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Evaluation of Plant Growth Promoting Traits of Mungbean Rhizobia

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Abstract Present study emphasized on plant growth promoting activities of rhizobial isolates isolated from mungbean nodule. Rhizobia has immense potential as a biofertilizer because its inoculation not only increase the plant growth attributes in all parameters but it also acts antagonist to biotic factors (plant pathogens). Thirty five rhizobial isolates were isolated using yeast extract mannitol medium and assessed for their plant growth activites. Out of thirty five almost solates showed plant growth promoting activites viz. ammonia excretion, siderophore production and ACC utilization. Three isolates MRH2, MRH3 and MRH4 showed maximum ammonia excretion, siderophore production and ACC utilization, make them suitable for further pot trials on the basis of plant growth promoting characteristics.

Keywords Mungbean, Rhizobia, Plant growth promoting traits, HCN production, ACC utilization.

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Introduction

Mungbean also kown as green gram is an important pulse crop of India. It is belonging to the family Leguminoceae and Papillonaceae. Mungbean (*Vigna radiata*) is under cultivation since ancient time in India. It serve are a major source of dietary protein for the vast majority of people. India is the largest producer of mungbean and account 54% of the world production and covers 65% of the world acreage. In India this crops are cultivated in three different seasons, viz., *kharif*, *rabi* and summer. Short maturity duration (<60 days) make the crop ideal for catch cropping, intercropping and relay cropping. It is grown during rainfed condition during *kharif* and on residual moisture during *rabi* in eastern and southern part of the country. However maximum area of its cultivation is under *kharif*, where intercropping with cotton, pearl-millet, castor, maize, sorghum, pigeonpea are also popular. In India it is cultivated under area of 2.92 million ha with a production of 1.42 million tonnes or with average productivity of 486 kg/ha. The major mungbean producing states in India are Andhra Pradesh, Haryana, Orissa, Madhya Pradesh, Maharashtra and Rajasthan accounting for 70% of total

Table 1. General characteristics of the test isolates.

Test isolates	Gram staining	Indole	Methylred	Vogus proskauer	Citrate	Oxidase	Catalase	H2S production	Starch hydrolysis
MRH1	- ve	–	+	+	–	+	+	+	+
MRH2	- ve	–	+	+	–	+	+	+	+
MRH3	- ve	–	+	+	–	+	+	+	+
MRH4	- ve	–	+	+	–	+	+	+	+
MRH5	- ve	–	+	+	–	+	+	+	+
MRH6	- ve	–	+	+	–	+	+	+	+
MRH7	- ve	–	+	+	–	+	+	+	+
MRH8	- ve	–	+	+	–	+	+	+	+
MRH9	- ve	–	+	+	–	+	+	+	+
MRH10	- ve	–	+	+	–	+	+	+	+
MRH11	- ve	–	+	+	–	+	+	+	+

country's production [1].

India is known as growing economic country but the credit limited to urban and suburban areas. More than 65% of world population lies in the villages. Hunger and poverty are there due to low job opportunities and low income level. Most of the population directly and indirectly depends on agriculture productivity benefits. But income from agriculture sources is fluctuating due low agriculture productivity. To feed poor and overgrown population need a sustained and environment friendly practices that help to increase productivity. These expectations cannot be met sustainably unless the soil fertility and productivity has been restored in the already degraded lands. Because with the use of chemical fertilizers increase the production cost and its hazardous nature led to resurgence of interest in the use of biofertilizers for enhanced the environment stability, lower cost production and better crop yield.

Among biofertilizers (plant growth promoting rhizobacteria (PGPR), phosphate solubilizing bacteria (PSB) and rhizobia), rhizobial isolates have shown potentials to be a promising technique in the practice of sustainable agriculture. Application of rhizobial isolates in the form of bioinoculants can be a potential substitute to harmful chemicals for enhancing growth and productivity of crop in ecofriendly manner. Rhizobia acts as a biofertilizers promotes plant growth in terms of biomass, nitrogen content, root and shoot length. Rhizobia can promote plant growth directly through biological nitrogen fixation, phytohormone production, phosphate solubilization, inhibition of ethylene level in response to biotic and abiotic stress or indirectly through inducing resistance to biotic agents (bacterial and fungal pathogen) [2]. Thus exploration mungbean nodulating rhizobial isolates give more promising rhizobial strain which can further use to improve agriculture productivity, soil erosion and prevents harmful effects of chemical fertilizers. Thus, present study emphasized on plant growth promoting activities of rhizobia isolated from mungbean root nodule.

Materials and Methods

Isolation of mungbean rhizobia

Plant (mungbean plant grown in CCSHAU farm field

Table 2. Plant growth promoting activities of *Rhizobium* isolates isolated from mungbean nodule.

Test isolates	ACC utilization	Ammonia production	Siderophore production
MRH1	+++	0.92	++
MRH2	+++	2.74	+++
MRH3	+++	3.70	+++
MRH4	+++	2.52	+++
MRH5	+++	1.60	++
MRH6	+++	0.25	–
MRH7	+++	0.52	–
MRH8	+++	–	–
MRH9	+++	0.75	++
MRH10	+++	0.26	++
MRH 11	+++	–	–

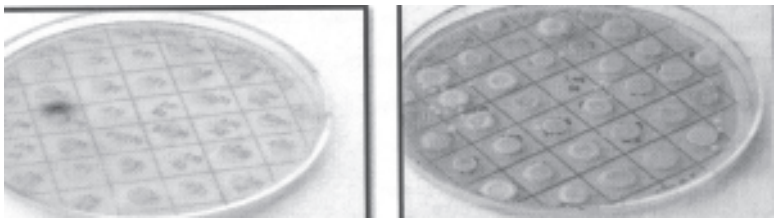

Fig. 1. ACC utilization by mungbean rhizobial isolate.

were uprooted carefully to get the intact nodule. Initially, detached nodules were washed under running tap water to remove the adhering soil porticles from nodule surface. Nodules were dipped in 70% ethanol and 0.1% of mercuric chloride ($HgCl_2$) solution for 30 seconds and later washed successively ten times with sterilized distilled water to remove the traces of toxic $HgCl_2$, surface sterilized nodules transferred in test tube containing 5 ml of sterilized distilled water. These nodules crushed with the help of sterilized rod to obtain a milky suspension of bacteriods. These were streaked on yeast extract mannitol agar media (YEMA) and further identify by gram's staning method [3].

Plant growth promoting traits

ACC utilization

All the rhizobial isolates were screened for utilization of ACC. Rhizobial cultures (3–5 days old grown) were spotted on the minimal medium plates supplemented with 2 mM ACC [4].

Siderophore production

Isolates were qualitatively detected using chrome azurol S (CAS) [5]. After 72 h of incubation, colonies were observed for orange halo zone formation.

Ammonia production

Rhizobial isolates were tested for ammonia excretion in peptone broth as per Chaney and Marbach [6]. After incubation, tubes were observed for the development of brown yellow color.

Results and Discussion

Rhizobial isolates were isolated from the nodule of mung bean plant grown in CCSHAU farm field. All the strains were gram- negative, did not absorb red color when cultured in YEMA containing congo red dye. *Rhizobium* colonies have a spherical shape, convex (convex), 2–4 mm diameter, high extracellular polysaccharide production, gummy (mucilaginous) and mostly yellow in the middle. Biochemical characteristics of selected rhizobial isolates described in Table 1.

Ethylene a phytohormone, synthesized from ACC is known to promote ripening of climaticeric fruits but, acts as inhibitor of nodulation in legumes. ACC deaminase is a microbial enzyme cleaves ACC to α ketobutyrate and ammonia, both of which are metabolized by most soil bacteria. Ultimately ACC producing rhizobia decreases ethylene level in host roots and thereby enhances nodulation. In most soil bacteria like rhizobia, a significant positive correlation was observed between ACC demainse activity and their ability to promote plant growth in various conditions, for example in wheat [7], Maize [8] and tomato [9]. ACC deaminase is usually present in bacteria at a low level, but they have the ability to grow on minimal medium containing ACC as a sole nitrogen source. ACC deaminase not only promotes plant growth but also protect plant from flooding, drought, organic contaminants and biotic factors (plant pathogen). A large number of mungbean isolates showed growth on plates supplemented with ACC and frequency of ACC utilizing isolates was found to be 80%, however, 32% of the isolates were high and 42% of the isolate

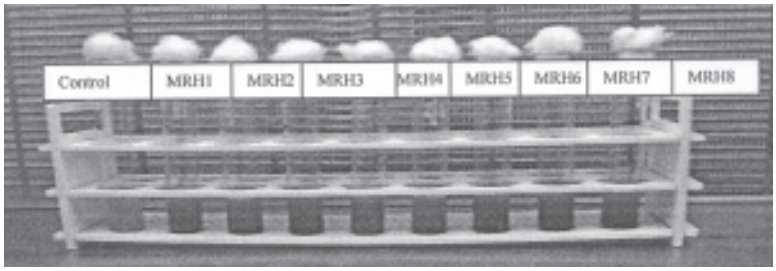

Fig. 2. Ammonia excretion by mungbean rhizobial isolate.

were moderate 6% of the isolates were low ACC utilizer (Fig. 1). These investigations are in line with studies of various research workers, Ma et al. [10] studied, reported five rhizobial isolates out of 13 produced characteristic activity of ACC utilization. Similar finding also reported by Alikhani and Yakhchali [11].

The ammonia released by microorganisms in soil is important because it benefits the crop. Accumulation of ammonia in soil may increase pH upto 9–9.5. It suppresses the growth of certain fungi and bacteria. The ammonia released by microoganisms play an important role in interaction between plant and rhizobia [12]. All the rhizobial isolates (eleven) tested for ammonia excertion. Most of the isolates were able to excrete ammonia, which varied from 0.92 to 3.70 µg in liquid medium after 4 days of incubation. High ammonia excretion was observed in MRH2, MRH3 and MRH4, low ammonia excretion was observed in MRH1, MRH5, MRH9 and rest of the isolates showed either negligible or did not show ammonia excretion (Table 2, Fig. 2). Similar results were also obtained by Deb et al. [13] who reported 20% rhizobial isolates isolated from *Cajanus cajan* L var were found to positive towards ammonia excretion.

Iron is fourth most abundant element on earth crust. Availability of soluble iron varies greatly in rhizosphere and soil. Most of the microorganisms have evolved specific high affinity mechanisms to acquire iron by producing extracellular siderophore. Siderophores is a bio-molecule of low molecular weight, which helps to plant in iron acquisition and also have antagonist effect against plant pathogenic microbes. Siderophore producing rhizobia not only promotes plant growth but also protect plant from

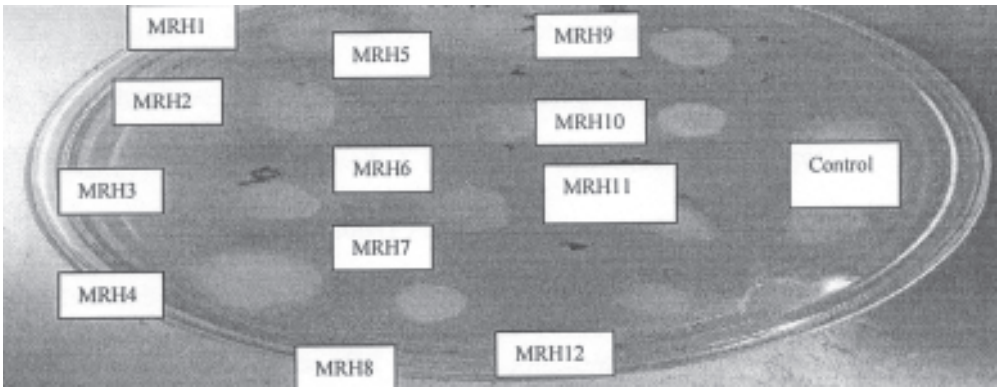

Fig. 3. Siderophore production (solid medium) by mungbean rhizobial isolate.

pathogenic microorganisms by acquisition of iron [14]. In the present study out of thirty five rhizobial isolates screened for siderophore production on CAS agar, only five showed orange color production and yellow-orange colored halo around the colonies, on CAS reagent overlaid on YEM agar (Table 2). Larger halo was formed around the colonies of strain MRH2, MRH3 and MRH4 in comparison to those of strain MRH8 and MRH9 after 72 h of incubation (Fig. 3). Similar results were reported by many researchers. Waheed et al. [15] found that 57% of rhizobial isolates obtained from pea plant were siderophore positive. Similar finding also reported by Bhargava et al. [16] able to found out siderophore positive strain from wild legumes grown in semi arid region of India.

Conclusion

We are successfully cultured thirty five rhizobial isolates on the YEMA media and on the basis of evaluation of plant growth promoting traits of different isolates it was concluded that novel rhizobial isolates particularly MRH2, MRH3 and MRH4 can be used for growth promotion in mungbean plant. Further, efficiency of these rhizobial isolates needed to be evaluated by pot trials for sustainable use of rhizobial isolate for agricultural purpose.

References

1. Singh AK, Singh SS, Prakash Kumar S, Dwivedi SK (2015) Pulses Production in India: Present Status, Bottleneck and Way Forward. J Agric Search 2 : 75—83.
2. Glick HR (2012) Plant growth promoting bacteria: Mechanism and applications. Scientilica 2012 : 1—15.
3. Vincent JM (1970) A manual for the practical study of root nodule bactera. Blackwell Scientific Publ, Oxford, UK, pp 164.
4. Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth promoting rhizobacteria. Pl Physiol 118 : 10—15.
5. Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160 : 47—56.
6. Chaney AL, Marbach EP (1962) In: Methods for evaluating biological nitrogen fixation. Bergerser FJ (ed). John Willey and sons, Chichester, New York. Clin Chem 8 : 130.
7. Zahir ZA, Farooq HM, Hussain M, Hussain A (2014) Application of ACC-deaminase containing rhizobacteria with fertilizers improves maize production under drought and salinity stress. Ind J Agric Biol 16 : 591—596.
8. Shaharoona B, Arashad M, Zahir ZA (2006) Effect of plant growth promoting rhizobacteria containing Acc-deaminase on maize (*Zea mays* L.) growth under axenic conditions and nodulation in mungbean (*Vigna radiata* L.). Lett Appl Microbiol 42 : 155—159.
9. Mayak S, Tirosh T, Glick BR (2004) Plant growth promoting bacteria confer resistance in tomatoes plants to salt stress. Pl Physiol Biochem 42 : 565—572.
10. Ma W, Guinel FC, Glick BR (2004) *Rhizobium leguminosarum* bv *viciae* I-aminocyclopropane 1-carboxylate deaminase promotes nodulation of pea plants. Appl Environ Microbiol 2 : 239—246.
11. Alikhani HA, Yakhchali B (2009) Potential use of Iranian rhizobial strains as plant growth-promoting rhizobacteria (PGPR) and effects of selected strains on growth characteristics of wheat, corn and alfalfa. Desert 14 : 27—35.
12. Martinez OV, Jorquera MA, Crowley DE, Gajardo G Mora, ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Pl Nutr 10 : 293—319.
13. Deb K, Deb P, Pandy P (2014) Screening the *Rhizobium* from *Cajanus cajan* for plant growth promoting factors. The Int J Sci & Technol 2 : 134—138.
14. Datta M (2011) Plant growth promoting rhizobacteria enhance growth and yield of chilli (*Capsicum annuum* L.) under field conditions. Aust J Crop Sci 5 : 531—536.
15. Waheed A, Afzal A, Sultan T, Rahman IU, Shah A (2014) Isolation and biochemical characterization of rhizobium from pea crop at Swabi. Int J Biosci 4 : 231—240.
16. Bhargava Y, Murthy JSR, Kumar RM, Narayana Rao (2016) Quantification of Phytohormones and ACC-Deaminase Activity in *Rhizobium* spp. Adv in Microbiol 6 : 1—12.