Madras Agric. J., 2019; doi:10.29321/MAJ 2019.000291

RESEARCH ARTICLE

Non-Rhizobial Nodule Associated Bacteria (NAB) From Blackgram (*Vigna mungo* L.) and their possible role in plant growth promotion

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Received : 10th June, 2019
Revised : 12th June, 2019
Accepted : 12th June, 2019

ABSTRACT

Rhizosphere engineering is one of the means to increase the competitive survivability of rhizobial bio-inoculants. This can be achieved by altering the rhizospheric community structure with a co-compatible and co-operative microbial partner. There exists a proto-cooperative mode of interaction between the non-rhizobial endophytes (NRE) and the endosymbiont *Rhizobium*, which can be unraveled to enhance their competitive survivability. In the present investigation, 8 isolates of nodule associated bacteria (NAB) and 1 rhizobial species were isolated from the root nodules of black gram (*Vigna mungo* L.,) cultivar VBN6. Among the NAB isolates, 75% were gram-positive bacilli belonging to the phylum firmicutes. The promising NABs that showed maximum PGP features were phylogenetically affiliated as *Bacillus subtilis* NANEB1 and *Paenibacillus taichungensis* TNEB6. Among them, *P.taichungensis* TNEB6 registered significant IAA production (15.39 $\mu g.ml^{-1}$), siderophore (10.7%), ammonia, HCN and also ACC deaminase activity (94.3 $mg^{-1} hr^{-1}$). Interestingly, 75 % of the NAB isolates could solubilize phosphorous. Furthermore, *P.taichungensis* TNEB6, when co-inoculated with *Rhizobium* sp showed significant plant growth promotion with respect to enhanced root and shoot length. The vigor index was also maximum in the treatment that received co-inoculation of *Rhizobium* sp and *P.taichungensis* (1812) Hence, the study suggests the scope of enhancing the yield potential and fitness of blackgram using a compatible co-inoculant, comprising a multifunctional NAB, *P. taichungensis* TNEB6 and *Rhizobium* sp.

Keywords: *nodules, nodule-associated bacteria, Blackgram, Paenibacillus, Rhizobium, Co-inoculant*

INTRODUCTION

The leguminous plants are symbiotically associated with rhizobia and their interaction plays a vital role in crop growth. Co-inoculation of rhizobia with other plant growth promoting rhizobacteria (PGPR) is one of the incredible means to enhance nutrient availability and growth for sustainable organic farming. Many rhizospheric Plant growth promoting rhizobacteria (PGPR) are already known to promote plant growth directly by the production of plant growth regulators and improvements in plant nutrient uptake or indirectly by the production of metabolites like antibiotics, siderophores, and antagonistic against phytopathogens. Moreover, simultaneous infection with rhizobia and rhizospheric bacteria increases nodulation and growth in a wide variety of legumes (Rajendran *et al*., 2012). Such nodule-assisting bacteria may be either free-living or endophytic. The endophytic bacteria resides intercellularly or intracellularly within host tissues and, therefore, more advantageous as compared to free-living counterparts by being protected from environmental stresses and microbial competitions (Sturz *et al*. 2000).

Root nodules form a rich niche for microbes and very few reports are available regarding the presence of other associated and endophytic entities. Nevertheless, some of the microbes perform a helper function for nodule- inducing rhizobia, while others can serve as opportunistic organisms in the nitrogen-rich habitat (Hoque et al., 2011). The presence of bacteria, other than *Rhizobium* in root nodules was first reported by Sturz et al., 1997 Several bacterial genera such as *Aerobacter*, *Pseudomonas*, *Agrobacterium*, *Bacillus*, *Chrysomonas*, *Curtobacterium*, *Enterobacter*, *Erwinia*, *Flavimonas*, *Sphingomonas* and *Exiguo bacterium* from diverse types of legumes have been reported (Pandya *et al*., 2013). However, these non-rhizobial endophytes (NRE) are unable to stimulate nodules, as they co-exist with nodulating rhizobia without making impairment to the hosts

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(Saini et al., 2015). It has been hypothesized that this NRE confers major ecological benefits, by producing biologically active metabolites and phytohormones such as indole–3 acetic acids, (IAA), gibberellins (GA), cytokinins and ACC deaminase (Rakholiya *et al.*, 2015). In addition, nodule endophytes render high beneficial uses to the legume crops by acquiring nutrients through nitrogen fixation, phosphate, Zn and K solubilization, *etc*. Also, these endophytes trigger ISR system *via* siderophore production and reduce the ethylene level in response to stress (Dudeja *et al*., 2012). More recently, Thanuja (2017) has reported that the microbial volatile organic compounds (mVOCs) produced by NRE assists nodule formation by Rhizobia.

Blackgram *(Vigna mungo L)* is the most important food legume and an annual pulse crop, widely cultivated in both tropical and sub-tropical countries. It is a cheap source of protein (17-34%) catering to the dietary requirements of the majority of the human population (Gour, 1993). In a global perspective, India is the largest producer and consumer of blackgram followed by Myanmar and Thailand. As an excellent source of plant protein blackgram is highly responsive to nitrogen and rhizobia was believed to be the only N_2-fixing symbiont of black gram nodules However, it may harbour other microbial inhabitants which are regarded as non-rhizobial endophytes (NRE) (De Meyer *et al*., 2015), nodule endophytes (Velazquez *et al*., 2013) or nodule- associated bacteria (NAB) (Rajendran *et al*., 2012) exhibiting negative nodulation. Some of the identified NRE isolates includes 71 genera, including *Dyella, Enterobacter, Pseudomonas* and *Steroidobacter* were observed in *Lespedeza* nodules (Busby *et al*., 2016); eight genera comprising *Paenibacillus, Bacillus, Klebsiella, Ensifer, Agrobacterium, Blastobacter, Dyadobacter* and *Chitinophaga* were isolated from the nodules of field-grown *Vignaradiata* (Pandya *et al*., 2013); and a wide variety of non-rhizobial bacteria (e.g., *Agrobacterium, Enterobacter, Paenibacillus*, and *Phyllobacterium*) can colonize *Glycyrrhiza* nodules (Li *et al*., 2012). With this background, the present investigation aimed to isolate and characterize the non-rhizobialendophytic bacteria from the nodules of black gram. The effect of the isolated NRE in nodule formation was evaluated to assess its role inside the nodules.

MATERIAL AND METHODS

Isolation of nre and rhizobia from the root nodules of black gram

Root nodules of blackgram were collected from the field of TNAU Eastern block (Latitude11°02" N, Longitude 76°57" E), to isolate the nodule endophytes. The root nodules were surface sterilized with 0.2% mercuric chloride and 70% of ethanol followed by four to six washes in sterile water to remove the chemicals (Vincent *et al*., 1970). The surface sterilized nodules were crushed and diluted with double sterilized water, and pour plating was done on TSA plates. The plates were incubated at 28±2°C for 3-4 days.The well isolated NRE colonies were picked from the plates and purified by repeated re-streaking. The isolates were named respectively according to the collection area. For authentication of NRE isolates from nodules, the uncrushed nodules are kept in TSA plates to ensure proper surface sterilization.

Morphological diversity of nodule – associated bacteria (nab)

All the NRE isolates from the root nodules of blackgram were observed for the colony morphology like colour, structure, texture, size and polysaccharide production. Cells of these NRE stained with Gram's staining method, and the stained cells were observed under the light microscope (100X).

Plant growth promoting attributes of nab

Siderophore production

The modified CAS assay was used to test the ability of rhizobial and non-rhizobial endophytic bacterial isolates to produce iron-binding compounds of siderophore type in a solid medium. CAS blue agar was prepared and Petri dishes were prepared with an appropriate medium. After solidifying the medium was cut into halves, one of which was replaced by CAS blue agar. The halves containing culture medium were inoculated with isolated cultures taken from stock cultures. The inoculum was placed as far as possible from the broad line between the two media and the plates were incubated in the dark at 28 ± 2° C for 10d. Uninoculated CAS agar plate served as control. The change in colour from blue to the orange or yellow colour indicated a positive reaction for siderophore production (Schwyn and Neilands, 1987).

Solubilization of phosphate

The ability of the endophytic isolates to solubilize insoluble phosphorus was observed by streaking or spot inoculation of the cultures on to Sperbergs hydroxyl appetite medium (Sperber, 1958) containing insoluble tri-calcium phosphate as a sole source of phosphorus. The efficiency of the isolates to solubilize phosphorus was determined by observing the clearing zone produced by phosphorus solubilization.

Determination of acc-deaminase activity

To determine the presence of ACC-deaminase, the ability of bacterial isolates to use ACC as N source was evaluated using the plate assays. The

ability to use ACC as N source is a consequence of the enzymatic activity of ACC-deaminase. Bacterial isolates were streaked on DF minimal salt agar media supplemented with 5.0mM ACC instead of $(NH_4)_2SO_4$ as N source and the plates were incubated at 28±2° C for 5d. After the incubation period, plates were observed for growth. The stock solution of ACC (M/s. Sigma, USA) filter sterilize through a 0.2µm membrane and frozen at -20°C (Penrose and Glick, 2003) was thawed and a 600 µl aliquot was added to 100 ml sterile DF mineral salts medium, just before the plating. The ability of a rhizobial and non- rhizobial endophytic bacterial isolates to utilize ACC was verified by maintaining the same isolate in control DF mineral salt supplemented with $(NH_4)_2 SO_4$.

Ammonia production

The ability of ammonia production was observed by all endophytic and rhizobial bacterial isolates inoculated with Peptone - water tubes and incubated at room temperature. After 2-7 d, a loopful of the endophytic and rhizobial isolates was added to a Nessler's reagent on a porcelain tile. The efficiency of the ammonia production was determined by the development of orange to brown colour.(Silpa *et al*.,2018)

HCN production

All the isolates were screened for the production of hydrogen cyanide. Briefly, the nutrient broth was amended with 4.4 g glycine and bacteria were streaked on a modified agar plate. A Whatman filter paper No. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of the plate. Plates were sealed with parafilm and incubated at 28±2°C for 4 d (Lorck 1948). Development of orange to the red color indicated HCN production.

Indole acetic acid (iaa) production

Indole acetic acid production was quantitatively measured for the NRE isolates. A hundred micrograms of a standard stock solution of IAA in 50 % ethanol was prepared and standard curve was prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of standard IAA solution in test tubes and bacterial cultures were grown in their respective broth and amended with tryptophan (5mM) for 2-3 d. Cultures were centrifuged at 10,000 rpm for 20 min. Two ml of supernatant was mixed with two drops of orthophosphoric acid and 4 ml of Salkowski reagent. Tubes were incubated at room temperature for 25 min under dark condition (Brick *et al*. 1991). The intensity of pink color was read at 530 nm in a Multimode Microplate reader (M/s.Molecular devices) and the amount of IAA produced was extrapolated from the standard curve.

Molecular characterization of nab

The genomic DNA from the three elite endophytic isolates was extracted using the standard protocol of hexadecyl-trimethyl ammonium bromide (CTAB) method with minor modifications(Porebski *et al*, 1997). The isolates were grown in nutrient broth and 96 h old culture of 10 ml quantity was centrifuged at 6,000 rpm for 5 min at 4°C. The supernatant was discarded; the pellet was suspended in 1 ml TE buffer, added with 0.5 ml of 1-butanol, vortexed well to mix with the cells followed by centrifuging at 5000 rpm for 5 min at 4°C. The supernatant was discarded and the pellet was resuspended in 2 ml of TE buffer and centrifuged again to remove all traces of butanol.

The pellet was resuspended in 1 ml TE buffer added with 100 µl lysozyme (10 mg ml^{-1} freshly prepared), and incubated at 28±2°C for 5min. After incubation, 100 µl of 10 per cent SDS and 25 µl of 100 µg ml^{-1} proteinase K were added, mixed well and incubated at 37°C for 1 h. To this, 200 µl of 5 M NaCl was added and mixed well. CTAB (10 per cent CTAB in 4.1 percent NaCl solution) at 150 µl was added, mixed well and incubated at 65°C for 10 min. The mixture was extracted with 1 ml of phenol: chloroform mixture and centrifuged at 6000 rpm for 15min at 4°C. The aqueous layer was transferred carefully to a new 2 ml microfuge tube and DNA was precipitated by adding 0.6 volume of ice-cold isopropanol, incubated for 1 h to overnight at -20°C. The DNA was pelletized by centrifugation at 12,000 rpm for 15 min at 4°C and the pellet was washed with 70 percent ethanol, dried under vacuum for 10 min and re-suspended in 50 µl of TE buffer. One µl of DNAse free RNAse (10 mg ml^{-1}) was added by swirling and incubated at 37°C for 30 min.

Full-length 16SrRNA gene using the primers pairs of 27F and 1492R was amplified (1500 bp) from the isolates by PCR. PCR amplification was performed in a thermo cycler (M/s. Eppendorf Master cycler, Germany) with following conditions: initial denaturation at 95°C for 1 min, 35 cycles consisting of 94°C for 1 min (denaturation), 60° C for 1 min (annealing), 72°C for 1 min (primer extension) and final extension at 72° C for 5 min. The amplified products were analyzed by electrophoresis in 1.5 percent agarose gel.

After separation of the PCR products in agarose gel, viewed and photographed using Alpha imager TM1200 gel documentation and analysis system. The band of the expected size was gel-purified using spin columns according to the manufacturer's instructions and eluted using sterile milli-Q water (Weisberg *et al*., 1991). The phylogenetic tree was constructed with existing 16S rRNA gene sequences from related eubacteria obtained from NCBI GenBank database by the neighbor-joining method (Saitou and Nei 1987) using MEGA 7.0 software (Tamura *et al*., 2007).

Influence of rhizobium and nab on blackgram by roll paper towel method

The selected rhizobial and NAB isolates were bio-assayed for their ability to promote or inhibit seedling growth (Ju *et al*.,2019). Seeds were surface sterilized with 0.02% sodium hypochlorite for 2 min and washed with distilled water for 4-5 min. Then the seeds were soaked for 20-30 min in 48h old selected bacterial endophytes broth cultures containing at least 10^8CFU.ml^{-1}. The seeds were kept on sterilized germination paper and incubated at 30°C. Seed germination, root and shoot lengths were recorded on the 8th day and the vigour index was calculated as follow:

VI (%) = (mean root length + mean shoot length) × germination %.

RESULTS AND DISCUSSION

A total of 8 nonrhizobial endophytic bacterial isolates were obtained from the root nodules of blackgram (35 DAS) with diversified morpho-traits.

Table 1.Endophytic bacterial count of blackgram nodules

Media	Population count (CFU *10^5 g^{-1} of fresh black gram nodules)
YEMA	3.6
TSA	5.6
NA	6.2

The intensity of nodule associated non-rhizobial endophytes(NRE) of legumes are very dense (Tariq *et al*., 2012). Struz *et al*., (1997) recorded7 bacterial genera and 15 species from the nodules of red clover plants. NAB populations were estimated as 4.0 x 10^4 CFU. g^{-1} nodule fresh weight. Uma Maheswari *et al.* (2013) also reported a population intensity of 7.6 x 10^3 and 8.6 x 10^3 CFU g^{-1} in nodules of blackgram on Tryptic Soy Agar (TSA) and Nutrient Agar (NA) plates respectively. The results of the present investigation also proved the existence of NAB inside the nodules of black gram. However, the blackgram nodules also registered almost the same population density (5.6 x 10^5 CFU g-1 to 6.2 x 10^5 CFU g^{-1} in fresh nodules of blackgram) that have been reported so far in other legumes (Table 1).

The morphological characteristics of all the 8 NAB isolates varied distinctly. It varied from gummy to non gummy, flat to raised, rough to smooth and the colony size varied from small to very large and exhibited different colors viz., brownish cream, pinkish white, milky white, cream, straw, and dirty yellow. Cell size varied from small to medium, cell shape also varied from cocci to rod. Out of 8 isolates from roots nodule endophytes, the majority were Gram-positive bacilli (75%), and were spore formers, belonging to the Phylum Firmicutes(Table 2). The results are in contrary with the findings of Rajendran *et al*., (2012), where the majority of putative NAB populations (64.7%) belonged to gram-negative cocco bacilli followed by gram-positive bacilli (29.4%) and 5.8% gram-positive cocci in fenugreek. The reason for the predominance of gram-positive bacilli in black gram nodules might be dictated by the genotypic nature of black gram. Further, it seems that the bacterial species entering into the plant tissue are dependent upon the type and availability of nutrients in a tissue, their abundance in the soil and environmental conditions prevailing in that region. This is in accordance with Saidi *et al* . (2011), who observed the existence of host specificity of endophytes in *Phaseolus vulgaris.* Saini *et al*. (2013) also reported the abundance of firmicutes in nodule tissue of chickpea.

Table 2. Morphological characters of isolates from blackgram nodules

Isolates	Colony color	Form	Margin	Elevation	Colony Diameter
TNEB1	White	Circular	Entire	Flat	0.2
TNEB2	White	Circular	Entire	Flat	0.2
TNEB3	Pale Yellow	Filamentous	Undulate	Flat	0.5
TNEB4	Dull White	Circular	Entire	Flat	0.4
TNEB5	Whitish Brown	Circular	Entire	Flat	0.2
TNEB6	Whitish Red	Circular	Entire	Raised	0.3
NANEB2	Dull White	Circular	Entire	Flat	0.2
NANEB3	White	Circular	Entire	Flat	0.5
YEMRB	White	Circular	Entire	Convex	0.4

When assessed for PGP traits, all NAB isolates produced significant levels of IAA in the range of 2.2 µg ml^{-1} to 28.3 µg ml^{-1}. Similar results were already achieved by Long *et al.* (2008) who reported the production of IAA in the range of 1.1 to 154 µg ml^{-1} by the endophytic isolates of *Solanumnigrum*. Tariq *et al*. (2012) studied the effect of *Bradyrhizobium* sp. for indole acetic acid production and the maximum IAA production was observed in *Bradyrhizobium*BR3 (4.95 µg ml^{-1}) where as the least was noticed in *Bradyrhizobium*BR2 (0.86 µg ml^{-1}). The results are presented in (Figure 1).

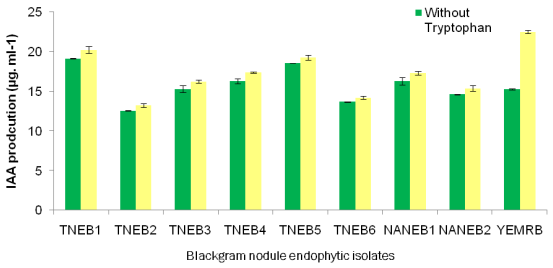

Figure 1. IAA production by blackgram endophytic nodule isolates. The culture were inoculated in the broth supplemented with or without tryptophan and incubated for 48h at dark condition.

The phosphate solubilizing efficiency of the isolates ranged from 13.3 to 34 %. The rhizobial isolate YEMRB (34%) and non-rhizobial isolate TNEB6 (21.3%) showed maximum solubilizing efficiency, which is evident by the presence of well distinct clearing zone compared to other strains (Figure 2).

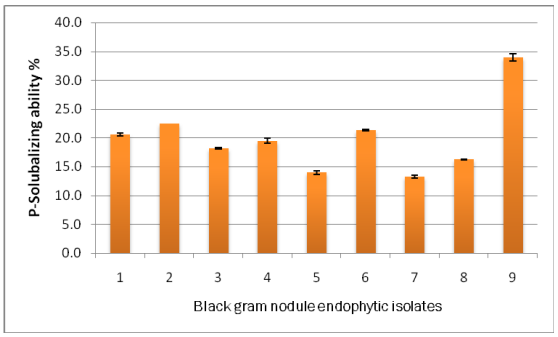

Figure 2. Phosphate solubilization activity of blackgramendophytic nodules

Several reports are available on the beneficial effect of phosphorus solubilizing microbe (PSM) as bio-inoculants, that enhances the P uptake in plants in term of increasing plant yields (Ahemad and Khan 2010; Jain and Khichi 2014).In this context, P solubilization by endophytes has added advantage because it imparts direct growth benefits to the associated host plants.

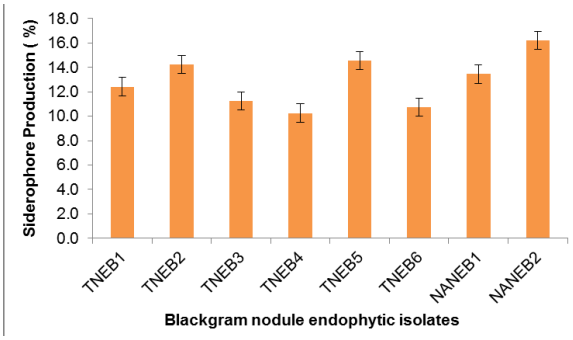

Figure 3. Percentage of siderophore production by blackgramendophytic nodule isolates in CAS medium

Siderophore production has a strong correlation with the inhibition potential against plant pathogens. In this context siderophore production was observed between 10.3 and 16.2 % for the NAB isolates, of which, the isolate NANEB2 recorded the highest siderophore production of 16.2%(Figure 3).

Table 3. Ammonia production ability and HCN Production of rhizobial and non-rhizobial isolates of blackgram nodules

Isolates	Ammonia Production	HCN Production
TNEB1	+	-
TNEB2	+	-
TNEB3	+	+
TNEB4	-	-
TNEB5	+	+
TNEB6	+	+
NANEB1	++	-
NANEB2	-	-
YEMRB	++	+

(++) - Very good; (+) - good and (-) – Absence of ammonia production based on color intensity

The results corroborate with the reports of Zhao *et al*., 2018, where the endophytic bacteria from nodules of soybean (*Glycine max* L.) exhibited antagonistic activity against phytopathogenic fungi *Pythium sojae* 01 and plant growth-promoting properties. Most of the *Bacillus* sp.

Table 4. Effect of co-inoculation of rhizobial and non-rhizobialendophytes on blackgram growth and nodule production

Treatment	Germination %	Vigour Index	No. of nodules
T1	66	1108	5
T2	85	1564	7
T3	92	1812	10
T4	90	1809	8
T5	88	1232	11

T1-Control,

T2-Rhizobium alone,

T3-Rhizobium+*Paenibacillustaichungensis*

T4-Rhizobium+ *Bacillus pumils,*

T5-Rhizobium + *Bacillus substills*

Produce siderophores, and the ability to utilize the siderophores of another organism is of selective advantage during iron-limited conditions. Hitherto, in this investigation also, the siderophores produced by the NAB isolate, NANEB2 could be cross-utilized by the *Rhizobium* sp. inside the nodule niche to overcome iron starvation. This interesting speculation was already proved by Khan *et al*. (2006), while exogenous siderophores stimulated the growth of *Rhizobium* sp.

Also, the present study showed that all the putative NAB isolates of black gram possess ACC-deaminase activity and are almost on par with each other except for the strain TNEB 3 (Figure 4). ACC-deaminase activity plays an important role in ethylene regulation and the nodulation process

of blackgram. Rhasid *et al*., 2012 isolated newer endophytes to utilize the plant compound ACC as a sole nitrogen source. In addition, these endophytes also have other growth promoting attributes IAA synthesis, siderophore production, phosphate

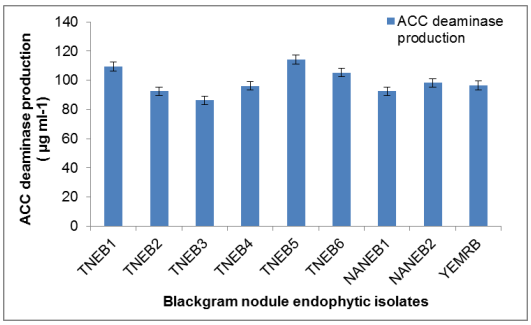

Figure 4. ACC deaminase activity by blackgramendophytic nodule isolates.

solubilization activity, optimal growth temperature, salt tolerance, and antibiotic sensitivity and proved to facilitate the growth of canola plant roots under controlled gnotobiotic conditions. However, no reports are available for ACC-deaminase in other putative NAB isolates studied in other legumes. The results also suggest that these NAB endophytes from blackgram aids in the survival of crop under moisture stress. The role of ACC-deaminase producing NAB and its interaction with the Rhizobial population has to be enunciated.

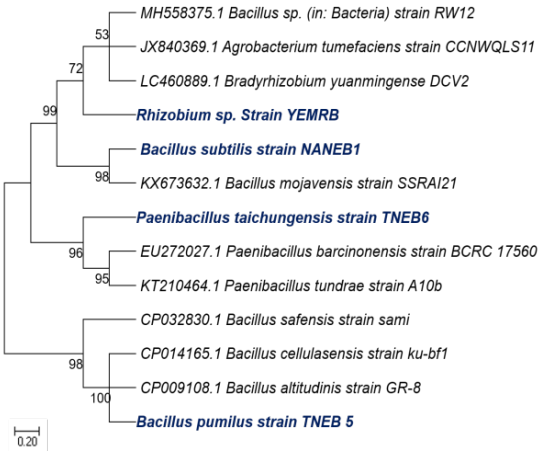

Figure 5. Phylogenetic tree based the 16S rRNA gene sequence from rhizobial and non-rhizobial endophytic bacterial isolates using Neighbor-joining method. Bootstraps values of 500 or more (from 1,000 replicates) are indicated at the nodes and the scale bar indicates one change per 100 bp nucleotides.

Another beneficial trait for a successful bio-inoculant is ammonia production, which helps the plants in N assimilation. While screening for the ability to produce ammonia of the eight putative NAB isolates, six (75%) of them showed the presence of ammonia production except for TNEB4 and NANEB3(Table 4). The production of ammonia by *Bacillus* sp. was reported by Silpa *et al*., 2018 with a positive role in the antagonistic activity. In the present study also many of NRE endophytes of blackgram produce secondary metabolites such as ammonia and HCN. Around fifty percent of blackgram nodule isolates produced hydrogen cyanide. The positive isolates are YEMRB, TNEB3, TNEB5 and TNEB6 respectively (Table 4).

In harmony with the phylogeny of 16S rRNA genes in the present study (Figure 5), the three most efficient NAB strains of blackgram were identified as *Bacillus* group. It was further demonstrated that the root nodules could be majorly occupied by these gram-positive bacilli as discussed above. These findings supported the results of other studies (Kumar *et al.,* 2016; Li *et al.,* 2008; Stajkovic *et al.,* 2009). In 2010, Palaniappan *et al*. reported that the bacterial strains were isolated and identified strains of three different phyla with nine genera *Arthrobacter, Bacillus, Bradyrhizobium, Burkholderia, Dyella, Methylobacterium, Micro bacterium, Rhizobium* and *Staphylococcus*. Similarly, other earlier reports also indicated major five genera of nodule endophytes of different legumes namely, *Acinetobacter*, *Bacillus*, *Enterobacter*, *Ochrobactrum* and *Pseudomonas* have been reported as nodule endophytes (Zakhia *et al*., 2006). The present findings are also in concordance with the above previous results.

Endophytes colonizing inside plant tissues contribute to the fitness of host and in return, they gain nutrient and protection from the host (Rosenbleuth and Martinez Romero, 2006). In the present study also, an investigation was conducted to evaluate the necessity of endophytes for legume growth as individual inoculum and co-inoculation with *Rhizobium*. As perceived earlier, the individual inoculum with (*Rhizobium* sp) showed least performance than co-inoculated cultures (*Rhizobium* sp + *P.taichungensis*) in terms of germination percentage,

Vigour index and nodule numbers(Table 4). Several reports are available on the benefits of co-inoculation on plant health especially in legumes on nodule development (Walpola and Yoon, 2013; Morel *et al*., 2012; Korir *et al*., 2017). In 2003, Bai *et al.* reported that co-inoculation of *Bacillus* strains in soybean plants with *Bradyrhizobium japonicum* provided the largest increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen, and grain yield. Thus it can be concluded that the co-operative interaction between rhizobia and nodule associated bacteria (NAB) is of relevance in the enhancement of nodulation and N_2 fixing efficiency in blackgram.

CONCLUSION

The present study clearly demonstrated the existence of putative nodule associated bacterial endophytes and there exists a co-operative mode of interaction. The phylum firmicutes are predominant in nodule niche and posses significant PGP traits such as IAA, siderophore, ammonia, HCN production, P solubilization and ACC-deaminase activity. The co-inoculation of promising NAB strains *Paenibacillus taichungensis* with the symbiont *Rhizobium* increased the vigor index of blackgram (8 DAS). Hence, the results paved a way to develop a co-inoculum of multifaceted highly co-operative endophytes, in order to increase pulse productivity and thereby sustains soil health

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