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1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing endophytic bacteria prolong vase life of cut roses

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Abstract

Cultivating rose as a cut flower is a profitable vending business. Preserving cut roses by keeping them fresh and long-lasting is difficult. One of the factors that shortens their lifespan is ethylene exposure. However, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase produced by some plant growth-promoting bacteria converts ACC, an intermediate precursor of ethylene biosynthesis, to ammonia and alpha-ketobutyrate. This study aims to evaluate the ACC deaminase activity of endophytic bacteria in extending the lifespan of cut roses. The isolates were screened on the Dworkin and Foster (DF) medium containing 0.25 mM ACC (ACC-DF) and tested for their efficiency in decreasing ACC by a colorimetric ninhydrin assay. All selected isolates reduced ACC concentration in the culture medium. *Acinetobacter* sp. SWUy05 could colonize in the stem of cut roses and significantly retarded bud opening and petal wilting or discoloration. Therefore, *Acinetobacter* sp. SWUy05 is a promising ACC deaminase endophytic bacterium, suitable for extending the vase life of cut rose.

Keywords: ACC Deaminase, *Acinetobacter*, Endophyte, Ethylene, Plant growth-promoting bacteria

1. Introduction

Rose is an economically viable flower and is produced in many countries. Loss in the quality of cut rose due to early senescence is a serious challenge in the production of cut rose. Most rose cultivars are sensitive to ethylene, which shortens their life cycle in a vase [1]. Ethylene is a phytohormone that plays a variety of roles in plants such as stress response, seed germination, fruit ripening, and senescence. Petal wilting is a characteristic of early senescence in cut rose, which is an effect of ethylene exposure. Another symptom of senescence is bent-neck, mainly caused by blockage of the xylem vessel due to microorganisms. Additionally, water loss in cut rose is correlated with ethylene sensitivity, resulting in vase life shortening [1,2].

Ethylene is usually maintained in plants at a low level. Varying from plant species to species, 1-aminocyclopropane-1-carboxylic acid (ACC) with concentration between 0.25 and 2 mM is needed to produce the maximal amount of ethylene [3]. However, it spikes during senescence. Ethylene is synthesized by the conversion of methionine to S-adenosylmethionine (SAM) by SAM synthetase, which is then further converted to ACC by ACC synthase. ACC is then converted to ethylene by ACC oxidase [4,5]. High level of ACC accumulation in a carnation cut flower is correlated with ethylene biosynthesis, resulting in high ethylene sensitivity and petal wilting [6]. Increase in ACC oxidase also causes vase life shortening of cut flower [1].

Plant growth-promoting bacteria (PGPB) promote root elongation in several ways. Some PGPB can produce indole-3-acetic acid (IAA) which is a plant hormone. Several PGPB inhabiting in the rhizosphere can consume ACC from root exudate as a nitrogen source [7]. PGPB can produce ACC deaminase enzyme which cleaves ACC to ammonia and alpha-ketobutyrate. Consequently, the ACC level in plant tissues is reduced to maintain an equilibrium between the level of ACC inside the plant tissue and the root exudate [8]. Some of the ACC deaminase producing bacteria are endophytes dwelling in plant tissue, consuming ACC [9]. Ali et al. [10] showed that ACC deaminase producing endophytic bacterium *Pseudomonas fluorescens* YsS6 and *P. migulae* 8R6, decelerate mini

carnation senescence for 3-4 days, compared with an uninoculated cut carnation. However, the utilization of ACC deaminase producing bacteria in prolonging vase life of another cut flower has been largely unexplored.

A variety of methods are now available for prolonging vase life of cut rose, such as adding silver thiosulfate to vase water as an ethylene inhibitor [11]. Sudaria et al recommended hydroxyquinoline sulfate supplemented with glucose to be added to vase water to maintain the vase life of cut rose for 4.5 days [12]. Rafi et al. [13] found that nano-silver can inhibit microorganism growth, which is the main cause of vessel blockage and also decreases stomatal opening and transpiration, thereby extending the vase life of cut rose. Additionally, Elgimabi showed that 30 ppm silver nitrate with 3% (w/v) sucrose effectively increased the vase life of cut rose [14]. However, the use of chemical substances such as silver thiosulfate or silver nitrate may hazard the user's health or cause skin irritation. The application of biological agents is an alternative method for extending the vase life of the cut flower. In this study, we focus on the evaluation of endophytic bacteria on ACC utilization for extending the vase life of cut roses.

2. Materials and methods

2.1 Isolation of ACC deaminase producing bacteria

Cut red rose (*Rosa hybrida* cv. hybrid tea 'Christian Dior') was used in this study which was purchased from a local market in Bangkok, Thailand. The rose was cut at 5 to 8 cm. below the flower bud to obtain the cut stem about 3 cm long. The cut stems were washed, and surface sterilized by 0.5% (v/v) NaOCl for 10 min, followed by washing in sterile water three times. One milliliter of the last washing water was poured plate in nutrient agar (NA) to assure the surface sterilization process. Both sides of the cut stem were trimmed for 1 cm each. The final cut about 1 cm long at the trimmed stem was shaken in 10 mL nutrient broth (NB) for 3 h and used for isolating endophytic bacteria. NB was then spread on to NA and incubated at room temperature (~30 ± 2°C) for 3 days. The colony-forming unit (CFU) in NA was collected and further purified by the cross-streak method.

To screen for ACC deaminase producing bacteria, the isolate was spot inoculated on three types of media. Dworkin and Foster (DF) medium with 0.2 % (w/v) $(NH_4)_2SO_4$ was used as the positive control while DF medium without $(NH_4)_2SO_4$ (N-free DF medium) was used as the negative control. DF minimal medium replacing $(NH_4)_2SO_4$ with 0.25 mM ACC (ACC-DF medium) as the sole nitrogen source was used as the treatment (i.e., for the isolation of ACC deaminase producing bacteria) [15-17]. Growth of isolates on ACC-supplemented plates was compared to the negative and positive controls. Experimental results show that the selected isolates on DF medium with $(NH_4)_2SO_4$ and ACC-DF medium were able to grow while those on N-free DF medium were not.

2.2 Biochemical characterization and motility of the isolates

Experiments were set up to screen for facultative anaerobic ACC deaminase producing bacteria according to characterization and motility of the isolates which could affect the vase life period. The isolate was first stabbed in ACC-DF semi-solid medium (0.4% (w/v) agar) in a deep tube and the isolate that grew all over the tube was selected. Glucose and sucrose fermentation ability of the isolate was tested since these sugars are typically used in vases to prolong the vase life of the cut flower. The glucose and sucrose broths with phenol red were used. The glucose and sucrose broths (Himedia^{TM}) contain 1 g/L beef extract, 10 g/L peptone, 5 g/L NaCl, 0.018 g/L phenol red, and 5 g/L glucose or sucrose, respectively. The pH of the broths was adjusted to 7 before they were sterilized by filtering through 0.22 PES membrane. Followed by the fermentation test's instruction, if glucose or sucrose is fermented, the culture broth color will change from red to yellow and gas formation could be detected in the Durham tube.

2.3 Colorimetric ninhydrin assay

The potential of the isolate in ACC utilization was evaluated by colorimetric ninhydrin assay, following the method in Li et al. [18]. Briefly, $1.5x10^8$ suspension cells of isolate in ACC-DF broth were centrifuged at 5000 x g for 5 min. The isolate was then resuspended in N-free DF broth to wash the cell and centrifuged and resuspended again in 10 mL ACC-DF broth. The isolate was cultured at room temperature with agitation at 150 rpm for 24 h. The cell-free culture broth was mixed with the ninhydrin reagent (1:2 v/v) and then was heated in boiling water for 30 min. The non-inoculated ACC-DF broth mixed with ninhydrin reagent was used as a positive control. The DF medium was used as a blank. The standard curve of ACC concentrations was generated by the colorimetric ninhydrin assay [18] with ACC-DF medium containing 0.1 mM to 0.4 mM ACC. The absorbance of the mixer at 570 nm was measured with a spectrophotometer to determine the ACC amount. The experiment was performed in three independent replicates. Analysis of Variance (ANOVA) and least significance difference (LSD) was used for statistical analysis and multiple comparisons, respectively.

2.4 Bacterial identification

Bacterial identification was performed by 16S rRNA gene analysis. A single colony of the isolate was used for colony polymerase chain reaction (PCR) with universal 27F primer (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R primer (5' TACGGYTACCTTGTTACGACTT 3'). The approximately 1,500 bp long purified PCR product was sequenced by Macrogen Inc. (Korea). The sequences generated were analyzed by BioEdit to generate an around 1,300 bp sequence to be analyzed by the BLAST program, against GenBank and EzBioCloud databases [19]. The phylogenetic tree based on the 16S rRNA gene was constructed using the Maximum Likelihood method, with the Tamura-Nei model [20]. Bootstrap analysis was of 1000 repeats. Evolutionary analyses were conducted in MEGA7 [21].

2.5 Cut rose colonization of the isolate

The ACC deaminase producing isolates were suspended to 10^2 cell/mL in 0.5% (w/v) sterile glucose. A 20 cm long cut rose was washed and sprayed with 70% (v/v) ethanol before being stabbed down to the cell suspension in a bottle closed with cotton plug for 5 days, at room temperature. The cut rose stabbed down in 0.5% (w/v) sterile glucose solution was used as the negative control. A 3 cm long stem was then cut at 5 cm below the apical bud and trimmed to 3 cm length of the cut rose stem as described above. After surface sterilization, using the same method as described in Section 2.1, the cut stem about 1 cm long was cut vertically and shaken in 1 mL sterile 0.85% (w/v) NaCl for 2 h. The cell suspension in 0.85% (w/v) NaCl was then plated on NA containing 35 mg/L nystatin to inhibit fungal growth. Colonies grown on the medium were counted. Six cut roses were used per treatment and the experiment was triplicated. All data from the triplicate experiments were pooled and subjected to ANOVA and the LSD was used for multiple comparisons.

2.6 Vase life prolonging experiment

Cut rose with red petals visible from the tight apical bud was used in this study. The stem of the cut rose was sprayed with 70% (v/v) ethanol and cut to 20 cm length. The cut rose was stabbed down to the cell suspension in a bottle closed with a cotton plug as described above, for 4 days. The cut roses were maintained at ambient temperature (30 ± 3°C) and photoperiodic lighting (12 h of light and 12 h of dark) during incubation with light intensity of 1280 lux. The cut rose stabbed down in 0.5% (w/v) sterile glucose solution without cell suspension was used as a control. After 4 days, they were observed for three characteristics of flower bud: bud opening, neck bending, and petal wilting or discoloration (Table 1). Six cut roses were used per treatment and the experiment was performed in triplicate. All data from triplicate experiments were pooled and subjected to Kruskal-Wallis and Dunn's test for multiple comparisons. A scoring scheme for each characteristic was defined as follows.

Table 1 Score of cut rose development.

Parameters	Score	Developmental stage
Flower bud opening	0	tight bud, red of petals just visible
	1	slightly open bud, outer petal split from top
	2	open bud, outer petal reflexed to stem
	3	fully open bud, outer petals reflexed over 135° to stem but anthers not
	4	exposed fully open bud, outer petals fallen or anthers are exposed
Bent-neck	0	no bent
	1	less than 30° bending
	2	30-60° bending
	3	60-90° bending
	4	more than 90° bending or U shape
Petal wilting or discoloration	0	no wilting or discoloration
	1	1 or 2 petals' rim showing wilting or discoloration
	2	more than 2 petals' rim showing wilting or discoloration
	3	1 or 2 full petals showing wilting or discoloration
	4	more than 2 full petals showing wilting or discoloration

3. Results and discussion

3.1 Isolation and biochemical characterization of ACC deaminase producing bacteria

We obtained 120 bacterial isolates from twelve stems of cut red roses purchased from four flower shops. The bacterial isolates might be endophytes because the last washing water from the surface sterilization process was sterile. All of them were screened for ACC utilization ability by ACC-DF medium which contains ACC as a sole nitrogen source. Eighty isolates could grow on ACC-DF agar medium which is an indicator of their ability to utilize ACC. Those isolates were further tested for their motility. Thirty-one out of eighty were motile and facultative anaerobes. Nine isolates that showed the widest colony in the ACC-DF agar medium were selected to identify and test for their potential in ACC utilization ability. The selected isolates were SWUa209, SWUa212, SWUb06, SWUb16, SWUb18, SWUc05, SWUw03, SWUw04, and SWUy05. The glucose and sucrose fermentation tests of the nine isolates are shown in (Table 2). All of them could ferment glucose and six isolates could ferment sucrose. The glucose fermentation capability might be important for bacterial survival in the vase.

Table 2 Glucose and sucrose fermentation, motility, and identification of isolates.

Isolate	ACC utilization ability activity	Motility	Glucose Fermentation	Sucrose Fermentation	Strain Identification	Genbank Accession No.	% Similarity
SWUa209	+	+	+	+	*Enterobacter* sp.	OP363182	98.31
SWUa212	+	+	+	+	*Enterobacter* sp.	OP363183	99.46
SWUb06	+	+	+	+	*Pantoea* sp.	OP363184	94.14
SWUb16	+	+	+	+	*Acinetobacter soli*	OP363185	100
SWUb18	+	+	+	+	*Acinetobacter baumannii*	OP363186	99.85
SWUc05	+	+	+	+	*Pantoae* sp.	OP363187	98.47
SWUw03	+	+	+	-	*Acinetobacter schindleri*	OP363188	99.38
SWUw04	+	+	+	-	*Acinetobacter indicus*	OP363189	99.92
SWUy05	+	+	+	-	*Acinetobacter* sp.	OP363191	98.24

+ and – indicate the presence and absence, respectively, of the capability for ACC utilization, motility, glucose, and sucrose fermentation. The 16S rRNA gene sequences of isolates were blasted against EzBioCloud database. The sequences were deposited to GenBank-NCBI and the accession numbers are listed.

3.2 Identification of ACC deaminase producing bacteria

Based on the 16S rRNA gene sequence compared against the EzBioCloud database, the nine selected isolates were similar to *Enterobacter cloacae* (JUZJ01000090), *Enterobacter* sp. R4-368 (CP005991), *Pantoea dispersa* (DQ504305), *Acinetobacter soli* (APPU01000012), *Acinetobacter baumannii* (ACQB01000091), *Pantoae coffeiphila* (KJ427829), *Acinetobacter schindleri* (APPQ01000011), *Acinetobacter indicus* (KI530754), and *Acinetobacter soli* (APPU01000012) with identity percentage of 98.31%, 99.46%, 94.14%, 100%, 99.85%, 98.47%, 99.38%, 99.92%, and 98.24%, respectively (Table 2). The phylogenetic trees of the selected isolates are shown in supplementary. As suggested by Junda and Abbott (2007), the similarity for bacterial identification using 16S rRNA gene analysis should be at least 99% for species identification [22]. Therefore, SWUa209, SWUb06, SWUc05, and SWUy05 was identified as *Enterobacter* sp., *Pantoea* sp., *Pantoea* sp., and *Acinetobacter* sp., respectively.

3.3 Evaluation of isolates for ACC utilization ability

We studied the ACC utilization ability of isolates by a colorimetric ninhydrin assay [18]. This assay determines the utilization of ACC in the medium by the isolate, with a known initial amount of ACC in the medium. The ninhydrin test is typically used for protein or amino acid analysis. The Ruhemann's purple was observed when ACC reacted with ninhydrin, measurable by absorbance spectrophotometry at 570 nm. As shown in (Figure 1), the absorbance at 570 nm obtained from the cultured medium of all isolates was significantly less than that of control. The estimated concentrations of ACC in culture medium of isolates ranges from 0.17-0.19 mM, obtained from the standard curve. This indicated that all of the nine selected isolates were capable of utilizing ACC as a

nitrogen source. However, there was no significant difference among isolates in ACC consumption. Therefore, we selected isolates that might not be pathogenic or opportunistic pathogenic bacteria for the subsequent study. As mentioned in bacterial identification result, SWUa209, SWUb06, SWUb18, and SWUw03 might be *E. cloacae*, *P. dispersa*, *A. baumannii*, and *A. schindleri*, respectively. Therefore, *Enterobacter* sp. SWUa212, *A. soli* SWUb16, *Pantoae* sp. SWUc05, *A. indicus* SWUw04, and *Acinetobacter* sp. SWUy05 were chosen.

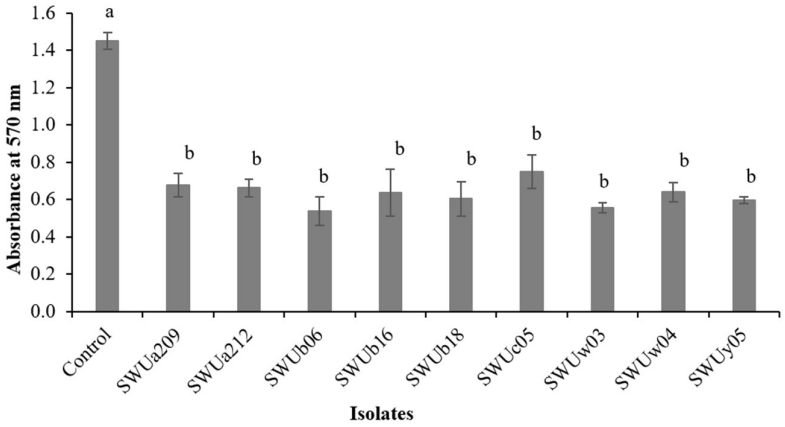

Figure 1 ACC utilization of isolates. Control was the ACC-DF medium. Bars represented mean with standard error. The difference letters above the bar represented significant differences ($p < 0.05$).

3.4 Colonization in the cut rose of isolates

ACC deaminase producing endophytic bacteria may effectively reduce ACC in cut rose. We hypothesized that the isolates which can colonize in the stem of cut rose can utilize ACC more easily than epiphytic bacteria. The bacterial colonies obtained from the uninoculated stem (control experiment) were natural bacterial endophytes. It should be noted that the bacterial colony was not found from the last washing water from the surface sterilization process. The cut rose inoculated with each isolate showed the larger number of bacteria inside the stem than that of the uninoculated stem. These observations could imply that all of the selected isolates except *A. soli* SWUb16 could colonize in the stem of cut rose as shown in Figure 2. Although we did not perform 16S rDNA sequencing analysis or PCR to identify the endophyte isolates, we rely on a hypothesis that the number of natural endophytic bacteria is relatively constant across different samples of cut rose stems. Therefore, the number of isolates (even though those isolates were not identified) that was larger than that of the control was most likely due to the effect of the inoculation.

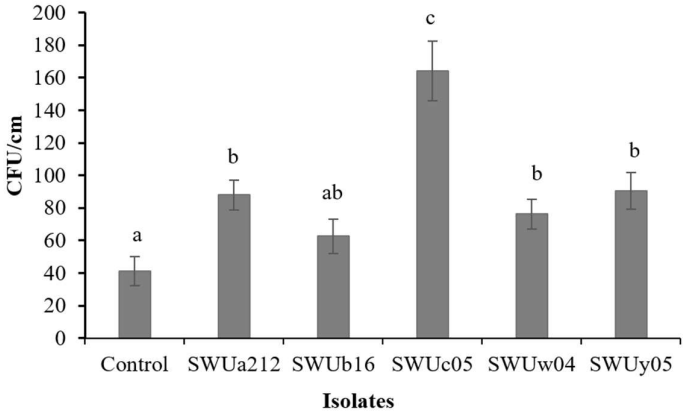

Figure 2 Endophytic bacteria in the cut-rose stem (CFU/cm). Bars represented mean with standard error. The difference letters above bar represented significant differences ($p < 0.05$).

3.5 Effects of the isolates on prolonging vase life

The criterion for selecting the best strain was based on the vase life of cut roses. Chamani and Wagstaff assessed the vase life period starting from when the treatment was applied until bent neck or wilting sign on all petals was observed [23]. Horibe and Makita defined the vase life period starting from the time of applying the treatment to petal wilting [24]. To quantify the vase life in this study, a scoring scheme was devised for each of the various bud characteristics of cut roses including bud opening, bent neck, and petal wilt (Table 1). The lower the score (closer to zero), the longer the vase life period, and the higher the score, the shorter the vase life period. We found that the use of isolates did not affect the bent-neck characteristic of the cut rose as shown in Table 3. Inoculating cut roses with *Enterobacter* sp. SWUa212 did not enhance the vase life period indicated by the same scores evaluated on uninoculated cut roses according to bud opening, bent neck, and petal wilt. A relatively lower score was achieved by inoculating cut roses with isolates in *Acinetobacter* genus including *A. soli* SWUb16, *A. indicus* SWUw04, and *Acinetobacter* sp. SWUy05. *Pantoae* sp. SWUc05 is the best performer achieving the lowest scores of the petal wilting characteristic; however, the bud opening score is not statistically different from the control. When the effect of isolates on bud opening and petal wilting characteristics is considered, *Acinetobacter* sp. SWUy05 was among the most promising ACC deaminase endophytic bacterium.

Table 3 Effects of isolates on flower bud characteristics.

Parameters	Control	SWUa212	SWUb16	SWUc05	SWUw04	SWUy05
Bud opening	2.22 ± 0.21^{a}	2.06 ± 0.19^{a}	1.89 ± 0.20^{ab}	1.83 ± 0.22^{abc}	1.44 ± 0.20^{bc}	1.33 ± 0.11^{c}
Bent neck	1.22 ± 0.13^{ns}	1.22 ± 0.10^{ns}	1.11 ± 0.08^{ns}	1.28 ± 0.14^{ns}	1.28 ± 0.11^{ns}	1.67 ± 0.20^{ns}
Petal wilting and discoloration	3.61 ± 0.14^{a}	3.22 ± 0.19^{abc}	2.89 ± 0.16^{cd}	2.67 ± 0.21^{d}	3.44 ± 0.12^{ab}	3.17 ± 0.15^{bcd}

Numbers showed scores with standard error of bud opening, bent neck and petal wilting and discoloration. The maximum score is four and the minimum score is zero. The different superscript letters show significant differences ($p<0.05$) of the score in the same row.

Cut rose senescence after harvesting results in dramatic economic losses. The senescence of cut rose is usually shown as petal abscission, wilting, and/or bent-neck. The bent-neck symptom of the cut rose is typically caused by blockage of vascular vessels. Either multiplication of microorganisms in the vascular system or air blockage induced by cutting leads to vascular occlusion [25]. The application of bactericide such as silver thiosulfate solution in vase water decreases bent-neck in cut rose and also acts as an ethylene inhibitor [12,26-27]. Contrary to this earlier study, we used endophytic bacteria to enhance the longevity of cut rose. These isolates are ACC deaminase producing endophytic bacteria isolated from a cut rose, which are typically symbiotic to the stem. Our hypothesis is that these isolates are not likely to block the xylem vessel, but decreased ethylene in the vessel, which in turn explains a deceleration of the cut rose senescence. The potential of ACC deaminase producing isolates on prolonging vase life was evaluated by scoring the inoculated cut roses based on three characteristics: bud opening, bent neck and petal wilting, and discoloration. However, the direct measurement of ethylene emission from plant by gas chromatography would be a more reliable method to validate the function of ACC deaminase produced from endophyte.

Ethylene accumulation in plant tissue is the main cause of cut flower senescence. Reduction in ethylene level in plant tissue can delay flower senescence. ACC is an intermediate precursor for ethylene production. Most researches have been focused on the transgenic cut flower and the impairment of ethylene production by reduction of ACC conversion. For example, transgenic carnation with ACC oxidase gene suppression produced a low level of ethylene and prolonged vase life by 2-folds [28]. Insertion of antisense copies of 1-aminocyclopropane-1-carboxylic acid oxidase gene reduced ethylene production in *Dendrobium* orchids [29]. Introducing sense or antisense ACC oxidase gene to the torenia plant could silence ACC oxidase gene expression and the transgenic can last two days longer than wild type [30]. However, the cultivation of transgenic plants is prohibited in many countries including Thailand. Thus, the use of ACC deaminase producing endophytic bacteria is a novel and promising strategy to prolong the vase life of cut flower, and is also easier and cheaper than transgenic flower cultivation. A variety of non-chemical preservation solutions could be used to extend the flower vase life efficiently [27]. Applying ACC-deaminase producing endophytic bacteria is also a non-chemical and environmentally friendly solution for extending the vase life of cut rose.

Interestingly, four of the nine selected isolates including *E. cloacae* SWUa209, *P. dispersa* SWUb06, *A. baumannii* SWUa18, and *A. schindleri* SWUw03 might be pathogenic or opportunistic bacterial species. This is probably because vase water used in the market or during transportation from the garden was contaminated with these bacteria as they naturally inhabit the soil and water in this region [31]. Bacteria in these genera are also endophytic bacteria. Kuan et al isolated PGPB from maize root and found that some isolates such as *Acinetobacter* sp. S3r2, *Klebsiella* sp. Br1 and *K. pneumoniae* Fr1 promote plant growth because they had nitrogen fixation, phosphate solubilization, and phytohormone-auxin production abilities [32]. The strains with the potential to be

pathogenic or opportunistic bacterial species must be aware of and a systematic approach to safety in the use of this application remains to be established.

Bacteria belonging to the genus *Acinetobacter* are often found in rhizospheric soil or plant. Moreover, many species promote plant growth by reducing plant stress by reducing ethylene levels. Huang et al isolated a variety of species of ACC deaminase producing bacteria including *Acinetobacter* sp. from soil and rhizospheric soil of carnation [33]. *Acinetobacter* sp. Q2BJ2, an ACC deaminase producing endophytic bacteria, could enhance *Commelina communis* plant biomass and Pb-uptake in Pb-polluted environment [34].

4. Conclusion

ACC deaminase producing bacterial endophytes isolated from cut rose could utilize ACC as their nitrogen source *in vitro*, and prolong vase life *in vivo*. Among other candidates in the *Acinetobacter* genus, *Acinetobacter* sp. SWUy05 is a promising species for prolonging the vase life of cut rose as evident from experimental results showing its effectiveness in extending bud opening and alleviating petal wilting.

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6. References

[1] In BC, Ha STT, Lee YS, Lim JH. Relationships between the longevity, water relations, ethylene sensitivity, and gene expression of cut roses. Postharvest Biol Tec. 2017;131:74-83.
[2] Mladenovic E, Cukanovic J, Tanjga B, Pavlovic L, Hiel K, Miric M. Selection of garden rose cultivars for use as a cut flower. Genetika. 2018;50:495-502.
[3] Khan AA. ACC-derived ethylene production, a sensitive test for seed vigor. J Am Soc Hort Sci. 1994;119:1083-1090.
[4] Gamalero E, Glick BR. Mechanisms used by plant growth-promoting bacteria. In: Maheshwari D, editor. Bacteria in Agrobiology: Plant Nutrient Management, Berlin: Springer; 2011. p. 17-46.
[5] Gepstein S, Glick BR. Strategies to ameliorate abiotic stress-induced plant senescence. Plant Mol Biol. 2013; 82:623-633.
[6] Overbeek JHM, Woltering EJ. Synergistic effect of 1-aminocyclopropane-1-carboxylic acid and ethylene during senescence of isolated carnation petals. Physiol Plant. 1990;79:368-376.
[7] Olanrewaju OS, Glick BR, Babalola OO. Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol. 2017;33:197.
[8] Arshad M, Saleem M, Hussain S. Perspectives of bacterial ACC-deaminase in phytoremediation. Trends Biotechnol. 2007;25:356-362.
[9] Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res. 2014;169:30-39.
[10] Ali S, Charles TC, Glick BR. Delay of flower senescence by bacterial endophytes expressing 1-aminocyclopropane-1-carboxylate deaminase. J Appl Microbiol. 2012;113:1139-1144.
[11] Wagstaff C, Chanasut U, Harren FJM, Laarhoven LJ, Thomas B, Rogers HJ, et al. Ethylene and flower longevity in Alstroemeria: relationship between tepal senescence, abscission and ethylene biosynthesis. J Exp Bot. 2005;56:1007-1016.
[12] Sudaria MA, Uthairatanakij A, Nguyen HT. Postharvest quality effects of different vaselife solutions on cut rose (*Rosa hybrida* L.). Int J Agric Life Sci. 2017;1:12-20.
[13] Rafi ZN, Ramezanian A. Vase life of cut rose cultivars 'Avalanche' and 'Fiesta' as affected by nano-silver and S-carvone treatments. S Afr J Bot. 2013;86:68-72.
[14] Elgimabi MENE. Vase life extension of rose cut flowers (*Rosa Hybirida*) as influenced by silver nitrate and sucrose pulsing. Am J Agric Biol Sci. 2011;6:128-133.
[15] Dworkin M, Foster JW. Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol. 1958;75:592-601.
[16] Ali SZ, Sandhya V, Rao LV. Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent *Pseudomonas* sp. Ann Microbiol. 2014;64:493-502.
[17] Penrose DM, Glick BR. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant. 2003;118:10-15.
[18] Li Z, Chang S, Lin L, Li Y, An Q. A colorimetric assay of 1-aminocyclopropane-1-carboxylate (ACC) based on ninhydrin reaction for rapid screening of bacteria containing ACC deaminase. Lett Appl Microbiol. 2011;53:178-185.

[19] Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, et al. Introducing EzBiocloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol. 2017;67:1613-1617.
[20] Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 1993;10:512-526.
[21] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870-1874.
[22] Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. J Clin Microbiol. 2007;45:2761-2764.
[23] Chamani E, Wagstaff C. Effects of postharvest relative humidity and various re-cutting on vase life of cut rose flowers. Int J Postharvest Technol Innov. 2019;6:70-82.
[24] Horibe T, Makita M. Promotion of flower opening in cut rose cultivars by 1-naphthaleneacetic acid treatment. Ornam Hortic. 2021;27:314-319.
[25] Balas J, Coronado PAG, da-Silva JAT, Jayatilleke MP. Supporting post-harvest performance of cut flowers using fresh-flower-refreshments and other vase-water-additives. In: Silva JAT, editor. Floriculture, ornamental and plant biotechnology: advances and topical issues. Isleworth: Global Science Books; 2006. p. 612-629.
[26] Ansari NA, Zangeneh M. Effects of cultivar, harvesting date and chemical treatments on the quality and soluble carbohydrate contents in rose (*Rosa hybrida*). Flori Ornam Biotech. 2008;2:1-4.
[27] Nguyen TK, Lim JH. Do eco-friendly floral preservative solutions prolong vase life better than chemical solutions? Horticulturae. 2021;7:415.
[28] Kosugia Y, Shibuya K, Tsurunoa N, Iwazakia Y, Mochizukib A, Yoshioka T, et al. Expression of genes responsible for ethylene production and wilting are differently regulated in carnation (*Dianthus caryophyllus* L.) petals. Plant Sci. 2000;158:139-145.
[29] Sornchai P, van-Doorn WG, Imsabai W, Burns P, Chanprame S. *Dendrobium* orchids carrying antisense ACC oxidase: small changes in flower morphology and a delay of bud abortion, flower senescence, and abscission of flowers. Transgenic Res. 2020;29:429-442.
[30] Aida R, Yoshida T, Ichimura K, Goto R, Shibata M. Extension of flower longevity in transgenic torenia plants incorporating ACC oxidase transgene. Plant Sci. 1998;138:91-101.
[31] World Health Organization. Guidelines for drinking-water quality Vol I. Geneva: WHO; 2008.
[32] Kuan KB, Othman R, Rahim KA, Shamsuddin ZH. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilization of maize under greenhouse conditions. Plos One. 2016;11:e0152478.
[33] Huang H, Huang M, Fan G, Liu X, Wang J, Duan Q, et al. Isolation and characterization of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing plant growth-promoting rhizobacteria from carnation soil and roots. Afr J Microbiol Res. 2013;7:5664-5668.
[34] Zhang YF, He LY, Chen ZJ, Zhang WH, Wang QY, Qian M, et al. Characterization of lead-resistant and ACC deaminase-producing endophytic bacteria and their potential in promoting lead accumulation of rape. J Hazard Mater. 2011;186:1720-1725.