Research Article
Volume 5:2, 2021

Journal of Microbiology and Pathology

Open Access

Evaluation of some Nodulation Genes found in Bambara Symbiotic Rhizobia Strains

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Abstract

Rhizobia are known to have specificity for their leguminous host . bambara groundnut although a legume has shown promiscuous ability nodulating with many Rhizobia spp. Bradhyrhizobia spp were recovered from selected Nigerian soils by trapping them in the nodules of Bambara groundnut(BG) using pot hose experiments. They were isolated from the nodules and authenticated in the screen house and on the field and six strains along-side USDA110 (control) were selected after they were found to be highly effective and efficient. The DNA was exyracted and Pcr was carried out to check for the presence of Nod A, NodB, NodzA and NodzB genes. Nod A and Nod B which are conserved in all Rhizobia spp were found to be present. NodzA was found to be present in five of them while Nodz B was found to be present in all six of them. NodzA and Nodz B which are functional genes in soybean symbiointic Rhizobia strains this therefore suggests that BG symbiotic rhizobia strains may be able to nodulate soybean legumes and that NodzA and Nodz B may possibly play a major role in the nodulation process of BG.

Keywords: Nodulation genes • Bambara groundnut • Symbiotic rhizobia

Introduction

Bambara nut [1] is a legume seed crop with African origin and is highly underutilized. It has its origin from the Sahelian region of West Africa from the Bambara tribe near Timbuktu in Mali It is currently now found in many parts of Asia, South America, and Oceania It has several natural agronomic advantages including a high nutritive value, drought tolerance, and ability to produce in soils that are considered intolerable [2]. Little is known on the biodiversity of the rhizobial nodulating Bambara nut i.e. its cross inoculating property. Sequence analysis revealed that Bambara is nodulated by diverse microorganisms that belong to both α and β rhizobia *spp*. [3,4] Also, Bradyrhizobium, Azorhizobium, mesorhizobium and ensifer which are members of α-proteobacteria have been listed as possible nodulators of Bambara [4,5].

The common *nodABC* genes is found in all *Azorhizobium*, *Rhizobium*, and *Bradyrhizobium* isolates which have been studied so far [6]. These genes are called common *nod* genes as they are structurally conserved and functionally interchangeable between *Rhizobium*, *Azorhizobium*, and *Bradyrhizobium* species without altering the host range. The common *nodABC* genes encode enzymes involved in the synthesis of the lipo-oligosaccharide core. Inactivation of the *nodABC* genes abolishes the ability to elicit any symbiotic reaction in the plant, including root hair curling (Hac2), infection thread formation (Inf2), cortical cell divisions, and nodule formation (Nod2), regardless of the host, the mode of infection, the type of nodule development, and the nodule location (Martinez *et al*., 1990 and Long, (1989)). NodB is a chito-oligosaccharide deacetylase, and NodA is required for the N-acylation of the amino-sugar backbone [6,7] NodZA and NodZB are two genes that have been shown to have specificity for soybean associated rhizobia and most Rhizobia spp. that have ability to nodulate soybean [8] as they play a major role in the nodulation of soybean. Bambara nut is a legume seed crop with African origin and is highly underutilized. It has its origin from the Sahelian region of West Africa from the Bambara tribe near Timbuktu in Mali It is currently now found in many parts of Asia, South America, and Oceania. It has several natural agronomic advantages including a high nutritive value, drought tolerance, and ability to produce in soils that are considered intolerable. Little is known on the biodiversity of the rhizobial nodulating Bambara nut i.e. its cross inoculating property. Sequence analysis revealed that Bambara is nodulated by diverse microorganisms that belong to both α and β rhizobia *spp*. Also, Bradyrhizobium, Azorhizobium, mesorhizobium and ensifer which are members of α-proteobacteria have been listed as possible nodulators of Bambara.

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Received 22 April 2020; **Accepted** 23 April 2020; **Published** 20 March 2021

Methodology

Trapping of Rhizobia within the roots of Bambara plant

Soil samples were collected from fields where legumes were previously planted and transferred to the Laboratory. Sterilize seeds (2) of Bambara groundnut were planted under sterile conditions in a screen house and thinned upon germination to one viable plant per pot. Plants were inoculated with the soil samples obtained and were allowed to grow for eight weeks to allow rhizobial in soil samples infect the root and form nodules. The plants were harvested and nodules were collected from their root.

Isolation of Rhizobia

Rhizobia were isolated from nodules collected from the roots of the plants used for the trapping experiment on Congo red agar [9] using spread plate method. Two undamaged nodules samples were picked from each plant and placed in sterile water for about 15 to 20 mins to rehydrate them after which they were surface sterilized using 3% sodium hypochlorite for 3 minutes. They were then be rinsed with sterile water after which they were further sterilized with 95% ethanol and then rinsed with six changes of sterile water [10]. The nodules were then be transferred into sterilize petri-dishes, crushed with flamed glass rod and mixed with a few drops of sterile water. A loop full of the crushed nodule will be streaked on Congo red agar and incubated at 28˚C for 5 - 7 days (Somasegaram and Hoben, 2012). Rhizobia isolates were identified and purified. Strains were authenthicated in field and Screen-house experiments to confirm their effectivetiveness.

DNA Extraction

Purified cultures of the six effective strains (RB1, RB 2, RB3, RB4, RB5 and RB6) and the control USDA110 strain were collected and inoculated in YM

broth, after which they were incubated in a rotatory shaker for 5-7 days at 28°C. Cells were pelletized and DNA was extracted from the bacterial cultures using ZYMO bacterial/ fungal kit. Extracted DNA was loaded electrophoretically through 1% agarose gel stained with ethidium bromide (10mg/ml) at 100v for 1 hour. The bands were observed and photographed under UV light using BioDoc-IT imaging system to confirm the presence of DNA.

DNA amplification and restriction

PCR was done to detect the presence of NodA, NodB, NodZA, and NodZB genes amplicon**.** For template preparation, extracted DNA. Aliquots (3 µl) of the resulting lysate were directly used for PCR without further purification by adding master mix 12 µl (One-Taq quick load 2x Master Mix with standard Buffer New England BioLabs Inc.), the primer 1 µl and nuclease free water 8µl was used for the reaction mix. The amplifications were performed in a GeneAmp PCR System 2400 (PE Biosystems) with the specific protocol required for each primer (see Table 1). After the reaction, aliquots (3 µl) of the PCR products was loaded electrophoretically in 1.5% agarose gel stained with ethidium bromide (10 mg/ml) at 120v for 1 hour 30 mins. The bands would be observed and photographed under UV light using BioDoc-IT imaging system.

Results

NODA

Nod A was 550kb was found to be present in all the six strains including USDA110 confirming that they are rhizobia strains as this nod gene is conserved/ found in all rhizobia and are functionally similar in Rhizobia spp. (Figure 1).

NODB

Nod B was found to be present in all the six strains confirming that they are rhizobia strains as this gene is found in all rhizobia and are functionally similar in Rhizobia spp (Figure 2).

NodZA

NodZA was found in all the strains except for RB1 where it was completely absent. The gene showed polymorphism having varying bands of varying sizes, this showed that the strains that had this gene can nodulate soybean strains (Figure 3).

Table 1: Sequence of primers used for PCR.

Primer	5'- 3'	Annealing temperature (°C)	
Nodz- A	GGTTTGGCGACTGTCTGTGGTC (F) TTCCACCATGTTGGAAAGAATGGTCC (R)	60.5 59.4	Branden et al., 2010
Nodz- B	TGATGCCTGCCTGATGTGGCG (F) ATCTTCGCCATTGCCATGCCG (R)	62.8 61.1	Branden et al., 2010
Nod- A	TTTGAGCCCGACCCCCGA (F) CCGTTTCGGTCGCTGATGGC (R)	62.0 61.5	Larguerre et al., 2001
Nod- B	ATCAGCGACCGAAACGGGACG (F) GGCCGATCAAACCGGACCCA (R)	62.1 62.6	Chambon et al., 2017

KEY- F- forward; R- reverse; G-glutamine; T- thiamine; A- adenosine; C- cytosine.

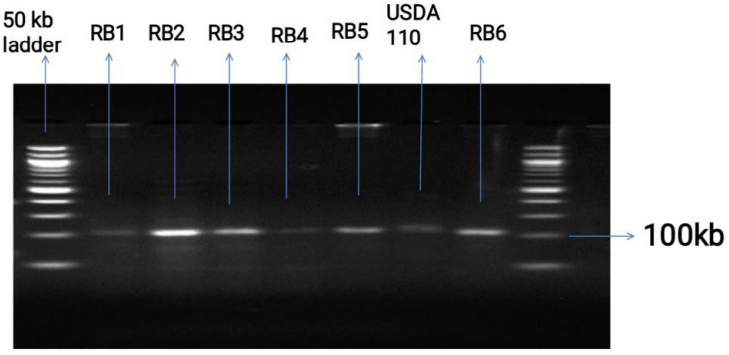

Figure 1: Amplicon of NodA gene.

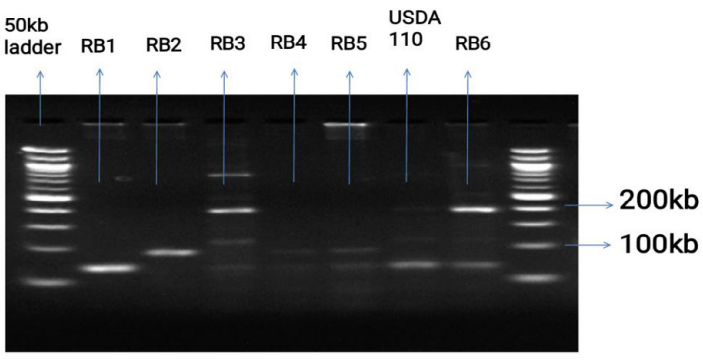

Figure 2: Amplicon of NodB gene.

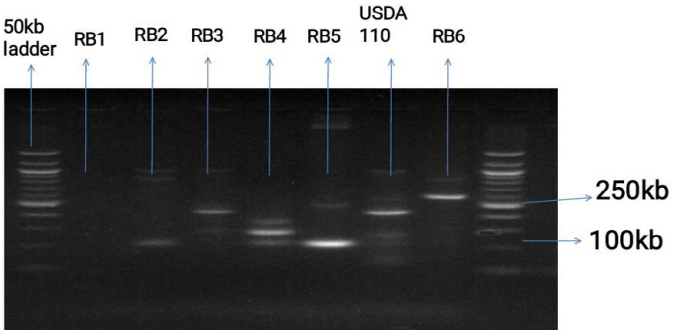

Figure 3: Polymorphic bands of amplicons of NodzA gene.

NodZB

NodZB was found in all the strains and showed polymorphism having 5 different bands of varying sizes, this showed that the strains can nodulate soybean (Figure 4).

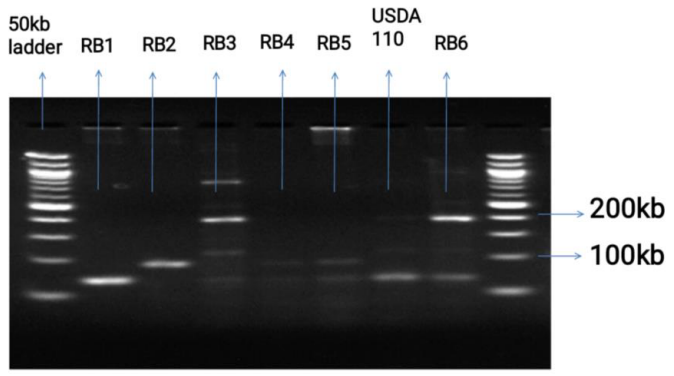

Figure 4: Polymorphic bands of amplicons of NodzB gene.

Discussion

Both Nod A and Nod B were found to be present in all the strains confirming that they are rhizobia strains as these two nod genes are found in all rhizobia and are functionally similar in Rhizobia spp. This is similar to the findings of [11,12] where both genes have been found to be conserved in Rhizobia spp. NodZA and NodZB were also found in all strains, but showing polymorphysism, this showed that the strains can nodulate soybean strains as these two genes are show to have specificity for soybean associated rhizobia because their products is the fucosyl group which a common product for the symbiont of soybean and most rhizobia spp that have ability to nodulate soybean as confirmed by [13] Five of the six strains used were confirmed to be Bradhyrhizobim sp particularly Bradhyrhizobim vignea. This is similar to the findings of where Bradhyrhizobim sp[14]. was listed as one of the nodulating species for Bambara groundnut [15].

Conclusion

The Molecular studies carried out showed that Bambara groundnut symbiotic strains also carry the NodzA and NodzB in addition to NodA and NodB. This suggests that these strains may also be used as inoculants for promiscious soybean varieties and that they may also play salient roles in the nodulation process of Bambara groundnut plants.

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How to cite this article: Ajayi OO, Dianda M, Fagade OE Adelowo. "Evaluation of some Nodulation Genes found in Bambara Symbiotic Rhizobia Strains." *J Microbiol Pathol* 5 (2021): 116.