**ORIGINAL ARTICLE** 

## **Streptomyces** sp. mitigates abiotic stress response and promotes plant growth

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#### **Abstract**

Coexisting microorganisms are abundant in nature. Plant growth promoting rhizobacteria (PGPR) is a group of beneficial microorganism living around the roots of plants which are able to confer beneficial effects on plant growth. Streptomyces sp. is a gram-positive bacteria as PGPR that can promote plant growth and enhance tolerance in adverse environment. This research was aimed to study the effects of plant growth promotion and stress tolerance of Streptomyces sp. in Arabidopsis and Brassica sp. The amount of indole-acetic acid (IAA) and phosphate solubility were assessed from isolated bacterial. Plant growth promotion was examined in 10-days old seedling with three independent experiments. Our results showed that Streptomyces sp. produced moderate levels of IAA and it was able to solubilize phosphate. Inoculation of Streptomyces sp. enhanced lateral root number, fresh weight and chlorophyll content in Arabidopsis thaliana. Moreover, the inoculation of Streptomyces sp. significantly increased vegetative growth on Arabidopsis and Brassica sp. by producing higher fresh weight and chlorophyll content. Streptomyces sp. also enhanced tolerance to abiotic stress in Arabidopsis and Brassica sp. by increasing fresh weight under condition of salt and heat stress. Under salt stress, inoculation of Streptomyces sp. in Arabidopsis induced activity of catalase enzyme and decreased hydrogen peroxide (H,O<sub>3</sub>) and malondialdehyde (MDA) production. In the molecular levels, Streptomyces sp. induced protein accumulations in Arabidopsis including nitrogen assimilation (GS1), carbohydrate metabolism (cFBPase), and the light-harvesting chlorophyll (Lhcb1) protein.

**Keywords:** indole-3-acetic acid (IAA), catalase enzyme, solubilize phosphate, malondial-dehyde (MDA) production, *Streptomyces* sp.

#### Introduction

Agriculture has strongly felt the effects of climate change occurring throughout the world. Climate change threatens food security throughout the developing countries (Mosttafiz et al. 2012). Global climate change accelerates the concurrence of a variety of abiotic (e.g., drought, salinity, heavy metals, and extreme temperatures) and biotic stresses (e.g., phytopathogens). The dependence of agriculture on chemical fertilizers and pesticides has also resulted in adverse impacts on the environment (Ma et al. 2019). Nowadays, plant growth promoting microorganisms (PGPM) are receiving increasing attention by agronomists and environmentalists as candidates to develop an effective,

eco-friendly, and sustainable alternative to conventional agricultural (e.g., chemical fertilizers and pesticides) and remediation (e.g., chelate-enhanced phytoremediation) methods employed to deal with these climate-change-induced stresses (Ma et al. 2011; Ma et al. 2016). Using microorganisms as biocontrol and organic fertilizers has become an alternative way to reduce the adverse effects of chemicals used in agriculture (Bhardwaj et al. 2014).

Plant growth promoting rhizobacteria (PGPR) is a group of beneficial microorganisms living around the roots of plants which are able to confer beneficial effects on plant growth without causing damage to the



host plant (Ahemad and Kibret 2014). Streptomyces sp. is a gram-positive bacteria that is known to have a high ability of producing numerous antimicrobial compounds for functioning as a biocontrol agent (Evangelista and Martínez 2013). Besides being a biocontrol agent, Streptomyces sp. is also considered to have great potential to promote plant growth (Olanrewaju and Babalola 2019). It has been found that Streptomyces sp. has the ability to produce indole acetic-acid (IAA) phytohormone, solubilize phosphates and fix nitrogen (Gopalakrishnan et al. 2013). Streptomyces sp. PM9 has been studied for its ability to promote root number and root length in Eucalyptus grandis and E. globules by its high production of IAA (Salla et al. 2014). Inoculation of Streptomyces sp. to wheat (Triticum aestivum) increased plant growth by increasing phosphate solubilization ability and indole-3-acetic acid production. *Streptomyces* sp. also enhanced plant defense in wheat through the production of siderophore and chitinase to inhibit Penicillium sp. pathogen (Jog et al. 2014). Gopalakrishnan et al. (2013) also reported that inoculation of Streptomyces sp. significantly enhanced grain yield in sorghum by enhanced nitrogen source and availability of P in rhizosphere soil. In addition, Srivastava (2015) has shown that Streptomyces sp. has a capacity to protect chickpea (Cicer arietinum L.) plant from Sclerotinia sclerotiorum pathogen infection by increasing accumulation of antioxidant compounds such as phenolic compounds and antioxidant enzymes like catalase. Hence, this bacterial isolate was selected for further study especially in abiotic stress response.

#### **Materials and Methods**

#### **Bacterial isolation**

The studied bacterial strain was isolated by Dr. K.J. Duan from Tatung University. To perform strain identification, the bacterial strain was cultured in the solid medium of YCED (Casamino Acids Yeast Extract Glucose Agar) containing yeast extract 0.3 g, casamino acids 0.3 g, D-glucose 0.3 g, agar 20 g in 1,000 ml (de Vasconcellos *et al.* 2010). A single colony of the isolated bacterium was cultured in a liquid culture of the R2YE medium containing sucrose 103 g, K<sub>2</sub>SO<sub>4</sub> 0.25 g, MgCl<sub>2</sub>.6H<sub>2</sub>O 10.12 g, glucose 10 g, casamino acids 0.1 g, yeast extract 5 g in 1,000 ml (Shepherd *et al.* 2010). The bacterial strains were grown for 1 week at 28°C, and subsequently used for genomic DNA extraction.

#### **Genomic DNA extraction**

Genomic DNA extraction was prepared from a week old bacterial culture following the procedure of Wilson (2001). Genomic DNA fragments were obtained by polymerase chain reaction (PCR) using primers fD1 and rD1. The fD1 forward sequence was AGAGTTGAT CCTGGCTCA, and rD1 reverse sequence was AAGAG GTGATCCAGCC. The PCR fragments were sequenced in both the sense and antisense directions using a 3730 DNA Analyzer (Applied Biosystems®; thermofisher.com). The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST)program (Altschul *et al.* 1990). A phylogenetic tree was constructed using the 16 s rDNA sequences of isolated bacterial strains by the ClustalX method (Larkin *et al.* 2007).

### Quantitative analysis of IAA production and phosphate solubility

The production of IAA from the bacterial isolate was tested with the colorimetric method described by Patten and Glick (2002). The bacterial isolate was inoculated in a test flask with 10 ml of R2YE culture medium containing one single colony of Streptomyces sp. with a supplement of 2 mg · ml<sup>-1</sup> L-tryptophan at room temperature for a week. Bacterial culture was centrifuged for 15 min at 11,000 rpm. Two milliliters of the supernatant was mixed with 2 ml of Salkowski's reagent containing 36% (v/v) H<sub>2</sub>SO<sub>4</sub> and FeCl<sub>3</sub>.6H<sub>2</sub>O 0.5 M, and incubated at room temperature for 30 min. The presence of IAA was determined by the development of a pink color and the IAA concentration was measured using a spectrophotometer at 530 nm and quantified according to an IAA standard curve. Phosphate solubilization bioassay was described by Tandon et al. (1968). Pikovskaya's (PVK) medium contained glucose  $10 \, \text{g} \cdot l^{-1}$ , ammonium sulphate  $0.5 \, \text{g} \cdot l^{-1}$ , sodium chloride  $0.2 \text{ g} \cdot l^{-1}$ , KCl  $0.3 \text{ g} \cdot l^{-1}$ , FeSO<sub>4</sub>.7H<sub>2</sub>O  $0.03 \text{ g} \cdot l^{-1}$ ,  $MnSO_4.4H_2O$  0.3 g · l<sup>-1</sup>,  $Ca(PO_4)_2$  5 g · l<sup>-1</sup>, yeast extract  $0.4 \text{ g} \cdot l^{-1}$ , and agar 15 g ·  $l^{-1}$ , with pH 7.0 was prepared. Streptomyces sp.was streaked on the middle surface of PVK medium and phosphate solubilizing activity was estimated after 1 to 2 weeks of incubation at room temperature for 60 min. The absorbance of the solution was determined at a wavelength of 420 nm. The phosphate concentration in the supernatant was calculated according to a standard curve created from known concentrations of KH<sub>2</sub>PO<sub>4</sub>.

### Analysis of the growth promotion effect of *Streptomyces* sp. on *Arabidopsis* seedlings

To investigate plants' responsiveness to growth-promoting effects of *Streptomyces* sp., 4-day-old *Arabidopsis thaliana* L. ecotype Columbia (Col-0) seedlings, grown in 1/2 Murashige and Skoog (MS) medium, were transferred to a new medium with one loop of bacterial inoculum and positioned approximately 2 cm below the root tips of seedlings. Petri dishes were placed vertically in a growth chamber at 23°C with a 16 h/8 h light/dark photoperiod. Ten-day-old



seedlings were analyzed for the growth-promoting effect of *Streptomyces* sp. Thirty plants were included in each treatment. The data are presented as the mean ± SE from three independent experiments.

# Analysis of the growth promotion effect of *Streptomyces* sp. and commercial biofertilizer in *Arabidopsis* and *Brassica* sp.

Two week-old *Arabidopsis* and 1-week-old *Brassica chinensis* L. seedlings in non-sterilized potting soil with fertilizer were preprared before treatment. An inoculant of 30 ml *Streptomyces* sp. with a density of  $1 \times 10^8$  colony forming units CFU·ml<sup>-1</sup> was utilized to treat *Arabidopsis* once a week for 3 consecutive weeks. A fertilizer (HYPONEX No. 2) with N-P-K ratio of 20-20-20 was foliar applied to both control and treated plants twice a week. Eight plants were included in each treatment. The data are presented as the mean  $\pm$  SE. from three independent experiments. Four-week-old *Arabidopsis* and *Brassica* sp. plants were analyzed for shoot growth and fresh weight.

### Analysis of the growth promotion effect of *Streptomyces* sp. under abiotic stress

To analyze salt stress, 2-week-old Arabidopsis and 1-week-old *Brassica* sp. seedlings in non-sterilized potting soil were preprared before treatment. Salt stress was treated by 125 mM NaCl for Arabidopsis, and 150 mM NaCl for *Brassica* sp. For the heat treatment, 1-week--old *Arabidopsis* were treated at 40°C for 7 h for 4 days. Each day, after 7 h heat treatment, they were moved to a growth chamber with a long photoperiod (16 h light and 8 h dark) at 23°C. After 4 days of heat treatment, the plants continued to be grown in a growth chamber with a long photoperiod (16 h light and 8 h dark) at 23°C. For both abiotic stress treatments, an inoculant of 30 ml Streptomyces sp., with a bacterial population of 10<sup>8</sup> · ml<sup>-1</sup> was applied twice a week to the plants. For the mock treatment, the same volume of water was applied to Arabidopsis seedlings. One day after the treatments, the water supply was withheld for 7 days until wilted seedlings appeared. At the end of the water withholding period, seedlings without wilted leaves were scored as survival plants. These water-restrained seedlings were re-watered, and the recovery fresh weight was measured 7 days after the water supply was renewed.

#### Determination of total chlorophyll content, hydrogen peroxide, lipid peroxidation level and antioxidant enzyme activity

To analyze chlorophyll content, leaf tissue of *Arabidopsis* mature plants (0.5 g) was homogenized in 20 ml chilled ETOH (95%) and stirred for 30 min.

The homogenate was centrifuged at 3,000 g for 5 min. The absorbance of the supernatant was recorded at 664 nm and 648 nm. Chlorophyll content (mg · ml<sup>-1</sup>) was estimated by the following formula 5.24 × OD664 + + 22.24  $\times$  OD648. The procedures were described by Lichtenthaler (1987). To quantify H<sub>2</sub>O<sub>2</sub> concentration, ferrous ion oxidation xylenol orange (FOX) solution was prepared following the procedures described by Delong et al. (2002). Fresh tissue (0.1 g) from mature soil-grown Arabidopsis was ground in 1 ml 80% ethanol, then centrifuged at 3,000 g for 10 min. One hundred microliters supernatant was added to FOX reagent and incubated at room temperature for 30 min in the dark. The absorbance of the solution at 560 nm was measured. The hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentration was calculated based on a standard curve generated from known concentrations of H<sub>2</sub>O<sub>2</sub>. To analyze the activity of catalase, 0.2 g of Arabidopsis leaf tissue was ground in liquid nitrogen and then added to 0.2 M potassium phosphate buffer (pH 7.8 with 0.1 mM EDTA). The supernatant of this extraction was used for the catalase analysis based on a previous publication (Aebi 1984) in which 0.1 ml of supernatant was added to 3 ml of analysis solution (0.1 mM EDTA, 0.1% H<sub>2</sub>O<sub>2</sub>, 100 mM potassium phosphate buffer pH 7.0). The catalase activity was expressed as micromole of H<sub>2</sub>O<sub>2</sub> decomposed within 1 min with 1 g of fresh weight tissue. The lipid peroxidation level was determined in terms of malondialdehyde (MDA) content. Leaf tissue (0.1 g) from mature soil-grown Arabidopsis was ground in liquid nitrogen. One milliliter of 0.1% trichloroacitic acid (TCA) was added to precipitate protein. One milliliter of supernatant was taken for analysis. Two and a half milliliter of 0.5% TBA (thiobarbituric Q) in 20% TCA (3 g TCA + 0.075 g TBA + + 15 ml ddH,O) and the supernatant were added. The mixture was heated in a 95°C waterbath for 15 min and cooled immediately on ice to stop the reaction. The supernatant was taken and the absorbance at OD 532 nm and 600 nm was measured. Molandialdehyde concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm using an absorbance coefficient (155 mM  $\cdot$  cm<sup>-1</sup>).

#### Protein extraction and western blot analysis

Total proteins extracted from mature plants were used as controls and those treated with *Streptomyces* sp. were prepared by homogenizing leaf tissues in ice cold buffer (50 mM Tris-Cl, 150 mM NaCl, 0.5% NP-40, 1 mM phenylmethanesulfonylfluoride (PMSF), 1 µl protease inhibitor cocktail (Sigma-Aldrich). Fifteen micrograms of total protein were loaded onto 10% SDS-PAGE gels. Afterwards, the membrane was incubated with the polyclonal antibodies including glutamine synthetase (GS-1), cytosolic fructose-1.6-



-bisphosphatase (cFBPase). All antibodies were purchased from Agrisera (Vännäs, Sweden). The hybridization images were captured with Chemi-Smart 5000 (VilberLourmat, France).

#### Statistical analyses

The data were statistically analyzed with the Statistical Analysis System (SAS version 9.2) using an ANOVA and Tukey's test in which a *p*-value less than 0.05 indicated a significant difference. Each measurement was repeated at least three times independently.

#### Results

### Strain identification and characterization of the isolated bacterium

The identification of bacterial strains was verified using the 16S rDNA sequence. A phylogenetic tree was constructed using the ClustalX method (Larkin *et al.* 2007) which confirmed that the bacterial strain was *Streptomyces* sp. (Fig. 1A). *Streptomyces* sp. is known to have great potential for promoting plant growth and as a biocontrol agent (Evangelista and Martínez 2013; Olanrewaju and Babalola 2019). Hence, this bacterial isolate was selected for further study especially for its abiotic stress response. In this study, we found that *Streptomyces* sp. induced catalase antioxidant enzyme production and was able to secrete large

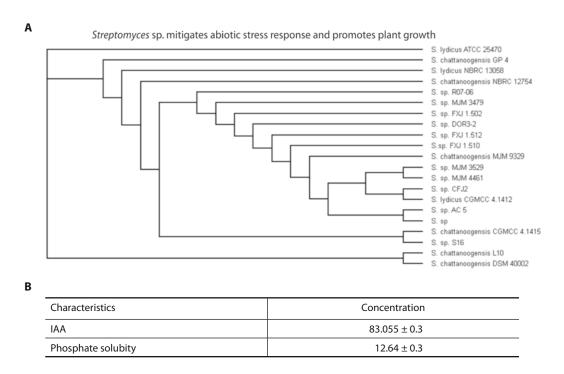
amounts of IAA 83.05  $\mu$ g · ml<sup>-1</sup> as well as solubilize phosphate 12.64  $\mu$ g · ml<sup>-1</sup> (Fig. 1B). At the molecular level we found that *Streptomyces* sp. changed the expression of GS1, cFBPase, and Lhcb1 genes which contributed to increased plant growth in *Arabidopsis* and *Brassica* sp.

### Analysis of the growth promotion effect of *Streptomyces* sp. on *Arabidopsis* seedlings

The bacterial isolates were co-cultured with the 4-day-old seedlings that had been grown in 1/2 Murashige and Skoog (MS) medium and transferred to a new medium with one loop bacterial inoculum positioned approximately 2 cm below the root tips of seedlings for 10-day-old seedlings. The *Streptomyces* sp. inoculation altered root architecture of *Arabidopsis* seedlings by significantly increased lateral root numbers of *Arabidopsis*. Moreover, the 3-week-old soil-grown *Arabidopsis* treated with *Streptomyces* sp. exhibited increases in fresh weight and chlorophyll contents (Figs. 2A and B).

## Growth promotion effect of *Streptomyces* sp. and commercial biofertilizer on *Arabidopsis* and *Brassica* sp.

This study found that *Streptomyces* sp. stimulated the development of lateral roots in *Arabidopsis* (Fig. 2A), which in turn caused an increase in the fresh weight in *Arabidopsis* and *Brassica* sp. (Figs. 3 and 5). To test whether isolated bacterial strains and biofertilizer can



**Fig. 1.** Characterization of *Streptomyces* sp. (A) The 16S rRNA sequences from various *Streptomyces* strains were used for construction of a phylogenetic tree using the ClustalX method. (B) Quantification of indole-acetic acid (IAA) and solubilizing phosphate produced by the activities of *Streptomyces* sp.

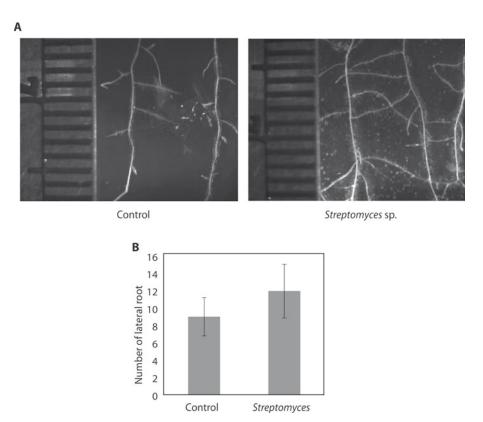


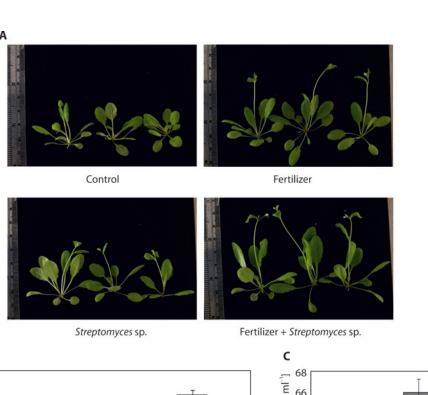
Fig. 2. Streptomyces sp. affected plant growth. Four-day-old Arabidopsis seedlings grown in 1/2 Murashige and Skoog (MS) medium and transferred to a new medium with one loop bacterial inoculum positioned approximately 2 cm below root tips of seedlings and grown for an additional 6 days. The 10-day-old seedlings exhibited changes in root architecture (A) and increased lateral root hair number (B)

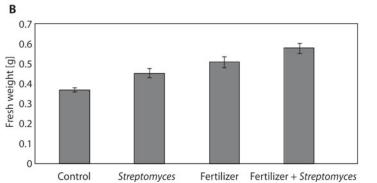
promote plant growth, Arabidopsis was treated with Streptomyces sp. and HYPONEX No. 2 biofertilizer. Fresh weight and chlorophyll content were investigated and showed significant increases compared to the control (Figs. 3B and C). At a molecular level Streptomyces sp. induced protein accumulation including nitrogen assimilation such as GS1, carbohydrate metabolism such as cFBPase, and the light-harvesting chlorophyll such as Lhcb1 protein (Fig. 4). Besides treating Arabidopsis, the application of Streptomyces sp. was tested in Brassica sp. as well. In the field there was increased vegetative growth of this vegetable after 3 weeks of treatment (Fig. 5A). Streptomyces sp. increased Brassica sp. fresh weight compared to the control (Fig. 5B). Furthermore, Streptomyces sp. and biofertilizer treatment can increase plant production compared to fertilizer only. Isolated bacterial strains and biofertilizer produced the highest fresh weight of Brassica sp.

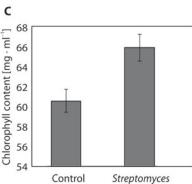
#### Streptomyces sp. increased tolerance to salt stress and heat stress

Under conditions of abiotic stress, salt stress treatment (125 mM NaCl) in Arabidopsis led to a high production of reactive oxygen species, H2O2 and MDA as a lipid peroxidation. Hydrogen peroxide and lipid peroxidation concentration under salt treatment were significantly

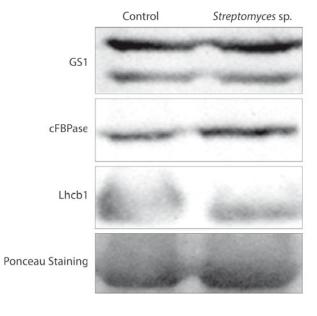
higher than those under other treatments (Fig. 6). However, as shown in Figures 6C and D hydrogen peroxide and lipid peroxidation concentration were significantly reduced as a result of inoculation of *Streptomyces* sp. Catalase enzyme may act as a scavenger of ROS for mitigating the injury in plants (Fig. 6E). Streptomyces sp. promoted salt stress tolerance in Brassica sp. as well. The effect of salt stress on Chinese cabbage has been observed. Salt stress treatment with 150 mM NaCl produced small shoot growth in Brassica sp. (Fig. 7). However, treatment with bacteria Streptomyces sp. improved the survival of this plant, demonstrating a protective effect of these bacteria. Recovery to a healthier phenotype and continued growth was poor in untreated plants while bacteria treated plants showed a good recovery after salt treatment. The results showed that inoculation of Streptomyces sp. had higher fresh weight than the control treatment, as seen by greater growth of shoots (Figs. 7A and B). The identification of a Streptomyces sp. effect on improving heat stress tolerance can be seen in Figure 8A. Under heat stress conditions, Arabidopsis shoot growth was smaller and more yellow after exposure to a temperature of 40°C for 7 h for 4 days. The ability of *Streptomyces* sp. to enhance plant tolerance toward heat stress was investigated. Under heat stress conditions, fresh weight and chlorophyll content of Arabidopsis was measured.





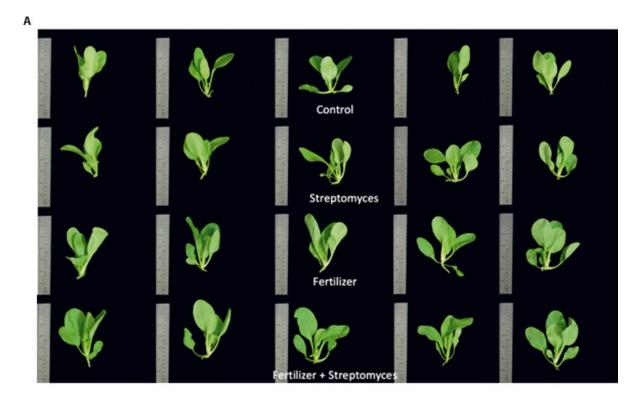


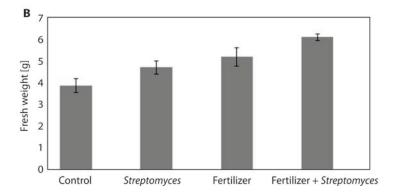
**Fig. 3.** Streptomyces sp. affected Arabidopsis plant growth. Two- and 1-week-old Arabidopsis and Brassica sp. seedlings grown in pots, respectively, were inoculated with  $1 \times 10^8$  colony forming units CFU  $\cdot$  ml<sup>-1</sup> Streptomyces sp. once a week for 3 consecutive weeks. A fertilizer (HYPONEX No 2) with N-P-K ratio of 20–20–20 was foliar applied to both control and treated plants twice a week. Four-week-old Arabidopsis plants were analyzed for shoot growth and fresh weight. (A) Streptomyces sp. promoted vegetative growth in Arabidopsis; (B and C) Streptomyces sp. increased fresh weight and chlorophyll content in Arabidopsis



**Fig. 4.** Streptomyces sp. induced protein accumulation in *Arabidopsis*. Total proteins isolated from *Arabidopsis* seedlings inoculated with bacteria inoculants were hybridized with antibodies derived from glutamine synthetase 1 (GS1), cytosolic fructose-1,6-bisphosphatase (cFBPase), and light-harvesting chlorophyll a/b binding (Lhcb1) protein accumulation in *Arabidopsis*. Ponceau staining of transferred proteins was used to check equal loading of protein samples

Windy Manullang and Huey-wen Chuang: Streptomyces sp. mitigates abiotic stress response...





**Fig. 5.** Streptomyces sp. affected Brassica sp. plant growth. One-week-old Brassica sp. seedlings grown in pots were inoculated with  $1 \times 10^8$  colony forming units CFU · ml<sup>-1</sup> Streptomyces sp. once a week for 3 consecutive weeks. A fertilizer (HYPONEX No 2) with a N-P-K ratio of 20–20–20 was foliar applied to both control and treated plants twice a week. Four-week-old Brassica sp. plants were analyzed for shoot growth and fresh weight. (A) Streptomyces sp. promoted vegetative growth in Brassica sp. (B) Streptomyces sp. increased fresh weight in Brassica sp.

Inoculation of *Streptomyces* sp. increased fresh weight (Fig. 8B) and chlorophyll (Fig. 8C) content in *Arabidopsis*.

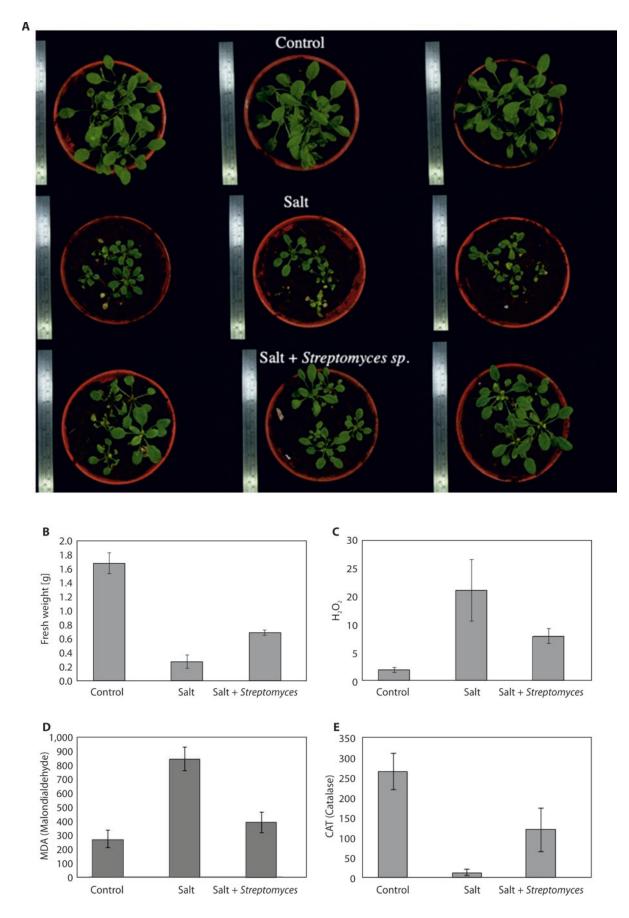
#### **Discussion**

Abiotic stress, in this scenario, salt and heat stress caused various physiological and biological changes in basil plants, one of which was the accumulation of reactive oxygen species in the cell. Reactive oxygen radicals are toxic and may result in a series of injuries to plant metabolism that affect the growth and

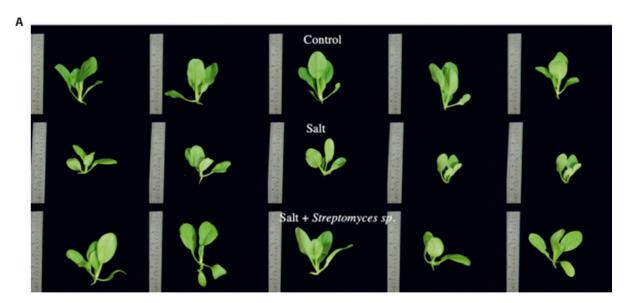
productivity of the plant. The results of this study showed that, salt and heat stress increased hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), malondialdehyde (MDA), and decreased the fresh weight and chlorophyll content. However, inoculation with *Streptomyces* sp. could efficiently be used to improve growth, antioxidant status and photosynthetic pigments under salt and heat stress. Under salt stress, *Streptomyces* sp. significantly improved catalase enzyme activity in the leaves, and increased fresh weight and plant vegetative growth. Under heat stress the chlorophyll content in leaves was increased by inoculation with *Streptomyces* sp.

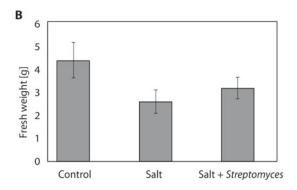
Under salt stress treatment (125 mM NaCl) in *Arabidopsis*, the production of H<sub>2</sub>O<sub>2</sub> and MDA were





**Fig. 6.** Streptomyces sp. promoted salt stress tolerance in Arabidopsis. Two-week-old seedlings grown in pots with 125 mM NaCl, inoculated with Streptomyces sp.  $(1 \times 10^8 \text{ CFU} \cdot \text{ml}^{-1})$  for 3 consecutive weeks, exhibited increments in several physiological systems including fresh weight (B), hydrogen peroxide (C), malondialdehyde (D) and catalase antioxidant enzymes (E)





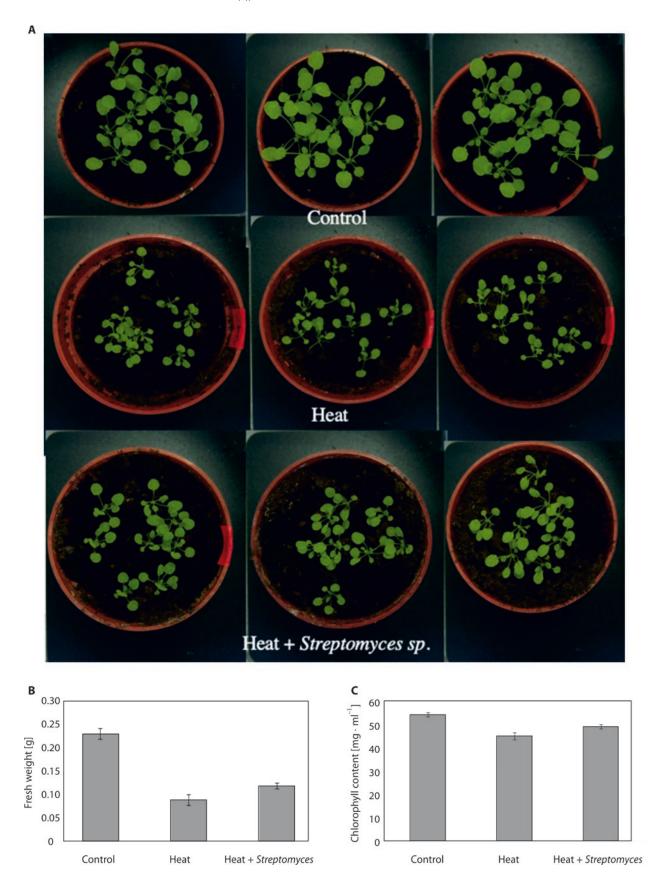
**Fig. 7.** Streptomyces sp. promoted salt stress tolerance in *Brassica* sp. (A) Streptomyces sp. promoted vegetative growth in *Brassica* sp. under salt stress. (B) One-week-old *Brassica* sp. seedlings grown in pots with 150 mM NaCl, inoculated with Streptomyces sp.  $(1 \times 10^8 \cdot \text{ml}^{-1})$  for 3 consecutive weeks, exhibited increments in fresh weight

significantly higher than under other treatments. However, these were significantly reduced as a result of inoculation with Streptomyces sp. (Figs. 6C and D). This study showed that *Streptomyces* sp. induced catalase enzyme production in Arabidopsis under salt stress treatment (Fig. 4E). This result suggests that catalase antioxidant enzyme activity by Streptomyces sp. isolates is able to confer abiotic stress tolerance and increase plant growth in Arabidopsis (Fig. 6) and Brassica sp. (Fig. 7). Catalase enzyme may act as a scavenger of ROS for mitigating the injury in plants (Fig. 6E). Catalase antioxidant enzyme prevented the formation of H<sub>2</sub>O<sub>2</sub> under abiotic stress in plants (Sharma and Ahmad 2014). There are many reports about the relationship between enhanced antioxidant enzyme activities and increased resistance to environmental stresses in several plant species, such as rice (Rossatto et al. 2017), vegetable crops (Kusvuran et al. 2016), sugar beet (Karagöz et al. 2018), wheat (Mutlu et al. 2018) and barley (Pakar et al. 2016).

In addition to its antioxidant production, PGPR's ability to increase plant growth is due to its ability to

secrete very functional hormones for plant growth. In this study, we found that *Streptomyces* sp. is able to secrete high amounts of IAA 83.05 μg · ml<sup>-1</sup> (Fig. 2) and can solubilize phosphate 12.64  $\mu g \cdot m l^{\text{--}1}$  (Fig. 3). The ability of this isolate to increase lateral root number in Arabidopsis is closely related to the IAA and phosphate solubilisation production, which was produced by Streptomyces sp. isolates (Fig. 2). Streptomyces sp. isolates that significantly promoted physiological responses such as fresh weight and chlorophyll content in Arabidopsis and Brassica sp. were able to solubilize phosphate (Fig. 3). Varying studies have found that IAA in PGPR can induce root growth in plants (Cassán et al. 2014; Poupin et al. 2016) and the production of phosphate solubilisation of inoculants simultaneously increases P uptake by the plant and crop yield (Kalayu 2019). The principal mechanism for mineral phosphate solubilization is the production of organic acids that are used in the mineralization of organic phosphorus in soil. This ability provides a promising alternative of phosphate biofertilizers in fields to reduce the use of phosphate chemical fertilizers.





**Fig. 8.** Streptomyces sp. promoted heat stress tolerance in *Arabidopsis*. One-week-old *Arabidopsis* were treated at 40°C for 7 h for 4 days. After 4 days of heat treatment, plants continued to be grown in a growth chamber with a long photoperiod (16 h light and 8 h dark) at 23°C. An inoculant of 30 ml *Streptomyces* sp. with a bacterial population of 10<sup>8</sup> · ml<sup>-1</sup> was applied twice a week to plants. One day after the treatments, the water supply was withheld for 7 days until wilted seedlings appeared. At the end of the water-withholding period, seedlings without wilted leaves were scored as survival plants. These water-restrained seedlings were re-watered, and their recovery fresh weight was measured 7 days after being rewatered. (A) *Streptomyces* sp. promoted vegetative growth in *Arabidopsis* under heat stress. (B and C) *Streptomyces* sp. increased fresh weight and chlorophyll content in *Arabidopsis* under heat stress



Moreover, at a molecular level Streptomyces sp. induced protein accumulation including nitrogen assimilation, such as GS1, carbohydrate metabolism, such as cFBPase, and the light-harvesting chlorophyll, such as Lhcb1 protein (Fig. 5). In this study we found changes in the expression of GS1, cFBPase, and Lhcb1 genes (Fig. 5). Glutamine synthetase (GS1) is a key enzyme in nitrogen assimilation and metabolism in higher plants. There is no doubt that nitrogen assimilation is an important process in the formation of organic nitrogen compounds from inorganic nitrogen compounds present in the environment. Nitrogen is a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide in photosynthesis. It is also a major component of amino acids, the building blocks of proteins. Cytosolic fructose-1, 6-bisphosphatase (cFBPase) is a key component in photosynthetic sucrose biosynthesis. Photoassimilated carbons are converted to sucrose in green plant leaves and distributed to provide carbon and energy. cFBPase overexpression lines exhibited enhanced growth with larger rosette sizes and increased fresh weights than with wild-type (WT) plants (Cho et al. 2012). Light--harvesting chlorophyll such as Lhcb1 protein is one of the most abundant proteins of the chloroplast in plants. It roughly accounts for half of the chlorophyll involved in photosynthesis. The main function of Lhcb1 is collecting and transferring light energy to photosynthetic reaction centers. The enhancement of GS1, cFBPase, and Lhcb1 gene protein expression levels by Streptomyces sp. treated plants contributed to increased plant growth in Arabidopsis and Brassica sp.

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