Endophytic *Burkholderia* sp. SSG as a potential biofertilizer promoting boxwood growth

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**Background.** *Burkholderia* sp. SSG is a bacterial endophyte isolated from boxwood leaves showing a resistant response to infection by the boxwood blight pathogen *Calonectria pseudonaviculata*. SSG acted as a protective and curative biocontrol agent for boxwood blight and as a bio-sanitizer of disease inoculum in the field. Many gene clusters involved in antibiotic production and plant growth promotion (PGP) were found in the genome, giving this endophyte great application potential as a treatment for plant protection. However, the PGP features have not been documented. This study investigated the plant growth promotion activity of SSG in boxwood. **Methods.** To determine whether SSG is a plant growth promoting bacterium, four PGP traits, auxin and siderophore production, nitrogen fixation and phosphate solubilization, were examined in the laboratory with colorimetric or agar plate assays. The plant growth promoting activity of SSG was tested on three boxwood varieties characterized by slow, intermediate and fast growth rates, namely Justin Brouwers, Buddy and Winter Gem, respectively. These plants were drenched with an SSG cell suspension or water and washed plant weight was compared before and after treatment to determine growth changes after 10 months. **Results.** The SSG culture was sustainable on nitrogen free media, suggesting that SSG may fix atmospheric nitrogen. It was also a strong phosphate solubilizer and a potent siderophore and indole-3-acetic acid (IAA) producer. Significant growth promotion was observed on boxwood cultivars Justin Brouwers, Buddy and Winter Gem 10 months after plant roots were drenched with SSG cells. The growth rate of treated plants was 76.1, 58.3, and 37.3% higher than that of the control, respectively. The degree of growth promotion was significantly different among plant varieties, notably more pronounced with the slow and intermediate growers. This study demonstrates that the SSG bacterium has multiple PGP traits and is a prospective plant biofertilizer.
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Introduction

Endophytes have recently received considerable attention because of their ability to promote plant growth and suppress plant pathogens (Díaz Herrera et al., 2016; Eljounaidi et al., 2016; Joy and Parke, 1994; Nejad and Johnson, 2000; Reinhold-Hurek and Hurek, 2011; Santoyo et al., 2016). *Burkholderia* sp. SSG was isolated from boxwood leaves showing a resistant response to infection by *Calonectria pseudonaviculata* (*Cps*): the initial water-soaked lesions which developed 48h after inoculation with *Cps* disappeared with no subsequent disease development (Kong and Hong, 2020b). As an environmental member of the *Burkholderia cepacia* complex (Bcc), SSG differs from the clinical strains involved in lung infections of immunocompromised patients (Vandamme et al., 1997) by the onion maceration test response, RecA restriction fragment length polymorphism and lack of the Burkholderia cepacia Epidemic Strain Marker (BCESM) (Kong and Hong, 2020b). Recent genome sequencing (Kong and Hong, 2020a) has confirmed that SSG does not have the cable pini subunit gene (*cblA*) for BCESM (Mahenthiralingam et al., 2000; Mahenthiralingam et al., 1997; Sajjan et al., 1995). It also reveals the absence of several multiloci that are used for Bcc typing (Baldwin et al., 2005). More interestingly, the SSG genome contains genes encoding traits that are uncommon in Bcc clinical strains, such as those involved in nitrogen fixation and production of bacteriocin (Bevivino et al., 1994; Gonzalez and Vidaver, 1979). These traits indicate a low human health risk and high potential of SSG as a biocontrol agent for plant diseases and biofertilizer for plant production.

Boxwood blight is a deadly disease of boxwood caused by *Cps* (Daughtrey, 2019; LeBlanc et al., 2018). Leaves inoculated with the pathogen can develop blight symptoms within 72 h (Kong and Hong, 2018). SSG provided nearly complete protection from the disease when used as a foliar treatment on boxwood plants before or shortly after plant infection by *Cps* (Kong and Hong, 2020b). Such protection is superior to any biocontrol product or other potential biocontrol agents evaluated to date (Kong, 2019; Kong and Hong, 2017; Kong and Hong, 2019; Yang and Hong, 2018; Yang and Hong, 2017). When used to treat diseased leaf debris in the field, SSG diminished production of inocula and mitigated disease development (Kong and Hong, 2020b).

Biocontrol agents for plant diseases are often plant growth promoters (Compant et al., 2005; Pal, 2006). This is particularly true for Bcc environmental strains (Batista et al., 2018; Bevivino et al., 1998; Germida and Walley, 1996; Ghosh et al., 2016; Sopheareth et al., 2013; Trân Van et al., 2000). Many of these Bcc strains were reported to have a high capacity for antibiotic production (Depoorter et al., 2016), as well as production of other metabolites that can promote plant growth through phosphate solubilization, ethylene regulation with 1-aminocyclopropane-1-carboxylate (ACC) deaminase and sequestering iron (Batista et al., 2018; Ghosh et al., 2016; Santoyo et al., 2016; Santoyo et al., 2012; Trân Van et al., 2000). Whole genome sequencing of SSG indicated greater capacity than other members of the environmental Bcc for antibiotic synthesis and production of other secondary metabolites beneficial for plant growth (Kong and Hong, 2020a). However, SSG has not been verified as a plant growth promoting (PGP) bacterium. This study aims to explore the potential of SSG as a biofertilizer. Four PGP traits: nitrogen fixation, phosphate solubilization and production of IAA (Indole-3-Acetic Acid) and...
siderophores were examined through colorimetric or agar plate assays. SSG was also evaluated for plant growth promotion on three boxwood varieties through drench application.

**Materials & Methods**

**SSG Culture growth conditions.** *Burkholderia* sp. SSG, from the Virginia Tech Collection of Phytophthora and Beneficial Microbes (VTC) of the World Data Center for Microorganism (WDCM1197), was grown and maintained on potato dextrose agar (PDA), nutrient agar (NA) or in nutrient broth (NB) (Becton, Dickinson and Company, Spark, MO, USA) at 25-28°C. For a fresh culture, a streak plate was prepared from the stored culture and incubated for 48 h.

**IAA production.** IAA production by SSG was determined quantitatively using the colorimetric method (Liaqat and Eltem 2016) with a minor modification. Specifically, 4 ml of NB containing 4 mg tryptophan was inoculated with a single colony from a 48-h SSG fresh culture plate. After a 72-h incubation at 28°C, 1.5 ml of SSG broth culture or the control, NB without SSG, was centrifuged at 13,523 g for five minutes. 0.5 ml of the supernatant was then mixed with 1 ml Salkowski’s reagent in a 1.5-ml tube and incubated at 23°C for 30 min. The reaction with SSG supernatant was then measured for absorbance at 530 nm after blanking with the control on a DU800® spectrophotometer (Beckman Coulter, Indianapolis, IN, USA). The assay was run in triplicate and repeated once. A standard curve constructed with an IAA dilution series (Sigma-Aldrich, St. Louis, MO, USA) at a range of 0.1 to 300 µg ml/l was used for quantification of IAA in the sample.

**Nitrogen fixation ability.** Nitrogen fixation was determined by growing SSG on nitrogen-free agar medium as described previously (Liaqat and Eltem 2016). Specifically, nitrogen-free agar plates were streaked with fresh SSG colonies from a PDA culture. Nutrient agar plates were used as a positive control. Plates were incubated at 25°C for 4 days and examined for bacterial growth. The assay was conducted in triplicate and repeated once.

**Phosphate solubilization.** The ability of SSG to solubilize phosphate was determined using the National Botanical Research Institute’s Phosphate (NBRIP) broth or agar medium and the colorimetric method (Nautiyal 1999; Pradhan and Raj Pokhrel 2013) with minor modifications. For the plate assay, three sterilized Whatman filter paper disks were placed on NBRIP agar plates at the points of an equilateral triangle. A 10-µl aliquot of SSG cell culture stock was pipetted onto each disk. Control disks received the same amount of nutrient broth without SSG. All plates were incubated at 27°C for seven days, then examined for development of a halo around the disks. For the broth colorimetric assay, 150 mg Ca$_3$(PO$_4$)$_2$ as an insoluble form of phosphate was added to 30-ml NBRIP broth, to which 0.3 ml of an overnight (16-18 h) SSG culture in NB or NB alone (the control) was added. After incubation on a shaker at 27°C for seven days, the culture was centrifuged at 13416 g for 10 min. The supernatant was autoclaved
for 20 min and stored at 4°C. To determine soluble phosphate release into the solution, 1 ml of
the supernatant or its dilution was added to 2 ml of 2.5% ammonium molybdate and 0.5 ml of 10
mol/l sulfuric acid, mixed with 1 ml of 0.5 mol/l hydrazine hydrate solution, then brought to 25
ml with SDW. The NB control was used as a blank and the SSG culture supernatant was
measured for absorbance at 840 nm on a DU800® spectrophotometer. When the absorbance of a
sample was one or smaller, soluble phosphate was calculated by sample absorbance /0.1235 +
0.0018. When the absorbance of a sample was one or greater, soluble phosphate was calculated
after a 100x dilution (Pradhan and Raj Pokhrel 2013). Both assays included three replicates and
were repeated once.

Siderophore production. Siderophore production by SSG was determined using blue agar
medium containing chrome azurol S (CAS) and the indicator hexadecyltrimethylammonium
bromide (Schwyn and Neilands 1987). Specifically, the media plates were streaked with SSG
and incubated at 25°C. Plate color change was examined after 48 h. Plates with a color change
from blue to yellow were recorded as positive. This assay included three replicate plates and the
assay was repeated twice.

Plant treatment and growth measurement. Three boxwood cultivars with different growth
rates, Buxus sempervirens ‘Justin Brouwers’ (slow), ‘Buddy’ (intermediate) and B. microphylla
var. japonica ‘Winter Gem’ (fast), were used in this study. Two plants were grown in 3.8-liter
containers and maintained in a greenhouse before use. One week before SSG treatment in
November 2018, plants were separated and rinsed with tap water to remove potting mix. Cleaned
individual plants were weighed after drying with a paper towel, then repotted in a mixture of
Scotts® Premium Potting Soil (Marysville, OH) and pine bark (Pacific Mulch Inc, Henderson,
NC) at 1: 2 in 3.8-liter containers. These plants were watered manually to saturate the soil,
followed by drip irrigation every other day for one min.

Plants were treated by drenching with an SSG cell suspension prepared by inoculating 3
flasks each containing 150 ml NB with 1 ml from a 5ml overnight broth culture. After incubation
at 28°C on a shaker for 40 h, each culture was pooled and centrifuged at 8,275 g for 15 min. The
cell pellets were resuspended in 500 ml dH2O after supernatant was removed. For treatment, a
50-ml aliquot of SSG resuspension at 10⁸ cfu/ml or the same volume of water without SSG was
evenly poured onto the potting mix around plants in containers. After treatment, containers were
arranged in a randomized complete block design and drip irrigation was resumed after two days.
In March 2019 plants were moved out of the greenhouse to a gravel pad with overhead irrigation.
In September 2019 plants were removed from containers, washed free of soil mix and weighed
as in November 2018. Plant growth was measured by the difference in plant weight between the
beginning and end of the experiments. The experiment was conducted three times with an
interval of a week.
Statistical analysis. Plant growth data from three repeated experiments were subjected to a homogeneity test and subsequently pooled for further analyses. Analysis of variance was conducted using the Statistical Analysis Software Version 9.4 (SAS Institute, Cary, NC). Treatment means were separated by boxwood cultivar according to the least significance difference at $P = 0.05$.

Results

Plant growth promotion traits of SSG

IAA was detected in the cell free supernatant two days after NB broth containing tryptophan was inoculated with SSG cells (Fig. 1a). The estimated yield was 2.9 – 4.5 µg/ml. The amount of IAA detected did not change with longer growth periods, suggesting limited use of tryptophan. No color change occurred in the control (Fig. 1b).

SSG grew on nitrogen-free medium (Fig. 1c) although not as well as on nitrogen- rich medium, NB (Fig. 1d).

Phosphate solubilization by SSG was confirmed by both plating and colorimetric methods. A clear halo developed around the SSG disks on NBRIP agar medium within three days. These halos enlarged with increasing incubation time. They were 14 mm ($\pm$ 0.3) in diameter by the 7th day (Fig. 1e). No halos formed on any of the control plates (Fig. 1f). The solubilized phosphate measured colorimetrically after 7 days was 206.4 ppm ($\pm$ 5.0), approximately 21% of the insoluble form of phosphate.

The blue agar chrome azurol S assay detected siderophore production by SSG. The agar turned yellow 48 h after the plate was streaked with SSG (Fig. 1g) and no color change occurred on the NB streaked control (Fig. 1h).

Effect of SSG on boxwood plant growth

The growth rate of three boxwood varieties was measured 10 months after drenching the container mix with an SSG cell suspension or water. There was no difference between three repeated experiments ($P = 0.6905$) nor interaction between cultivar and treatment ($P = 0.2121$), cultivar and experiment ($P = 0.1366$) and between treatment and experiment ($P = 0.2434$). However, there was significant difference between treatments with and without SSG and the difference varied with cultivar ($P < 0.0001$). SSG consistently promoted plant growth of all three boxwood cultivars when compared to the control (Fig. 2). Specifically, the growth increase in SSG treated plants was 58%, 76% and 37% greater than that of the control in Buddy ($P = 0.0236$).

Discussion

This study investigated the plant growth promotion activity of SSG on boxwood. Although SSG was isolated from leaves, it stimulated plant growth when applied as a root treatment. 76%
growth increase was observed in the slower growing ‘Justin Brouwers’ cultivar used in a
previous study evaluating disease suppression by SSG (Kong and Hong, 2020b). In that study, an
increase in leaf number was observed when SSG culture was used to treat diseased leaf debris
added to containers with healthy plants. However, since boxwood blight incidence also
decreased with the treatment, it was not certain whether the leaf increase was a result of normal
plant growth after disease reduction. This study confirms the plant growth promotion ability of
SSG and suggests that the increase in leaf number observed previously may be attributed to the
treatment. The current study revealed a trend that slower growing cultivars ‘Justin Brouwers’ and
‘Buddy’ benefited more from SSG treatment than the fast-growing cultivar ‘Winter Gem’. All
three showed a significant increase in growth after SSG treatment compared to their controls. It
is not clear why SSG was more effective on the slow and intermediate than the fast-growing
cultivar, which one possibility is that the effect of SSG may be overruled by other genetic factors
in the faster growing cultivar which may be less dependent on environmental conditions for
growth. SSG has been shown to be able to survive in soil and rhizosphere (Kong and Hong,
2020b). However, how it behaves in the rhizosphere and how it responds to plant genetic factors
remain to be further studied.

SSG is a plant growth promoting bacterium. IAA is the basic and most potent auxin natively
occurring and functioning in plants and it regulates leaf and flower development (Benková et al.,
2003; Ludwig-Müller, 2011). IAA was detected in SSG cell free culture supernatant. To our
knowledge, SSG is the first leaf endophytic burkholderial bacterium producing IAA, as other
IAA-producing *Burkholderia* are found in the stem, root and rhizosphere (Mendes et al., 2007;
Weiharter et al., 2011). IAA production by SSG was relatively low, 2.9 – 4.5 µg/ml, compared
to some non-*Burkholderia* bacterial endophytes that produce 9.6 - 43 µg/ml (Liaqat and Eltem,
2016). However, it is not clear whether such yield is common in IAA producing *Burkholderia*
due to lack of quantitative data. Interestingly, genes encoding tryptophan-2-monoxygenase or
tryptophan transaminase were not found in the SSG genome (Kong and Hong, 2020a). These
enzymes play important roles in the pathways of tryptophan-dependent IAA biosynthesis in
bacteria (*Pseudomonas* and *Agrobacterium*) and plants (Zhao, 2010; Zhao, 2012). It is not
understood how IAA was produced without these genes, although there are genes for tryptophan
production. Whether SSG may use a different pathway for IAA production is still a question to
be answered.

Another distinctive trait of SSG is nitrogen fixation as indicated by SSG growth on nitrogen-
free medium. Nitrogen fixation has been found in various endophytic bacteria (Estrada-De Los
Santos et al., 2001; Ghosh et al., 2016; Liaqat and Eltem, 2016; Trân Van et al., 2000), but it is
uncommon for Bcc (Gonzalez and Vidaver, 1979). SSG is the second member of Bcc that can fix
nitrogen, following *B. vietnamiensis* (Gillis et al., 1995). This ability of SSG corresponds well
with its genome compacity for the trait. Many genes involved in nitrogen fixation and regulation
have been found in the SSG genome (Kong and Hong, 2020a). These genes include the
nitrogenase gene (eg. *NifQ*) (Hoffman et al., 2014), the *hglE* cluster, heterocyst glycolipid
synthase-like PKS involving nitrogen fixation in cyanobacteria heterocyst (Campbell et al.,
1997; Fan et al., 2005), and genes for nitrogen fixation and regulation such as \textit{pstN} and \textit{glnB} (Fan et al., 2005; Michiels et al., 1998). With this capacity, SSG can modulate nitrogen acquisition and metabolism.

Treatment of seed or soil with phosphate-solubilizing bacteria can improve crop yield by releasing insoluble and fixed forms of phosphorus such as rock phosphate (Khan et al., 2007; Qureshi et al., 2012; Reijnders, 2014). Weak phosphate-solubilizing bacteria do not produce a halo in the plate assay (Nautiyal, 1999). The halo formed by SSG suggests that this bacterium is a potent phosphate solubilizer. The amount produced as quantified with the colorimetric method (Pradhan and Raj Pokhrel, 2013) is similar to that reported for some strong phosphate solubilizing bacterial endophytes including \textit{Burkholderia} spp. (Ghosh et al., 2016; Liaqat and Eltem, 2016; Qureshi et al., 2012). Optical density of the supernatant of phosphate-solubilizing bacterial culture in NBRIP with Ca$_3$(PO$_4$)$_2$ has been used to measure soluble form of phosphorus in other studies (Ghosh et al., 2016; Liaqat and Eltem, 2016). However, since there are no comparative studies on these methods, values of soluble form of phosphorus by these bacteria from different research may not be comparable.

Siderophores from microorganisms can be used by a plant for iron nutrition, soil heavy metal stress alleviation and plant pathogen suppression (Glick 2012). SSG was a potent siderophore producer as shown by the plating method. This is consistent with the data from SSG genome sequencing revealing more than 100 genes involved in siderophore biosynthesis, assembly and metabolism (Kong and Hong 2020a). However, it is not clear whether SSG may be different from other plant growth promoting Bcc in terms of siderophore composition and number due to limited research on plant growth promoting Bcc species.

**Conclusions**

This study confirms that the potent biocontrol agent, boxwood endophytic \textit{Burkholderia} sp. SSG, is also a plant growth promoter. Plant growth increased by 37 – 76% when the bacterium was applied as a drench to containerized boxwood. Four important plant growth promoting traits predicted by SSG genome sequencing were also verified in the laboratory. IAA production, nitrogen fixation, phosphorus solubilization and siderophore production were confirmed in this endophyte. These traits along with other features such as potent antagonism against pathogens and low human health risk demonstrate its potential as a highly beneficial biofertilizer. To understand more \textit{Burkholderia} sp. SSG as a super biofertilizer, future studies should include more genomic prospection of the bacterium, such as acquisition, transfer and metabolism of the growth hormone, nitrogen, phosphorus and iron, as well as protein secretion systems, especially the Type VI Secretion Systems that are widespread in \textit{Burkholderia} spp. and very powerful to suppress bacterial or eukaryotic cells. To promote application of SSG in crop production and health, assessment of its biocontrol spectrum for plant pathogens and development of effective formulations are warranted.
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References

Baldwin, A., Mahenthiralingam, E., Thickett, K.M., Honeybourne, D., Maiden, M.C.J., Govan, J.R., Speert, D.P., LiPuma, J.J., Vandamme, P., Dowson, C.G., 2005. Multilocus sequence typing scheme that provides both species and strain differentiation for the Burkholderia cepacia complex. Journal of Clinical Microbiology 43, 4665-4673.

Batista, B.D., Lacava, P.T., Ferrari, A., Teixeira-Silva, N.S., Bonatelli, M.L., Tsui, S., Mondin, M., Kitajima, E.W., Pereira, J.O., Azevedo, J.L., Quecine, M.C., 2018. Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. Microbiological Research 206, 33-42.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G., Friml, J., 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115, 591-602.

Bevivino, A., Sarrocco, S., Dalmastri, C., Tabacchioni, S., Cantale, C., Chiarini, L., 1998. Characterization of a free-living maize-rhizosphere population of Burkholderia cepacia: effect of seed treatment on disease suppression and growth promotion of maize. FEMS Microbiology Ecology 27, 225-237.

Bevivino, A., Tabacchioni, S., Chiarini, L., Carusi, M.V., Del Gallo, M., Visca, P., 1994. Phenotypic comparison between rhizosphere and clinical isolates of Burkholderia cepacia. Microbiology 140, 1069-1077.

Campbell, E., Cohen, M., Meeks, J.C., 1997. A polyketide-synthase-like gene is involved in the synthesis of heterocyst glycolipids in Nostoc punctiforme strain ATCC 29133. Archives of Microbiology 167, 251-258.

Compant, S., Duffy, B., Nowak, J., Clément, C., Barka, E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Applied and Environmental Microbiology 71, 4951-4959.

Daughtrey, M.L., 2019. Boxwood blight: Threat to ornamentals. Ann Rev Phytopathol 57, 189-209.

Depoorter, E., Bull, M.J., Peeters, C., Coenye, T., Vandamme, P., Mahenthiralingam, E., 2016. Burkholderia: an update on taxonomy and biotechnological potential as antibiotic producers. Applied Microbiology and Biotechnology 100, 5215-5229.

Díaz Herrera, S., Grossi, C., Zawoznik, M., Groppa, M.D., 2016. Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of Fusarium graminearum. Microbiological Research 186-187, 37-43.

Eljounaidi, K., Lee, S.K., Bae, H., 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases – Review and future prospects. Biological Control 103, 62-68.

Estrada-De Los Santos, P., Bustillos-Cristales, R.o., Caballero-Mellado, J., 2001. Burkholderia, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Applied and Environmental Microbiology 67, 2790.
Fan, Q., Huang, G., Lechno-Yossef, S., Wolk, C.P., Kaneko, T., Tabata, S., 2005. Clustered genes required for synthesis and deposition of envelope glycolipids in *Anabaena* sp. strain PCC 7120. Molecular Microbiology 58, 227-243.

Germida, J.J., Walley, F.L., 1996. Plant growth-promoting rhizobacteria alter rooting patterns and arbuscular mycorrhizal fungi colonization of field-grown spring wheat. Biology and Fertility of Soils 23, 113-120.

Ghosh, R., Barman, S., Mukherjee, R., Mandal, N.C., 2016. Role of phosphate solubilizing *Burkholderia* spp. for successful colonization and growth promotion of *Lycopodium cernuum* L. (*Lycopodiaceae*) in lateritic belt of Birbhum district of West Bengal, India. Microbiological Research 183, 80-91.

Gillis, M., Van Van, T., Bardin, R., Goor, M., Hebbar, P., Willems, A., Segers, P., Kersters, K., Heulin, T., Fernandez, M.P., 1995. Polyphasic taxonomy in the genus *Burkholderia* leading to an emended description of the genus and proposition of *Burkholderia Vietnamiensis* sp. Nov. for N2-fixing isolates from rice in Vietnam. International Journal of Systematic and Evolutionary Microbiology 45, 274-289.

Gonzalez, C.F., Vidaver, A.K., 1979. Bacteriocin, plasmid and pectolytic diversity in *Pseudomonas cepacia* of clinical and plant origin. Microbiology 110, 161-170.

Hoffman, B.M., Lukoyanov, D., Yang, Z.-Y., Dean, D.R., Seefeldt, L.C., 2014. Mechanism of nitrogen fixation by nitrogenase: the next stage. Chemical Reviews 114, 4041-4062.

Joy, A.E., Parke, J.L., 1994. Biocontrol of *Alternaria* leaf blight on American ginseng by *Burkholderia cepacia* AMMD. In: Bailey, W.G., Whitehead, C., Proctor, J.T.A., Kyle, J.T., Eds.), The Challenge of the 21st Century. Proc. Inc. Ginseng Conf. Simon Fraser University, Vancouver, BC, pp. 93-100.

Khan, M.S., Zaidi, A., Wani, P.A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review. Agronomy for Sustainable Development 27, 29-43.

Kong, P., 2019. Evaluation of a novel endophytic *Pseudomonas lactis* strain for control of boxwood blight. Journal of Environmental Horticulture 37, 39-43.

Kong, P., Hong, C., 2017. Biocontrol of boxwood blight by *Trichoderma koningiopsis* Mb2. Crop Protection 98, 124-127.

Kong, P., Hong, C., 2018. Host responses and impact on the boxwood blight pathogen, *Calonectria pseudonaviculata*. Planta 249, 831-838.

Kong, P., Hong, C., 2020a. Complete genome sequence of a boxwood endophyte *Burkholderia* sp. SSG with broad biotechnological application potential. Biotechnology Reports https://doi.org/10.1016/j.btre.2020.e00455.

Kong, P., Hong, C., 2020b. A potent *Burkholderia* endophyte against boxwood blight caused by *Calonectria pseudonaviculata*. Microorganisms 8(2), 310.

Kong, P., Hong, C.X., 2019. Utilization of plant endophytes for control of boxwood blight. Proc. South Nurs. Assoc. Res. Conf. 63, 115-117.

LeBlanc, N., Salgado-Salazar, C., Crouch, J.A., 2018. Boxwood blight: an ongoing threat to ornamental and native boxwood. Applied Microbiology and Biotechnology 102, 4371-4380.

Liaqat, F., Eltem, R., 2016. Identification and characterization of endophytic bacteria isolated from *in vitro* cultures of peach and pear rootstocks. 3 Biotech 6, 120-120.

Ludwig-Müller, J., 2011. Auxin conjugates: their role for plant development and in the evolution of land plants. Journal of Experimental Botany 62, 1757-1773.

Mahenthiralingam, E., Bischof, J., Byrne, S.K., Radomski, C., Davies, J.E., Av-Gay, Y., Vandamme, P., 2000. DNA-based diagnostic approaches for identification of *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, *Burkholderia cepacia* genomovars I and III. Journal of Clinical Microbiology 38, 3165-3173.

Mahenthiralingam, E., Simpson, D.A., Speert, D.P., 1997. Identification and characterization of a novel DNA marker associated with epidemic *Burkholderia cepacia* strains recovered from patients with cystic fibrosis. Journal of Clinical Microbiology 35, 808-816.
Manuscript to be reviewed

Mendes, R., Pizzirani-Kleiner, A.A., Araujo, W.L., Raaijmakers, J.M., 2007. Diversity of cultivated endophytic bacteria from sugarcane: Genetic and biochemical characterization of Burkholderia cepacia complex isolates. Applied and Environmental Microbiology 73, 7259-7267.

Michiels, J., Van Soom, T., D’Hooghe, I., Dombrecht, B., Benhassine, T., de Wilde, P., Vanderleyden, J., 1998. The Rhizobium etli rpoN locus: DNA sequence analysis and phenotypical characterization of rpoN, ptsN, and ptsA mutants. Journal of Bacteriology 180, 1729-1740.

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters 170, 265-270.

Nejad, P., Johnson, P.A., 2000. Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. Biological Control 18, 208-215.

Pal, K.K.a.B.M.G., 2006. Biological control of plant pathogens. The Plant Health Instructor. Am. Phytopath. Soc., 10.1094/PHI-A-2006-1117-02.

Pradhan, S., Raj Pokhrel, M., 2013. Spectrophotometric Determination of Phosphate in Sugarcane Juice, Fertilizer, Detergent and Water Samples by Molybdenum Blue Method.

Qureshi, M.A., Ahmad, Z.A., Akhtar, N., Iqbal, A., Mujeeb, F., Shakir, M.A., 2012. Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. Journal of Animal and Plant Sciences 22, 204-210.

Reijnders, L., 2014. Phosphorus resources, their depletion and conservation, a review. Resources, Conservation and Recycling 93, 32-49.

Reinhold-Hurek, B., Hurek, T., 2011. Living inside plants: bacterial endophytes. Current Opinion in Plant Biology 14, 435-443.

Sajjan, U.S., Sun, L., Goldstein, R., Forstner, J.F., 1995. Cable (cbl) type II pili of cystic fibrosis-associated Burkholderia (Pseudomonas) cepacia: nucleotide sequence of the cblA major subunit pilin gene and novel morphology of the assembled appendage fibers. Journal of Bacteriology 177, 1030.

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiological Research 183, 92-99.

Santoyo, G., Orozco-Mosqueda, M.D., Govindappa, M., 2012. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of Bacillus and Pseudomonas: a review. Biocontrol Science and Technology 22, 855-872.

Sopheareth, M., Chan, S., Naing, K.W., Lee, Y.S., Hyun, H.N., Kim, Y.C., Kim, K.Y., 2013. Biocontrol of late blight (Phytophthora capsici) disease and growth promotion of pepper by Burkholderia cepacia MPC-7. The plant pathology journal 29, 67-76.

Trần Van, V., Berge, O., Ngô Kê, S., Balandreau, J., Heulin, T., 2000. Repeated beneficial effects of rice fertility sulphate acid soils of Vietnam. Plant and Soil 218, 273-284.

Vandamme, P., Holmes, B., Vancanneyt, M., Coenye, T., Hoste, B., Coopman, R., Revets, H., Lauwers, S., Gillis, M., Kersters, K., Govan, J.R.W., 1997. Occurrence of multiple genomovars of Burkholderia cepacia in cystic fibrosis patients and proposal of Burkholderia multivorans sp. nov. International Journal of Systematic and Evolutionary Microbiology 47, 1188-1200.

Weilharter, A., Mitter, B., Shin, M.V., Chain, P.S.G., Nowak, J., Sessitsch, A., 2011. Complete genome sequence of the plant growth-promoting endophyte Burkholderia phytofirmans strain PsJN. Journal of Bacteriology 193, 3383.

Yang, X., Hong, C., 2018. Biological control of boxwood blight by Pseudomonas protegens recovered from recycling irrigation systems. Biological Control 124, 68-73.

Yang, X., Hong, C.X., 2017. Evaluation of biofungicides for control of boxwood blight on boxwood. Plant Disease Management Reports 11, OT023.

Zhao, Y., 2010. Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol 61, 49-64.

Zhao, Y., 2012. Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. Molecular Plant 5, 334-338.
Figure 1

SSG plant growth promoting traits as shown in a colorimetric or plate assay.

(a) Light pink color produced at 2 days showing IAA production; (c) Growth on nitrogen free media at 4 days showing nitrogen fixation; (e) Halo produced around disks at 7 days showing phosphate solubilization and (g) Yellow color change at 3 days showing siderophore production. (b), (d), (f) and (h) are images of the control tube or plate for a, c, e, and g, respectively.
Figure 2

Boxwood plant growth of three cultivars – Buddy (intermediate), Justin Brouwers (slow) and Winter Gem (fast) as affected by SSG cell suspension (SSG) or control (dH₂O) drench over a 10-month period.

Each column is a mean of 9 replicate plants from three repeated experiments. Standard error bars are presented on top of the columns. Columns within each cultivar topped with different letters differed according to the least significant difference at $P = 0.05$. 

![Boxwood Cultivar & Treatment Graph]