0.05$).

and the differences in disease incidence between the susceptible variety with or without the WoRs-501 strain were not statistically significant. On the other hand, when a moderately resistant variety (‘Snowden’) was planted in pots containing the scab-infested soil, the disease incidence score was reduced by 52% compared to the susceptible variety ‘Irish Cobbler’. In combination with ‘Snowden’, the inoculation of WoRs-501 even at lower populations ($2.0 \times 10^5$ and $2.0 \times 10^6$ CFU/g dry soil) significantly reduced the disease incidence score by 91% and 65%, respectively, compared to the untreated control of ‘Irish Cobbler’, though the disease-suppressive effect of WoRs-501 varied in the inoculant populations. The difference in disease incidence between the moderately resistant variety with or without WoRs-501 was statistically significant at $2.0 \times 10^5$ CFU/g dry soil, but not statistically significant at $2.0 \times 10^6$ CFU/g dry soil.

When WoRs-501 suspended in 0.1% CMC ($1.1 \times 10^7$ CFU/mL) was applied to seed tubers (cv. ‘Irish Cobbler’) before planting in nonwoven fabric pots containing *S. turgidiscabies*-infested soil ($2.0 \times 10^4$ CFU/g dry soil), the disease incidence score was significantly reduced by 71% compared to the untreated control (Fig. 3), which was the nearby suppression observed in treatment using NEBIJIN powder (by 78%). Even tuber treatment with 0.1% CMC reduced the score by 61% compared to the untreated control, though the difference was not statistically significant. The difference in disease incidence between treatments of CMC with or without the WoRs-501 strain was not statistically significant.

When lopsided oat was grown and incorporated into *S. turgidiscabies*-infested soil ($2.0 \times 10^4$ CFU/g dry soil) in nonwoven fabric pots before planting potatoes (cv. ‘Nishiyutaka’), the disease incidence score was significantly reduced by 80% compared to the untreated control (Fig. 4). When WoRs-501 suspended in SDW ($2.2 \times 10^5$ CFU/mL) was applied to lopsided oat seed, the degree of disease score reduction further increased (by 94%), although the difference in disease incidence scores of potato between lopsided oat seeds treated with and without the WoRs-501 strain was not statistically significant. When WoRs-501 was applied to a pot without lopsided oat seed, the disease incidence score was reduced by 24% compared to the untreated control, and the difference was not statistically significant.

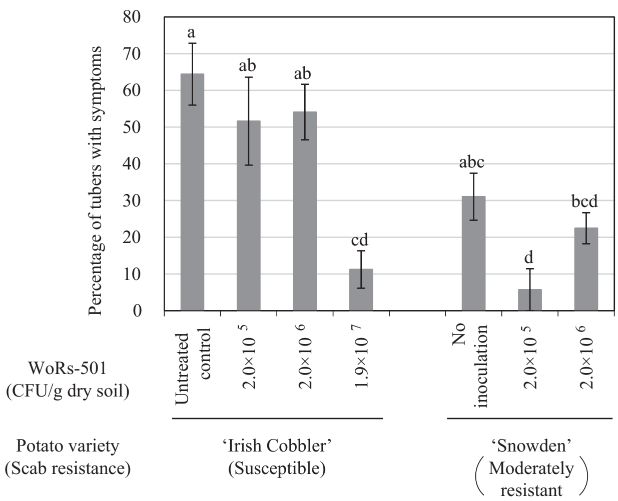


**Fig. 2. Suppressive effect of *Streptomyces* sp. strain WoRs-501 on incidence of potato scab in different potato varieties.**
Tubers of two potato varieties harboring different resistance levels to common scab were planted in nonwoven fabric pots containing a soil mixture inoculated with WoRs-501 ($2.0 \times 10^5$, $2.0 \times 10^6$, and $1.9 \times 10^7$ CFU/g dry soil) and $2.0 \times 10^4$ CFU/g dry soil of *S. turgidiscabies*, and then were planted and grown in a field. *Vertical bars* indicate standard errors of the mean of five replicates. *Different letters* indicate statistical significance according to Tukey’s HSD test ($P = 0.05$).

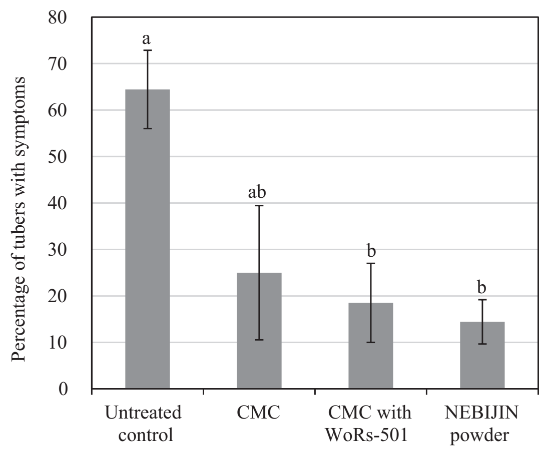


**Fig. 3. Suppressive effect of inoculation of potato seed tubers with *Streptomyces* sp. strain WoRs-501 on incidence of potato scab.**
Potato tubers (cv. ‘Irish Cobbler’) soaked in 0.1% sodium carboxy methyl cellulose (CMC) or WoRs-501 suspension in 0.1% CMC ($1.1 \times 10^7$ CFU/mL) were planted in nonwoven fabric pots containing $2.0 \times 10^4$ CFU/g dry soil of *S. turgidiscabies*, and then were planted and grown in a field. Potato tubers were also planted in *S. turgidiscabies*-infested soil treated with NEBIJIN powder (an agricultural chemical). *Vertical bars* indicate standard errors of the mean of three replicates of the CMC control or five replicates of the other treatments. *Different letters* indicate statistical significance according to Tukey’s HSD test ($P = 0.05$).

## Discussion

In a field pot trial using two potato varieties differing in resistance to common scab (Fig. 2), the disease incidence score in the susceptible variety 'Irish Cobbler' was significantly reduced by soil application of $1.9 \times 10^7$ WoRs-501 CFU/g dry soil ($3.4 \times 10^{10}$ CFU/plant) as compared to the untreated control. When the moderately resistant variety 'Snowden' was planted, soil application of $2.0 \times 10^5$ and $2.0 \times 10^6$ WoRs-501 CFU/g dry soil ($3.7 \times 10^8$ and $3.7 \times 10^9$ CFU/plant), 1/92 and 1/9.2, respectively, of the inoculant population needed to suppress the disease for the susceptible potato variety, could significantly reduce the disease incidence score compared to the untreated control of the susceptible variety, although the disease-suppressive effects tended to be reversed between the two inoculant populations of WoRs-501 in this trial. The results suggest that treatment with WoRs-501 populations at $10^5$ or $10^6$ CFU/g dry soil could sufficiently suppress the potato scab in combination with the cultivation of moderately resistant or resistant potato varieties.

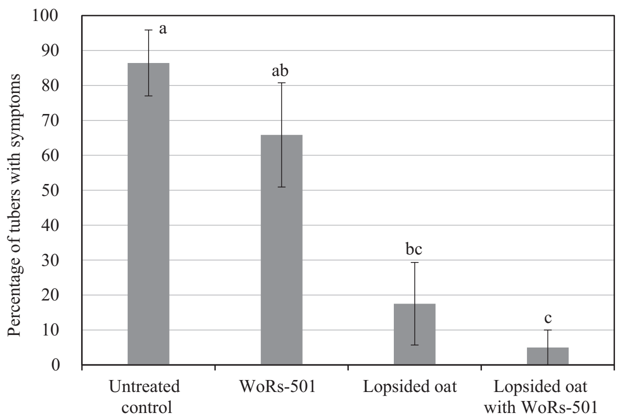


**Fig. 4. Suppressive effect of *Streptomyces* sp. strain WoRs-501 on incidence of potato scab combined with incorporation of green manure into soil.**
Seeds of lopsided oat (cv. 'Hayoats') that were either treated with or without WoRs-501 suspension in SDW ($2.2 \times 10^5$ CFU/mL) were planted in nonwoven fabric pots containing $2.0 \times 10^4$ CFU/g dry soil of *S. turgidiscabies*. Other controls consisted of pots in which no lopsided oats had been sown or the WoRs-501 suspension had been dropped in a seeding hole without lopsided oat seeds. All control and treatment pots were planted in the field. After incorporating the lopsided oat green manure into the soil, potato tubers (cv. 'Nishiyutaka') were planted in all pots. *Vertical bars* indicate standard errors of the mean of four replicates. *Different letters* indicate statistical significance according to Tukey's HSD test ($P = 0.05$).

Susceptible varieties such as 'Irish Cobbler' and 'May Queen' are the leading potato varieties for Japan's fresh vegetable market. Even in a susceptible variety, potato scab could be suppressed by combining WoRs-501 treatment with paper pot transplanting or lopsided oat cultivation (Figs. 1, 4).

Common scab disease can suppressed by transplanting potato seedlings grown in paper pots (Naito 2001). The paper pot prevents the potato plant from coming into direct contact with the scab pathogen during tuber formation when actively growing tubers become infected with the pathogen. Nakagawa et al. (2002) reported that the effectiveness of this method varies in Nagasaki. However, the application of an arbuscular mycorrhiza fungus or *Trichoderma* biological control materials to soil in paper pots improves and stabilizes the control effect. In our trial conducted in Hokkaido, the application of WoRs-501 to soil in paper pots also tended to increase the scab control effect of the paper pot transplanting method (Fig. 1).

In our previous field study conducted in 2009 (Kobayashi et al. 2015), WoRs-501 suppressed disease incidence by 40% when 1.5 L of WoRs-501 inoculum was applied to each potato plant ($2.9 \times 10^{11}$ CFU/plant). However, in combination with transplanting in paper pots, only 40-45 mL of the inoculum (approx. 5.3-5.9 × 109 CFU/plant) or approximately 1/35 of the volume (or 1/52 of the inoculant population) used for treatment in the previous study reduced disease incidence by 52%.

The WoRs-501 inoculum contains an antibiotic produced by the strain in the vermiculite-oatmeal mix (Kobayashi et al. 2015). When the inoculum is applied to soil, the antibiotic could decrease scab pathogen populations in the soil, and thereby suppresses scab disease incidence. After transplanting in the field, the growing tubers break through the paper pots and come into contact with the scab pathogen in soil around the paper pots. The antibiotic from WoRs-501 might decrease scab pathogen populations in soil around the paper pots, and thereby suppress scab disease. However, the treatment of seed tubers with only a mycelial suspension of WoRs-501, which did not contain the antibiotic, also had a suppressive effect on the disease (Fig. 3). Therefore, during paper pot cultivation, the WoRs-501 strain might establish high populations around the growing tubers, which then might prevent the scab pathogen from colonizing tubers after they break through the paper pots.

The cultivation of lopsided oat as green manure controls potato scab in Hokkaido, where growing lopsided oat plants are incorporated into field soil twice per year from spring to autumn, and potato is planted the following year (Sakuma et al. 2011). The treatment with green manure of lopsided oat also suppressed scab disease in our trial conducted in Miyazaki, even though lopsided oat did not actively grow from May to July due to high temperatures, and was incorporated into the soil only once before planting potato (Fig. 4). Moreover, the inoculation of lopsided oat seed with WoRs-501 suspended in SDW ($2.2 \times 10^5$ CFU/mL) tended to increase the disease-suppressive effect of lopsided oat in Miyazaki, although WoRs-501 was isolated in Hokkaido. Green manure increases the organic matter and nutrients in soil, and controls weeds. In addition, lopsided oat cv. 'Hayoats' suppresses a species of nematode (*Pratylenchus penetrans*) in soil (Yamada 1998). Thus, this green manure has several advantages in addition to scab suppression.

The disease-suppressive effect of lopsided oat was confirmed in a study from Argentina that reported an enhanced disease-suppressive effect of inoculating seed tubers with a *Bacillus subtilis* strain from alfalfa (*Medicago sativa*) when these tubers were planted in field soil treated with green manure from lopsided oat (Yossen et al. 2011). In our field pot trials, the inoculation of potato tubers with WoRs-501 reduced the scab disease incidence score (Fig. 3). Therefore, if seed tubers inoculated with WoRs-501 are planted in soil treated with green manure as the method reported by Yossen et al. (2011), a synergetic disease-suppressive effect of WoRs-501 with the green manure can be expected. However, the WoRs-501 strain was initially isolated from the rhizosphere soil of lopsided oat, suggesting that its population would be expected to increase in soil treated with lopsided oat before planting potato. Thus, in combination with lopsided oat, it would be possible to reduce the inoculant amount of WoRs-501 for scab suppression. Indeed, when lopsided oat was seeded in pots, approximately 650 μL of the WoRs-501 suspension was applied to a pot, and the population of the strain was $1.4 \times 10^5$ CFU/pot, or approximately 1/240,000 of the inoculant population required for suppressing scab disease in the susceptible variety (Fig. 2). The WoRs-501 strain might reduce or suppress scab pathogen populations in soil either by producing antibiotics in the soil or by competing with scab pathogens for nutrients or space. Further studies are necessary to clarify the mechanism of disease suppression by WoRs-501.

Seed tuber inoculation with WoRs-501 suspended in 0.1% CMC ($1.1 \times 10^7$ CFU/mL) significantly reduced scab disease incidence to almost the same level as did NEBIJIN powder (Fig. 3). When 3 mL of the WoRs-501 suspension was applied to a seed tuber, the population of the strain was $3.3 \times 10^7$ CFU/plant, or 1/1030 of the inoculant population required for suppressing scab disease in the susceptible variety (Fig. 2). CMC is used as an adhesive agent for some biocontrol agents such as the *Trichoderma* species used for the control of peanut brown rot caused by *Fusarium solani* or the *Pseudomonas* species used for the control of potato scab caused by *S. scabiei* (Rojo et al. 2007, Singhai et al. 2011). Though a disease-suppressive effect of CMC has not been reported, the potential of CMC for suppressing common scab was suggested by comparing the untreated control and CMC control without microorganisms (Fig. 3). Therefore, the disease-suppressive effect of the WoRs-501 inoculant could be considered a synergetic effect of the WoRs-501 strain and CMC, though the disease-suppressive effect of WoRs-501 without CMC was not examined. Around the tubers, CMC might increase the populations of WoRs-501 or indigenous cellulose-decomposing microorganisms because *S. turgidiscabies*-infested soil was formulated using a non-sterile soil mixture, which might prevent scab pathogens from colonizing tubers.

CMC is inexpensive and readily available as a stable material. Moreover, CMC does not pollute the environment or impair human health. Therefore, CMC might be a useful new candidate agent for disease control. The potential disease-suppressive effect of CMC at different concentrations and the mechanism of disease-suppression by CMC should be further studied.

As described above, the disease-suppressive effect of several methods commonly used to control common scab of potato can be increased by employing these methods in conjunction with a small inoculation population of WoRs-501. In particular, the cultivation of WoRs-501-coated lopsided oat as green manure would be a practical method of producing a significant disease-suppressive effect. When the use of green manure is difficult to include in planting systems due to the cost of the practice without concomitant income, seed tuber inoculation with WoRs-501 is a practical, effective, and relatively inexpensive method of suppressing common scab.

## Acknowledgments

The authors wish to thank Dr. Y. Nishi from the Kagoshima Prefectural Institute for Agricultural Development and Dr. S. Naito for supplying the scab pathogens, Dr. A. Oyanagi and K. Mori for their helpful suggestions, and N. Taira, T. Sugimatsu, K. Hatanaka, N. Matsuzaki, N. Tokuchi, K. Yoshidome, T. Miike, T. Ashida, S. Souma, A. Shintani, H. Suzuki, H. Ohnuma and K. Sakamoto for their technical assistance.

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