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Impact of pre-inoculating soil with *Streptomyces* sp. GanoSA1 on oil palm growth and *Ganoderma* disease development

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ABSTRACT

Basal stem rot (BSR) or *Ganoderma* disease is a huge problem for palm oil producers worldwide because it severely damages infected palms and significantly affects yield. Previous studies have indicated that actinomycetes have potential applications as biological control agents against a range of phytopathogens. The aim of this study was to assess whether an application of actinomycetes could potentially reduce the disease incidence in oil palm plantations. In total, 129 *Streptomyces*-like isolates were purified from oil palm rhizospheric soils and tested against *Ganoderma boninense* PER71 *in vitro*. Of these, isolate GanoSA1 exhibited the strongest mycelial inhibition of *G. boninense* PER71. Molecular identification using 16S rRNA gene sequence analysis indicated that GanoSA1 had a high level of sequence similarity to *Streptomyces nigrogriseolus*. GanoSA1 was able to tolerate a wide range of temperatures, pH levels, and NaCl concentrations, and tests for the production of hydrolytic enzymes, siderophores and phosphate solubilization were positive. In tests for tolerance to commercial herbicides, GanoSA1 exhibited tolerance to field application levels of glyphosate isopropylamine at all concentrations tested (i.e., 1.0%, 0.5%, and 0.25%). Seedlings grown in soil mixed with a GanoSA1 vermiculite powder formulation (10^8 colony forming units/g) showed a higher vegetative growth index and lower disease incidence (48.89% versus 91.11%), decreased severity of foliar symptoms, and lower mortality six months after treatment than untreated control seedlings. GanoSA1 enhanced the growth of oil palm seedlings and reduced the incidence of BSR, suggesting that GanoSA1 may have a potential use as a biological control agent.

1. Introduction

Plant disease control is necessary to reduce the damage caused by plant pathogens and to reduce the economic impact of plant diseases. Like other plants, oil palm (*Elaeis guineensis*) is vulnerable to pests and diseases (Parveez et al., 2020). Basal stem rot (BSR) disease caused by *Ganoderma boninense* is widely recognized as a serious threat to the oil palm industry, especially in Southeast Asia (Kushairi et al., 2018), where it causes estimated losses of nearly RM 1.5 billion (~US$480 million) (Arif et al., 2011). In 2016–2017, the incidence of BSR disease in Malaysia was 7.4% with 221,000 ha affected (Idris, 2019). Assis et al. (2016) estimated that the potential yield was reduced by 43.32% due to the disease. As part of efforts to tackle this issue and to reduce chemical application, our attention has focused on assessing the potential of microbial inoculation technologies, such as biological control, as a means of suppressing plant diseases. Our overall goal is to identify a biological control agent (BCA) that could be used to minimize the source of *Ganoderma* inoculum and to reduce the disease incidence either at replanting or within the existing planting. Numerous research studies have demonstrated the antagonistic activity of microbes such as *Hendersonia* sp., *Phlebia* sp., *Trichoderma* spp., *Diaporthe* sp., *Penicillium citrinum*, arbuscular mycorrhizal fungi (AMF), endophytic *Pseudomonas* sp., *Burkholderia* sp. and actinomycetes against *Ganoderma* and have suggested their potential use as BCAs against *Ganoderma* (Shariffah-Muzaimah et al., 2015, 2018; Nurrashyeda et al., 2018; Kamarudin et al., 2017; Nur Azura et al., 2016; Ramli et al., 2016; Ting and Jioe, 2016; Sundram et al., 2015; Anuar et al., 2015; Nurrashyeda et al., 2018, 2018).

Actinomycetes are spore-forming Gram-positive bacteria that belong to the Actinomycetales within the phylum Actinobacteria. Members of the actinomycetes have a diverse morphology, including filamentous fungal-like aerial hyphae (Barka et al., 2016). Actinomycetes are found

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in a range of diverse habitats but are predominantly found in soils. Their spore-forming ability helps them to survive in a semi-dormant state under various, unfavorable soil conditions and explains their abundant presence in the natural soil microflora. Their ability to adapt to diverse environmental conditions, such as high temperatures and acidic, alkaline and saline soils, is one of the main reasons for their distribution in so many diverse ecosystems (Binayke et al., 2018; Zenova et al., 2011). Actinomycetes are an important part of the rhizosphere owing to their ability to produce a wide range of antimicrobial products such as antifungal metabolites (Wonglom et al., 2019; Lim et al., 2018), volatile compounds (Cordovez et al., 2015; Boukaew et al., 2013; Wang et al., 2013; Li et al., 2010), cell-wall-degrading enzymes (Mun et al., 2020; Wonglom et al., 2019; Gopalakrishnan et al., 2011; Sadeghi et al., 2012), and siderophores (Zeng et al., 2018), and to induce resistance (Ansari et al., 2020; Dias et al., 2017; Awla et al., 2017; Senthilraja, 2016). The most prevalent genus of Actinomycetes in the soil is *Streptomyces*. Owing to their ability to synthesize various types of secondary metabolites, *Streptomyces* spp. have been reported to reduce plant pathogen infections and have the capacity to be developed as BCAs (Jacob et al., 2016; Jones and Elliot, 2017; Law et al., 2017; Newitt et al., 2019). Due to their huge potential, the aim of this study was to investigate the effectiveness of *Streptomyces* spp. isolated from the rhizosphere of oil palm in the management of BSR disease of oil palm seedlings *in vitro* and *in vivo*.

2. Materials and methods

2.1. Isolation of Streptomyces spp. and Ganoderma culture and growth conditions

Soil samples were collected from the rhizosphere of oil palms growing in an oil palm plantation (that was more than 20 years old) located in Peninsular Malaysia that had been reported to have a high incidence of BSR. The collection and treatment of samples was as described in Shariffah-Muzaimah et al. (2015). *Streptomyces* spp. were isolated from the samples using a serial dilution spread-plate method. Aliquots from the three lowest dilutions (i.e., 10^{-4}, 10^{-5} and 10^{-6}) were spread on four different isolation agar media: yeast extract–malt extract (YME) agar, inorganic salt-starch agar, humic acid vitamin-agar and yeast casamino acid-dextrose agar, which had been supplemented with 50 ppm of nystatin, 50 ppm of cycloheximide and 20 ppm of nalidixic acid to minimize the growth of fungi and bacteria. *G. boninense* PER71 was obtained from a stock culture at the Plant Pathology and Biosecurity Laboratory, Malaysian Palm Oil Board (MPOB), Bangi, Malaysia. Working cultures of *G. boninense* PER71 and *Streptomyces* spp. were prepared by subculturing the *G. boninense* PER71 stock culture onto potato dextrose agar (PDA) and subculturing isolates of *Streptomyces* spp. onto YME agar. All plates were incubated at 28 ± 2 °C for 7 days. Spore and mycelial suspensions of *Streptomyces* spp. were maintained in 20% (v/v) glycerol at −80 °C for long-term preservation.

2.2. Dual culture assay

Pure cultures of *Streptomyces* spp. were tested for antagonistic activity against *G. boninense* PER71 using a modified plug method (Getha and Vikineswary, 2002; Shariffah-Muzaimah et al., 2015) on PDA. A mycelial disc was cut from a 7-day-old culture of a *Streptomyces* sp. using a 5-mm borer and placed in the center of an assay plate. After 5 days of incubation at 28 ± 2 °C, a 5-mm diameter mycelial plug of *G. boninense* was placed on either side of the *Streptomyces* mycelial disc at a distance of 2 cm from the edge of the assay plate. A plate without a mycelial disc of a *Streptomyces* sp. served as a control. The plates were incubated for a further 7 days at 28 ± 2 °C. The ability of a *Streptomyces* sp. to inhibit the growth of *G. boninense* PER71 was expressed as the percentage inhibition of radial growth (PIRG), which was calculated according to the following formula: $\text{PIRG}\ (\%) = \frac{(A-B)}{A} \times 100$, where A is the radial growth of *Ganoderma* in the absence of a *Streptomyces* sp. and B is the average radial growth of *Ganoderma* in the presence of a *Streptomyces* sp.

2.3. Molecular identification and morphological and physiological characterization of a Streptomyces sp. with strong antagonistic activity toward Ganoderma boninense PER71

Based on the antagonistic activity of *Streptomyces* isolate GanoSA1 in the dual culture assay, sequence analysis of the 16S rRNA gene was performed to determine the molecular identification of this isolate. DNA extraction was performed using a DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocol. The lysate (DNA) obtained was used in a polymerase chain reaction (PCR). PCR and purification of the PCR product was conducted as described in Shariffah-Muzaimah et al. (2018). The PCR amplified sequences obtained were compared to published actinomycete sequences by performing a basic local alignment search tool (BLAST) search in the NCBI GenBank database (BLASTn, http://blast.ncbi.nlm.nih.gov/). Multiple sequence alignments of *Streptomyces* sp. isolate GanoSA1 and additional sequences obtained from GenBank were performed using the Clustal W Multiple Sequence Alignment program (version 1.8). Sequences of *Streptomyces indigoferus* strain NCBI 67307 (KY 407747.1), *Streptomyces nigrogriseolus* strain NCBI 285470 (MG 984076.1), *Kitasatospora herbaricolor* strain NCBI 68217 (MT 081107.1), *Kitasatospora xanthocidia* strain NCBI 83382 (MN 585739.1), *Streptomyces* sp. NCBI 1931 (LC 475423.1) and *Kitasatospora aburaviensis* strain NCBI 67265 (MK 942686.1) were used as reference sequences.

The maximum likelihood method was used to construct a phylogenetic tree using MEGA version 6 (Tamura et al., 2011). Bootstrap analysis was conducted to estimate the statistical confidence of tree branches by performing 1000 resamplings. Known phytopathogenic actinomycetes, such as Streptomyces scabiei NCBI 1930 (MG856055.1) and Streptomyces ipomoea NCBI 103232 (MF077114.1), pathogenic actinomycetes, such as Actinomyces urogenitalis NCBI 103621 (AJ243791.1), Actinomyces israelii NCBI 1659(NR_114401.1) and Nocardia asteroids NCBI 1824 (X53205.1), non-Streptomyces actinomycetes, such as Micromonospora sp. NCBI 1738021 (KR780409.1) and Actinoplanes regularis NCBI 52697 (X93188.1), and actinomycetes used as biocontrol agents, such as Streptomyces griseoviridis NBRC 12874 (NR 112313.1) and Streptomyces lydicus NCBI 47763 (FJ799181.1), were included as outgroups.

International Streptomyces Project (ISP) media (yeast malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts-starch agar (ISP4) and glycerol-asparagine agar (ISP5)) were used to study the cultural and morphological characteristics of GanoSA1, as recommended by Shirling and Gottlieb (1966). The color of the aerial spore mass of mature colonies and of the soluble pigments produced on each medium was recorded after 7 days of incubation in the dark at 28 °C. Other characteristics, such as spore-bearing hyphae and spore chains, were assessed using the methods described in Li et al. (2016). *Streptomyces* sp. GanoSA1 was streaked on YME agar and then sterile microscope slide covers were placed on the medium near to the culture streaks. After 7 days of incubation in the dark at 28 ± 2 °C, the slide covers were removed using sterile forceps and placed onto microscope slides with one drop of lactophenol with methyl-blue and examined under a binocular microscope (Olympus, Tokyo, Japan).

The physiological responses of *Streptomyces* sp. GanoSA1 to a range of temperatures, pH levels, and salinity levels were investigated using a streak plate technique. *Streptomyces* sp. GanoSA1 was streaked on YME agar and incubated in the dark at 28 °C, 30 °C, 40 °C or 50 °C for 7 days. The GanoSA1 isolate was also grown on YME agar with pH levels ranging from 4.5 to 8.5 and on YME supplemented with sodium chloride (NaCl) at concentrations of 0, 2, 4, 6, 8, 10 or 12% (w/vol) to assess the effect of pH and salinity on the growth of GanoSA1. Plates were

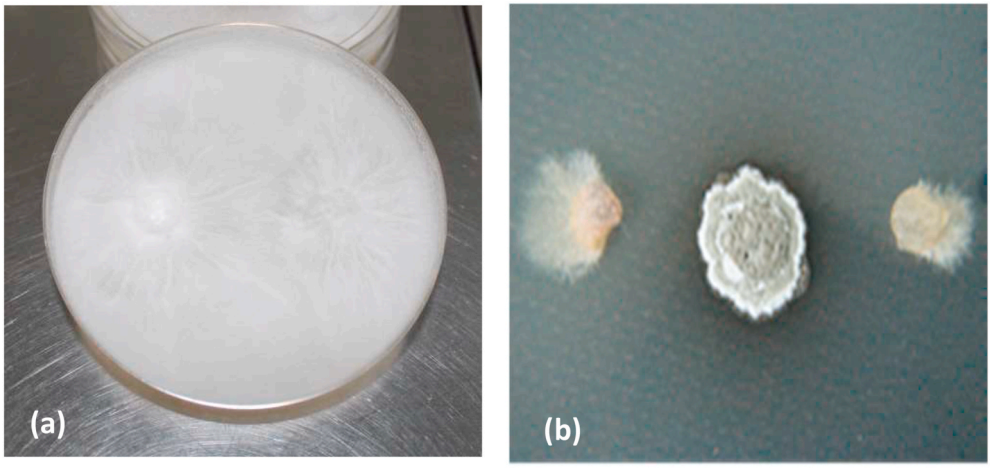

Fig. 1. *In vitro* antagonism of *Streptomyces* sp. GanoSA1 toward *Ganoderma boninense* PER71 in a dual culture assay on potato dextrose agar: (a) Seven-day-old colony of *G. boninense* PER71 grown in the absence of a *Streptomyces* sp. GanoSA1 inoculum disc (control); (b) Inhibition of 7-day-old *G. boninense* PER71 inoculum discs (left and right) when transferred to the PDA plate 5 days after the *Streptomyces* sp. GanoSA1 inoculum disc (center).

incubated in the dark at 28 ± 2 °C and growth was observed and recorded after 7 days of incubation. All plates were incubated in an inverted position to avoid evaporation.

The physiological characteristics of *Streptomyces* sp. GanoSA1 were assessed by placing a 5-mm-diameter agar plug of well-sporulated culture onto lipase medium, CM-cellulose agar, colloidal chitin agar, a double-layered chrome azurol S agar, and phosphate solubilization agar (Pikovskayas agar) to screen for the production of lipase, cellulase, chitinase, and siderophores and phosphate solubilization, respectively (Gopalakrishnan et al., 2013; Sahilah et al., 2010; Hu and Xu, 2011). β-1, 3-glucanase activity was determined as described by Singh et al. (1999) and indole acetic acid production was determined as described by Liu et al. (2016). Four replicates of each assay were performed.

The sensitivity of GanoSA1 to commercial herbicides was investigated using an agar plate dilution method. Standard minimal salt agar (pH 6.5) plates supplemented with paraquat (Capayam®, Syngenta Corporation Sdn. Bhd., Malaysia), glyphosate isopropylamine (Roundup®, Monsanto) or glufosinate-ammonium (Basta® 15, Bayer Crop Sciences) were spot inoculated with a 5-mm agar plug of 7-day-old GanoSA1. Herbicide concentrations that were 0.25, 0.5 and 1 times the recommended field application rates of 400 g a. i. ha^{-1} for paraquat and glufosinate-ammonium and 800 g a. i. ha^{-1} for glyphosate were assessed in this study, as described in Zain et al. (2013). Seven days after incubation in the dark at 28 ± 2 °C, GanoSA1 growth was scored as follows: (−) no growth; (±) little growth with no aerial mycelia; (+) little growth; (++) medium growth. The highest concentration of each herbicide that did not inhibit the growth of *Streptomyces* sp. GanoSA1 was defined as the maximum tolerance level.

2.4. Application of the Streptomyces sp. GanoSA1 treatment under nursery conditions, disease assessment and seedling growth

A vermiculite powder formulation of GanoSA1 was prepared according to Shariffah-Muzaimah et al. (2012). The effectiveness of the GanoSA1 treatment *in vivo* was evaluated using commercial oil palm seedlings (*dura* × *pisifera* (D × P) cross) at leaf stages three to four. An unsterilized soil mixture of commercial topsoil and sand was used to imitate the soil that palm oil seedlings experience under natural conditions. Based on the findings of a previous *in vitro* assay that showed that 10^6–10^8 CFU/g of a vermiculite powder formulation of *Streptomyces* GanoSA1 effectively inhibited *Ganoderma* and that 10^8 CFU/g resulted in a PIRG of 100% (data not shown), each seedling was pretreated with 50 g (10^8 CFU/g) of the vermiculite powder formulation mixed with soil in a 15 × 23 cm polypropylene bag. Fourteen days after the pretreatment, each seedling was transferred to a bigger polypropylene bag (38 × 46 cm) and then inoculated artificially with *G. boninense* PER71 using a rubber wood block (RWB) sitting technique (Ramli et al., 2016). In general, three roots of each seedling were each placed on an RWB that had been completely colonized by *G. boninense* PER71, and then covered with soil mixed with a further 50 g of the GanoSA1 vermiculite powder formulation. Two types of treatments were applied: seedlings were either treated or not-treated with the vermiculite powder formulation of *Streptomyces* sp. GanoSA1. Each treatment consisted of three replicates with five seedlings per biological replicate (i.e., a total of 15 seedlings per treatment). In each treatment, seedlings were split into two groups: 15 seedlings were kept pathogen-free and 15 were inoculated with *G. boninense* PER71. All seedlings used in this trial were maintained in a shaded nursery under standard nursery practices (Esnan, 2019).

On a monthly basis, *Ganoderma* disease monitoring was performed by assessing the percentage of disease incidence (DI, %) and the severity of foliar symptoms (SFS, %). DI and SFS were assessed visually. The infected seedlings were classified based on the development of chlorotic and necrotic leaves and the development of *Ganoderma* white mycelia, a white button or a fruiting body. DI and SFS were calculated using the method described by Campbell and Madden (1990). To calculate the disease severity (DS) caused by *Ganoderma* in each treatment, each seedling was scored based on a disease scale ranging from 0 to 4 (Sundram, 2013). A disease progression curve was also plotted using the DS data to monitor the disease progress over time. Areas under the plotted disease progression curve (AUDPC) were assessed to calculate the proportion of disease reduction (DR, %) (Nur Ain Izzati and Abdullah, 2008; Sapak et al., 2008; Sundram et al., 2008). All the seedlings were harvested at the end of the experiment to assess the internal disease severity caused by *Ganoderma*.

The efficacy of the GanoSA1 powder as a plant growth promotor was assessed at monthly intervals by measuring vegetative growth parameters, i.e., seedling height, stem diameter measurement, and the number of fronds produced. Three readings of the relative chlorophyll content of each leaflet along the mid-rib were recorded using a SPAD chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan). Photosynthetic rate (P_N), intercellular CO_2 concentration (ci), stomatal conductance (g_s) and water-use efficiency (WUE) were determined using a portable photosynthesis system (LI-6400, Li-Cor Biosciences, Lincoln, NE, USA) 8 months after treatment. Measurements of gas exchange parameters were made on fully opened, matured and healthy leaves under bright sunlight between 09.00 to 11.00 a.m. Eight months after the treatments were applied to the seedlings, all seedlings were harvested to determine the

Table 1
Morphological and physiological characteristics of *Streptomyces nigrogriseolus* GanoSA1 after 7 days of incubation.

Morphological characteristic				
	Growth of *Streptomyces nigrogriseolus* GanoSA1	Reverse color	Diffusible pigment	Spore formation
	Aerial surface color			
YME	Light-bluish gray	Brown	–	++
ISP3	Gray	Gray	–	++
ISP4	Light gray	Brown	–	+
ISP5	White	Brown	–	±
Spore formation	Rectiflexibiles/ Retinaculiaperti			
Physiological characteristic				
➢ β-1,3-glucanase	+			
➢ Chitinase	+			
➢ Cellulase	–			
➢ Lipase	+			
➢ Siderophores	+			
➢ Phosphate solubilization	+			
➢ Indole acetic acid	–			
Temperature effect				
➢ 28 °C	++			
➢ 30 °C	++			
➢ 40 °C	+			
➢ 50 °C	–			
pH				
➢ 4.5	++			
➢ 5.5	++			
➢ 6.5	++			
➢ 7.5	++			
➢ 8.5	++			
NaCl				
➢ 2%	++			
➢ 4%	++			
➢ 6%	++			
➢ 8%	+			
➢ 10%	–			
➢ 12%	–			
Pesticides	Pesticide strength (%)			
	1	0.5	0.25	
➢ Glyphosate isopropylamine	±	+	++	
➢ Paraquat dichloride	–	–	±	
➢ Glufosinate ammonium	–	–	–	

Streptomyces growth: (−) no growth; (±) little growth with no aerial mycelia; (+) little growth; (++) medium growth.

number of roots and root mass.

2.5. Statistical analysis

All experiments were laid out in a completely randomized design. To confirm the reproducibility of data, the glasshouse experiments were repeated thrice. Data from three experiments were analyzed for experiment × treatment interactions. Given that there was no experiment × treatment interaction, data were pooled for statistical analysis. Data for disease incidence were arcsine-transformed before the statistical analysis (Student's *t*-test). Data that failed a normality test were analyzed using the Mann–Whitney rank sum test.

3. Results

3.1. Isolation of Streptomyces spp. with antagonistic activities against G. boninense PER71

Pure cultures of 129 *Streptomyces*-like isolates were recovered from soil samples collected from the rhizosphere of oil palms growing in a plantation with a high incidence of BSR disease. These isolates were assayed for antagonistic activity against *G. boninense* PER71. Out of the 129 *Streptomyces* spp. isolated, 10 isolates inhibited the growth of *G. boninense* PER71 and had a PIRG value of more than 80%. Of these, isolate GanoSA1 showed stronger mycelial inhibition of *G. boninense* PER71 than the other isolates, with new mycelial growth from the inoculum plug inhibited for the first 3–7 days after inoculation in dual culture with GanoSA1 (Fig. 1).

3.2. Characterization of Streptomyces sp. GanoSA1

Morphological and physiological characteristics of *Streptomyces* sp. GanoSA1 are summarized in Table 1. Gram staining of *Streptomyces* sp. GanoSA1 confirmed that it was a Gram-positive bacterium with a filamentous structure. Extensive branches were observed; however, the aerial mycelium was not fragmented. Spore-bearing hyphae observed under a light microscope were characterized as Rectiflexibiles and Rectinaculiaperti (Fig. 2a). At 1–3 days after streaking, white colonies with a smooth surface were observable on all plates. From day 5 onwards, colonies developed powdery to dusty sporulating aerial mycelium, indicating abundant spore formation (Fig. 2b). The aerial mycelium was light-to bluish-gray on ISP2, ISP3 and ISP4 media, and white on ISP5. After 7 days of incubation, the colonies that had developed on ISP3 and ISP5 media were smaller than those on ISP2 and ISP4 media. The substrate mycelium of GanoSA1 was brown with no pigmentation. This strain exhibited good growth with observable abundant spore formation on YME when incubated at temperatures ranging from 28 °C to 40 °C and when grown on YME with pH levels ranging from 4.5 to 8.5. When grown on YME supplemented with 2, 4 or 6% NaCl, GanoSA1 grew abundantly and the appearance of the colony was unchanged; GanoSA1 tolerated media with up to 8% NaCl. However, no growth was observed on YME plates with a NaCl concentration of more than 8%. Assays for chitinase, β-1,3-glucanase, lipase, phosphate solubilization and siderophore production were positive. This strain also showed tolerance to glyphosate ammonium at all tested concentrations, slight tolerance to paraquat dichloride at 0.25% but was susceptible to glufosinate ammonium at all concentrations (1.0, 0.5 and 0.25%) when tested at field application levels.

Initial identification based on morphological characteristics indicated that GanoSA1 belonged to the genus *Streptomyces*, which was confirmed by partial sequencing of the 16S rRNA gene sequence. The GanoSA1 PCR amplification product showed a single band of between 500 bp and 1000 bp on an electrophoresis gel (Fig. 2c). The partial 16S rRNA gene sequence of GanoSA1 showed a high level of similarity to the *Streptomyces nigrogriseolus* NCBI 285470 (MG984076.1) sequence (Fig. 2d).

3.3. Suppression of basal stem-rot disease by Streptomyces sp. GanoSA1

For up to 3 months after treatments were applied, no visible symptoms of disease were observed on any seedlings. Observable external symptoms, such as the desiccation of leaves, were first observed 3 months after artificial inoculation with the pathogen. At this time, a white mycelial mass was observed on the lower basal area of the seedling, which later developed into a button-like fruiting body. Disease development on seedlings treated with GanoSA1 was slower than on untreated seedlings. The DI, SFS and DS of GanoSA1-treated seedlings were also lower than those of untreated seedlings, indicating disease suppression by GanoSA1. Six months after untreated seedlings were

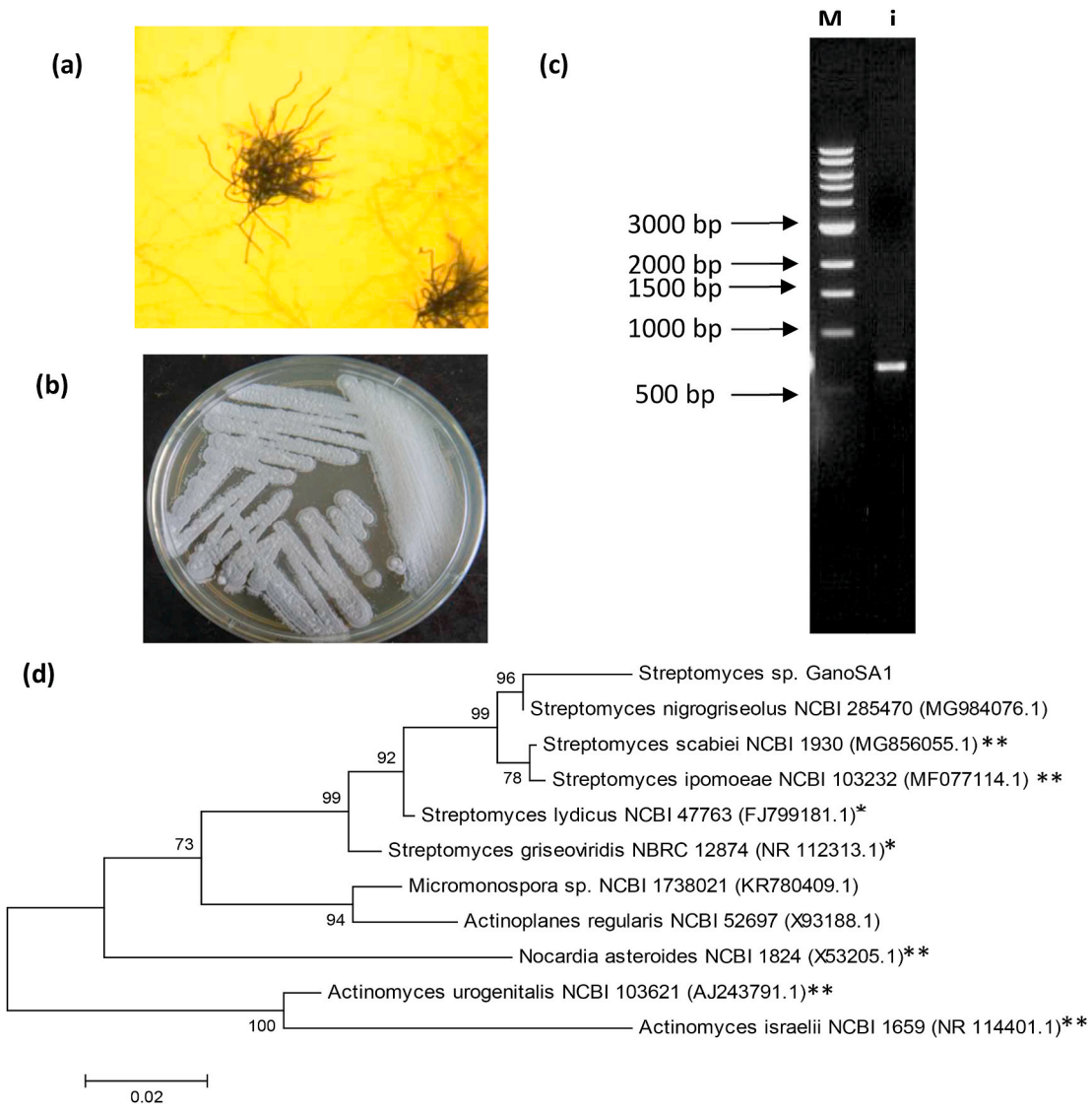

Fig. 2. Morphological and molecular identification of *Streptomyces nigrogriseolus* strain GanoSA1. (a) Sporulating aerial mycelium at × 40 magnification under a light microscope. (b) Colony morphology at 7 days old on yeast malt extract agar. (c) Gel electrophoresis image of the *S. nigrogriseolus* strain GanoSA1 PCR amplification product (M, universal marker; i, GanoSA1). (d) Phylogenetic tree generated using the maximum-likelihood method showing the relationship between the partial 16S rRNA gene sequence of the *Streptomyces* strain GanoSA1 investigated in this study and other actinomycetes. Single asterisks denote species with known biological control activity. Double asterisks denote known pathogenic actinomycetes. Triple asterisks denote phytopathogenic actinomycetes. The scale bar indicates 0.02 nucleotide substitutions per nucleotide position. GenBank accession numbers are indicated in parentheses. Bootstrap values (1000 replicate runs) are indicated. Streptomyces scabiei, Streptomyces ipomoea, Actinomyces urogenitalis, Actinomyces israelii, Nocardia asteroids, Micromonospora sp., Actinoplanes regularis, Streptomyces griseoviridis and Streptomyces lydicus were used as outgroups.

artificially inoculated with *G. boninense* PER71, a DS value of 75.00% was recorded for these seedlings (Table 2, Fig. 3). Seedlings treated with *Streptomyces* sp. GanoSA1 showed an average AUDPC value of 52.22 unit^2 compared with 151.94 unit^2 for untreated seedlings. Based on these AUDPC values, seedlings treated with an application of *Streptomyces* sp. GanoSA1 at a concentration of 10^8 CFU/g powder formulation showed a 63.01% reduction in disease severity.

Destructive sampling of diseased seedlings at the end of the experiment revealed extensive internal root rot that extended into the stems of untreated seedlings. By contrast, seedlings treated with GanoSA1 showed no penetration by fungal mycelium. Necrotic lesions extended further up the bole of untreated seedlings than those of treated seedlings, indicating severe bole infection due to *G. boninense*. Analysis of the internal DS revealed that seedlings treated with GanoSA1 had a DS value of 43.32% whereas untreated seedlings had a DS value of 88.89%.

3.4. Effect of *Streptomyces* sp. GanoSA1 on oil palm seedling growth

There was no adverse effect on the growth of oil palm seedlings treated with *Streptomyces* sp. GanoSA1 powder: all treated seedlings showed healthy and normal growth. Monthly observations of the vegetative growth revealed that *Streptomyces* sp. GanoSA1-treated seedlings showed significantly increased plant height, stem diameter, and relative leaf chlorophyll content compared with the control seedlings. Root and shoot length and biomass values of treated seedlings were significantly greater than those of untreated seedlings from 1 to 6 months after GanoSA1 application (Fig. 4). Oil palm seedlings showed significantly higher growth values 8 months after treatment with *Streptomyces* sp. strain GanoSA1 than untreated seedlings ($P < 0.05$) (i.e., plant height, 116.27 cm versus 106.44 cm; leaf number, 13 versus 11; plant diameter, 58.73 cm versus 50.37 cm; and root mass, 165.52 g versus 130.58 g). However, the leaf mass of treated seedlings was not

Table 2
The effect of *Streptomyces nigrogriseolus* GanoSA1 on disease and vegetative growth of oil palm.

Treatment	Control	*S. nigrogriseolus* GanoSA1 powder
Disease assessment		
Disease incidence (%)	91.11 ± 4.29[a]	48.89 ± 7.54[b]
Severity of foliar symptoms (%)	83.11 ± 3.29	42.45 ± 5.57[b]
Dead seedlings (%)	80.00 ± 6.03[a]	31.11 ± 6.98[b]
Disease severity (external, %)	75.00 ± 2.79[a]	31.11 ± 1.57[b]
Disease severity (internal, %)	88.89 ± 2.91[a]	43.32 ± 4.94[b]
AUDPC (unit^2)	151.94 ± 24.07[a]	52.22 ± 7.03[b]
Disease reduction (%)	–	63.01 ± 4.99[a]
Vegetative growth		
Number of fronds	11.67 ± 0.28[a]	13.278 ± 0.38[b]
Plant height (cm)	106.44 ± 2.32[a]	116.27 ± 2.25[b]
Stem girth (cm)	50.37 ± 1.53[a]	58.73 ± 1.35[b]
Root mass (g)	130.58 ± 1.1[a]	165.52 ± 0.67[b]
Leaf mass (g)	255.59 ± 1.78[a]	272.6 ± 3.35[a]

Ganoderma disease development and oil palm seedling growth parameters expressed as the means of three trials ± the standard deviation of the means. Values followed by different letters show a significant difference between treatments according to the least significant difference (LSD) test ($P < 0.05$). AUDPC: area under the curve.

significantly greater than that of untreated seedlings ($P > 0.05$) (Table 2). Except for c_i, all the gas exchange parameters (i.e., P_N, g_s, T_r, WUE and relative chlorophyll content) for seedlings treated with GanoSA1 powder were significantly greater than those obtained for untreated seedlings (Fig. 5).

4. Discussion

The first challenge when attempting to control a plant disease with the aid of a BCA is the selection of a suitable BCA. The potential BCA was selected using a bioactivity-based selection method based on antagonism against *G. boninense*. Endophytic bacteria, such as *Burkholderia* spp., *Pseudomonas* spp., *Bacillus* spp. and *Serratia* spp. (Sapak et al., 2008; Bivi et al., 2010; Ramli et al., 2016), chitinolytic *Enterobacter* spp. and *Bacillus* spp. (Suryanto et al., 2012) have been reported to show antagonistic activity against *G. boninense* with PIRG of more than 70%. Similarly, several fungi, such as *Trichoderma* spp. (Siddiquee et al., 2009; Sundram, 2013; Musa, 2017), *Hendersonia* sp. (Nurrashyeda et al., 2018), *Amphinema* sp. (Nurrashyeda et al., 2012a), *Phlebia* sp. (Nurrashyeda et al., 2012b), *Neonothopanus nambi*, *Schizophyllum commune* and *Ganoderma orbiforme* (Naidu et al., 2016), have also been demonstrated to inhibit the growth of *G. boninense* in *in vitro* assays. Furthermore, *Streptomyces* spp. and some non-*Streptomyces* actinomycetes and/or their metabolites have been reported to affect the growth of *Ganoderma* and cause lesions on its mycelia (Sujarit et al., 2020; Queendy and Roza, 2019; Lim et al., 2018; Nur Azura et al., 2016; Pithakkit et al., 2015; Shariffah-Muzaimah et al., 2015; Ting and Jioe, 2016). In this study, the actinomycete strain GanoSA1 strongly inhibited *G. boninense* PER71 mycelial growth *in vitro*, with no formation of new mycelia observed on the *Ganoderma* plug when challenged with this strain. GanoSA1 showed a slight variation in morphology depending on the composition of the agar media. Similar results have been reported by Zhu et al. (2007), Prasad et al. (2013) and Charousova et al. (2015). Analysis of the GanoSA1 16S rRNA gene sequence revealed that this isolate showed a high level of sequence similarity to that of *Streptomyces nigrogriseolus*. This species has been reported to positively inhibit other pathogenic microbes such as *Aspergillus niger*, *Aspergillus wentii*, *Candida albicans*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* (Ellaiah et al., 2002). To date, there is no report of the use of this strain as a BCA against *G. boninense* on oil palm.

In this experiment, secondary metabolites produced by GanoSA1 showed antifungal activity toward *Ganoderma*; however, further analysis and characterization of these potential metabolites was not undertaken in this study. As well as the production of bioactive metabolites, *Streptomyces nigrogriseol*us GanoSA1 showed hydrolytic enzyme activity (chitinase and glucanase) related to fungal cell wall degradation. Numerous studies have demonstrated the importance of hydrolytic

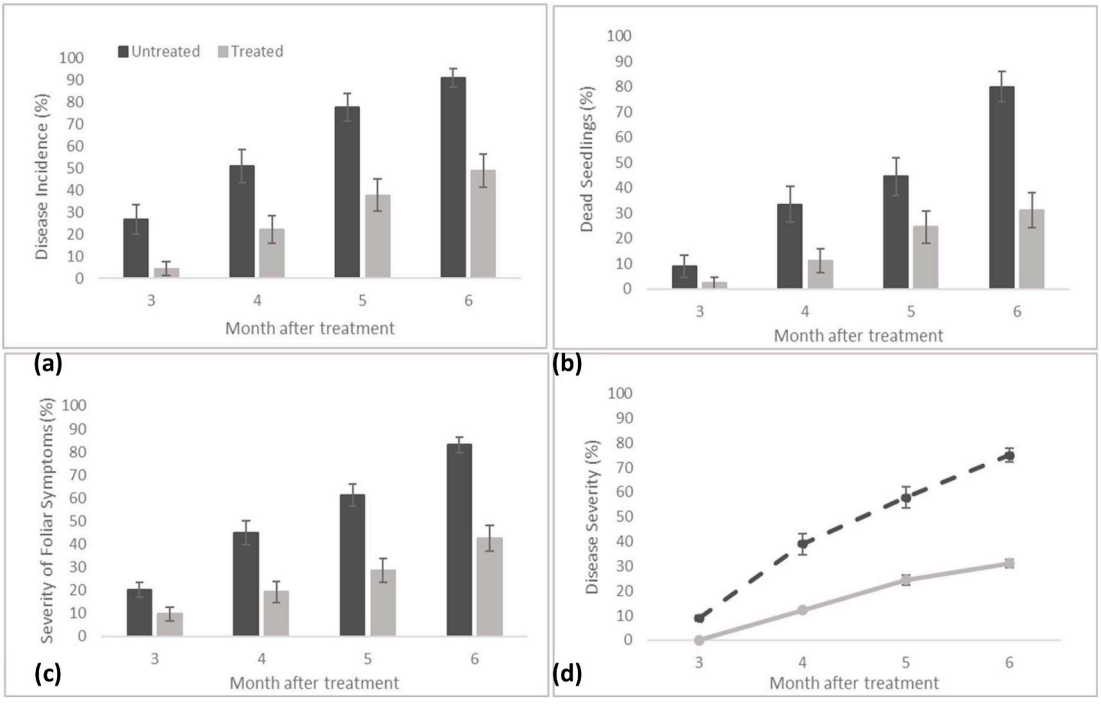

Fig. 3. Effect of *Streptomyces nigrogriseolus* GanoSA1 on *Ganoderma* disease development up to sixth months after inoculation. (a) Disease incidence; (b) Dead seedlings; (c) severity of foliar symptoms; (d) disease progression. Values are the means of three trials; the vertical bars represent the standard errors of the means.

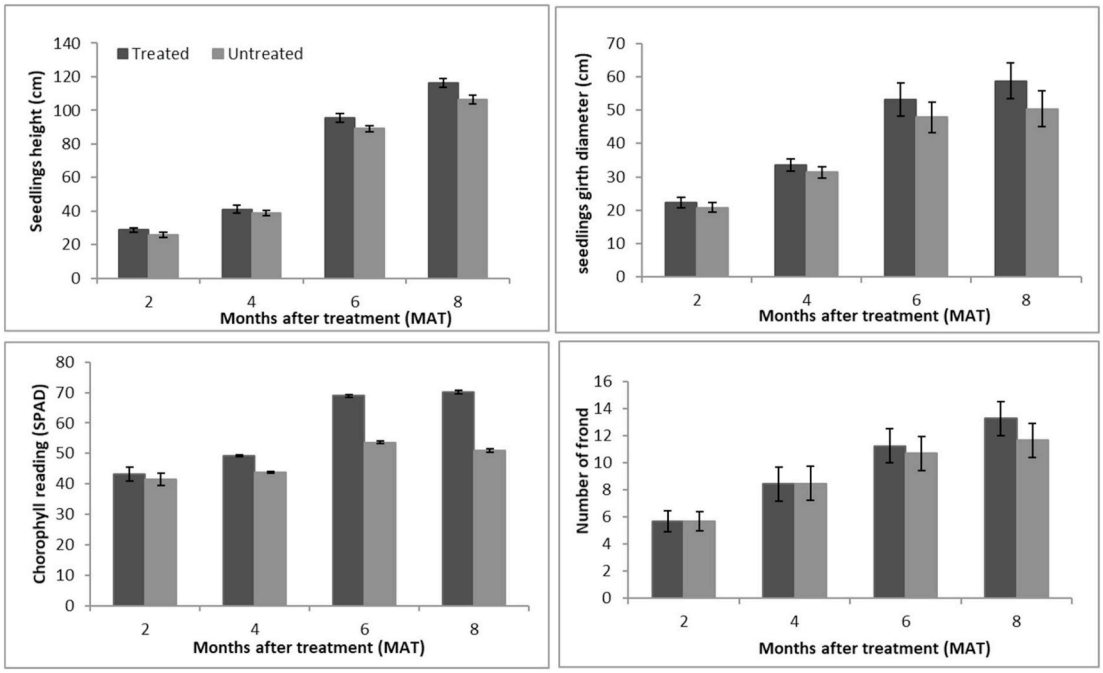

Fig. 4. Effect of *Streptomyces nigrogriseolus* GanoSA1 on vegetative growth of oil palm seedlings up to eight months after inoculation. (a) Seedling height, cm; (b) girth diameter, cm; (c) chlorophyll reading (SPAD) (d) number of frond. Values are the means of three trials; the vertical bars represent the standard errors of the means.

enzymes produced by *Streptomyces* spp. in biological control activity (Mun et al., 2020; Wonglom et al., 2019; Jadhav et al., 2017; Janaki et al., 2017; Gopalakrishnan et al., 2015; Pithakkit et al., 2015; Tang-Um and Niamsup, 2012). *Streptomyces* spp. have also been shown to secrete a wide spectrum of antimicrobial compounds that inhibit the growth of plant pathogens, indicating their potential use as BCAs against plant diseases (Wonglom et al., 2019; Lim et al., 2018; Shen et al., 2016; Boukaew et al., 2013) and possibly as plant growth promoters (Liotti et al., 2019; Amaresan et al., 2019; Awla et al., 2017; Jog et al., 2016; Nur Azura et al., 2016).

Soil conditions are one of the factors that can influence the BCA or plant growth promoter activity of bacterial inoculants. *S. nigrogriseolus* GanoSA1 grew well at temperatures ranging from 28 °C to 40 °C and in media with pH values ranging from 4.5 to 8.5 or saline concentrations of up to 8%. This suggests that this strain could be applied as a treatment in acidic, alkaline or saline soils. Extensive research on the use of actinomycetes as BCAs and bio-fertilizers for various agricultural crops has been established in recent years as part of efforts to develop greener and more sustainable crop protection methods (Goudjal et al., 2014). Studies on the potential usage of actinomycetes for the biocontrol of plant diseases have been published and some of these BCAs, such as *S. lydicus* WYEC 108, *Streptomyces* K61, *S. colombiensis* and *S. kasugaensis*, have been developed into commercial biological control agents (Vurukonda et al., 2018).

Apart from biological control activity, other characteristics, such as susceptibility or tolerance to chemical herbicides and pesticides that are used in oil palm plantations, are also important factors when selecting a potential BCA because they will affect the establishment of the BCA in the soil when applied as a treatment. *S. nigrogriseolus* GanoSA1 showed tolerance to glyphosate at all the concentrations tested, slight tolerance to paraquat dichloride and was susceptible to glufosinate ammonium at all concentrations when tested at field application levels using an *in vitro* direct exposure technique. Similar *in vitro* tests have shown that herbicides have strong inhibitory effects on fungi such as *Mucor* sp., *Penicillium* sp. and *Aspergillus* sp. (Zain et al., 2013) and on actinomycetes (Šantrić et al., 2016); however, these inhibitory effects are weaker in soil treatments or *in vivo* studies (Zain et al., 2013; Šantrić et al., 2016). The inhibitory effects of herbicides are weaker in soil because biological, chemical and physical processes that occur in the soil can degrade herbicides (Zain et al., 2013). Furthermore, no significant effects have been observed on microbial community structure, soil fluorescein diacetate hydrolysis or β-glucosidase activity in response to recommended application rates of glyphosate (Ratcliff et al., 2006; Dennis et al., 2018), glufosinate, paraquat, or paraquat-diquat (Dennis et al., 2018). However, further studies will be undertaken to establish the tolerance of GanoSA1 to commercial herbicides when applied under field conditions.

In vitro dual culture assays are a good way of initially distinguishing potential isolates that are antagonistic toward a pathogen; however, under natural conditions, the competitive ability and potential of an antagonistic strain to produce antimicrobial compounds may be altered. Therefore, *in plantae* experiments in the greenhouse and field are essential to assess the plant protection potential of isolates. To date, there have been few reports of greenhouse experiments or field trials to assess the effect of *Streptomyces* spp. on oil palm growth promotion and protection against *Ganoderma*. In our nursery experiment, we demonstrated that 6 months after applying the *S. nigrogriseolus* GanoSA1 powder formulation to the soil, the disease incidence of treated seedlings was approximately half that of the untreated seedlings. The rate of disease development in terms of DI, SFS and DS was significantly slower in seedlings inoculated with GanoSA1 powder (i.e., 48.89, 42.45 and 31.11%, respectively) compared with that observed for the untreated seedlings (i.e., 91.11, 83.11 and 75.00%, respectively). Furthermore, 6 months after treatment, the disease severity of seedlings treated with GanoSA1 powder was 63.01% lower than that of untreated seedlings ($P \leq 0.05$).

Recently, Sujarit et al. (2020) reported that prior to inoculation with *Ganoderma*, oil palm seedlings treated three times with a spore suspension of *Streptomyces palmae* CMU-AB204^T in sterile soil or encapsulated within alginate beads exhibited lower DS values (13.7%) than the *Streptomyces* sp. GanoSA1 isolate used in this study. However, the application of *Streptomyces hygroscopicus* subsp. *hygroscopicus* AGA347 in a vermiculite powder formulation resulted in a DS of 30.0%, which was similar to the level of control obtained by applying GanoSA1 in this study (Shariffah-Muzaimah et al., 2018). Other microorganisms that have also been reported to control BSR disease in oil palms include

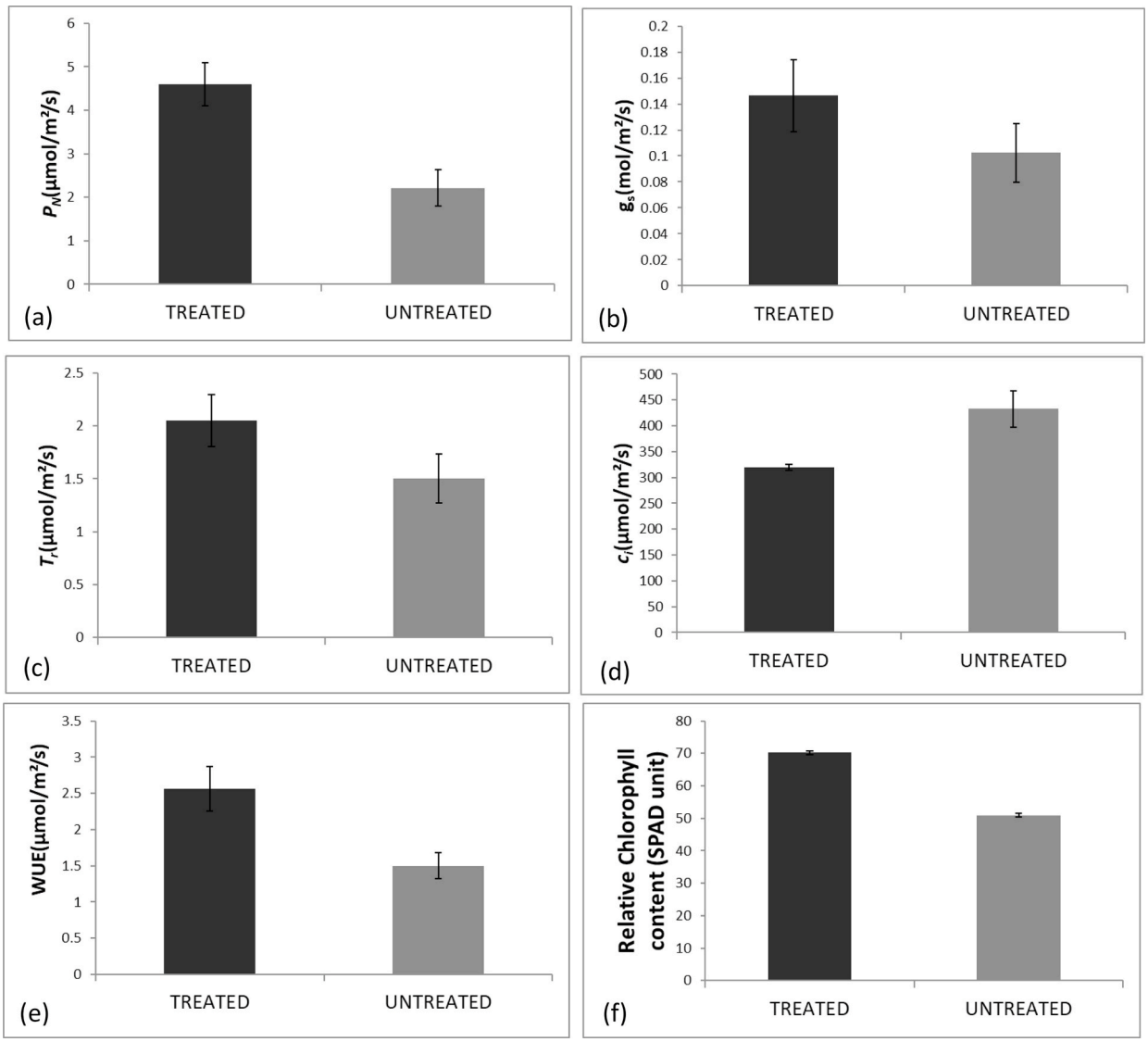

Fig. 5. Gas exchange parameters and chlorophyll content of seedlings treated with *Streptomyces nigrogriseolus* GanoSA1 compared with the untreated control at eight months after treatment. (a) Photosynthesis rate, P_N; (b) stomatal conductance, g_s; (c) transpiration rate, T_r; (d) intercellular CO_2 concentration, c_i; (e) water use efficiency, WUE; and (f) relative chlorophyll content. Vertical bars represent the standard errors of the means.

Trichoderma harzianum, AMF, *Pseudomonas syringae* and *Pseudomonas aeruginosa*. The application of *Trichoderma* T29-PFP to a surface mulch at six application times resulted in a DS of 46.67% (Sundram, 2013). Meanwhile, the application of AMF (*Glomus intraradices* UT126 and *Glomus clarum* BR152B) in combination with *P. aeruginosa* UPMP3 resulted in significantly lower DS (38.75%) than that of the untreated control seedlings (Sundram et al., 2015). These results suggest that the timing of the BCA application and the frequency of the applications may influence the effectiveness of the BCA. Sundram (2013) indicated that a single conidial application of *Trichoderma* in soil was not sufficient to significantly reduce BSR disease. Thus, repeated applications of the BCA were required. This may be related to the establishment of the BCA population in the soil, which will affect the competition for nutrients in the rhizosphere and interactions between the microbial community. Better knowledge of the plant microbiome and of the microbial interactions that occur under natural ecosystems should help to improve the effectiveness of BCAs. Based on the results of the present study, we suggest that two applications of the GanoSA1 isolate are required to control BSR disease in oil palm nurseries and plantations.

The GanoSA1 treatment not only influenced disease progression in oil palm seedlings but also significantly increased the vegetative parameters of oil palm seedlings, such as height, stem diameter, and the relative leaf chlorophyll content of treated seedlings compared with those of untreated control seedlings. The highest growth values were obtained 6 months after treatment (i.e., 116.27 cm (plant height), 13 (number of leaves), 58.73 cm (plant diameter) and 165.52 g (root mass) and 272.6 g (leaves mass)). Our results are in line with the findings of Nur Azura et al. (2016) who reported that oil palm seedlings inoculated with *Streptomyces sanglieri* strain AUM 00500 developed increased height, root length, number of secondary roots, wet weight and number of leaves. Similarly, other studies have also reported the promotion of plant height, plant dry weight and grain yields of various crop plants in response to inoculation with *Streptomyces* spp. (Liotti et al., 2019; Awla et al., 2017; Toumatia et al., 2016; Nur Azura et al., 2016; Laid et al., 2016; Goudjal et al., 2014; Shahbazi et al., 2014; Gopalakrishnan et al., 2013; Hastuti et al., 2012). Furthermore, Dias et al. (2017) reported that *Streptomyces* spp. produced siderophores and ACC deaminase, solubilized phosphate and produced volatile organic compounds that promoted tomato seedling growth. *Streptomyces griseocarneus* R132 has been shown to control the growth of phytopathogens and to promote the growth of pepper (*Capsicum annuum*) (Liotti et al., 2019). In general, improvements in plant growth may be triggered by the activities of the

BCA, either through direct growth-promoting activities and/or control of plant pests or pathogens, or in combination with (or without) plant-mediated control of plant pests and pathogens.

Based on our findings, the BSR incidence in oil palm seedlings was reduced when soil was treated with *S. nigrogriseolus* GanoSA1. These biocontrol activities may be related to several different modes of action, such as the production of secondary metabolites or volatile organic compounds, the secretion of hydrolytic enzymes or the production of siderophores. *Streptomyces* spp. have also been reported to trigger induced systemic resistance and the upregulation of defense-related genes in relation to phytopathogen infection (Ansari et al., 2020; Dias et al., 2017; Awla et al., 2017; Senthilraja, 2016). Thus, applying GanoSA1 to the soil when seedlings are transplanted at the nursery stage and to the planting hole when seedlings are planted out at the field stage may potentially protect oil palm seedlings from infection by *Ganoderma*. Further studies are needed to investigate the mechanisms of *Streptomyces* sp. GanoSA1 biocontrol action, and to evaluate the level of biocontrol activity in the field to determine whether biocontrol and growth promotion activities occur in oil palm under natural conditions.

Ethical approval

This article does not contain any studies with human participants or animal performed by any of the authors.

Declaration of interest

The authors declare no conflicts of interests.

CRediT authorship contribution statement

Syed Aripin Shariffah-Muzaimah: Conceptualization, Methodology, Writing - original draft, Investigation, Formal analysis, Writing - review & editing. **Abu Seman Idris:** Conceptualization, Methodology, Funding acquisition, Project administration. **Ramli Nur-Rashyeda:** Writing - review & editing, Validation, Formal analysis. **Yuvarani Naidu:** Software, Writing - review & editing. **Nur H. ZainolHilmi:** Software, Writing - review & editing. **Kamarudin Norman:** Funding acquisition, Project administration, Writing - review & editing.

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References

Amaresan, N., Kumar, K., Naik, J.H., Bapatla, K.G., Mishra, R.K., 2019. *Streptomyces* in plant growth promotion: mechanism and role. In: Gupta, V.G., Pandey, A. (Eds.), New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier B.V., pp. 125–135. https://doi.org/10.1016/b978-0-444-63994-3.00008-4

Ansari, W.A., Krishna, R., Zeyad, M.T., Singh, S., Yadav, A.K., 2020. Endophytic actinomycetes-mediated modulation of defense and systemic resistance confers host plant fitness under biotic stress conditions. In: Singh, R., Manchanda, G., Maurya, I., Wei, Y. (Eds.), Microbial Versatility in Varied Environments. Springer, Singapore, pp. 167–180. https://doi.org/10.1007/978-981-15-3028-9_10.

Anuar, E.N., Nulit, R., Idris, A.S., 2015. Growth promoting effects of endophytic fungus *Phlebia* GanoEF3 on oil palm (*Elaeis guineensis*) seedlings. Int. J. Agric. Biol. 17 (1), 135–141.

Assis, K., Chong, K.P., Idris, A.S., Ho, C.M., 2016. Economic loss due to *Ganoderma* disease in oil palm. World Acad. Sci. Eng. Technol. 18, 683–688.

Arif, M.S., Roslan, A., Idris, A.S., 2011. Proceedings of the Third MPOB-IOPRI International Seminar: Integrated Oil Palm Pests and Diseases Management. Kuala Lumpur: Convention Centre. Economics of Oil Palm Pests and *Ganoderma* Disease and Yield Losses.

Awla, H.K., Kadir, J., Othman, R., Rashid, T.S., Hamid, S., Wong, M.Y., 2017. Plant growth-promoting abilities and biocontrol efficacy of *Streptomyces* sp. UPMRS4 against *Pyricularia oryzae*. Biol. Contr. 112, 55–63. https://doi.org/10.1016/j.biocontrol.2017.05.011.

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., Clément, C., Ouhdouch, Y., van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol. Mol. Biol. Rev. 80, 1–43. https://doi.org/10.1128/MMBR.00019-15.

Binayke, A., Ghorbel, S., Hmidet, N., Raut, A., Gunjal, A., Uzgare, A., Patil, N., Waghmode, M., Nawani, N., 2018. Analysis of diversity of actinomycetes from arid and saline soils at Rajasthan, India. Environ. Sustain. 1 (1), 61–70.

Bivi, M.R., Farhana, M.S.N., Khairulmazmi, A., Idris, A., 2010. Control of *Ganoderma boninense*: a causal agent of basal stem rot disease in oil palm with endophyte bacteria *in vitro*. Int. J. Agric. Biol. 12 (6), 833–839.

Boukaew, S., Plubrukam, A., Prasertsan, P., 2013. Effect of volatile substances from *Streptomyces philanthi* RM-1-138 on growth of *Rhizoctonia solani* on rice leaf. BioControl 58, 471–482. https://doi.org/10.1007/s10526-013-9510-6.

Campbell, C.L., Madden, L.V., 1990. Introduction to Plant Disease Epidemiology. John Wiley and Sons, Inc, New York.

Charousova, I., Javorekova, S., Wink, J., 2015. Isolation and characterization of *Streptomyces rishiriensis* (vy31) with antibiotic activity against various pathogenic microorganisms. J. Microbiol. Biotechnol. Food Sci. 4, 23–28. https://doi.org/10.15414/jmbfs.2015.4.special1.23-27.

Cordovez, V., Carrion, V.J., Etalo, D.W., Mumm, R., Zhu, H., Van Wezel, G.P., Raaijmakers, J.M., 2015. Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. Front. Microbiol. 6, 1081.

Dennis, P.G., Kukulies, T., Forstner, C., Orton, T.G., Pattison, A.B., 2018. The effects of glyphosate, glufosinate, paraquat and paraquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity. Sci. Rep. 8 (1), 2119.

Dias, M.P., Bastos, M.S., Xavier, V.B., Cassel, E., Astarita, L.V., Santarém, E.R., 2017. Plant growth and resistance promoted by *Streptomyces* spp. in tomato. Plant Physiol. Biochem. 118, 479–493.

Ellaiah, P., Bapi Raju, K.V.V.S.N., Adinarayana, K., Adinarayana, G., Saisha, V., Madhavi, S., Premkumar, J., 2002. Bioactive actinomycetes from krishna river sediments of Andhra Pradesh. Hind. Antibiot. Bull. 44, 8–16.

Esnan, A.G., 2019. Bab 10: penyediaan kawasan dan penanaman sawit. In: Kushairi, A., Parveez, G.K.A., Norman, K. (Eds.), Perusahaan Sawit di Malaysia: Satu panduan. Jilid 1. Malaysian Palm Oil Board. Kuala Lumpur, Malaysia, pp. 121–132.

Getha, K., Vikineswary, S., 2002. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. cubense race 4: indirect evidence for the role of antibiosis in the antagonistic process. J. Ind. Microbiol. Biotechnol. 28 (6), 303–310. https://doi.org/10.1038/sj/jim/7000247.

Gopalakrishnan, S., Pande, S., Sharma, M., Humayun, P., Kiran, B.K., Sandeep, D., Vidya, M.S., Deepthi, K., Rupela, O., 2011. Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of Fusarium wilt of chickpea. Crop Protect. 30 (8), 1070–1078. https://doi.org/10.1016/j.cropro.2011.03.006.

Gopalakrishnan, S., Srinivas, V., Vidya, M.S., Rathore, A., 2013. Plant growth-promoting activities of *Streptomyces* spp. in sorghum and rice. SpringerPlus 2 (1), 574. https://doi.org/10.1186/2193-1801-2-574.

Gopalakrishnan, S., Srinivas, V., Alekhya, G., Prakash, B., Kudapa, H., Rathore, A., Varshney, R.K., 2015. The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum *Streptomyces* sp. in chickpea. SpringerPlus 4 (1), 31.

Goudjal, Y., Toumatia, O., Yekkour, A., Sabaou, N., Mathieu, F., Zitouni, A., 2014. Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. Microbiol. Res. 169 (1), 59–65.

Hastuti, R.D., Lestari, Y., Suwanto, A., Saraswati, R., 2012. Endophytic *Streptomyces* spp. as biocontrol agents of rice bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. oryzae). HAYATI J. Biosci. 19 (4), 155–162.

Hu, Q., Xu, J., 2011. A simple double-layered chrome azurol S agar (SD-CASA) plate assay to optimize the production of siderophores by a potential biocontrol agent *Bacillus*. Afr. J. Microbiol. Res. 5 (25), 4321–4327. https://doi.org/10.5897/AJMR11.238.

Idris, A.S., 2019. Integrated *Ganoderma* management in oil palm plantations. Planter 95, 255–262.

Jacob, S., Sajjalaguddam, R.R., Kumar, K.V.K., Varshney, R., Sudini, H.K., 2016. Assessing the prospects of *Streptomyces* sp. RP1A-12 in managing groundnut stem rot disease caused by *Sclerotium rolfsii* Sacc. J. Gen. Plant Pathol. 82 (2), 96–104.

Jadhav, H.P., Shaikh, S.S., Sayyed, R.Z., 2017. Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: an overview. In: Mehnaz, S. (Ed.), Rhizotrophs: Plant Growth Promotion to Bioremediation. Springer, Singapore, pp. 183–203. https://doi.org/10.1007/978-981-10-4862-3.

Janaki, T., 2017. Enzymes from actinomycetes–review. Int. J. Chem. Res. 10 (2), 176–182.

Jog, R., Nareshkumar, G., Rajkumar, S., 2016. Plant growth promoting actinobacteria: a new avenue for enhancing the productivity and soil fertility of grain legumes. In: Gopalakrishnan, S., Sathya, A., Vijayabharathi, R. (Eds.), Plant Growth Promoting Actinobacteria: A New Avenue for Enhancing the Productivity and Soil Fertility of Grain Legumes, pp. 1–298. https://doi.org/10.1007/978-981-10-0707-1.

Jones, S.E., Elliot, M.A., 2017. Streptomyces exploration: competition, volatile communication and new bacterial behaviours. Trends Microbiol. 25 (7), 522–531.

Kamarudin, A.N., Lai, K.S., Lamasudin, D.U., Idris, A.S., Balia Yusof, Z.N., 2017. Enhancement of thiamine biosynthesis in oil palm seedlings by colonization of endophytic fungus *Hendersonia toruloidea*. Front. Plant Sci. 8, 1799.

Kushairi, A., Loh, S.K., Azman, I., Hishamuddin, E., Ong-Abdullah, M., Izuddin, Z.B.M.N., Razmah, G., Sundram, S., Parveez, G.K.A., 2018. Oil palm economic performance in Malaysia and R&D progress in 2017. J. Oil Palm Res. 30 (2), 163–195.

Laid, B., Kamel, K., Mouloud, G., Manel, S., Walid, S., Amar, B., Hamenna, B., Faiçal, B., 2016. Effects of plant growth promoting rhizobacteria (PGPR) on *in vitro* bread wheat (*Triticum aestivum* L.) growth parameters and biological control mechanisms. Adv. Microbiol. 6, 677–690. https://doi.org/10.4236/aim.2016.69067.

Law, J.W.F., Ser, H.L., Khan, T.M., Chuah, L.H., Pusparajah, P., Chan, K.G., Goh, B.H., Lee, L.H., 2017. The potential of *Streptomyces* as biocontrol agents against the rice blast fungus, *Magnaporthe oryzae* (*Pyricularia oryzae*). Front. Microbiol. 8, 3.

Li, Q., Ning, P., Zheng, L., Huang, J., Li, G., Hsiang, T., 2010. Fumigant activity of volatiles of *Streptomyces globisporus* JK-1 against *Penicillium italicum* on *Citrus microcarpa*. Postharvest Biol. Technol. 58, 157–165. https://doi.org/10.1016/j.postharvbio.2010.06.003.

Li, Q., Chen, X., Jiang, Y., Jiang, C., 2016. Morphological Identification of Actinobacteria. Actinobacteria-Basics and Biotechnological Applications, vol. 11. InTech, Rijeka, Croatia, pp. 59–86. Feb.

Lim, P., Gansau, J.A., Chong, K.P., 2018. *Streptomyces* spp. a potential biocontrol agent against *Ganoderma boninense* of basal stem rot. J. Oil Palm Res. 30 (2), 265–275.

Liotti, R.G., da Silva Figueiredo, M.I., Soares, M.A., 2019. *Streptomyces griseocarneus* R132 controls phytopathogens and promotes growth of pepper (*Capsicum annuum*). Biol. Contr. 138, 104065. https://doi.org/10.1016/j.biocontrol.2019.104065.

Liu, M., Liu, X., Cheng, B.S., Ma, X.L., Lyu, X.T., Zhao, X.F., Ju, Y.L., Min, Z., Zhang, Z.W., Fang, Y.L., 2016. Selection and evaluation of phosphate-solubilizing bacteria from grapevine rhizospheres for use as biofertilizers. Span. J. Agric. Res. 14 (4), 26.

Mun, B.G., Lee, W.H., Kang, S.M., Lee, S.U., Lee, S.M., Lee, D.Y., Shahid, M., Yun, B.W., Lee, I.J., 2020. *Streptomyces* sp. LH 4 promotes plant growth and resistance against *Sclerotinia sclerotiorum* in cucumber via modulation of enzymatic and defense pathways. Plant Soil 448, 87–103. https://doi.org/10.1007/s11104-019-04411-4.

Musa, H., 2017. Efficacy of *Trichoderma spp.* Consortium as Potential Biocontrol Agents of *Ganoderma* Disease of Oil Palm, and Involvement of Antioxidant Enzymes and Total Phenolic Content, Doctoral Dissertation. School of Graduate Studies, Universiti Putra Malaysia, p. 119.

Naidu, Y., Idris, A.S., Madihah, A.Z., Kamarudin, N., 2016. *In vitro* antagonistic interactions between endophytic basidiomycetes of oil palm (*Elaeis guineensis*) and *Ganoderma boninense*. J. Phytopathol. 164 (10), 779–790.

Newitt, J.T., Prudence, S.M., Hutchings, M.I., Worsley, S.F., 2019. Biocontrol of cereal crop diseases using streptomycetes. Pathogens 8 (2), 78.

Nur Ain Izzati, M.Z., Abdullah, F., 2008. Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. Plant Protect. Sci. 44, 101–107.

Nur Azura, A.B., Yusoff, M., Tan, G.Y.A., Jegadeesh, R., Appleton, D.R., Vikineswary, S., 2016. *Streptomyces sanglieri* which colonised and enhanced the growth of *Elaeis guineensis* Jacq. seedlings was antagonistic to *Ganoderma boninense* in *in vitro* studies. J. Ind. Microbiol. Biotechnol. 43 (4), 485–493.

Nurrashyeda, R., Idris, A.S., Maizatul, S.M., Madihah, A.Z., Kushairi, A., 2012a. *Phlebia* GanoEF3 powder as biological control of *Ganoderma* in oil palm. MPOB TT Inf. Ser. 510.

Nurrashyeda, R., Idris, A.S., Maizatul, S.M., Madihah, A.Z., Kushairi, A., 2012b. *Amphinema* GanoEF2 powder as biological control of *Ganoderma* disease in oil palm. MPOB TT Inf. Ser. 509.

Nurrashyeda, R., Semanb, I.A., Zairunc, M.A., Mohamedd, M.S., Sebrane, N.H., Itam, O.J. A., Bangi, K.I., 2018. Biocontrol of basal stem rot (BSR) disease of oil palm using endophytic fungus, *Hendersonia* sp. Int. J. Pure Appl. Math. 118 (24).

Parveez, G.K.A., Hishamuddin, E., Loh, S.K., Ong-Abdullah, M., Salleh, K.M., Izuddin, Z. B.M.N., Sundram, S., Hasan, Z.A.A., Idris, Z., 2020. Oil palm economic performance in Malaysia and R&D progress in 2019. J. Oil Palm Res. 30 (2), 163–195.

Pithakkit, S., Petcharat, V., Chuenchit, S., Pornsuriya, C., Sunpapao, A., 2015. Isolation of antagonistic actinomycetes species from rhizosphere as effective biocontrol against oil palm fungal diseases. Walailak J. Sci. Technol. 12 (5), 481–490.

Prasad, P., Singh, T., Bedi, S., Kumari, S., 2013. Molecular characterization and phylogenetic analysis of cellulase producing *Streptomyces griseorubens* (Strain St-1) isolated from Indian soil. Jordan J. Biol. Sci. 6, 239–242. https://doi.org/10.12816/0001541.

Queendy, V., Roza, R.M., 2019. Aktivitas antifungi isolat aktinomisetes arboretum Universitas Riau terhadap jamur *Fusarium oxysporum* f. Sp *lycopersici* dan *Ganoderma boninense*. Al-Kauniyah: J. Biol. 12 (1), 73–88.

Ramli, N.R., Mohamed, M.S., Seman, I.A., Zairun, M.A., Mohamad, M., 2016. The potential of endophytic bacteria as a biological control agent for *Ganoderma* disease in oil palm (Potensi Bakteria Endofit sebagai Agen Kawalan Biologi untuk Penyakit *Ganoderma* pada Pokok Sawit). Sains Malays. 45, 401–409.

Ratcliff, A.W., Busse, M.D., Shestak, C.J., 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Appl. Soil Ecol. 34, 114–124. https://doi.org/10.1016/j.apsoil.2006.03.002.

Sadeghi, A., Karimi, E., Dahaji, P.A., Javid, M.G., Dalvand, Y., Askari, H., 2012. Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. World J. Microbiol. Biotechnol. 28, 1503–1509. https://doi.org/10.1007/s11274-011-0952-7.

Sahilah, A.M., Tang, S.Y., Zaimawati, M.N., Rosnah, H., Uni Kalsum, M.S., Son, R., 2010. Identification and characterization of actinomycetes for biological control of bacterial wilt of *Ralstonia solanacearum* isolated from tomato. J. Trop. Agric. Food Sci. 38, 103–114.

Šantrić, L., Radivojević, L., Gajić-Umiljendić, J., Sarić-Krsmanović, M., Đurović-Pejčev, R., 2016. Effects of herbicides on growth and number of actinomycetes in soil and *in vitro*. Pestic. Fitomedicina 31 (3–4), 121–128.

Sapak, Z., Sariah, M., Ahmad, Z.A., 2008. Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. Int. J. Agric. Biol. 10, 127–132.

Senthilraja, G., 2016. Induction of systemic resistance in crop plants against plant pathogens by plant growth-promoting actinomycetes. In: Subramaniam, G., Arumugam, S., Rajendran, V. (Eds.), Plant Growth Promoting Actinobacteria. Springer, Singapore, pp. 193–202.

Shahbazi, P., Musa, M.Y., Tan, G.Y.A., Avin, F.A., Teo, W.F.A., Vikineswary, S., 2014. *In vitro* and *in vivo* evaluation of *Streptomyces* suppressions against Anthracnose in chili caused by *Colletotrichum*. Sains Malays. 43, 697–705.

Shariffah-Muzaimah, S., Idris, A., Madihah, A., Kushairi, A., 2012. Streptomyces GanoSA1 powder as biological control of *Ganoderma* in oil palm. MPOB Inf. Series. 506, 593.

Shariffah-Muzaimah, S.A., Idris, A.S., Madihah, A.Z., Dzolkhifli, O., Kamaruzzaman, S., Cheong, P.C.H., 2015. Isolation of actinomycetes from rhizosphere of oil palm (*Elaeisguineensis* Jacq.) for antagonism against *Ganoderma boninense*. J. Oil Palm Res. 27, 19–29.

Shariffah-Muzaimah, S.A., Idris, A.S., Madihah, A.Z., Dzolkhifli, O., Kamaruzzaman, S., Maizatul-Suriza, M., 2018. Characterization of *Streptomyces* spp. isolated from the rhizosphere of oil palm and evaluation of their ability to suppress basal stem rot disease in oil palm seedlings when applied as powder formulations in a glasshouse trial. World J. Microbiol. Biotechnol. 34 (1), 15. https://doi.org/10.1007/s11274-017-2396-1.

Shen, T., Wang, C., Yang, H., Deng, Z., Wang, S., Shen, B., Shen, Q., 2016. Identification, solid-state fermentation and biocontrol effects of *Streptomyces hygroscopicus* B04 on strawberry root rot. Appl. Soil Ecol. 103, 36–43. https://doi.org/10.1016/j.apsoil.2016.02.016.

Shirling, E.B., Gottlieb, D., 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16, 313–340.

Siddiquee, S., Yusuf, U.K., Hossain, K., Jahan, S., 2009. *In vitro* studies on the potential *Trichoderma harzianum* for antagonistic properties against *Ganoderma boninense*. J. Food Agric. Environ. 7 (3&4), 970–976.

Singh, P.P., Shin, Y.C., Park, C.S., Chung, Y.R., 1999. Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology 89, 92–99. https://doi.org/10.1094/PHYTO.1999.89.1.92.

Sujarit, K., Pathom-aree, W., Mori, M., Dobashi, K., Shiomi, K., Lumyong, S., 2020. *Streptomyces palmae* CMU-AB204^{T}, an antifungal producing-actinomycete, as a potential biocontrol agent to protect palm oil producing trees from basal stem rot disease fungus, *Ganoderma boninense*. Biol. Contr. 148, 104307.

Sundram, S., 2013. The effects of *Trichoderma* in surface mulches supplemented with conidial drenches in the disease development of *Ganoderma* basal stem rot in oil palm. J. Oil Palm Res. 25, 314–325.

Sundram, S., Abdullah, F., Ahmad, Z.A.M., Yusuf, U.K., 2008. Efficacy of single and mixed treatments of *Trichoderma harzianum* as biocontrol agents of *Ganoderma* basal stem rot in oil palm. J. Oil Palm Res. 20, 470–483.

Sundram, S., Palm, M., Board, O., Meon, S., Putra, U., Othman, R., Putra, U., 2015. Application of arbuscular mycorrhizal fungi with *Pseudomonas aeruginosa* UPMP3 reduces the development of *Ganoderma* basal stem rot disease in oil palm seedlings. Mycorrhiza 25, 387–397. https://doi.org/10.1007/s00572-014-0620-5.

Suryanto, D., Wibowo, R.H., Siregar, E.B.M., Munir, E., 2012. A possibility of chitinolytic bacteria utilization to control basal stems disease caused by *Ganoderma boninense* in oil palm seedling. Afr. J. Microbiol. Res. 6 (9), 2053–2059.

Tamura, K., Paterson, D., Paterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA 6: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony method. Mol. Biol. Evol. 28, 2731–2739. https://doi.org/10.1093/molbev/msr121.

Tang-Um, J., Niamsup, H., 2012. Chitinase production and antifungal potential of endophytic *Streptomyces* strain P4. Maejo Int. J. Sci. Technol. 6, 95–104.

Ting, A.S.Y., Jioe, E., 2016. *In vitro* assessment of antifungal activities of antagonistic fungi towards pathogenic *Ganoderma boninense* under metal stress. Biol. Contr. 96, 57–63.

Toumatia, O., Compant, S., Yekkour, A., Goudjal, Y., Sabaou, N., Mathieu, F., Sessitsch, A., Zitouni, A., 2016. Biocontrol and plant growth promoting properties of *Streptomyces mutabilis* strain IA1 isolated from a Saharan soil on wheat seedlings and visualization of its niches of colonization. South Afr. J. Bot. 105, 234–239.

Vurukonda, S.S.K.P., Giovanardi, D., Stefani, E., 2018. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. Int. J. Mol. Sci. 19 (4), 952.

Wang, Z., Wang, C., Li, F., Li, Z., Chen, M., Wang, Y., Qiao, X., Zhang, H., 2013. Fumigant activity of volatiles from *Streptomyces alboflavus* TD-1 against *Fusarium moniliforme* Sheldon. J. Microbiol. 51, 477–483. https://doi.org/10.1007/s12275-013-2586-y.

Wonglom, P., Suwannarach, N., Lumyong, S., Ito, S.I., Matsui, K., Sunpapao, A., 2019. *Streptomyces angustmyceticus* NR8-2 as a potential microorganism for the biological control of leaf spots of *Brassica rapa* subsp. *pekinensis* caused by *Colletotrichum* sp. *and Curvularia lunata*. Biol. Contr. 138, 104046.

Zain, N.M.M., Mohamed, R.B., Sijam, K., Morshed, M.M., Awag, Y., 2013. Effect of selected herbicides *in vitro* and in soil on growth and development of soil fungi from oil palm plantation. Int. J. Agric. Biol. 15, 820–826.

Zeng, J., Xu, T., Cao, L., Tong, C., Zhang, X., Luo, D., Han, S., Pang, P., Fu, W., Yan, J., Liu, X., 2018. The role of iron competition in the antagonistic action of the rice endophyte *Streptomyces sporocinereus* OsiSh-2 against the pathogen *Magnaporthe oryzae*. Microb. Ecol. 76 (4), 1021–1029.

Zenova, G.M., Manucharova, N.A., Zvyagintsev, D.G., 2011. Extremophilic and extremotolerant actinomycetes in different soil types. Soil Sci. 44 (4), 417–436.

Zhu, H.H., Guo, J., Yao, Q., Yang, S.Z., Deng, M.R., Phuong, L.T.B., Hanh, V.T., Ryan, M. J., 2007. *Streptomyces vietnamensis* sp. nov., a streptomycete with violet-blue diffusible pigment isolated from soil in Vietnam. Int. J. Syst. Evol. Microbiol. 57, 1770–1774. https://doi.org/10.1099/ijs.0.64774-0.