Times New Roman 14 Capitalized Each word (Centred)

1.5 cm margin on all sides (Top, Bottom, Left and Right side) of the page for whole manuscript

ISSN 2091-2609

## Characterization and Optimization of 1-Aminocyclopropane-1-Carboxylate Deaminase (Accd) Activity in Different Rhizospheric PGPR along with Microbacterium sp. Strain ECI-12a

Times New Roman, font size 12, Lower case, Bold, Centred

**Umesh Prasad Shrivastava1\* and Ashok Kumar2**

Asterisk (\*) for the corresponding author

Superscript for indicating affiliation of author and co-authors

1\*Department of Botany, Tribhuvan University, TRM Campus, Birganj, Nepal

2School of Biotechnology, Banaras Hindu University, Varanasi-221005, India

Times New Roman, font size 10, Lower case and centred for affiliation of author and co-authors

Superscript for indicating affiliation of author and co-authors

\*Corresponding author email: [upshrivastava@gmail.com](mailto:upshrivastava@gmail.com)

Asterisk (\*) for the corresponding author

Abstract should be maximum 250 words, Times New Roman, font size- 9, single column, Justified, Line spacing - Multiple 1.15

Times New Roman, font size-12, Bold for all main subheadings

**Abstract**

A total of nine strains of plant growth promoting rhizobacteria were analyzed for ACC deaminase activity, where highest ACC deaminase activity was found in *Klebsiella* sp strain ECI-10A (539.1nmol α-keto butyrate/ mg protein/h) and lowest in *Microbacterium* sp strain ECI-12A (122.0 nmol α-keto butyrate/ mg protein/h). Although *Microbacterium* sp strain ECI-12A showed lowest level of ACC deaminase activity, but, the species of *Microbacterium* isolated from rhizosphere is the first report. *Microbacterium* sp strain ECI-12A was also analyzed under varying conditions of time, amount of 1-Aminocyclopropane-1-carboxylate (ACC), and temperature for optimization of the ACC deaminase activity. The optimum activity was recorded with the supplementation of 5mM ACC at 30oC temperature after 24h of culture growth. All the nine strains showed *acdS* gene in the PCR amplification of that gene. No any rhizospheric *Microbacterium* species showing ACC deaminase activity have been reported earlier, therefore, we report here ACC deaminase activity in *Microbacterium* sp ECI-12A isolated from rice rhizosphere is a novel finding.

3-6 key words, separated by semi colon (;)

**Key words:** ACC deaminase activity; rhizospheric bacteria; *Microbacterium* sp; PGPR.

References to the literature cited for the manuscript should be written as shown here. In case of multiple references, they should be separated by (;) sign

Manuscript (except abstract) should be Times New Roman, font size-10, line spacing: multiple-1.15, 6 pt before and 6 pt after paragraph, justified

## **Introduction**

1-Aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme capable of hydrolyzing ACC, the immediate precursor of ethylene was firstly reported in 1978, which was isolated from *Pseudomonas* sp. strain ACP (Honma and Shimomura, 1978). The ACC deaminase has been detected in the fungus, *Penicillium citrinum* and in a number of bacteria (Ma *et al.,* 2003; Blaha *et al.,* 2006; Madhaiyan *et al.,* 2006; Belimov *et al.*, 2007). The gene responsible for ACC deaminase activity (*acdS*) has been recently found in *Azopirillum*., *Burkholderia cepacia* genomovars (which include PGPR, phytopathogens and opportunistic human pathogens), and *Agrobacterium* genomovars (Blaha *et al.,* 2006). These microorganisms were identified by their ability to grow on minimal medium containing ACC as its sole nitrogen source (Honma and Shimomura, 1978; Belimov *et al.,* 2007; Ma *et al.*, 2003).

The plant growth-promoting bacterium, *Pseudomonas putida* GR12-2, whichcontains the enzyme ACC deaminase, stimulates root elongation (Glick *et al.*, 1994) andsignificantly reduces the level of ACC in emerging rootsand shoots. Three separate mutants of *Pseudomonas**putida*GR12-2, deficient in ACC deaminase activity, were reported to lose the ability to promote canolaroot elongation under gnotobiotic conditions (Glick*et al*., 1994)**.** The ACC in the exudates may betaken up by the bacteria andsubsequently hydrolyzed by the enzyme, ACC deaminase, to ammonia andα-ketobutyrate. The uptake andcleavage of ACC byplant growth-promoting bacteria decrease the amount of ACC outside the plant. Increasing amounts of ACCare exuded by the plant in order to maintain the equilibrium between interna1 andexternal ACC levels. It is proposed that plant growth-promoting bacteria that possess the enzymeACC deaminase and are bound to seeds or roots of seedlings can reduce the amount of plant ethylene and thus cause inhibition of root elongation. Thus, theseplants should have longerrootsandpossibly longer shoots as well, in as muchas stem elongation, except in floodingresistant plants, is also inhibited byethylene (Abeles *et al*., 1992). Soil bacteria that have ACC deaminase activity should then have a selective advantage over other soi1bacteria in situations in whichthe mainbacterial nutrients are fromexudates of plants (Shah *et al.*, 1998).It should also beborne in mindthat soil bacteria may acquire ACC deaminase genes bymechanisms other than fortuitous mutation-transfer of sucha gene fromanother soil bacterium is another possible mechanism. Theregulation of ethylene production in plants, especially to prevent increased ethylene production and accumulation, may reduce many of the inhibitory effects of this hormone (Jacobson *et al.*, l994). Many agricultural andhorticulturalcrops are particularly sensitive to ethylene levels which regulate fruitripening and control the deleterious effects of senescence in vegetables and flowers (Sisler andSerek, 1997). The bacterial enzyme, ACC deaminase, ispotentially a valuable tool for controlling the levels, and hence the effects of ethylene in plants. ACC deaminase hasalready been used to substantially reduce ethylene levels in transgenic tomato plants which have exhibited a prolonged ripeningphase (Klee*et al.,* 1991) and to lower stress ethylene levels following infection by bacterial and fungalpathogens (Lund*et al.*, 1998). Strainsof plant growth-promoting bacteria thatcontain ACC deaminase are known to reduce ACC, andhence ethylene, levels in canola seedlings (Penrose and Glick, 2003), promote rootelongation in a variety of plants (Hall *et al.*, 1996), decrease the deleterious effects of floodingon tomato plants, and prolong the shelf-life of ethylene sensitive cutflowers (Klee*et al.,* 1991). Therefore, plant growth promoting bacteria containing ACC deaminase activity can be utilized for the improvement of crop yields. Based on our knowledge, there is no any report of *Microbacterium* having ACC deaminase activity present in the rhizosphere of plants, whereas, only a few endophytes has been reported for this activity. The objective of the present study was to evaluate the activity of the ACC deaminase enzyme in rhizospheric plant growth promoting rhizobacteria with special reference to *Microbacterium* in the various conditions. In addition to above objective, we attempted to find out the presence of *acdS* gene in all the strains used in this study.

## **Materials and Methods**

## ***Bacterial strains and growth conditions***

Bacterial strains and their culture conditions of rhizospheric plant growth promoting bacteria showing nitrogen fixation, IAA production, siderophore production and P-solubilization properties were previously reported (Shrivastava, 2012). With an objective to optimize of various growth conditions for the estimation of ACC deaminase activity and amplification of *acdS* gene, nine most efficient strains isolated from rice rhizosphere of Indo-Nepal border region (1 *Microbacterium* sp strain ECI-12A; 3 *Klebsiella* sp strains ECI-10A, AF-4C and BN-4A; 2 *Agrobacterium* sp strains AF-1D and BN-2A; 2 *Pseudomonas* sp strains AF-4B and PN-4D and 1 *Serratia* sp strain AF-5A) were taken.

## ***Estimation of 1-Aminocyclopropane-1-Carboxylate Deaminase (ACC Deaminase) Activity***

For the measurement of ACC deaminase activity, selected isolates were grown overnight in 10 mL of NB medium and thereafter harvested by centrifugation. The pellet was washed with normal saline and suspended in 7.5 mL of JNFb- medium containing 5 mM of 1-aminocyclopropane-1-carboxylate (ACC). Tubes were incubated at 28oC with shaking (120 rpm) for growth. ACC served as the sole source of nitrogen in the medium. After 24h of growth, cells were centrifuged at 8000 rpm at 4oC for 10 min. The pellet was suspended in 1 mL of 0.1M Tris-HCl (pH 7.6) and again centrifuged at 15000 rpm for 15 min. Pellet was collected and supernatant discarded. The pellet was re-suspended in 600 μL of 0.1 M Tris-HCl (pH 8.5). 30 μL of toluene was added to the cell suspension and vortexed at higher setting for 30 s. Tube was kept at 4oC for 1h and then centrifuged at 1200 rpm for 10 min at 4oC. The thin layer of toluene was aspirated by micro-pipette gently. Now, the toluenized cells were equally distributed in two eppendroff tubes. First part was stored at 4oC for protein assay and other part was used for ACC deaminase assay immediately. 200 μL of toluenized cells was transferred in a fresh 1.5 mL microcentrifuge tube and 20 μL of 0.5 M ACC was added to the suspension. It was briefly vortexed and incubated at 30oC for 15 min. 1.0 mL of 0.56 M HCl was added, vortexed and centrifuged for 10 min at 12000 rpm. Now, 1mL of the supernatant was taken in another tube and 800 μL of 0.56 M HCl was added and vortexed briefly. Thereafter 300 μL of 2,4, dinitrophenylhydrazine (2 % w/v) was added to the tube. It was mixed properly by vortexingand incubated at 30oC for 30 min. 2μL of 2M NaOH was added and after mixing absorbance was recorded at 540 nm. The amount of μmol of α-ketobutyrate produced by this reaction was determined and compared with a standard curve of α-ketobutyrate ranging between 0.1 and 1.0 μmol. For the purpose of standard curve generation a sock solution of 100 mM α-ketobutyrate (Sigma-Aldrich Co., USA) was prepared in 0.1M Tris-HCl pH 8.5 and stored at 4oC. Enzyme activity was expressed as μ mol/mg protein/h.

## ***Amplification of acdS gene***

## The primers 5’- GGCAAGGTCGACATCTATGC-3’ and 5’-GGCTTGCCATTCAGCTATG-3’ (Duan et al., 2009) were used to amplify acdS gene. The thermal profile for amplification was 2-min initial denaturation at 94oC, 35 cycles of 1-min denaturation at 92oC, 50-s primer annealing at 58oC, and 1 min of elongation at 72oC. The amplified products were visualised with ethidium bromide stained agarose gel electrophoresis.

**Result and Discussions**

## ***Estimation of ACCD activity in selected isolates***

ACCD activity is indirectly responsible for growth promotion in plants; therefore, its activity was measured. Out of nine isolates tested highest activity was found in *Klebsiella* sp strain ECI-10A followed by *Pseudomonas* sp strain AF-4B. All the isolates showed activity in the range of 122 – 539.1 nmol α-ketobutyrate/mg protein /h (Table 1).

***Time course assay of ACCD activity***

Since all the isolates tested showed appreciable level of ACCD activity, it was desirable to test time course appearance of activity. Accordingly the *Microbacterium* sp strain ECI-12A was incubated in JNFb- medium containing 5mM ACC and ACCD activity was measured at desired time intervals (0, 4, 8, 12, 24 and 48h). It is evident from the data of Fig.1 that there was presence of some basal activity at 0 h which increased with time of incubation. Activity showed linear increase from 8 h, the maximum level was attained at 24 h (316.0 nmol α-keto butyrate/mg protein/h). Beyond 24 h there was no significant increase; it was almost constant after 24 h (Fig. 1)

***Do varying concentrations of ACC affect activity?***

Effect of varying concentrations of ACC was tested so as to find out the optimal concentration required for the ACCD activity. It is evident from the result of Fig. 2 that there was negligible activity without ACC addition to culture, but activity started appearing with the addition of as low as 0.1mM ACC in the culture medium. The level of enzyme activity increased with the increasing concentrations of ACC and maximum increase was attained with 5mM ACC. Further increase in ACC concentration did not show any increase in activity (data not shown).

## ***Effect of varying temperature on ACCD activity***

Since temperature plays important role in the regulation of plant growth promoting features of any bacteria, it was desirable to test ACCD activity at varying temperature. Keeping this objective in mind, ACCD activity at varying temperature was tested in *Microbacterium* sp strain ECI-12A. It is evident from the data of Fig. 3 that maximum ACCD activity was observed at 30oC and thereafter there was decrease. Only 33.2% activity was left at 35oC (Fig. 3).

## ***Amplification of acdS gene***

The PCR amplification of *acdS* gene responsible for ACCD activity was done in all the nine isolates. It is evident from the gel photograph (Fig. 4) that all the isolates showed amplicon of *acdS* gene (~ 1.0 kb).

Amelioration of salt stress has been reported in canola (*Brassica napus* L.) growth by the ACCD containing fluorescent pseudomonads (Jalili *et al.*, 2009). Prevalence of ACC deaminase activity in various PGPR including *Enteobacter cloacae* (Belimov *et al.*, 2005), *Pseudomonas* sp ACP (Sheehy *et al.*, 1991), *Serratia quinivorans* SUD165 (Belimov *et al.*, 2005), *Pseudomonas putida* strain UW4 (Shah *et al.*, 1998) and *Klebsiella pneumonie* strain Kp 342 (Iniguez *et al.*, 2005) as well as certain yeast and fungi (Yao *et al.,* 1995) has been reported. Results of the present finding are in agreement with above reports since isolates reported here in also belong to the genus *Klebsiella* sp (AF-4C and BN-4A), *Pseudomonas* sp (AF-4B and PN-4D), *Serratia* sp (AF-5A) and *Agrobacterium* sp (AF-1D and BN-2A). However presence of ACC deaminase activity in the *Microbacterium* species has not been reported from rhizosphere of any plant except a few reports from endophytic bacteria. Endophytic *Microbacterium* sp G16 isolated from rape (*Brassica napus*) roots showed ACC deaminase activity (Sheng *et al*., 2009), whereas ACC deaminase activity showing *Microbacterium* sp ECI-12A isolated from rice rhizosphere is a first report. Time course study of the activity suggests that the presence of ACC in the medium is prerequisite for optimal activity of the enzyme. ACC deaminase activity plays vital role in maintaining ethylene level in any plants. ACC released exudates by roots of plants may be utilized as nitrogen source by root associated bacteria if they possess ACC deaminase enzyme. It has been reported that plants treated with ACC deaminase containing bacteria have longer roots and can show resistance to inhibitory effects of ethylene stress on plant growth. Several stresses such as flooding, heavy metals, salinity as well as pathogens are known to induce ethylene stress. Under such stresses PGPR possessing ACC deaminase activity would be useful to counteract the inhibitory effects imposed by ethylene. Furthermore, results of this study clearly show that all the nine isolates could be exploited to manage the ethylene stress if they establish colonization in root region of any plants.

**Acknowledgement**

The first author is thankful to ICCR, Government of India for financial supports and Tribhuvan University, Nepal for study leave. This study was partly supported by a research grant sanctioned to Prof. Ashok Kumar (the second author), ICAR, New Delhi (No. NBAIM/AMAAS/MD (19)/AK/BG, New Delhi).

**References**

Abeles FB, Morgan PW and Saltveit ME (1992) Ethylene in Plant Biology. Academic press, San Diego.

Belimov AA, Dodd IC, Safronova VI, Hontzeas N and Davies WJ (2007) *Pseudomonas brassicacearum* strain AM3 containing 1-aminocyclopropane-1-carboxylate deaminase can show both pathogenic and growth-promoting properties in its interaction with tomato. *J. Exp. Bot.* **58**:1485-1495. DOI: [10.1093/jxb/erm010](http://dx.doi.org/10.1093/jxb/erm010)

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S and Glick BR (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol. Biochem.* **37**:241-250. DOI: [10.1016/j.soilbio.2004.07.033](http://dx.doi.org/10.1016/j.soilbio.2004.07.033)

References should be Times New Roman, font size- 9, Indentation: special hanging 1.0 cm, single spacing, 6 pt before and 6 pt after paragraph. Each reference should be written with hyperlinked DOI (If available) at last without underlined & font color automatic.

**For Journal Reference**  
Shrivastava UPand Kumar A (2011) A Simple and Rapid Plate Assay for the Screening of Indole-3-acetic Acid (IAA) Producing Microorganisms. *Int. J. Appl. Biol. Pharma. Technol.* **2**: 120-123.  
Jha PN andKumar A (2009) Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. *Microbial Ecol*. **58**: 179-188. **For Chapter in Book Reference**  
Kumar A, Rai AK, Shrivastava UP**,** Tyagi MB, and Kumar A (2011) Microcystins, a Novel Class of Toxins from Cyanobacteria. In: Roy BK and Chandary BR (Eds) *Plant Genome; Conservation, Manipulation and Diversity.* ***Chapter 11***, Narosa, New Delhi, 129-139.  
**For Book Reference**  
Benson HJ (1998) Microbiological applications: A laboratory manual in general microbiology. McGraw-Hill College.   
**For Patent Reference**Aviv H, Friedman D, Bar-Ilan A and Vered M (1996) Submicron emulsions as ocular drug delivery vehicles, U.S. Patent US 5496811.

Blaha D, Prigent-Combaret C, Mirza MS and Moenne-Loccoz Y (2006) Phylogeny of the 1-aminocyclopropane-1-carboxylic acid deaminase-encoding gene *acdS* in phytobeneficial and pathogenic proteobacteria and relation with strain biogeography. *FEMS Microbiol. Ecol.* **56**:455-470. DOI: [10.1111/j.1574-6941.2006.00082.x](http://dx.doi.org/10.1111/j.1574-6941.2006.00082.x)

Duan J, Muller KM, Charles TC, Vesely S and Glick BR (2009) 1-aminocyclopropane-1-carboxylic acid deaminase genes in Rhizobia from southeren Saskatchewan. *Microb. Ecol.* **57**: 423-436. DOI: [10.1007/s00248-009-9493-0](http://dx.doi.org/10.1007/s00248-009-9493-0)

Fallik E, Okon Y, Epstein E, Goldman A and Fischer M (1989) Identification and quantification of IAA and IBA in *Azospirillum-brasilense*-inoculated maize roots. *Soil. Biol. Biochem.* **21:**147-153. DOI: [10.1016/0038-0717(89)90024-2](http://dx.doi.org/10.1016/0038-0717(89)90024-2)

Glick BR, Jacobson CB, Schwarze MMK and Pasternak JJ (1994) 1-aminocyclopropane-1-carboxylic acid deaminase mutants of the plant-growth promoting rhizobacterium *Pseudomonas putida* GR12-2 do not stimulate canola root elongation. *Can. J. Microbiol.* **40:**911-915. DOI: [10.1139/m94-146](http://dx.doi.org/10.1139/m94-146)

Glick BR, Liu CP, Ghosh S and Dumbroff EB (1997) Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Soil Biol. Biochem.* **29**:1233-1239. DOI: [10.1016/S0038-0717(97)00026-6](http://dx.doi.org/10.1016/S0038-0717(97)00026-6)

Hall JA, Peirson D, Ghosh S and Glick BR (1996) Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Israel J. Plant Sci.* **44:**37-42. DOI: [10.1080/07929978.1996.10676631](http://dx.doi.org/10.1080/07929978.1996.10676631)

Honma M and Shimomura T (1978) Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric. Biol. Chem.* **42**:1825-1831. DOI: [10.1271/bbb1961.42.1825](http://dx.doi.org/10.1271/bbb1961.42.1825)

Iniguez AL, Dong YM, Carter HD, Ahmer BMM, Stone JM and Triplett EW (2005) Regulation of enteric endophytic bacterial colonization by plant defenses. *Mol Plant-Microbe Interact.* **18**:169-178. DOI: [10.1094/MPMI-18-0169](http://dx.doi.org/10.1094/MPMI-18-0169)

Jacobson CB, Pasternak JJ and Glick BR (1994) Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant-growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Can. J. Microbiol.* **40:**1019-1025. DOI: [10.1139/m94-162](http://dx.doi.org/10.1139/m94-162)

Jalili F, Khavazi K, Pazira E, Nejati A, Rahmani HA, Sadaghiani HR and Miransari M (2009) Isolation and characterization of ACC deaminase-producing fluorescent Pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. *J. Plant. Physiol.* **166**:667-674. DOI: [10.1016/j.jplph.2008.08.004](http://dx.doi.org/10.1016/j.jplph.2008.08.004)

Klee HJ, Hayford MB, Kretzmer KA, Barry GF and Kishore GM (1991) Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *Plant Cell* **3:**1187-1193. DOI: [10.2307/3869226](http://dx.doi.org/10.2307/3869226)

Lund ST, Stall RE and Klee HJ (1998) Ethylene regulates the susceptible response to pathogen infection in tomato. *Plant Cell* **10:**371-382. DOI: [10.1105/tpc.10.3.371](http://dx.doi.org/10.1105/tpc.10.3.371)

Ma WB, Sebestianova SB, Sebestian J, Burd GI, Guinel FC and Glick BR (2003) Prevalence of 1-aminocyclopropane-1-carboxylate deaminase in *Rhizobium* spp. *Antonie Van Leeuwenhoek*  **83**:285-291. DOI: [10.1023/A:1023360919140](http://dx.doi.org/10.1023/A:1023360919140)

Madhaiyan M, Poonguzhali S, Ryu J and Sa T (2006) Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. *Planta* **224**:268-278. DOI: [10.1007/s00425-005-0211-y](http://dx.doi.org/10.1007/s00425-005-0211-y)

Patten CL and Glick BR (1996) Bacterial biosynthesis on indole-3-acetic acid. *Can. J. Microbiol* .**42**:207-220. DOI: [10.1139/m96-032](http://dx.doi.org/10.1139/m96-032)

Penrose DM and Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiologia Plantarum* **118**:10-15. DOI: [10.1034/j.1399-3054.2003.00086.x](http://dx.doi.org/10.1034/j.1399-3054.2003.00086.x)

Shah S, Li JP, Moffatt BA and Glick BR (1998) Isolation and characterization of ACC deaminase genes from two different plant growth-promoting rhizobacteria. *Can. J. Microbiol.* **44**:833-843. DOI: [10.1139/w98-074](http://dx.doi.org/10.1139/w98-074)

Sheehy RE, Honma M, Yamada M, Sasaki T, Martineau B and Hiatt WR (1991) Isolation, sequence, and expression in *Escherichia-coli* of the *Pseudomonas* sp strain ACP gene encoding 1-aminocyclopropane-1-carboxylate deaminase. *J. Bacteriol.* **173**:5260-5265.

Sheng XF, He LY, Zhou L, and Shen YY (2009) Characterization of *Microbacterium* sp. F10a and its role in polycyclic aromatic hydrocarbon removal in low-temperature soil. *Can J Microbiol* **55**: 529–535. DOI: [10.1139/W09-005](http://dx.doi.org/10.1139/W09-005)

Shrivastava UP (2012)Molecular Study of Rice Plant Rhizobacteria of Indo-Nepal Border. LAP LAMBERT Academic Publishing GmbH & Co. KG, Heinrich-Bӧcking-Str. 6-8, 66121 Saarbrücken, Germany

Sisler EC and Serek M (1997) Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum* **100:**577-582. DOI: [10.1111/j.1399-3054.1997.tb03063.x](http://dx.doi.org/10.1111/j.1399-3054.1997.tb03063.x)

Xie H, Pasternak JJ and Glick BR (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* CR12-2 that overproduce indoleacetic acid. *Curr Microbiol* **32**:67-71. DOI: [10.1007/s002849900012](http://dx.doi.org/10.1007/s002849900012)

Yao M, Horiuchi A, Tanaka I and Honma M (1995) Crystallization of 1-aminocyclopropane-1-carboxylic acid deaminase from yeast. *Protein Peptide Lett.* **2**:305-30.



Fig. should be labeled below the fig., font size-10, single spacing

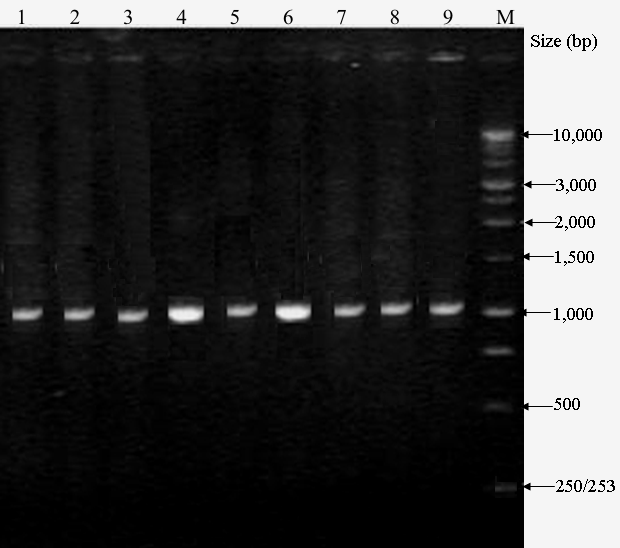
Fig. 1: Time course induction of ACCD activity in *Microbacterium* sp strain ECI-12A. Culture was grown with 5 mM ACC and activity was measured at desired time intervals. Data shown is the average of two independent experiments performed separately in identical condition.



**Fig. 2:** ACCD activity in *Microbacterium* sp strain ECI-12A in the presence of varying concentrations of ACC. Data shown is the average of two independent experiments performed separately in identical condition.

## 

Fig. 3: ACCD activities at varying temperature in the Microbacterium sp strain ECI-12A.



Photograph must be clear and sharp and follow the following guidelines:

* 300 dpi or higher size
* JPEG, GIF, TIFF and PDF formats are preferred

Fig. 4: Agarose gel photograph showing acdS gene fragment (1.0 kb) in nine selected isolates. Lane 1 to 9: *acdS* gene fragment of various strains (*Klebsiella* sp strain ECI-10A, *Microbacterium* sp strain ECI-12A, *Agrobacterium* sp strain AF-1D, *Pseudomonas* sp strain AF-4B, *Serratia* sp strain AF-5A, *Klebsiella* sp strain AF-4C, *Pseudomonas* sp strain PN-4D, *Agrobacterium* sp strain BN-2A, and *Klebsiella* sp strain BN-4A); M: 1.0 kb DNA marker.

Table 1:ACC deaminase activity in PGP strains

|  |  |
| --- | --- |
| **Bacterial strains** | **ACC deaminase activity**  **(nmol α-keto butyrate/ mg protein/h)** |
| *Klebsiella* sp strain ECI-10A | 539.1 |
| *Microbacterium* sp strain ECI-12A | 122.0 |
| *Agrobacterium* sp strain AF-1D | 237.3 |
| *Pseudomonas* sp strain AF-4B | 435.2 |
| *Klebsiella* sp strain AF-4C | 171.9 |
| *Serratia* sp strain AF-5A | 305.7 |
| *Pseudomonas* sp strain PN-4D | 358.4 |
| *Agrobacterium* sp strain BN-2A | 316.0 |
| *Klebsiella* sp strain BN-4A | 261.9 |

Data shown is the average of two independent experiments performed in identical conditions. The induction of ACCD activity was tested with 5mM ACC

Foot note of table should be font size-8, single spacing