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First report of *Rhizobium pusense* within Voandzou (*Vigna subterranea* (L.) Verdc.) rhizosphere in Côte d'Ivoire

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Abstract

There is currently no information on the diversity of indigenous rhizobial populations associated with bambara groundnuts (*Vigna subterranea* L. Verdc.) in Côte d'Ivoire soils. The aim of this study was to assess the diversity of microsymbionts nodulating a Bambara groundnut landrace in north of Cote d'Ivoire. For this purpose, a local bambara groundnut accession was used to trap root nodules bacteria from three soils obtained from farmers' fields in North Côte d'Ivoire. It was shown that all the isolates had very fast growth rates on YEMA and not absorbing Congo red. All isolates were gram negative rods and able to grow in a TY medium at pH ranging from 5.8 to 8.8 and at temperatures up to 45°C. The 16S RNA gene phylogeny showed three main clades including *Rhizobium sp.,* an unclassified species and *R. pusense*. 16S RNA gene identification of the strain K39 as *R. pusense* was congruently confirmed by a*tp*D gene phylogeny. Strain K39 did not nodulate bambara groundnut plants. This is the first report of the presence of *R. pusense* in *Vigna subterranea* (L.) Verdc.) rhizosphere in Côte d'Ivoire.

Keywords: Bambara groundnut, root nodule bacteria, *Rhizobium pusense*

Résumé

Il n'existe actuellement aucune information sur la diversité des populations rhizobiennes autochtones associées à l'arachide bambara (*Vigna subterranea* L. Verdc.) dans les sols de la Côte d'Ivoire. Le but de cette étude était d'évaluer la diversité des microsymbiontes nodulant une variété locale d'arachide Bambara au nord de la Côte d'Ivoire. À cette fin, une variété locale d'arachide bambara a été utilisée pour piéger les bactéries de trois sols provenant de champs d'agriculteurs situés au nord de la Côte d'Ivoire. Il a été démontré que tous les isolats avaient des vitesses de croissance très rapides sur le YEMA et n'absorbaient pas le rouge Congo. Tous les isolats étaient des bâtonnets Gram négatif. Tous les isolats ont pu croître dans un milieu TY à pH variant de 5.8 à 8.8 et à des températures allant jusqu'à 45°C. La phylogénie du gène de l'ARN 16S révélait trois clusters principaux, dont Rhizobium sp., une espèce non classée et *R. pusense*. La nature de la souche K39 en tant que *R. pusense* revélée par la phylogénie de l'ARN 16S a été confirmée par la phylogénie du gène atpD. Les tests de nodulation ont confirmé que la souche K39 ne nodule pas les plants d'arachide bambara. C'est la première fois que *R. pusense* est mise en évidence dans la rhizosphère de *Vigna subterranea* (L) Verdc en Côte d'Ivoire.

Mots-clés : Arachide bambara, bactéries nodulatrices, *Rhizobium pusense*

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Introduction

Improving agricultural productivity and soil fertility are two priority objectives of agricultural policies in West Africa (CORAF/WECARD, 2008). While in developed countries, farmers rely extensively on industrial fertilizers to maximize crop productivity, this is not the case in developing countries. Indeed because of the industrial fertilizers financial and environmental costs, industrial fertilizers are not a solution for developing countries (Sanchez, 2002). Consequently one major challenge in West Africa agriculture, particularly in Côte d'Ivoire, is to be able to ensure high agricultural productivity while preserving the environment. This ecological intensification implies a sustainable increase of production by substituting industrial factors of production by biological processes or by improving the integration of the components of the production systems. Legumes often referred to as "green manure" have a clear cut potential contribution to soil fertility and plant productivity (Bationo and Ntare 2000, Fening et al., 2011). For these reasons, legumes are increasingly integrated into cropping systems due to their multiple benefits (Stagnari et al., 2017). Indeed, legumes are important protein resources and are also able to improve soil fertility. In addition, legumes have the property of fixing atmospheric nitrogen due to symbiotic relationship with soil bacteria collectively known as rhizobia (Faye et al., 2006, Herrmann et al., 2012). Among these legumes, Bambara groundnuts (*Vigna subterranea* L. Verdc.), commonly known as voandzou, is the third most important food legume in Africa after groundnut and cowpea, both for consumption and cultivation. It is widely grown in Nigeria and other African countries such as Ghana, Cameroon, Côte d'Ivoire and Togo (Klu et al., 2001). The edible grain of this crop is rich in protein (20.6%) and carbohydrate (56.5%). It contains 6.6% fat and 6.3% fiber, making it a complete meal (Mazahib et al., 2013). In Africa, it is grown by smallholders either in monoculture or in rotation with cereals or mixed with cereals (Doku 1995). In Côte d'Ivoire, it is mainly cultivated in the northern region where a sound management of soil conditions, mineral nutrients and water resources together with measures to prevent soil erosion are the prerequisites for a sustainable agriculture. Bambara groundnuts (*Vigna subterranea* L. Verdc.), that has interesting ecological characteristics, including drought resistance, and grows in nutrient-poor soils (Linnemann and Azam-Ali, 1993 ; Linnemann, 1994) could be used in production systems to improve soil fertility and crop productivity in Northern Côte d'Ivoire. An inoculation technology using rhizobia associated could be initiated. For a better integration of bambara groundnuts into ecological intensification systems, it will be necessary to characterize the diversity of bacteria associated with it in Côte d'Ivoire. The diversity of rhizobia associated with legumes such as peanut, soybean, cowpea, pigeon pea has been well characterized in Africa (Belane and Dakora 2009, Pule-Meulenberg and Dakora 2009, Fossou et al., 2016). However, study on the diversity of symbiotic and non-symbiotic rhizobia of bambara groundnuts is scarce (Onyango et al., 2015). Until recently, about 40 rhizobia species belonging to the seven genera of Alphaproteobacteria including Rhizobium, Sinorhizobium, Azorhizobium, Bradyrhizobium, Allorhizobium, Mesorhizobium, Bradyrhizobium, and Methylobacterium have been identified (Lemaire et al. 2015). In addition, root nodule bacteria have recently been identified that are generally unable to form nodules or fix nitrogen. The newly defined species, *R. pusense*, isolated from *Cicer arietinum* roots (Panday et al., 2011, Mousavi et al., 2015) belongs to this group. The *R. pusense* ecological role is not well defined since several strains have been found in human wounds and body fluids (Aujoulat et al., 2015). Here we report for the first time the presence of *R. pusense* in the rhizosphere of bambara groundnuts in Côte d'Ivoire.

Materials and Methods

Soil sampling procedures and analysis

Soil sampling was done at bambara groundnut fields, in Korhogo (9°27'28"N, 5°37'46"W), Katiola (8°08'15"N, 5° 06'07"W) and Odienné (9°30'05"N, 7°33'45"W) located in the North of Côte d'Ivoire. At each sampling point, 2 kg of soil were collected from a depth of 0-20 cm at bambara groundnut rhizosphere using a shovel. To prevent a contamination of the samples, the shovel was cleaned between sampling with 5% sodium hypochlorite solution, then rinsed with water three times and dried using a sterile cloth. Within each site, three replicate samples were collected at a distance of 20 km by randomly sampling. In the laboratory, the soil samples from each field were spread onto a sturdy plastic bag before being thoroughly mixed and allowed to dry at room temperature for five days. The soil samples were placed in brown paper bags and subsequently used for soil analysis and for bacteria trapping.

Soil chemical analysis was conducted in the Pedology laboratory of Institut National Polytechnique Félix Houphouët Boigny, Yamoussoukro (INP-HB) in Cote d'Ivoire using standard methods (Anderson and Ingram, 1993).

Trapping and isolation of root nodulating bacteria

A local landrace of bambara groundnut with cream colored red spotted seeds, preferred by farmers in the region because of early maturity, superior cooking quality and taste, was chosen for this study. This landrace was used to trap indigenous root nodule bacteria symbiotic to Bambara groundnuts from the collected soil samples. The seeds were germinated in perforated plastic pots containing 1.5 kg of autoclaved soil and 200mg of soil samples as inoculum. The seeds were incubated for germination in sterile Petri dishes containing sterilized distilled water for three days at 28°C. After germination, two seedlings were sown in each plastic pots. Plants were watered with nutritive solution and harvested six weeks after sowing. The plants with soil and roots were thoroughly cleaned with a stream of fresh water. Sterilization of the surface of the nodules was performed using an initial incubation of 3 min in 70% EtOH (v/v) followed by 3 min in a 4% (w / v) sodium hypochlorite solution and complete washing with sterile ddH2O. Each nodule was then crushed in a drop of sterile ddH2O using a sterile toothpick and streaked on Tryptone Yeast extract Agar (TYA). All plates were incubated at 28°C and bacterial growth was monitored daily. When nodule isolates began to develop, a sample was used to repeat successive streaks. Thus, all the isolates described in this study were purified from a single colony. The isolates were maintained in growth chamber at 4 ± 2°C for further study or stored at -80°C with addition of 20% glycerol for long-term preservation.

DNA extraction and PCR amplification

Genomic DNA of each isolate was extracted from 2 mL of TY broth culture in the log phase. The isolates were collected by centrifugation at 6000×g. The CTAB method (Li et al., 2012) was used to extract the genomic DNA, which was then stored at −20◦C until analysis. DNA was quantified and assessed for purity using a NanoDrop 2000 spectrophotometer (Thermo Scientifc, USA). DNA concentration was determined by absorbance at 260 nm and DNA purity was estimated by the 260:280 ratio and the 260:230 ratio. The 16S RNA encoding gene, was amplified from genomic DNA.

The PCR using primers FGPS6 (5'-GGAGAGTTAAGATCTTGGCTCA-3') and FGPS 1509 (5'-AAGGAGGGGATCCAGCCGGA-3')(Normand and al., 1992) was performed in a GeneAmp System 9700 thermocycler (Applied Biosystem, Foster City, California, USA) in a total volume of 25 µL consisting 10 ng of genomic DNA, 12.5 µL of master Mix ''One Taq Quick Load 2X Master Mix with standart buffer'' (New England BioLabs, USA), 10 µM of the Forward Primer, 10 µM of the Reverse Primer. The amplifications were carried out with the following PCR conditions: denaturation step at 94°C for 5 min, followed by 35 amplification cycles of denaturing at 94°C for 30 s, annealing at 55 °C for 30 s and elongation at 72°C for 1 min 30s. The final extension was at 72°C for 7 min followed by a soaking temperature of 4°C. PCR products were separated by electrophoresis in agarose gel at 0.8 % stained with ethidium bromide. The housekeeping gene atpD was also amplified from selected strains using primers atpD-F (5'-ATCGGCGAGCCGGTCGACGA -3') and atpd-R (5'-GCCGACACTTCCGAACCNGCCTG -3') (Gaunt et al., 2001). The amplifications were carried out with the following PCR conditions: denaturation step at 95°C for 2min, followed by 35 amplification cycles of denaturing at 95°C for 30 s, annealing at 66 °C for 30 s and elongation at 68° C for 2 min. The final extension was at 68°C for 5 min followed by a soaking temperature of 4°C. PCR products were separate by electrophoresis in agarose gel at 1 % stained with ethidium bromide.

Sequencing and phylogenetic analyses of 16S-rDNA and atpD genes

In a first approach the 16S RNA gene amplicons were subjected to PCR-RFLP fingerprinting. An aliquot (10 µL) of the PCR products was digested separately with the restriction endonuclease *Rsa*I (New Englend BioLabs, USA) according to the manufacturer's instructions. The restricted bands were then separated by electrophoresis in 2.5% (w/v) agarose gel, and the patterns were visualized as described previously (Wang et al., 1999). The restriction fragment length fragment (RFLP) patterns obtained were identified as genotype. Each 16S rDNA type was purified by Favour/Prep PCR purification kit (FAVORGEN, Sigma, USA). The purified samples were

sequenced (Macrogen, France), and the quality of all sequences checked using BioEdit 6.8.3 software (Ref). Assembled sequences were transferred to MEGA® (Molecular Evolutionary Genetics Analysis) version 6 software and aligned using CLUSTAL W (Tamura et al., 2013). Sequences were submitted to the NCBI BLAST portal (www.ncbi.blast.lnm.nih.gov) for a sequence similarity search, and sequences with greater than 94% similarity were retrieved for phylogenetic analysis. Evolutionary histories were inferred using the Neighbor-Joining (NJ) and Maximum-likelihood (ML) method and genetic distances computed based on the p-distance method implemented in Mega software (Tamura et al., 2013). Bootstrap tests (1000 replicates) were used to cluster associated taxa and replicate. Amplicons of the atpD gene were also sequenced and phylogenetic analyses were performed using the same method as described above.

Nodulation tests

16S RNA gene RFLP types were selected for determination of symbiotic status. Bambara groundnut seeds were surface sterilized in 95% ethanol for 1 min, followed by 2% sodium hypochlorite solution for 30 s and finally rinsed in five exchanges of sterile water. The seeds were ncubated in dark at 25°C for germination for 3 days. Plastic pots of 1.5 L divided in two parts and used as jars were prepared by sterilizing in 5% sodium hypochlorite solution for 15 min. The sea sand was sterilized at 121°C for 1 h and then filled in the jars. Two seedlings were aseptically transferred into the sea sand and watered using sterile N-free nutrient solution (Lafay and Burdon, 2006). The inoculum was prepared by streaking each isolate on freshly prepared TYA plates. 1 mL of the pure culture was aseptically taken, re-suspended into sterile bottle containing 10 mL of TY broth and left on a shaker overnight. Four replicates per isolate were inoculated directly around seedling hypocotyls three days after establishment using sterile disposable pipette tips. Each time, 3 mL (about 108 cells) of bacterial suspension were used to inoculate each seedling. Test seedlings and the negative control were irrigated twice per week with a nitrogen-free nutrient solution alternating with ddH2O sterile, while the positive control was irrigated with 0.05% KNO3 solution to supply the plants with nitrogen. After six weeks, the root systems of individual plants were washed separately for observation of nodules.

Phenotypic characterization of isolates

Isolates were characterized on the basis of their microscopic, morphological and physiological characteristics using standard methods. For comparison, the four strains based on the RFLP types were used (Table 1). Phenotypic characteristics were determined on YMA and CR-YMA (Vincent, 1970). Growth of rhizobial strains was compared at different pH (4.8, 5.8, 6.8, 7.8 and 8.8) in TY broth. Hydrochloric acid (HCl) was used to adjust lower pH and sodium hydroxide (NaOH) was used to adjust high pH in medium. For the analysis, each isolate was cultured in TY broth and 1 mL of the culture containing about 108 rhizobial cells was used to inoculate 50 mL of TY broth flask, adjusted to different pH. The inoculated broth cultures were incubated at 28°C and kept at 105 rev. min-1 in an incubator shaker. Growth was determined by measuring the optical density (OD) at 540 nm after 24 h of incubation using a spectrophotometer. The growth of the isolates at different temperatures (4°C, 20°C, 28°C, 30°C, 45°C and 50°C) was also assessed on TY medium. The growth results were recorded after 24 h incubation in plates.

Data analysis

The STATISTICA version 7.1 software was used for ANOVA at one factor at 5% threshold. The averages were ranked according to the Tukey LSD test. Numerical calculations and graph construction were performed by the Excel software.

Table 1. Frequencies of different genotypes

Code	Strain type	Genotype percentage
Genotype 1	K39	7.89%
Genotype 2	O12	71.05%
Genotype 3	T65	18.42%
Genotype 4	O41	2.63%

Results

Chemical analysis of soils

Soils chemical properties of the three study sites are given in Table 2. All three sampling sites had predominantly acidic soils with a pH ranging from 4.3 at Odienné to 5.0 at Korhogo. Soil organic matter ranged from 1.48% in Odienné to 0.61% in Katiola. The available P content was low at 50 ppm. However, the Korhogo soil was slightly more saturated than the others, and the Odienné soil was richer in organic matter with a relatively greater mineralization of organic matter.

Diversity of nodulating bacteria associated to *Vigna subterranea* (L.) Verdc.)

In total, 152 bacteria were isolated from the nodules. The PCR-amplified 16S-rDNA genomic region of each of the 152 isolates yielded a single band of approximately 1500 bp. The isolates exhibited variable banding patterns of the 16S-rDNA gene when digested with the restriction endonuclease RsaI. Overall four genotypes were identified, and isolates K39, O12, T65 and O41 selected as their representative strains (Table 1). The pylogenetic analyses of the 16S RNA gene (Figure 1) grouped the four genotypes in three clades including R. pusense (strain K39), Rhizobium sp. (strains O12, T65) and a non identified strain (O41). All four representative isolates were rod shaped, Gram negative, not absorbing the congo red and fast-growing bacteria (visible growth within 2 days). The rhizobial isolates evaluated were able to grow in TY media with the pH ranging from 5.8 to 8.8. The best growth rate was observed at pH 6.8 with an ODxx of up 1.6 for isolat 6 (Figure 2). At pH 4.8, there was almost no growth, rates were lowest for isolate 2 with 0.009 OD. Isolates tested showed significant differences in their ability to grow at different pH. Furthermore, thermotolerance variability was noted among rhizobia isolates, which were able to grow up to 45° C (Table 3).

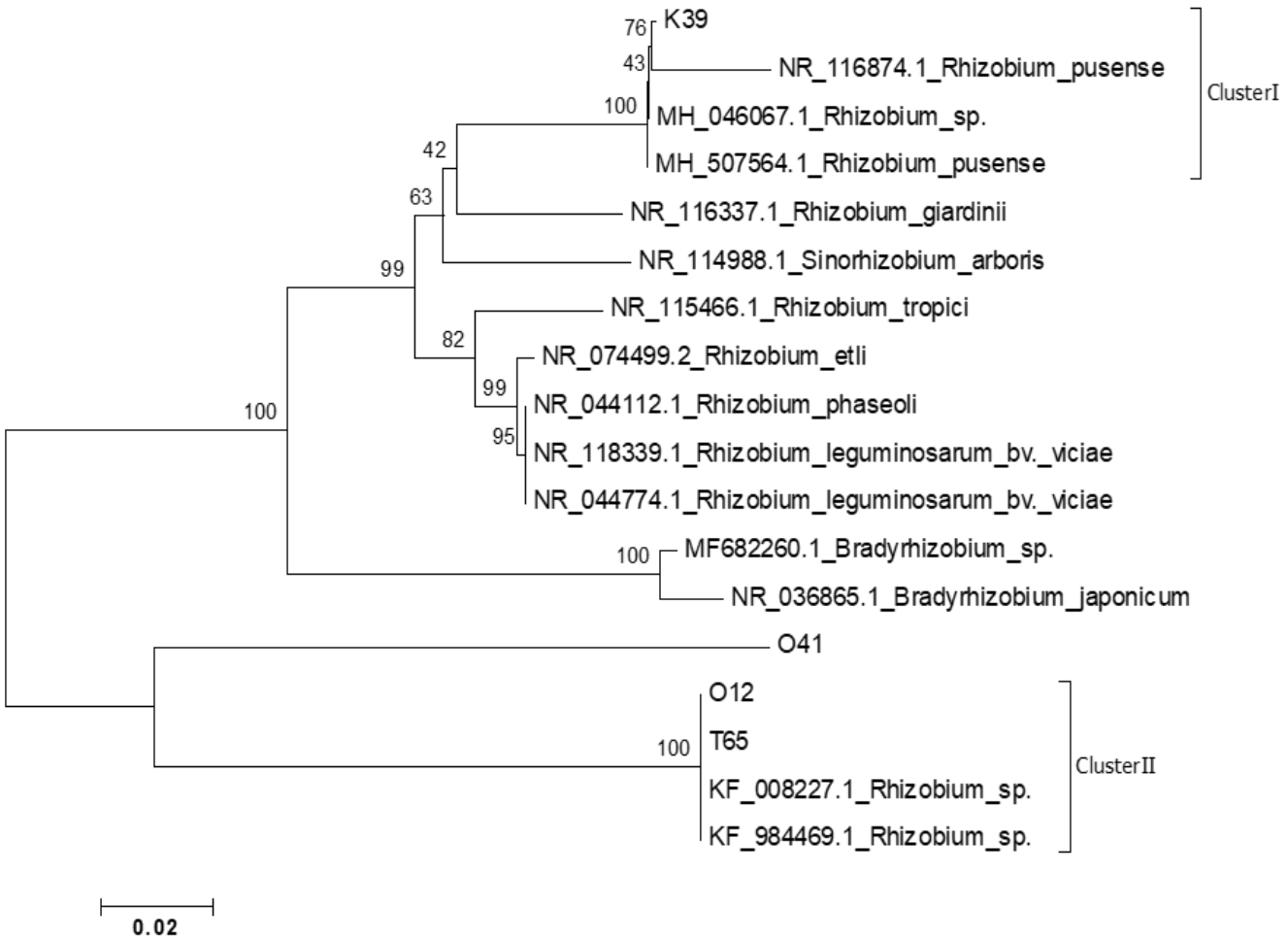

Figure 1. Neighbor-Joining (NJ) phylogenetic tree of 16S rRNA gene sequences showing the relation between the bacteria isolated from bambara groundnut nodules and related reference strains. Analysis used the JC model, a total of 1300 positions (1300 bp) and 1,000 replicates for the bootstrap.

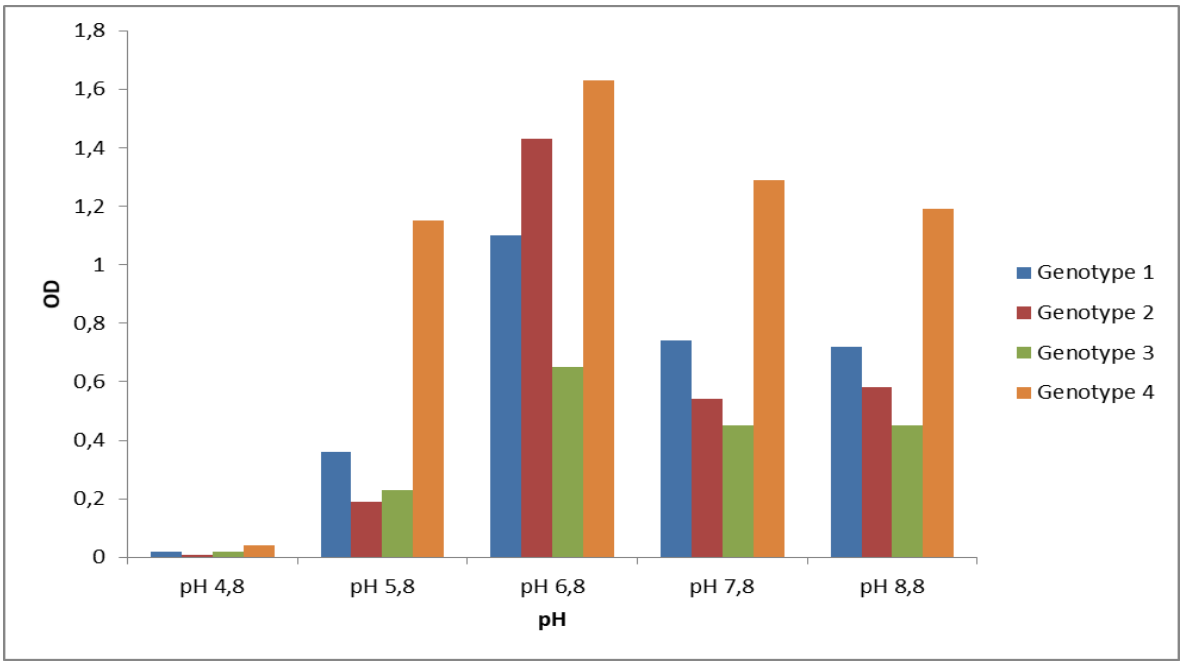

Figure 2. Effect of pH on growth on bacteria isolated from bambara groundnut nodules

Table 2. Chemical characteristics of soils collected from three sites within North of Cote d'Ivoire.

Soil chemical analysis											
	pH	M.O (%)		N-NH4+	Ass. P	Absorbent Complex (cmol.k-1)					
Sites	H2O	C	N	g/kg	Ppm	CEC	Ca2+	Mg2+	K+	Na+	S
Katiola	4,4	0,61	0,06	0,19	47	4,40	1,276	0,521	0,139	0,037	1,97
Korhogo	5,0	0,89	0,08	0,26	48	6,48	2,450	0,490	0,140	0,040	3,12
Odienné	4,3	1,48	0,11	0,37	49	7,76	1,666	0,553	0,128	0,038	2,39

Table 3. Phenotypic characteristics of rhizobia isolates. +, growth or positive reaction; –; no growth or negative reaction

Characteristics		1	2	3	4
Tempera-ture	4°C	+ (after 6 days)	+ (after 6 days)	+ (after 6 days)	+ (after 6 days)
	20°C	+	+	+	+
	28°C	+	+	+	+
	30°C	+	+	+	+
	40°C	+	+	+	+
	45°C	+	+	+	+
	50°C	-	-	-	-
pH 4.8-8.8		+	+	+	+

The identified R. pusense did not nodulate *Vigna subterranea* (L.) Verdc.)

In order to confirm the 16S RNA gene identification of strain K39 as R. pusense, the atpD gene was amplified by PCR. The PCR amplification resulted in a single 550 bp fragment approximatively. The phylogenetic tree based on the atpD gene confirmed the K39 strain as R. pusense (Figure 4). The atpD gene sequence similarity between strain K39 and the type strains of reference Rhizobium species are given in Table 5. Strain K39 exhibited level of atpD and 16S RNA gene sequence similarity of respectively 98.8%, and 100% identity to R. pusense S1 (PVWT01000008.1) and R. pusense CCGM11 (MAPG01000613.1) (Tables 4 and 5). When nodulating tests were conducted, strain K39 did not nodulate while the other three genotypes gave nodules.

Table 4: Levels of similarity between 16S rRNA gene sequences of K39 and references

1	**Isolate K39**	100									
2	*R. pusense* S1 (PVWT01000005.1)	**100**	100								
3	*R. pusense* CCGM11 (MAPG01000666.1)	**100**	100	100							
4	*R. pusense* KCJK7997 (QAYR01000011.1)	**100**	100	100	100						
5	*R. pusense* LMG 25623 (FNBB01000023.1)	**100**	100	100	100	100					
6	*R. pusense* NRCPB10^T (MRDJ01000001.1)	**100**	100	100	100	100	100				
7	*A. tumefaciens* S33 (CP014260.1)	**100**	100	100	100	100	100	100			
8	*A. tumefaciens* KCJK1736 (LXPS01000020.1)	**100**	100	100	100	100	100	100	100		
9	*A. salinitolerans* YIC 5082^T (MRDH01000011.1)	**100**	100	100	100	100	100	100	100	100	
10	*A. deltaense* CNPSo 3391^T (RRZI01000041.1)	99.3	99.3	99.3	99.3	99.3	99.3	99.3	99.3	99.3	100

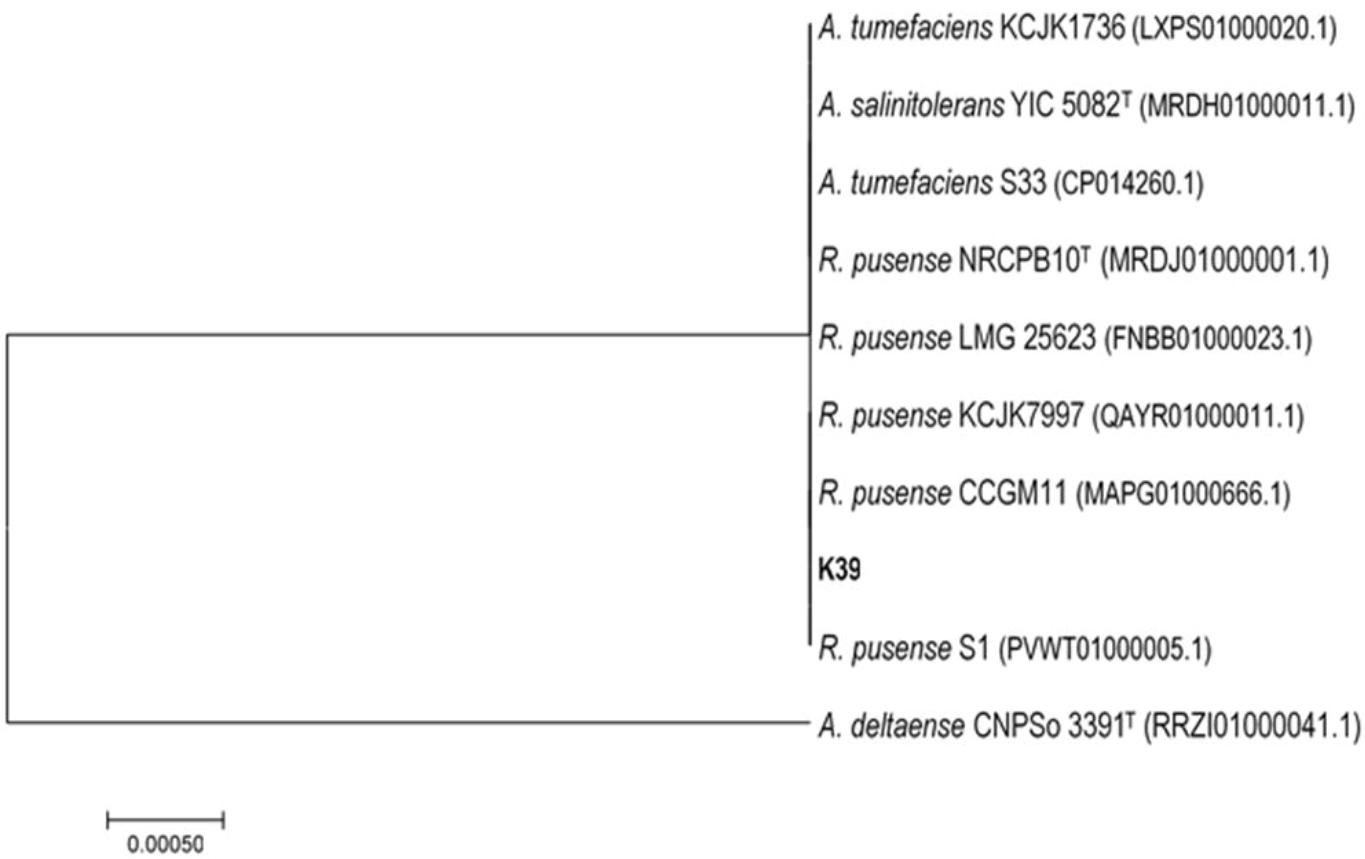

Figure 3: Maximum-likelihood (ML) phylogenetic tree of 16S rRNA gene sequences showing the relation between isolate K39 and related reference strains. Analysis used the JC model, a total of 1300 positions (1300 bp) and 1,000 replicates for the bootstrap

Table 5. Levels of similarity between atpD gene sequences of K39 and reference strains

	Strain									
1	**Isolate K39**	100								
2	*R. pusense* S1 (PVWT01000008.1)	**98.8**	100							
3	*R. pusense* CCGM11 (MAPG01000613.1)	**98.8**	100	100						
4	*R. pusense* KCJK7997 (QAYR01000008.1)	98.3	99.0	99.0	100					
5	*R. pusense* LMG 25623 (FNBB01000010.1)	98.1	98.8	98.8	99.0	100				
6	*R. pusense* NRCPB10^T (MRDJ01000014.1)	98.1	98.8	98.8	99.0	100	100			
7	*A. tumefaciens* S33 (JFFS01000747.1)	97.9	98.7	98.7	99.2	98.7	98.7	100		
8	*A. tumefaciens* KCJK1736 (LXPS01000010.1)	96.0	96.3	96.3	96.9	96.3	96.3	96.5	100	
9	*A. deltaense* CNPSo 3391^T (RRZI01000004.1)	94.4	95.2	95.2	95.0	94.4	94.4	95.0	94.4	100
10	*A. salinitolerans* YIC 5082^T (MRDH01000039.1)	94.2	95.0	95.0	95.6	95.4	95.4	95.2	94.4	94.2	100

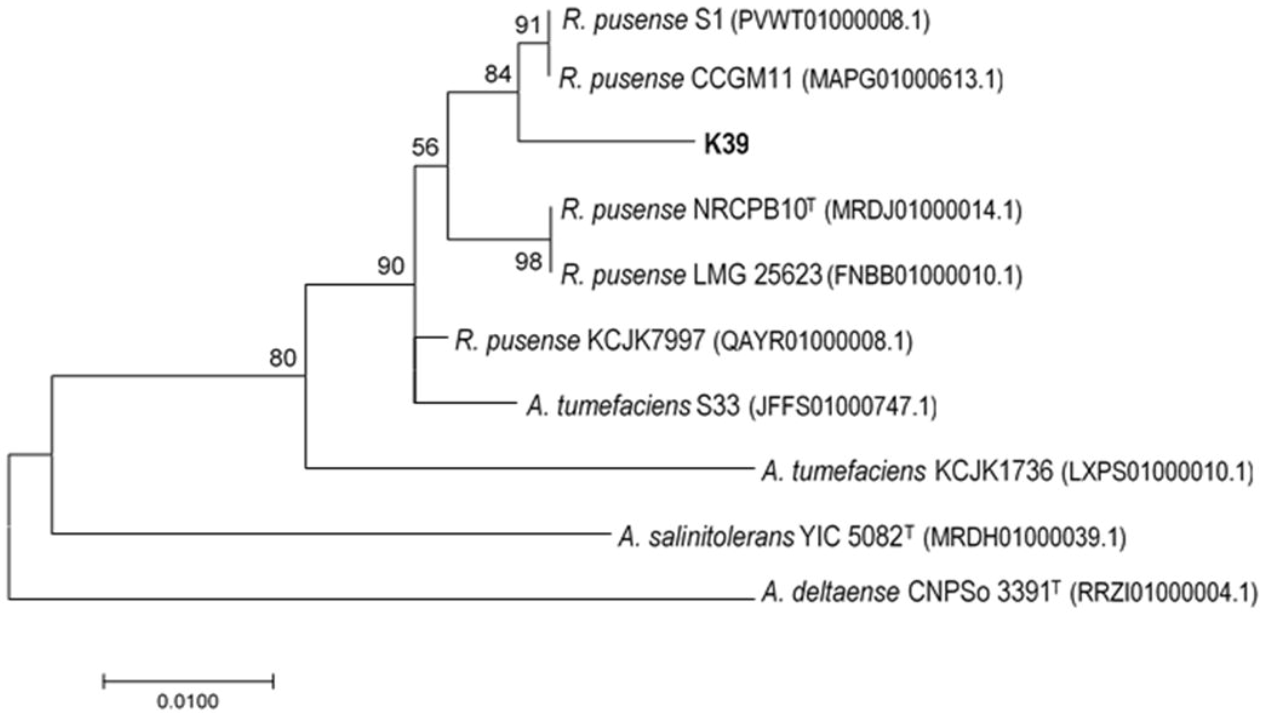

Figure 4. Maximum-likelihood (ML) phylogenetic tree of atpD gene sequences showing the relation between isolate K39 and related reference strains. Analysis used the TN93+G model, a total of 519 positions (519 bp) and 1,000 replicates for the bootstrap.

Discussion

The objective of this work was to describe the diversity of nodulating bacteria associated to Vigna subterranea (L.) Verdc. A total of 152 indigenous bacteria were isolated from bambara groundnut nodules after inoculation of plantlets with soils from three sites within North of Côte d'Ivoire. The soils of the study sites were globally unsuitable for agriculture. Indeed, they had a high level of acidity (4.3 to 5), a low CEC (4.40 to 7.76) and an average rate of assimilable P. However, due to its ability to grow in poor soils (Linnemann and Azam ali, 1993, Linnemann, 1994), cultivation of bambara groundnut in these soils could revitalize them after cultivation.

The PCR-RFLP analysis of the 16S RNA gene grouped the 152 strains in four genotypes that were Gram-negative, fast grower, and did not absorb the congo red (Somasegaran and Hoben 1994; Hungria et al. 2016). Such results were obtained in Kenya where 70% of bacteria isolated from bambara groundnut had very fast growth rates on YEMA (Onyango et al. 2015). Additionally, the genotypes appeared to be varying in their pH tolerance in TY broth. In the current investigation, at pH 4.8, the isolates showed although very poor growth. This trend might be related to the acid pH that characterizes most of the origin soils of the tested isolates. From the 16S RNA gene phylogenetic tree, three main clades were identified including Rhizobium sp, R. pusense and an unidentidied clade. The diversity found in Côte d'Ivoire is less than the one in Kenya where Rhizobium sp., *Bradyrhizobium sp., Burkholderia sp*. and *Agrobacterium sp.* were identified (Onyango et al. 2015). Two native rhizobia genotypes clustered together with the putative Rhizobium sp. Whether these two genotypes of this cluster belong to taxonomically distinct species remains to be determined. These two genotypes belonging to Rhyzobium sp., within the rhizobial population studied could be the symbiotics strains most numerous in the rhizosphere of bambara groundnut. Indeed, Oyango et al.

(2015) isolated 18 bambara groundnut nodule strains in Kenya, seven of which were identified as *Rhizobium sp.*

Out of the four genotypes, one strain K39 was congruently identified as R. pusense by 16S RNA and atpD gene analyses. Indeed, phylogenetic analysis based on ATP atpD gene sequences, in agreement with 16S rRNA gene sequence phylogeny, demonstrated the close relationship of strain K39 with *R. pusense* S1, *R. pusense* CCGM11 and *R. pusense* NRCPB10T strains. We found the occurrence of R. pusense in two of three soils tested indicating the high distribution of this group within the region of north of Cote d'Ivoire. Yet the phylogenetic relationship between these strains has to be evidenced. Early studies that used genomic comparison demonstrated that *R. pusense* strains obtained from bean nodules were different (Aguilar et al., 2016). This study is the first to report the presence of *R. pusense* within bambara groundnut rhizosphere in Côte d'Ivoire. *R. pusense* strains may represent an interesting link between the evolution from symbiosis to pathogenicity or the evolution from pathogenicity to symbiosis .

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