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Screening and Identification of ACC Deaminase Producing Endophytic Bacteria

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Abstract

Aminocyclopropane-1-carboxylate deaminase (ACCD) producing endophytic microorganisms support plant growth under a variety of biotic and abiotic stress conditions such as drought, soil salinity, flooding, heavy metal pollution and phyto-pathogen attack. The application of plant growth promoting endophytes (PGPE) is an alternative strategy for improving plant fitness under stressful conditions. The 1-aminocyclopropane 1-carboxylate (ACC) deamianse producing PGPE offer drought stress tolerance by regulating plant ethylene levels. The aim of the present study was to evaluate the effect of ACC producing bacteria. Total five endophytic organisms were selected as plant growth promotion amongst them two were found to be 1-Amino Cyclopropane-1-Carboxylic acid (ACC) deaminase positive having the ability to use ACC, the precursor of ethylene, as sole nitrogen source. These endophytes also showed growth on DF minimal salt medium as well as liquid broth culture medium. The sequences were submitted to NCBI GenBank and their phylogenetic tree analysis was also carried out using MEGA 4.0 software.

Key words: Endophytic microorganisms; drought; ACC; stress tolerance; PGPE

Introduction

The gaseous hormone ethylene (C_2H_4) synthesized in plants tissues from the precursor 1-aminocyclopropane-1-carboxylic acid (ACC) is involved in multiple physiological and developmental processes in plants (Bleecker and Kende 2000). Ethylene also regulates plant responses to biotic and abiotic stresses (Abeles et al., 1992). Under ambient conditions, plants produce required levels of ethylene, conferring beneficial effects on plant growth and development; however, in response to biotic and abiotic stresses there is an often a significant

increase in the endogenous ethylene production that has adverse effects on plant growth and is thought to be responsible for senescence in plants (Woltering and Van Doorn 1988; Ali et al., 2012).

Plant growth promoting endophytes are a group of bacteria associated within the plants or different parts of plants. Endophytes are unique in their adaptation to specific chemical environment of host plant (Strobel 2003). On the basis of limited evidence, endophytic plant growth promoting bacteria, ACC deaminase activity is a key factor in their ability to promote the growth of plant (Hardoim et al., 2008). The decrease of plant ethylene levels relies on the ability of the ACC deaminase positive bacteria to take up ACC before its oxidation by the plant ACC oxidase. In this context, bacterial endophytes with high locally induced ACC deaminase activities might be excellent plant-growth promoters, because they ameliorate plant stress by efficiency blocking ethylene production (Hardoim et al., 2008).

Materials and Methods

Screening for ACC deaminase activity

Screening for ACC deaminase activity of drought tolererant endophytic isoltes were done based on their ability to use ACC as a sole source of nitrogen. All the five drought tolerant isolates were grown in DF (Dworkin and Foster) salt minimal medium (Dworkin and Foster, 1958) per litre: 4.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 2.0 g glucose, 2.0 g gluconic acid and 2.0 g citric acid with trace elements: 1 mg $FeSO_4.7H_{2O}$, 10 mg H_3BO_3, 11.19 mg $MnSO_4.H_2O$, 124.6 mg $ZnSO_4.7H_2O$, 78.22 mg $CuSO_4.7H_2O$, 10 mg MoO3, pH 7.2 and 2.0 g $(NH_4)_2SO_4$ as a nitrogen source. Following an incubation of 24 h 1 ml aliquot was removed from this culture and transferred to 50 ml sterile DF salts minimal medium in a 250 ml flask containing 3.0 mM ACC instead of $(NH_4)_2SO_4$ as the sole source of nitrogen. Following inoculation, the cultures were placed in a shaker for 24 h at 30°C. Dilutions of these cultures were plated on to solid DF salts minimal medium and incubated for 48 h at 30°C.

16S rRNA sequence analysis

Identification of isolates were carried out by 16S rRNA sequencing. Sequence data was aligned and analyzed by BLAST analysis with the NCBI database for finding closest homology.

Phylogenetic tree based on 16S rRNA using MEGA 4.0

Phylogenetic analysis based on 16S rRNA gene sequence constructed by using the software Mega version 4.0 (Tamura, 2007).

Results and Discussion

Screening for ACC deaminase

All the five drought tolerant isolates were screened for ACC deaminase based on the enrichment method, where ACC was used the sole nitrogen source. Among five isolates, two isolates MGT9 and MGT16 grew well on DF salt minimal medium (Fig. 1) with ammonium sulfate as the sole nitrogen source. Isolate MGT9 gave positive result with DF salt minimal medium containing 3.0 mM ACC serving as the sole nitrogen source (Fig. 2)

Fig 1: Growth on solid DF medium

Fig 2: Growth on medium containing 3.0 mM AC

Ali et al. (2013) found that isolate SorgP4 utilized ACC as a sole source of nitrogen by the production of ACC deaminase enzyme activity. Jha et al. (2011) found that MSA1 and MSA2 grew well on DF salt minimal medium containing ACC.

16S rRNA sequence analysis

Sequence obtained reported in present study has been deposited in the NCBI GenBank nucleotide sequence database under the accession numbers MG734139 for MGT9 and MG734141 for MGT16 (Table 1)..

No.	Isolates	Accession Number	Name of Organism	Solid DF salt medium	Liquid culture broth	Medium containing ACC
1.	MGT9	MG734139	*Paraburkholderia megapolitana*	+ve	+ve	+ve
2.	MGT16	MG734141	*Alcaligenes faecalis*	+ve	+ve	+ve

Table 1: Isolates showing growth on different medium containing ACC

Phylogenetic tree based on 16S rRNA using MEGA 4.0

Phylogenetic analysis based on 16S rRNA gene sequence constructed after multiple alignment of data by cluster. Distances and clustering with the neighbor-joining method was performrd by using the software packages Mega version 4.0 (Fig 3.).

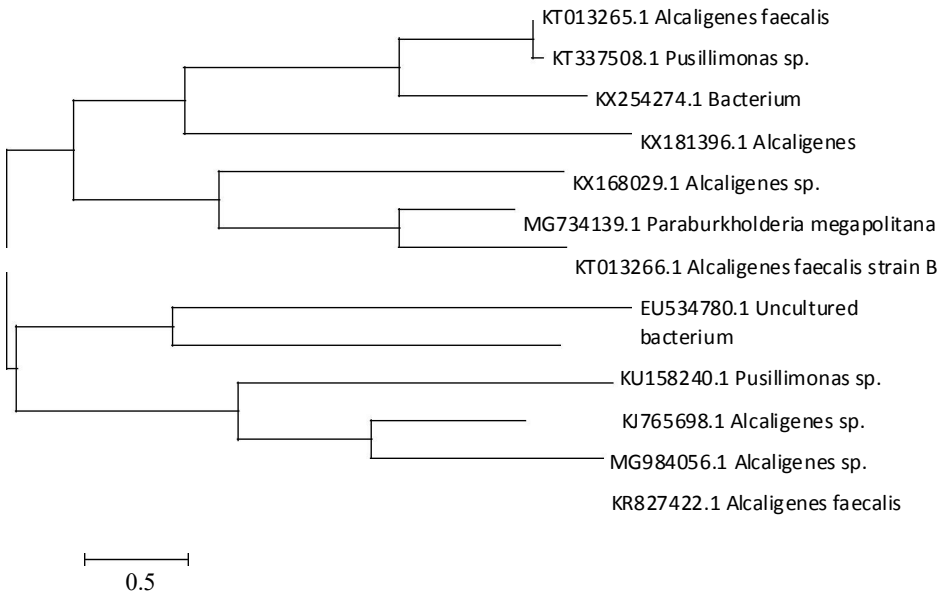

Fig 3: Phylogenetic analysis of MGT9 based on 16S rRNA

Conclusion

Isolate MGT9 and MGT16 are growing on solid DF salt medium, liquid culture broth as well as media containing ACC.

Isolates are able to grow were submitted to NCBI GenBank for accession number.

MGT9 and MGT16 are identified as *P. megapolitana,* and *A. faecalis.*

Phylogenetic tree was carried out using MEGA 4.0 software.

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