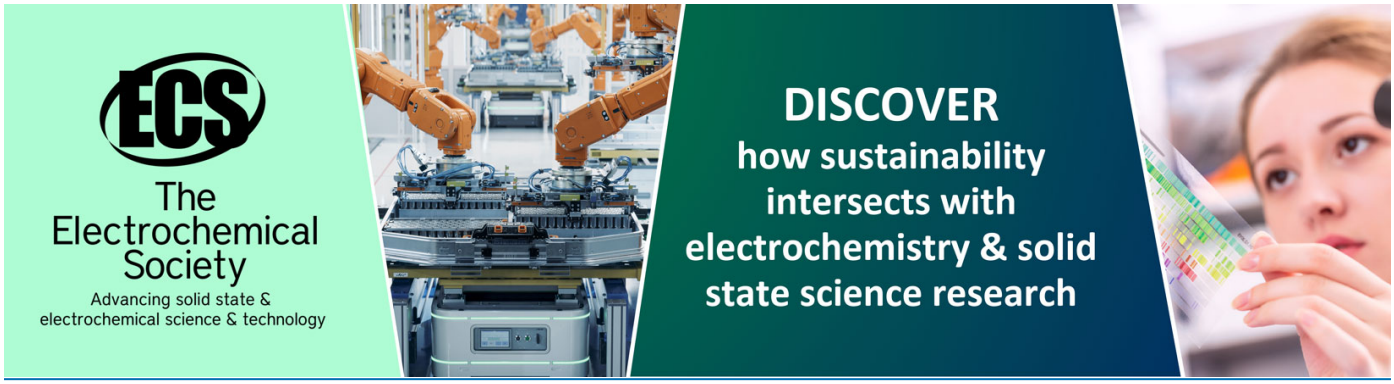
# Characterization of Plant Growth-Promoting Rhizobacteria (PGPR) From Saline Soil in Taiwan





# Characterization of Plant Growth-Promoting Rhizobacteria (PGPR) From Saline Soil in Taiwan

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**Abstract**. The agricultural sector is continuously facing myriad of problems such as yield loss due to abiotic stress as well as phytopathogen infestation. There is well established evidence that the use of plant growth-promoting rhizobacteria (PGPR) would overcome those problems and in turn improve plant growth and crop yield. In this research bacteria strains were isolated from root surface and inner tissue of plants grown in highly saline soils. The characterization includes the ability to produce aminocyclopropane-1-carboxylate (ACC), siderophore, chitinase as well as their antifungal ability. Those abilities are considered the most reliable traits for promoting plant growth. A total of 32 strains were isolated and examined, with 16 surface isolates and 16 endophytes. Thirty bacteria isolates showed capability to produce ACC deaminase with a range of values around 1.8 – 29.6 µmol/mg/h. Thirty isolates were found to secrete siderophore that resulted in halo zones around 1.5 mm - 15 mm after 7 days. Moreover, the chitinase assay showed 13 isolates having the ability to degrade chitin. Interestingly, two isolates with the highest chitinase activity also showed superior ability to inhibit fungal growth. To conclude, a procedure has been demonstrated that rhizobacteria with PGPR traits can be isolated from saline soil. Furthermore, some of these PGPR candidates showed potential to not only promote plant growth but also to control fungal pathogen. This type of dual-functional stains could serve as an environmentally friendly biofertilizer and meanwhile also a biocontrol agent in sustainable agriculture.

## 1. Introduction

The agricultural sector is continuously facing myriad of problems such as yield loss due to abiotic stress as well as phytopathogen infestation. Salt stress is one of the rapidly growing environmental stresses all over in the world, globally 22% of the cultivation and 33 % of the total irrigated agricultural areas are subjected to salt stress, and the affected area is increasing by an average of 10 % per year [1]. Saline soil in Taiwan is mainly caused by acid rain and chemical fertilization [2]. Moreover, the tropical climate of Taiwan serves as an ideal environment for phytopathogens such as bacteria, fungi and viruses.

Soil salinity can induce stress symptoms, decrease germination rates, lower biomass and plant height as well as photosynthesis in plants [3, 4]. These stress responses can be ameliorated by some rhizospheric bacteria by mechanisms such as lower ethylene levels, increasing growth hormone, enhancing total soluble sugars content and antioxidant enzymes that prevent reactive oxygen species (ROS)-induced oxidative damage in plants [5]. These plant growth-promoting rhizobacteria (PGPR) have the potential to overcome salinity problems and in turn improve plans growth and crop yield.

Furthermore, a large body of knowledge has been shown that PGPR could inhibit the growth of phytopathogenic fungi either under *in vitro* or in field condition [6]. Reported PGPR species mainly belong to *Bacillus* sp. [7], *Pseudonomas* sp. [8], *Azotobacter* sp. [9], and any other various genera [10-12], but possible new indigenous strain of PGPR could be found across different type of plant plantation.

Their antifungal properties are achieved by several synergistic activities including secreting lytic enzymes and producing antifungal compounds [6, 13]. PGPR secretes several enzymes that degrade fungal cell wall components such as chitin and cellulose. The active fungus-inhibiting compounds include phenolics, organic acids, polyamine and peptides such as benzoic acid as well as polyamine [14]. For instance, in the presence of *Macrophomina phaseolina* fugal pathogens *B. amyloliquefaciens* was shown to synthesize different lipopeptides such as surfactin, iturin, fengycin and polymyxin [6]. The objective of this study is to isolate novel PGPR-candidate bacteria that is capable of promoting plant growth and also protect plants from fungal pathogens.

## 2. Material and Methods

### *2.1. Screening Bacteria from Rhizospheric Roots*

Soil samples were taken from different locations that suffer from high soil salinity in Taiwan. Rhizospheric bacteria were isolated from root surface for epiphytic strains and from internal tissue for endophytes.

#### *2.1.1. Isolation from Root Surface (Epiphyte)*

The root fragments found in the soil samples were collected and separated from the rhizosphere soil. A total sum 1 g of roots was placed into 100 ml of King B liquid medium (15 ml glycerol, 20 g peptone, 1.15 g $K_2HPO_4$, 1.50 $MgSO_4$) containing 2 % NaCl, followed by overnight incubation with shaking (180 rpm) at 25 $^0$ C.

#### *2.1.2. Isolation from Root Internal Tissue (Endophyte)*

Isolated roots (1 g) were soaked in 100 ml of 70 % ethanol for 15 min to sterilize the surface. In order to release endophytic bacteria, the surface-sterilized roots were grounded with a homogenizer. The ground root sample was put into 100 ml of King B liquid medium and incubated in the same condition as above.

### *2.2. Selection of rhizobacteria to use ACC as a sole nitrogen*

Bacterial culture (1 ml) was transferred into 2 ml tube and then centrifuged for 15 min at 6,000 rpm and the supernatant was discarded. Each bacterial pellet were taken (2 µl) and inoculated in 50 ml DF salt liquid medium (4.0 g $KH_2PO_4$, 6.0 $Na_2HPO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 2.0 g glucose, 2.0 g gluconic acid, 2.0 citric acid, 0.1 ml solution 1 (10 mg $H_3BO_3$, 11.9 mg $MnSO_4 \cdot H_2O$, 124.6 mg $ZnSO_4 \cdot 7H_2O$, 78.22 mg $CuSO_4 \cdot 5H_2O$, 10 mg $MoO_3$ in 100 ml $DDH_2O$ ), 0.1 ml solution 2 (100 mg $FeSO_4 \cdot 7H_2O$ dissolved in 10 ml $DDH_2O$) containing nitrogen and carbon sources (+N, +C). The solution was incubated at 25$^0$ C for 24 hr, 180 rpm. Following incubation, the cultures were centrifuged and the obtained 2 µl pellets was inoculated into a new 50 ml DF (-N, +C) liquid media with ACC as the only nitrogen source (3.0 mM). This solution was incubated at 25$^0$ C for 24 hr, 180 rpm, and the overnight grown culture was pelleted. Pellets were spread onto DF agar containing ACC (30 µmol plate$^{-1}$) and incubated for 72 hr at 25$^0$ C, during the incubation the medium were checked daily.

### *2.3. 1-Aminocyclopropane- 1- carboxylic acid deaminase assay*

For measurement of ACC deaminase activity of rhizobacterial isolates was carried out using modified methods from Penrose and Glick [15]. α-ketobutyrate amount was measured by comparing the absorbance at 540 nm of sample to a standard curve of α-ketobutyrate ranging between 0.1 and 1.0 µmol. A stock solution of 100 mM α-ketobutyrate was prepared in 0.1 M Tris- HCl (pH 8.5) and stored at 4$^0$ C. The bacterial cell pellets were prepared of each suspension in 5 ml of DF containing 60 µl of ACC as the only nitrogen-containing nutrient, followed by incubation at 25$^0$ C for 24 hr, 200 rpm. The

pellets were harvested by centrifugation at 4,000 xg for 10 min, washed using 1 ml of 0.1 M Tris-HCl (pH 7.8) and transferred to a 1.5 ml microcentrifuge tube. The pellet was suspended in 600 µl of 0.1 M Tris-HCl (pH 8.5). Thirty microlitres of toluene was added to the cell suspension and vortexed vigorously for 30 s, and 100 ml aliquot of "toluenized cells" was set aside and stored at $4^0$ C for protein assay at a later time. The remaining toluenized cell suspension was used for assay of ACC deaminase activity. For ACC deaminase assay all samples were measured and carried out in triplicates. Two hundred microlitres of the toluenized cells were placed in a new 1.5 ml microcentrifuge and 20 µl of 0.5M ACC were added to the suspension, briefly vortexed and incubated at $30^0$ C for 15 min. Following the addition of 1 ml of 0.56 M HCl 35%, the mixture was vortexed and centrifuged for 5 min at 13,000 xg at room temperature. Then, 300 µl of the 2,4-dinitrophenyl hydrazine reagent (0.2 % 2,4 dinitrophenylhydrazine in 2 M HCl) was added to the glass tube, vortexed and incubated for 30 min at $30^0$ C. Following the addition and mixing of 2 ml of 2 N NaOH, the absorbance was measures at 540 nm. The absorbance of the reaction including the substrate, ACC, and the bacterial extract was determined with spectrophotometer. After the indicated incubations, the absorbance at 540 nm of the assay reagent in the presence of ACC was used as a reference for the spectrophotometric reading; it was substrate from the absorbance value calculated above.

### *2.4. Siderophore assay*

#### *2.4.1. Screening for siderophore production*

Siderophore production from all rhizobacterial isolates were tested qualitatively by Chrome Azural S (CAS) plate assay following Schwyn and Neiland [16-19]. Previously isolated strains were inoculated on CAS agar plates and incubated at $28^0$ C. The orange color around the colony was observed daily until 7 days. Siderophore production was confirmed by the presence of orange color halo zone around the CAS agar plates.

#### *2.4.2. Quantification of siderophore production*

To quantify siderophore production**,** 500 µl of culture was grown in 50 ml succinate acid medium and incubate for 48 hr at $28^0$ C with constant shaking 120 rpm [19, 20].After the incubation cell free supernatant was collected by centrifugation 15 min at 5000 xg at $25^0$ C, 0.5 ml supernatant was mixed with 0.5 ml of CAS solution and incubated at room temperature for 5 min. The absorbance of each reaction was determined at 630 nm against a reference consisting of 0.5 ml of uninoculated broth and 0.5 ml of CAS reagent. The siderophore level was determined using the following formula: % siderophore unit = [(Ar- As)] / Ar x 100, where Ar is the absorbance at 630 nm of reference (CAS assay solution + uninoculated media) and As is the absorbance at 630 nm of the sample (CAS assay solution + cell free supernatant).

### *2.5. Chitinase assay*

#### *2.5.1 Preparation of Colloidal Chitin*

The colloidal chitin was prepared from the chitin powder using modified methods described previously [21, 22]. In brief, chitin powder (5 g) was slowly added with 40 ml of pure HCl 37 % and kept for 50 min with vigorous stirring. Afterwards, the acidified chitin was added into 200 ml of $DDH_2O$ at 5-$10^0$C and precipitated as a colloidal suspension. The suspension was collected by filtration with vacuum suction on a coarse filter paper and rinsed by 500 ml $DDH_2O$ three times or until pH of the suspension was 3.5

#### *2.5.2 Colloidal chitin agar medium (CCA)*

The colloidal chitin medium was prepared by adding 5 g of colloidal chitin in 1 L of the agar medium (per liter: 0.7 g $K_2HPO_4$, 0.3 g $KH_2PO_4$, 0.5 g $MgSO_4$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4$, 0.001 g MnCl, 20 g agar). Before autoclaving the medium was homogenized by vigorous stirring for 30 min until the colloidal chitin is evenly suspended in the medium. The medium pH was then adjusted to 8.0 with 5N sterile NaOH. The melted medium was poured into petri dishes.

*2.5.3 Chitinase activity*

The chitinase activity was tested by placed 20 µl of bacterial suspension was added into paper dish. Incubated for 48 hr in $28^0$ C and then the medium agar was colored with 2 ml of 0.1 % Congo red (1g Congo red in 100 ml $DDH_2O$). The colloid chitin agar medium was used to determine chitin degradation by bacterial chitinase, for control placed 20 µl liquid medium (no bacteria) into paper dish and make same treatment above.

*2.6 Antifungal activity Test*

The *in vitro* inhibition of mycelium growth of *Botrytis cinerea* by rhizobacteria were tested on Potato Dextrose Agar (PDA; per liter: 4.0 g potato starch, 20 g dextrose and 15 g agar). For each PDA plate, an agar disc containing *B. cinerea* mycelium was placed in the center, and one bacterial strain was streaked as a straight line on the right and left side of the mycelium agar. Plates were incubated at $25^0$ C for 3 until 7 days, the radii of the fungal colonies towards and away from the bacterial were recorded. The percentage growth inhibition was calculated using this formula: % Inhibition = [(R-r)/R)*100%][23]. Where, r is diameter of sample and R is diameter control.

## 3. Results and Discussion

The present study showed the preliminary work to isolate and characterize plant growth promoting rhizobacteria (PGPR) candidates from both root surface (epiphyte) and internal root tissue (endophyte) in saline soil conditions. Thirty two rhizobacteria were isolated and screened for their ability to produce ACC deaminase, siderophore, chitinase as well as their ability as an antifungal agents. It was observed that the isolates differed in their potential for ACC deaminase activity greatly. SBE1 isolate showed a greater amount of ACC deaminase activity (29.6 µmol α-ketobutyrate µmol/mg/h) when compared with previous studies 5.0 α-ketobutyrate µmol/mg/h [24], 2.6 α-ketobutyrate µmol/mg/h [25] and 0.8 α-ketobutyrate µmol/mg/h [26]. Hence, SBE1 serves as a promising candidate as a PGPR to improve plant growth in soil salinity because salt stress can result in the production of inhibitory levels of ethylene and rhizobacteria with high levels of ACC deaminase could lower ethylene production in salt condition and in turn improve plant growth [27-29]. A fraction of the newly synthesized ACC is suspend from seeds or plant roots taken up by the PGPR and converted to ammonia and *α*-ketobutyrate by the enzyme ACC deaminase [30]. As an effect of this enzyme activity, the amount of salt-induced ethylene in the plant is reduced, resulting in increased plant biomass [31]. Some of gram positive and negative PGPR have been reported to colonize the root of the plants [32] and minimize the effect of salinity by different direct and indirect mechanism [33].

*3.1 Characterization of isolates plant growth promoting rhizobacteria (PGPR) candidate*

The rhizobacteria isolates were screened for different PGPR traits. The isolates showed a great variety in ACC-deaminase activity, siderophore production, chitinase and antifungal activities (Table. 1).

Iron is an essential element for plant growth and is abundant in earth. Yet, the bioavailability of iron is often limited due to the very low bioavailability of the $Fe^{3+}$ ion, the predominant form of iron in non-acidic oxygenated condition. Microorganisms secret siderophores to scavenge iron by forming siderophore-$Fe^{3+}$ complexes that can be taken up by plants. In this study the siderophore-producing ability of rhizobacteria was determined by two types of CAS assays; one by halo zone formation on the CAS agar plate and the other by spectrophotometry readings of the liquid culture supernatant. The best siderophore-producing strains identified by these two methods, however, were different. The highest isolates identified by the CAS agar method are SGS3 with (15.0 mm) followed by SKS2 (14.5 mm) and SCE1 (11.5 mm), but according to the spectrophotometry method the best performers were SHE1 (35.5%), SIS1(33.6%) and SGS1 (32.3%). These results could be due to the fact that siderophore production depends on some factors such as iron content in medium, and others minerals also influence its production; for example, $Zn^{2+}$ and $Cu^{2+}$ increased siderophore production and $Ni^{2+}$ promoted the production of yellow pigment or siderophore [34]. In addition, conditions such as pH, temperature, carbon source, nitrogen source, organic acids, and cell biomass could contribute to different measurement of siderophore production [20].

**Table 1**. Isolates showing different plant growth promoting rhizobacteria (PGPR) activities.

| Bacterial Origin | Isolates | ACC deaminase | Siderophore | Chitinase | Antifungal activity |
|---|---|---|---|---|---|
| Root surface (Epiphyte) | SAS1 | +++ | ++++ | + | ++ |
| | SBS1 | +++ | ++++ | - | +++ |
| | SCS1 | + | ++++ | + | + |
| | SDS1 | ++ | ++++ | - | ++ |
| | SES1 | + | ++ | - | ++ |
| | SFS1 | ++ | + | - | ++ |
| | SGS1 | +++ | + | - | ++++ |
| | SGS2 | ++++ | +++ | + | ++++ |
| | SGS3 | - | ++++ | - | ++++ |
| | SHS1 | ++ | +++ | - | ++++ |
| | SIS1 | +++ | +++ | + | ++++ |
| | SJS1 | + | + | + | ++++ |
| | SJS2 | +++ | +++ | + | ++++ |
| | SJS3 | ++ | ++ | + | + |
| | SKS1 | + | +++ | - | ++++ |
| | SKS2 | ++ | ++++ | - | + |
| | SLS1 | ++ | ++ | + | ++++ |
| Root internal tissue (Endophyte) | SAE1 | ++++ | + | - | +++ |
| | SAE2 | ++ | ++ | - | ++ |
| | SBE1 | ++++ | - | + | + |
| | SCE1 | ++++ | ++++ | - | +++ |
| | SCE2 | ++++ | ++ | - | ++++ |
| | SDE1 | - | ++ | + | ++ |
| | SEE1 | ++++ | +++ | - | +++ |
| | SFE1 | ++ | +++ | + | +++ |
| | SGE1 | ++++ | + | - | +++ |
| | SGE2 | ++ | ++ | - | +++ |
| | SHE1 | +++ | +++ | - | +++ |
| | SIE1 | ++ | + | - | ++ |
| | SJE1 | +++ | ++++ | - | ++ |
| | SKE1 | +++ | ++ | + | ++ |
| | SLE1 | ++++ | - | + | + |

The presence of an activity is indicated by "+" whereas the absence is indicated by "–".

*3.2 1-Aminocyclopropane- 1- carboxylic acid deaminase activity*

The ACC deaminase measurement was conducted to know the ability of plant growth promoting rhizobacteria (PGPR) to producing ACC deaminase, the high activity means ACC deaminase can be metabolized to lower the ethylene level and ethylene-induced stress. This uptake of ACC deaminase decreases the amount of ethylene inside the plant and thereby alleviated ethylene induced stress. Deamination of ACC into α-ketobutyrate by the enzyme ACC deaminase was determined in a biochemical assay. Among the 32 rhizobacterial isolates both from root surface and root internal tissue, ACC deaminase activity was detected in 30 isolates, ranging from 1.8 – 29.6 µmol/mg/h (Fig. 1). SBE1 isolate showed the highest activity of 29.6 µmol/mg/h, followed by SLE1 (12.0 µmol/mg/h) and SGS2 (11.1 µmol/mg/h).

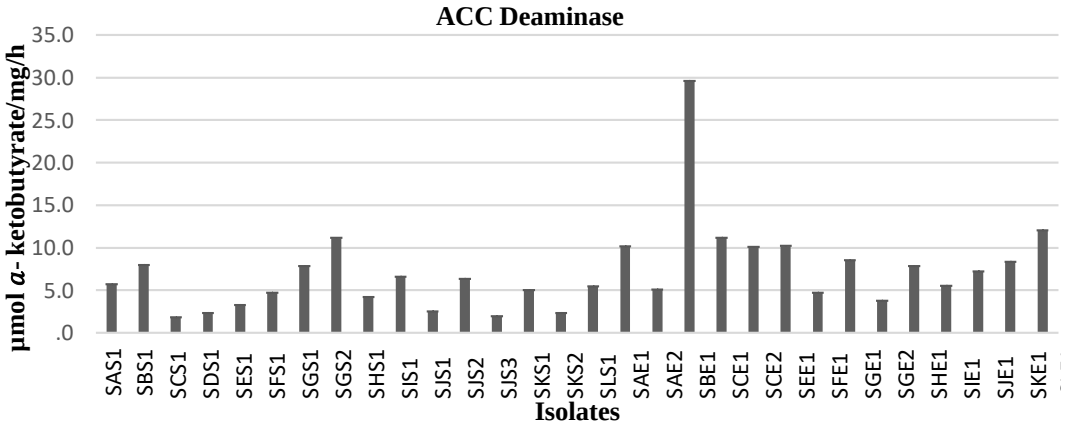


**Figure 1**. ACC deaminase activity from rhizobacterial isolates. Value are mean of three replications.

*3.3 Siderophore assay*

*3.3.1 Screening siderophore production*

The siderophore positive isolates were screened by using the CAS agar plates. The orange halo zone is formed when the colorimetric test using chrome azurol S (CAS) assay to detects siderophores independent of their structure. The siderophores scavenge iron from an Fe-CAS-hexadecyltrimethylammonium bromide complex, and the subsequent release of the CAS dye results in a color change from blue to orange. From 32 rhizobacterial isolates, 30 isolates were positive for siderophore production, ranging from of 1.5 mm - 15 mm after 7 days. Interestingly, SBE1 and SLE1 did not show visible halo zones (Fig 3). In the highest halo zone in day 7$^{th}$ were SGS3 with (15 mm) followed by SKS2 (14.5 mm) and SCE1 (11.5 mm). From each observation showed an average increased size of the halo zone from day 1 until day 7.

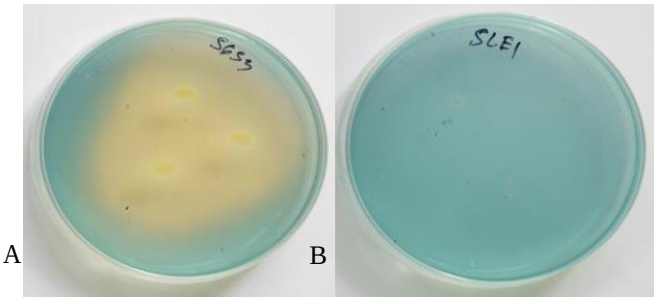


**Figure 2**. The CAS agar plate siderophore production assay. A). Halo zone from the highest of siderophore production (SGS3), B). The SLE1 isolate did not produce halo zones. The orange color indicating siderophore production.

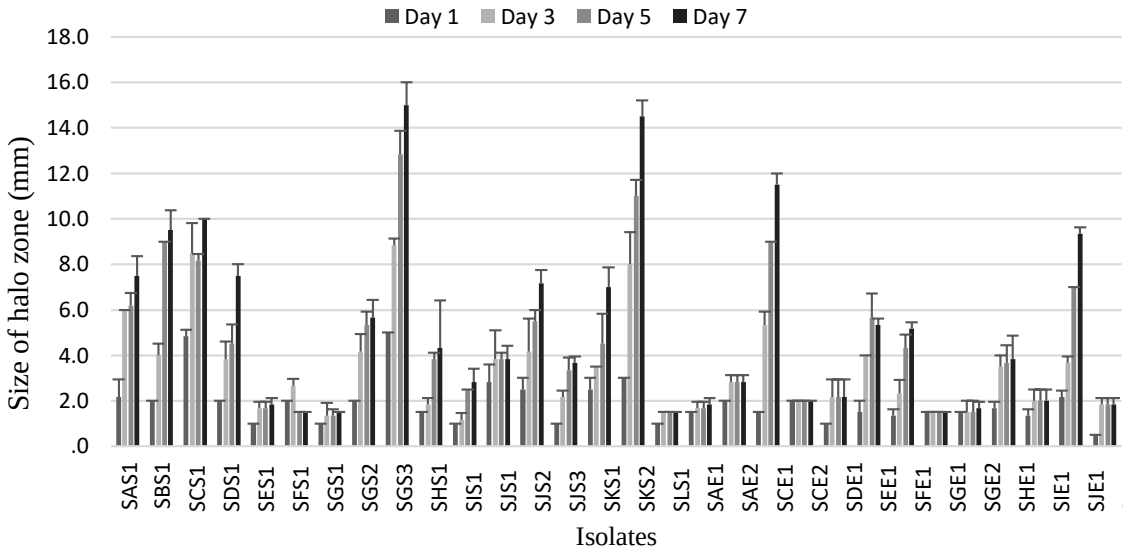


**Figure 3**. Comparison of qualitative siderophore production from rhizobacterial isolates. Value are mean of three replications.

*3.3.2 Quantification of siderophore produced by the rhizobacterial isolates*

To better compare the siderophore production levels among bacterial strains, a quantitative CAS liquid medium assay was performed after 48 hr of incubation. In this assay, isolate SHE1 showed the highest siderophore production level (35.5%), followed by SIS1(33.6%) and SGS1 (32.3%). Interestingly, this quantification method gave rise to different siderophore production levels from the results determined by measuring the halo zone size on CAS agar plates (Fig. 3).

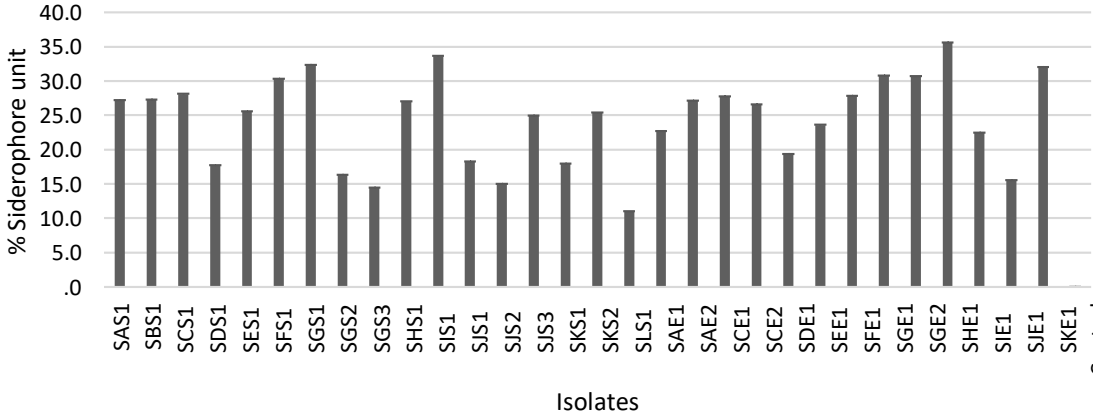


**Figure 4**. Quantification of siderophore production in liquid media after 48h incubation.

*3.4 Chitinase assay*

The chitinase assay was performed to determine whether the isolated rhizobacteria could degrade chitin as a substrate. Since chitin is a main component of the fungal cell wall, high chitinase production could serve as an indicator for the antifungal activity of a strain. From 32 isolates, only 13 (SAS1(2.1 mm), SCS1(1 mm), SGS2 (2.8 mm), SIS1 (2.1 mm), SJS1(5 mm), SJS2 (2.1 mm) , SJS3 (1.5 mm) , SLS1(1.8 mm), SBE1(2.8 mm), SDE1 (1.3 mm), SFE1(2 mm), SKE1(1 mm), SLE11.1 mm)) rhizobacteria showed the ability to degrade chitin and form transparent zones where colloidal chitin had been degraded. By contrast, in the control plate the chitin colloids remained intact and were strained red by Congo red.

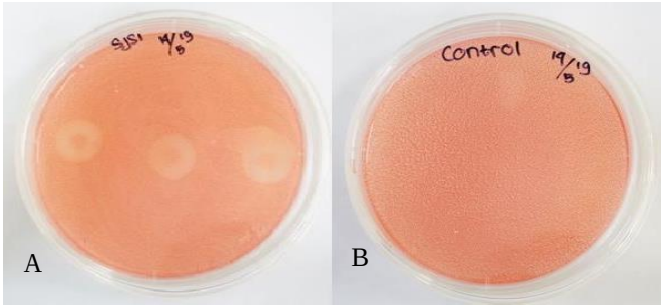


**Figure 5**. The chitinase assay. A) Clear zones formed around the filter paper disk innoculated with SJS1. B) Control (medium culture without bacteria inoculation).

*3.5 Antifungal activity of the rhizobacteria*

Rhizobacteria isolates were tested for their ability to control the fungal pathogen *Botrytis cinerea*. The growth inhibition effect on *B. cinerea* was observed. Inhibition of mycelia by rhizobacteria was done by two streaking methods (single-streak and double-streak) in the antagonistic assay (Fig 6a and 6b). On the left, the same inhibition was observed between the single-streak and double-streak methods. By contrast, on the right of the plates where the rhizobacterium SJS1 was absent or present, the fungal mycelia grew well without SJS1; whereas on the double-streak plate the expansion of mycelia were clearly impeded by the presence of SJS1. The control showed fast expansion of *B. cinerea* mycelia without the rhizobacterial inoculation (Fig 6c). The greatest *B. cinerea* growth inhibiton effect was observed with SJS1 (48.14 %) followed by SHS1(46.6 %) and SGS2 (44.4 %). It is noteworthy to point out that the greatest fungal growth inhibition was sobserved with SJSI isolate, which also showed superior chitin-degrading ability. The SGS2 also can degrading the chitin but the antagonist result showed more highest SJS1.

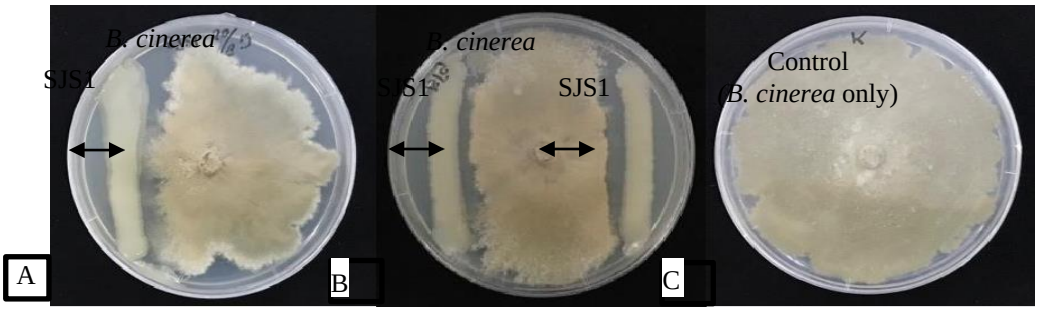


**Figure 6**. Antagonistic assay to determine the antifungal activity of rhizobacterial isolates. A) Inhibition of SJSI isolates againt *B. cinerea* with one side streak. B) Inhibition of SJSI isolates againt *B. cinerea* with double side streaks. C) Growth of *B. cinerea* without rhizobacteria isolates.

If compare of each rhizobacteria isolates had a different ability in PGPR traits (Table 1.), SBE1 showed the highest isolate in ACC deaminase but did not get good result in chitinase as well as other traits, SGS3 was showed the best isolates in siderophore also had ability in Antifungal. Interesting the results show the same that the best SJS1 isolates in chitinase also show the best results in antifungal, this shows the correlation between the two parameters.

It is interesting to point out that SJSI and SGS2 isolates, with high chitin-degrading ability, also showed great inhibition effect of *B. cinerea*. Chitinolytic bacteria are capable of hydrolyzing of chitin progressively to produce N-acetylglucosamine monomer [35], not only that the cellulase and proteinase activity also good indicators for antifungal activity. In addition, chitinases inhibit spore germination and germ tube elongation of the phytopathogenic fungi [36]. Since *B. cinerea* is a common plant pathogen, the antifungal activity exhibited by SJS1 and SGS2 sugguests that the application of these two strains

may be a promising biocontrol method, as reported by other recent studies [37, 38]. In the antagonistic assay (Fig 6a and 6b), on the left the same inhibition was observed between the single-streak and double-streak methods. By contrast, on the right of the plates where the rhizobacterium SJS1 was absent or present, the fungal mycelia grew well without SJS1; whereas on the double-streak plate the expansion of mycelia were clearly impeded by the presence of SJS1. This comparison indicated that the antifungal ability of rhizobacteria was effective only when the pathogen and the bacterial strain are in close vicinity.

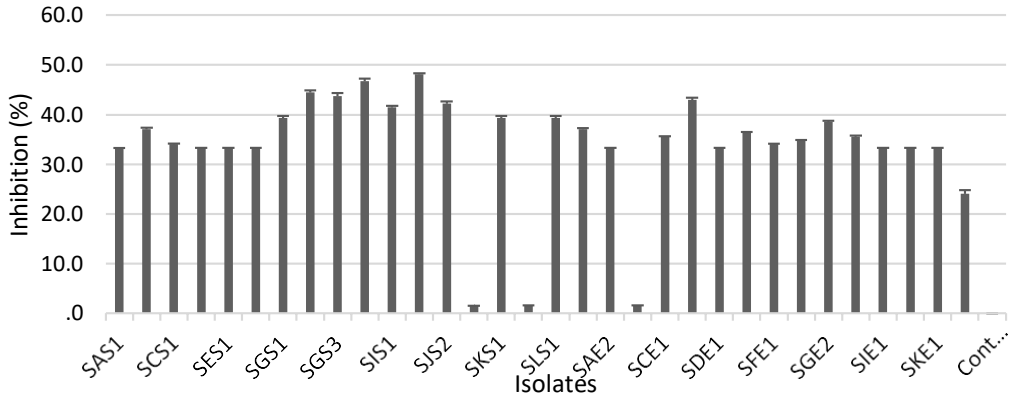


**Figure 7**. Inhibition of pathogenic fungus *B. cinerea* growth due to the presence of rhizobacteria isolates on 7 days.

**Table 2**. Summarized the highest performance PGPR traits of each isolates.

| PGPR Traits | 1st | 2nd | 3rd | 4th | 5th |
|---|---|---|---|---|---|
| **ACC deaminase** | SBE1 | SLE1 | SCE1 | SGS2 | SEE1 |
| **Siderophore production** | SGS3 | SKS2 | SCE1 | SCS1 | SJE1 |
| **Chitinase activity** | SJS1 | SGS2 | SBE1 | SJS2 | SIS1 |
| **Antifungal activity** | SJS1 | SHS1 | SGS2 | SGS3 | SCE2 |

The best five strains for each PGPR trait are summarized in Table 2. Among tested isolates, no one strain showed the best performance in all traits. For example, SBE1 showed the highest ACC deaminase activity but did not have high chitinase levels. Likewise, SGS3 showed the highest siderophore production and antifungal activity but did not perform well in other traits. Although we did not find a super isolate that is good in all four PGPR traits, the use of combination of multiple strains is a possible way to take advantages of these isolated strains. More research is in need to know these isolates growth promotion effect on plants in the field and their ability to improve plant growth in saline conditions.

## 4. Conclusion

This study was carried out to characterize bacterial strains isolated from both root surface and root internal tissue from plant rhizosphere collected from different locations of highly soil salinity in Taiwan. Isolates that showed high activities in SBE1, SGS3, SGS2, and SJS1 were selected as potential PGPR. The application of these strains could be a promising way to control the fungal pathogens and to improve plant growth in stress for sustainable agriculture.

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