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Original Research Paper

Production and metabolism of IAA by *Enterobacter* spp. (Gammaproteobacteria) isolated from root nodules of a legume *Abrus precatorius* L.

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ABSTRACT

Indole acetic acid is a phytohormone that plays a central role in plant growth and development. The present work was undertaken to shed some light on IAA metabolism in roots, nodules and non-rhizobial bacteria in nodule of *Abrus precatorius* L. Three IAA producing bacterial strains (A3CK, A7CK and A27CK) were isolated from the root nodule of legume *A. precatorius* L. and identified as *Enterobacter* spp. (Gammaproteobacteria) based on biochemical characteristics and 16S rDNA sequence homology. The root nodules of this plant contain higher amount of tryptophan, IAA and total phenol than the non-nodulated roots; but IAA metabolizing enzymes IAA oxidase, IAA peroxidase (E.C. 1.11.1.7) and polyphenol oxidase (E.C. 1.14.18.1) content were reversed. The *Enterobacter* spp. were found to produce copious amount of IAA in tryptophan supplemented YEM medium. The IAA production was enhanced with the optimization of the suitable carbon as mannitol, nitrogen as L-asparagine and vitamin as biotin sources in culture medium. The IAA production was also found to be stimulated by protocatechuic acid and ferulic acid in culture medium for A3CK and A7CK strain, respectively, but inhibited by rest of the phenolics tested. The presence of PGPR traits including ACC deaminase (E.C. 4.1.99.4) activity might be essential for plant microbe interaction, nodule function and plant growth promotion.

1. Introduction

The Fabaceae is second agriculturally important family after Poaceae as 25% of the world's major crop derived from the members of this family and more than one-third of nutritional nitrogen requirement comes from legume seeds (Rivas et al., 2009). Legumes are also very important ecologically as they are responsible for a substantial global flux of nitrogen from atmosphere. Among the large number of legume species and varieties, only few nodule bacteria are studied. Until 2001, the nodule bacteria isolated from legume plants were classified as members of the order Rhizobiales of Alphaproteobacteria, including the genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium and Sinorhizobium* and were collectively known as rhizobia (Rivas et al., 2009). Sy et al. (2001) showed that *Crotalaria* spp. could be nodulated by a non-rhizobial Alphaproteobacterium *Methylobacterium* sp. Since then, *Devosia* sp.—nodule bacterium of 'non-rhizobial' Alphaproteobacteria has been reported in *Neptunia natans* (Rivas et al., 2002). Also in the other member of legume plants, *Aspalathus carnosa* and *Machaerium lunatum* are nodulated by *Burkholderia* spp. (members of the Betaproteobacteria) (Moulin et al., 2001) and scientists reported different Betaproteobacteria from several legume plants (Leelahawonge et al., 2010; Gehlot et al., 2013). Besides, different genera of the member Gammaproteobacteria have been reported from different legume plants by different scientists (Ghosh et al., 2015a). Unlike the members of Alphaproteobacteria and Betaproteobacteria, the members of Gammaproteobacteria are unable to produce nodule under in vitro condition and makes their role in nodule formation is questionable.

Legume plant–bacterial symbiotic interactions are of continued interest because the establishment of symbiotic relationship and development of nodule are important phenomena required for N_2 fixation. However, hormone content of the root nodule has drawn attention for their involvement in successful symbiosis and in the development of nodules. Rhizobia producing IAA in culture medium are isolated from the root nodule of leguminous plants (Datta and Basu, 1998; Shridevi and Mallaiah, 2007; Ghosh et al., 2008; Mandal et al., 2009; Ghosh et al., 2015b).

Abrus precatorius L. is a slender, perennial climber with enormous medicinal value (Mendes, 1986). The present study reports

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Table 1
Characteristic features bacterial strains isolated from the nodule of *Abrus precatorius* L.

Isolated strains	A3CK	A7CK	A27CK
Morphological characters			
Colony	Rough, mucoid, cream colour	Smooth, mucoid, white colour	Smooth, mucoid, white colour
Shape of cell	Straight rod	Straight rod	Straight rod
Physio-biochemical characteristic			
Acid gas production (glucose, sorbitol, sucrose)	+	+	+
Gelatinase	+	+	+
Nitrate reductase	+	+	+
Citrate utilization	+	+	+
Catalase test	+	+	+
Growth on glucose peptone medium	−	−	−
3-Ketolactose test	−	−	−
Lysine decarboxylase test	−	−	−
Indole test	−	−	−
Urease test	−	−	−
Nitrate reductase	+	+	+
VP test	−	−	−
IAA Production (μg/ml)	155.0 ± 1.73	142.6 ± 1.15	77.0 ± 1.52
Molecular Characteristics			
16SrDNA sequence length (bp)	1116	1403	1419
16SrDNA accession number	KJ664812	KC914336	KF220490

Symbol + means positive result; − means negative result.

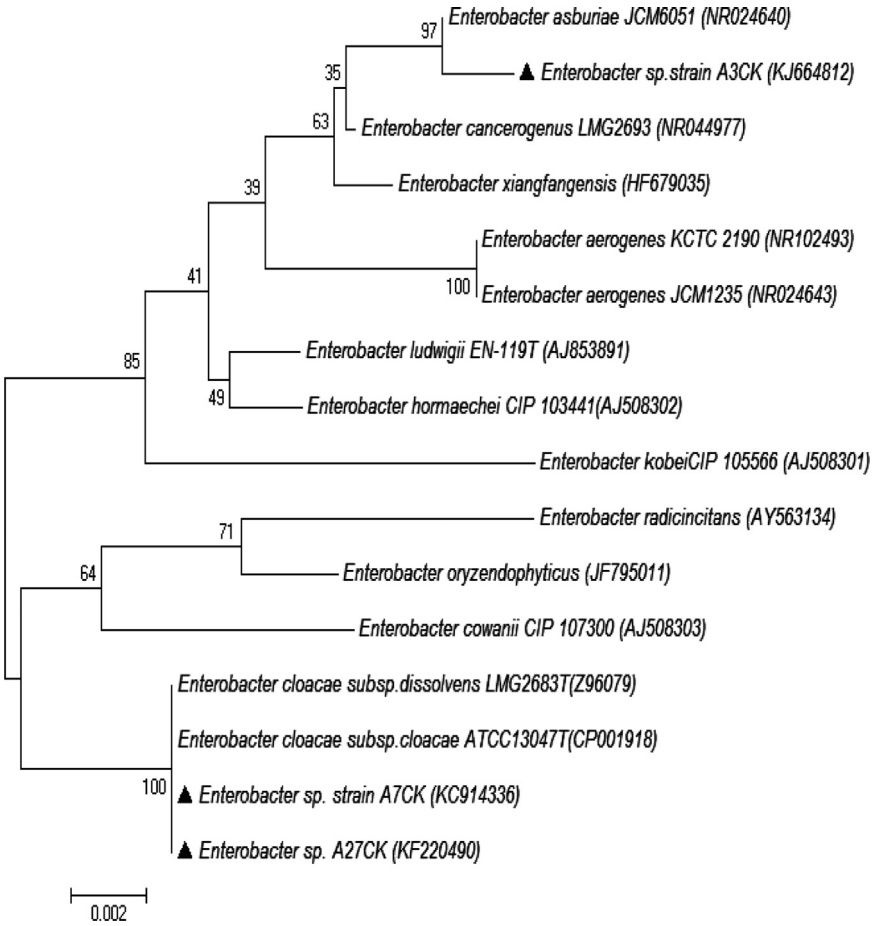

Fig. 1. Neighbour joining phylogenetic tree based on the Kimura 2-parameter model showing the position of *Enterobacter* sp. strain (A3CK, A7CK and A27CK) among the related taxa based on 16S rRNA gene sequences. Bootstrap values expressed as percentages of 1000 replications are given at branch points. Accession numbers are in parentheses. Bar 0.002 substitutions per nucleotide position.

the IAA producing Gammaproteobacterial strains (*Enterobacter* spp.) isolated from the root nodule of this medicinal legume and identification of their plant growth promoting traits. Attempts were also made to study the production and metabolism of IAA in root, nodules and its symbionts which might play interactive role legume–bacteria interaction.