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Plant-growth promoting traits of bacterial strains isolated from button mushroom (*Agaricus bisporus*) media

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ABSTRACT: A diverse group of plant-growth promoting bacteria were isolated in button mushroom (*Agaricus bisporus*) media to investigate the plant-growth promoting traits of compounds including indole acetic acid (IAA), ammonia, 1-aminocyclopropane-1-carboxylic acid deaminase, siderophore, and hydrogen cyanide. Twenty-one bacterial strains showing positive effects for all the test traits were selected and classified to confirm bacterial diversity in the media habitat. Plant-growth promoting traits of the isolates were also assessed. All strains produced IAA ranging from 20 μg/mL to 250 μg/mL. Most of the isolates produced more than 80% siderophore. Four strains (*Pantoea* sp., PSB-08, *Bacillus* sp., PSB-13, *Pseudomonas* sp., PSB-17, and *Enterobacter* sp., PSB-21) showed outstanding performances for all the tested traits. In a bioassay of these four strains using mung bean plant, the best growth performances (23.16 cm, 22.98 cm, 2.27 g/plant, and 1.83 g/plant for shoot length, root length, shoot dry weight, and root dry weight, respectively) were obtained from the plants co-inoculated with *Bacillus* sp., PSB-13. The resultant data indicate that button mushroom media have got a diverse group of bacteria with plant growth promoting abilities. Thus, the media could be a good recycling resource for using to an effective bio-fertilizer.

KEYWORDS: Bacteria isolation, Bioassay, Button mushroom media, Plant growth promotion traits

INTRODUCTION

Media for cultivating the button mushroom (*Agaricus bisporus*) was traditionally prepared with the fermented rice straw as main nutrient in Korea. The variety of microorganisms could be inhabited or succeed in rice straw media during fermentation process. Therefore, it was known well for button mushroom media (BMM) to be a habitat of the useful microorganisms such as bacteria, yeast, and fungi. Due to the fact that direct or indirect beneficial or detrimental activities, microbial communities are considered to be a key component of the soil (Hartmann *et al*., 2015). They enhance the growth of plants through various processes such as decomposition, nutrient immobilization and mineralization, nitrogen fixation, denitrification, plant hormone production and phosphate solubilization etc. In Korea farm, the BMM which include various microbes as well as nutrient has been used as an alternative biofertilizer and compost for growing plant.

A diverse group of plant-growth promoting bacteria (PGPB) in soil was reported to be involved in facilitating the growth of plants by stimulating the efficiency of (a) nitrogen fixation (Sahin *et al*., 2004); (b) accessibility of other trace elements (Mittal *et al*., 2008); (c) ability to produce plant hormones such as auxins (Egamberdiyeva, 2005), cytokinins (Gracia de Salamone *et al*., 2001), gibberellins (Gutierrez *et al*., 2009); (d) antagonism against phytopathogenic microorganisms by production of siderophore (Wani *et al*., 2007a), vitamin excretion (Streit *et al*., 1996), the synthesis of antibiotics (Lipping *et al*., 2008), enzymes and/or fungicidal compounds and competition with detrimental microorganisms such as chitinase (Hamdali *et al*., 2008).

Application of microbial biofertilizer could be an

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effective solution to improve a pshyco-chemical properties of soil as well as plant growth (Stark *et al*., 2007). Accordingly, these microbial communities either as single form (Poonguzhali *et al*., 2008) or in combination with other rhizosphere microbes (Vikram and Hamzehzarghani, 2008) have shown substantial measurable effects on plants in conventional agronomic soils. Therefore, PGPBs are shown to have direct impacts on soil conditions, nutrient availability, plant growth, and yield.

This study was performed to investigate the diversity of PGPBs inhabiting in button mushroom media and to assess the plant growth promotion traits of the bacterial isolates, i.e. indoleacetic acid (IAA), ammonia, ACC deaminase, siderophore, hydrogen cyanide (HCN) and bioassay evaluation.

MATERIALS AND METHODS

Isolation of plant-growth promoting bacteria

Plant-growth promoting bacteria were isolated from button mushroom compost at Chungchugnam-do province, Buyeo-Gun area in South Korea. Serially diluted aliquots of compost samples were inoculated on National Botanical Research Institute's phosphate (NBRIP) agar for phosphate solubilizing organisms or nutrient agar plates containing 0.1% tryptophan for indole acetic acid (IAA) producing strain (Yu and Yoon, 2019). Positive colonies on each medium were picked up and further purified by repeated sub-culturing. Pure cultures were maintained as a glycerol suspension (30% v/v) at -80°C until use.

Production of indole acetic acid

The strains distinguished with large pink color halo on agar plates were screen for qualitative assay of IAA production, IAA production of the isolates was done with method described by Gutierrez *et al*. (2009). Culture supernatant mixed with the Salkowski's reagent (2% 0.5M $FeCl_3$ in 35% perchloric acid) was incubated in the dark at 30°C for 30 minutes. Development of pink color indicated the IAA production and then the optical density was taken at 530 nm using UV spectrophotometer.

Production of ammonia

The bacterial isolates were tested for the production of ammonia in peptone water. Nessler's reagent (0.5 mL) was added to each culture tube. Development of brown to yellow colour was considered as being a positive test for ammonia production (Cappucino and Sherman, 1992).

ACC deaminase activity

Bacterial strains were assayed for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity by testing their ability to grow on DF minimal medium supplemented with 3 mmol ACC as the sole source of nitrogen (Penrose and Glick, 2003). Solid DF minimal medium containing ACC was inoculated with 10 µL of starter culture (grown overnight at 30°C). Plates were incubated at 30°C in dark and colony emergence was checked daily for up to 3 days.

Production of Hydrogen Cyanide

HCN production was tested by method of Donate-Correa *et al*. (2005). Filter paper soaked in picric acid and Na_2CO_3 (0.5% and 2% respectively) solution was fixed to the underside of the lids of plates and incubated for 5 days at 30°C. A change in filter paper color from yellow to orange-brown was considered as the indication of HCN production.

Production of siderophore

Siderophore production was assayed qualitatively using chrome azurol S (CAS) blue agar according to Schwyn and Neilands (1987). Quantitative estimation of siderophore was done by CAS-shuttle assay (Payne, 1994). Culture supernatant (0.5 mL) was mixed with same amount of CAS reagent (0.5 mL) and absorbance was measured at 630 nm against a reference consisting of equal volume of uninoculated broth and CAS reagent. Siderophore content (%) in the aliquots were calculated using following formula; Siderophore units(%) = $A_r - A_s / A_s \times 100$, where A_r : Absorbance of reference,

A_s : Absorbance of sample

Plant growth promotion bioassay with mung bean

Mung bean (*Vigna radiate* var. paiyur 1) seeds sterilized with 0.1% sodium hypochlorite solution were soaked in bacterial culture suspensions prepared in nutrient broth about 30 min prior to plant. Pots filled with soil were provided with the basal doses of nitrogen (50 mg/kg soil) and potassium (120 mg /kg soil) in the form of urea and potassium chlorite, respectively. The soil from 15 mm depth was removed from earthen pots and five seeds were placed at equal distance. Growth

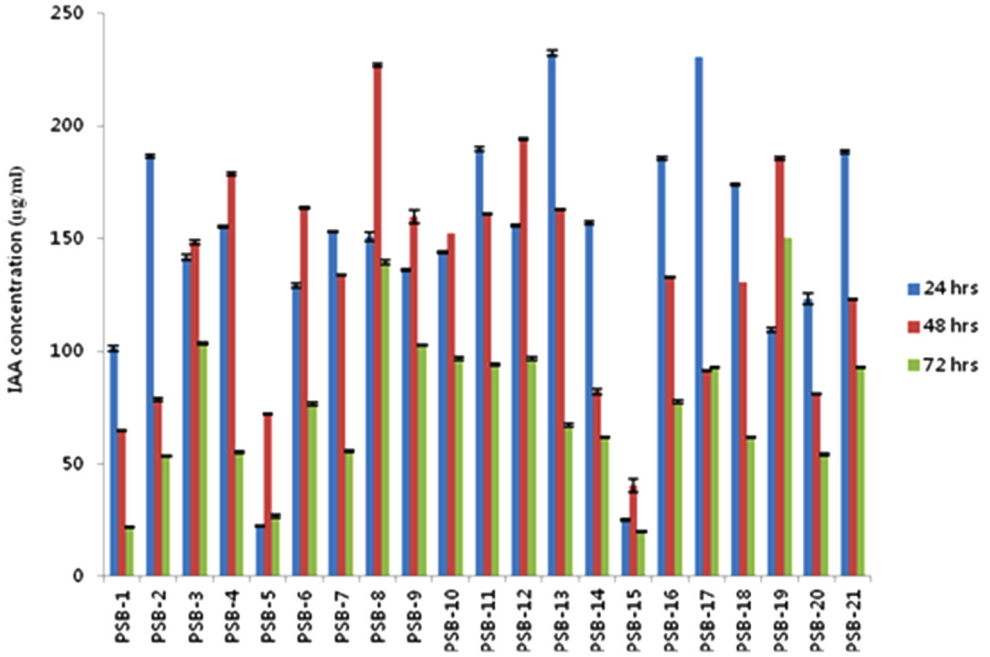

Fig. 1. IAA production of the isolated plant growth promoting bacterial strains.
Values are the means ($n = 3$) ± standard deviation.

promotion effects of bacterial treatment were assessed by measuring shoot and root length of mung bean plants.

Statistical analysis

Values were given as means ± SD for triplicate samples. The data were subjected to analysis of variance (ANOVA) using SAS package (SAS, 1999). The Duncan's Multiple Range Test (DMRT) was applied to test the significance of treatment means at $P \leq 0.05$.

RESULTS AND DISCUSSION

Isolation of plant-growth promoting bacteria

Out of 35 strains isolated from button mushroom media based on the results of IAA production and phosphate solubilization, 21 bacterial strains (PSB 1 to PSB 21) showed all positive response for both screen methods. The selected bacteria were classified by 16S rRNA gene sequence analysis as genus *Bacillus*, *Pseudomonas*, *Burkholderia*, *Klebsiella*, *Pantoea*, *Acidobacteria, Arthrobacter* and *Enterobacter etc* and used for estimating other plant-growth promoting traits.

Production of Indole Acetic Acid

All the strains produced IAA ranging from 20 µg/ mL to 250 µg/ mL The IAA production in *Pantoea* sp., PSB-8, *Bacillus* sp., PSB-13, and *Pseudomonas* sp., PSB-17 was found to be remarkably higher (more than 230 µg/ mL) than the other strains (Fig. 1). The majority of the present strains were shown to produce more IAA than the range (3.0-20 µg/mL) previously reported by Banerjee *et al*. (2010). All the isolates exhibited the highest IAA production within first 24-48 h followed by reduction as time progressed (Fig. 1). Microbial IAA production plays a significant role in the host plant's growth by stimulating both a rapid response (e.g. cell elongation) and a long-term response (e.g. cell division and differentiation) in plants (Ahmad *et al*., 2010). The resent results thus broadened the understanding of the ability of microbes to produce IAA.

Production of ammonia

Production of ammonia is an important trait of plant growth promoting bacteria that influences plant growth indirectly (Wani *et al*., 2007a). Production of this secondary metabolite was found in all the studied isolates and among them *Bacillus* sp., PSB-13 showed the highest ammonia production followed by PSB-3, PSB-6, PSB-8, PSB-13, PSB-16, PSB-19, PSB-20, and PSB-21 (Table 1).

Production of Hydrogen Cyanide

HCN is a secondary metabolite associated with plant

Table 1. Response of plant growth promoting bacteria to qualitative assay of plant growth promoting traits

Strain	Ammonia	HCN	ACC deaminase	Siderophore	IAA	Genus Name
PSB-1	2	1	1	1	2	*Bacillus*
PSB-2	2	1	1	1	2	*Pseudomonas*
PSB-3	3	1	1	1	2	*Arthrobacter*
PSB-4	1	1	3	_	2	*Bacillus*
PSB-5	1	_	3	3	2	*Acidobacteria,*
PSB-6	3	_	+	2	3	*Paraburkholderia*
PSB-7	1	_	3	_	2	*Burkholderia*
PSB-8	3	3	3	3	3	*Pantoea*
PSB-9	1	3	1	1	2	*Pseudomonas*
PSB-10	1	_	1	_	1	*Enterobacter*
PSB-11	1	2	3	3	2	*Lactobacillus*
PSB-12	1	1	1	_	1	*Klebsiella*
PSB-13	3	3	3	3	3	*Bacillus*
PSB-14	1	_	2	_	1	*Burkholderia*
PSB-15	3	3	3	3	3	*Enterobacter*
PSB-16	3	1	2	_	2	*Burkholderia*
PSB-17	3	2	3	3	3	*Pseudomonas*
PSB-18	2	2	3	3	2	*Pantoea*
PSB-19	3	2	3	3	2	*Arthrobacter*
PSB-20	3	2	3	2	2	*Bacillus*
PSB-21	3	3	3	3	3	*Enterobacter*

(1): Less response (2): Moderate response (3): Strong response (-): No response

protection. Thus the ability to produce HCN is a desired quality of plant growth promoting organisms. Based on the color change from yellow to orange-brown, 15 isolates were shown to exhibit HCN production. Among them, PSB-9, PSB-15, PSB-20, and PSB-21 were identified as being strong HCN producers (Table 1). The presence of HCN in the soil could inhibit plant disease development through strengthening the host's disease resistance mechanism (Banerjee *et al*., 2010).

ACC deaminase activity

ACC deaminase, an enzyme produced by many plant growth promoting microorganisms is involved in the stimulation of root elongation in seedlings (Lie *et al*., 2000). Giving the ability that they are capable of growing in N-free basal medium, all the isolates showed positive response for ACC deaminase activity (Table 1). Especially, PSB-8, PSB-19, PSB-20, and PSB-21 were identified as being strong ACC deaminase producers (Table 1). Microbial IAA also promotes root growth either directly by stimulating plant cell elongation or cell division or indirectly by its influence on the ACC deaminase activity (Kim and Yoon, 2018).

Production of siderophore

Siderophore producing bacteria are good candidates for plant growth promotion especially in neutral to alkaline soils and showing antifungal activity (Bultreys *et al*., 2001). The siderophore producers which exhibited large orange halos on CAS agar were selected and followed by estimating siderophore content on liquid culture media (Fig. 2). The highest siderophore production of most of the strains was recorded within 24-48 h after inoculation. However, PSB-4, PSB-21, PSB-7, and PSB-8 showed delayed start of siderophore production (24-48 h after incubation). Except PSB-10, PSB-12, PSB-13 and PSB-14, all the other isolates produced more than 80% siderophore units (Fig. 2).

Plant growth promotion bioassay with mung bean

Four strains (*Pantoea* sp., PSB-08, *Bacillus* sp., PSB-13, *Pseudomonas* sp., PSB-17 and *Enterobacter* sp.,

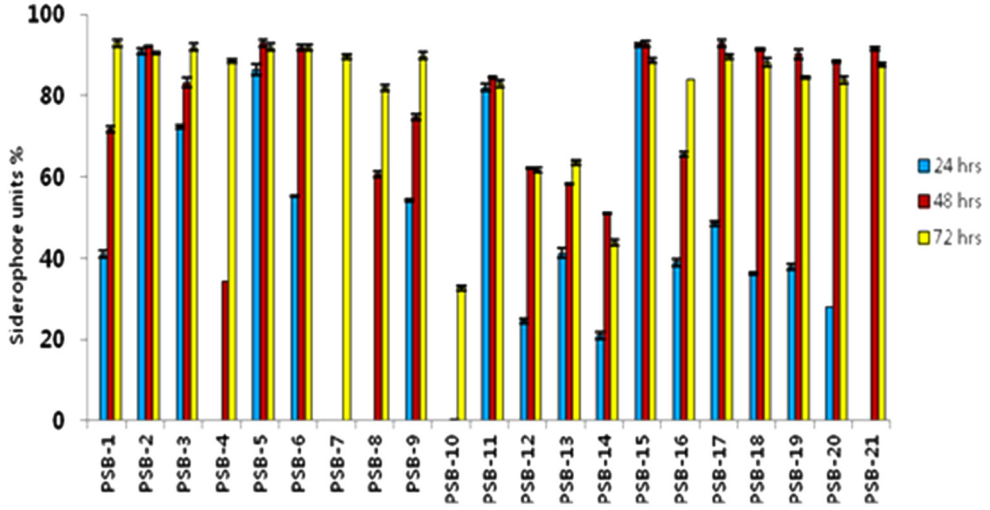

Fig. 2. Siderophore production of the isolated phosphate solubilizing bacterial strains. Values are the means (n = 3) ± standard deviation.

PSB-21) which showed the outstanding performance for all the tested traits were selected to estimate the growing effect of mung bean. According to the results of plant growth promotion assay, the strains could significantly promote the shoot and root growth of mung bean as compared with uninoculated seeds (Table 2), the inoculated seedlings recorded 14.16% and 16.36% higher shoot and root lengths respectively compared to uninoculated control. Increased shoot length, root length and shoot and root dry weight of mung bean plants were recorded from the seedlings raised with the PGPBs inoculated seeds (Table 2). The best growth performances (23.16 cm, 22.98 cm, 2.27 g/plant and 1.83 g/plant respectively, for shoot length, root length, shoot dry weight and root dry weight) were recorded from the plants co-inoculated with *Bacillus* sp., PSB-13 (Table 2). Though the inoculation of PGPBs resulted in better effect for plant growth compared to un-inoculated treatments, no significant differences in shoot length, root length and shoot-root dry weight were observed between each strain.

Soil bacteria are known to be involved in plant-growth promotion through biological nitrogen fixation, iron chelation, phosphate solubilization, producing plant hormones such as auxins, cytokinins, and producing ACC deaminase, which lowers plant ethylene levels, antagonism against phytopathogenic microorganisms by producing siderophores, hydrogen cyanide, and fungicidal enzymes like chitinase, etc (Wani *et al*., 2007; Lipping *et al*., 2008; Mittal *et al*., 2008). Therefore, it is worth to isolate and screen the bacteria producing other plant growth promoting substances in order to use them as effective bio-inoculants. In the present study, 21 bacterial strains were isolated from button mushroom media and screened for plant growth promoting traits. Based on the results of phosphate solubilization and IAA production, most of the isolated bacteria showed the outstanding performances for plant growth promoting characteristics tested in this study.

Though bacteria are known to exhibit several plant

Table 2. Effect of plant growth promoting bacteria on growth of mung bean plants

Treatment	Shoot length (cm)	Root length (cm)	Shoot dry matter (g/plant)	Root dry matter (g/plant)
Control	18.36^{b}±0.9	19.35^{b}±1.0	2.02^{a}±0.15	1.47^{b}±0.2
Pantoea PSB-08	22.35^{a}±1.2	21.51^{a}±1.1	2.19^{a}±0.23	1.67^{ab}±0.2
Bacillus PSB-13	23.16^{a}±1.1	22.98^{a}±0.9	2.27^{a}±0.20	1.83^{a}±0.2
Pseudomonas PSB-17	22.19^{a}±1.0	22.64^{a}±1.2	2.21^{a}±0.24	1.71^{ab}±0.1
Enterobacter PSB-21	20.22^{b}±0.9	21.57^{a}±1.1	2.24^{a}±0.19	1.69^{ab}±0.1

Values are the means (n = 3) ± standard deviation. Within each column, means followed by same letter(s) are not significantly different at $P \leq 0.05$

growth promoting characteristics, the production of phytohormones such as IAA is considered to be one of the most commonly reported direct plant growth promotion mechanism. Furthermore, it involves in development of root structures such as lateral roots and root primordia which in turn could facilitate high root surface area for better uptake of water and nutrients from soil (Kim and Yoon, 2018). As proved by the increased growth performances of mung bean plant, the isolated strains through its ability to produce IAA, may form a beneficial association with the host plants. The findings of the present investigation highlighted that the isolated strain has great potential to enhance soil fertility and plant growth through plant growth promoting. However, assessment of other plant growth promoting characteristics and further studies under field conditions would be ideal in confirming the present findings and also in recommending the strains as bio-inoculants.

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