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Genetic Characterization of Rhizosphere Bacteria that Inhabit Common Bean Nodules in Western Kenya Soils

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Abstract

Background: With the increasing world population, there is increasing demand for food. This has led to overuse of agricultural farms causing reduced soil fertility and accumulation of phytopathogens. Inorganic fertilizers and pesticides have been extensively used in response to these challenges. Extensive integration of inorganic fertilizers and pesticides in the farming system has contributed to soil and water pollution worsening the eutrophication in rivers lake waters. Alternative farming methods are therefore necessary to address this problem. Recent studies have found that rhizobacteria that colonize nodules of leguminous plants are capable of increasing yield and health of the tested plants. Their plant growth promoting ability depends on the rhizobacteria type, soil properties, and climatic conditions. The aim of this study, therefore, was to genetically characterize rhizobacteria that closely associate with common bean nodules by analyzing the nucleotide sequence of 16SrRNA gene.

Results: The 16SrRNA gene analysis revealed that common bean nodule associated bacteria in Western Kenya soils are genetically diverse as indicated by the evolutionary genetic distances. Not even organisms in the same species had zero genetic distance though they formed independent groups on the phylogenetic tree. The isolates belonged to the genus *Pseudomonas, Providencia, Rhizobia, Klebsiella, Enterobacter, Delfitia* and *Acinetobacter* as identified through nucleotide BLAST at the NCBI GenBank database.

Conclusion: Rhizobacteria that colonize common bean nodules are genetically diverse. Those found in this study may be adaptable to Western Kenya soils and further tests are required to determine their plant growth promoting efficiency.

Keywords: Rhizobacteria; Nodule associated bacteria; Nitrogen fixation; Phylogenetics

Background

A large proportion of the population in Western Kenya is involved in agricultural production and the common bean is one of the major crop grown [1]. Due to a rapid increase in population growth, there is a high demand for food production, hence farms are repeatedly used. This habit has greatly reduced soil fertility and bred more phytopathogens resulting to very low yield [2]. In order to increase the crops yields, farmers have therefore resorted to the intensive use of inorganic fertilizers in an attempt to boost fertility in their farms and use of pesticides to reduce damage by phytopathogens. Inorganic fertilizers and pesticides may increase the accumulation of heavy metals in the soil and plant systems [3]. Inorganic fertilizers mainly contain ammonia, phosphates, potassium and nitrate salts. These salts reach the water bodies through leaching, drainage, and flow. Water pollution by these inorganics constitutes a major concern globally as it may lead to the onset of many fatal diseases in humans, direct killing of aquatic animals, eutrophication in water bodies and bioaccumulation of these toxic compounds in food trophic levels [4].

The economic consequences of the application of inorganic fertilizers together with their negative impacts on the environment have become a concern globally thus, there is a need for farmers to shift to the farming practices that are sustainable [5]. Studies have shown that the use of plant growth promotion rhizobacteria (PGPR) can significantly increase the yield of common bean [6-9] and hence it is a potential alternative to heavy dependency on inorganic fertilization and use of pesticides. The mechanisms by which these soil microorganisms promote plant growth are not well elucidated but nitrogen fixation [10-12], phosphorus solubilization [13,14] and inhibition of phytopathogens growth [15,16] are thought to be a possible explanation for this effect. The belief that only rhizobia colonize nodules of leguminous plants is disputable. Researchers have been isolating other microorganisms besides rhizobia as bona fide members of nodules in the legumes [6,17]. They have demonstrated the isolation of bacteria of several genera; *Pseudomonas, Aerobacter, Agrobacterium, Chryseomonas, Bacillus, Curtobacterium, Erwinia, Enterobacter, Sphingomonas*, and *Flavimonas*. The presence of these bacteria in the nodules is not accidental. Available reports over time have shown that co-inoculation of rhizobia with other rhizobacteria tremendously increased the yield of common beans compared to when

rhizobia were the only inoculant in terms of increased number of pods per plant, the number of seeds per pod, weight of pods per plant and total dry matter of the shoot [18]. Rajendran et al. [19] reported an increased nodulation and root weight in greenhouse conditions when common beans were co-inoculated with rhizobia together with other nodule associated bacteria. Nodule associated bacteria that so far have been co-inoculated with rhizobia include *Azospirillum* [20], *Azotobacter*, *Bacillus* [21] and *Pseudomonas* [13]. All these experiments resulted in increased yields due to improved nutrient availability and plant health.

Various studies have found that plant growth-promoting rhizobacteria (PGPR) strains vary widely in different soils and their ability to promote growth may be highly specific to particular species, cultivar, soil and genotype [17]. Under such circumstances, knowledge of native bacterial population and their identification is important for understanding their distribution and diversity [22]. It is important to explore and identify region-specific microbial strains which can be used as potential plant growth promoters to achieve higher yields under specific ecological and environmental conditions. There are no published studies that characterized the rhizobacteria in the soils of Western Kenya associated with nodules of *Phaseolus vulgaris* and therefore efforts to establish inoculants that are specific for these soils have been elusive. Information concerning the genetic diversity and distribution of these important microbes is thus necessary for the production of PGPR inoculants specific for this region.

Molecular techniques have successfully been used in examining microbial identity and diversity [23]. Mostly these studies have utilized sequence analysis of 16SrRNA gene which is highly conserved in all prokaryotes [24,25]. The conservation of this gene has enabled synthesis of primers that target various taxonomic groups but have enough variations to give phylogenetic comparisons of different microbial communities [26,27].

The composition of microbial communities can be analyzed based on profiles generated from physical separation of 16SrRNA gene sequences on the gel [23]. These techniques detect different sizes of PCR-amplified 16SrRNA gene fragments. Direct sequencing of 16SrRNA gene has been employed in establishing genetic relationships and characterization of strains at the generic or higher level [28]. Sequencing techniques have increased tremendously due to the invention of next-generation sequencing that has reduced the cost of sequencing [29] making the technique affordable even to low income researchers.

Methods

Study site

Nodules of common beans were collected from farmers' fields in which there is no history of inoculation with any nodule associated bacteria but in which common bean has been grown frequently. Nodules were collected from the slopes of Mt. Elgon, shores of Lake Victoria at Kisumu and Kakamega. At the shores Lake Victoria, nodules were collected from farm A (S 00° 08.729'; E 034° 69.596'), Farm B (S 00° 08.828'; E 034° 69.654'), Farm C (S 00° 08.852'; 034° 69.654') and Farm D (S 00° 09.094; E 034° 69.715'), all in Korando sub-location in Kisumu County. At Mt. Elgon region, soils were collected from Farm A (S 00° 79.209'; E 034° 63.688'), Farm B (S 00° 77.913'; E 034° 64.030'), Farm C (S 00° 81.852'; 034° 61.654') and Farm D (S 00° 82.094; E 034° 59.715'), all in Kapkateny sub-location in Bungoma County. At Kakamega, soils and nodules were collected from Farm A (S 00° 19.570'; E 034° 65.921'), Farm B (S 00° 20.779'; E 034° 65.663'), Farm C (S 00° 18.982'; 034° 68.534') and Farm D (S 00° 18.715; E 034° 68.607'), all in Kakamega South sub-county in Kakamega County. Collection strategy employed the randomized technique in which nodules were collected six meters apart following a W pattern running across the whole plot. Three common bean plants were collected from each site. The uprooted plants were packed in khaki bags and transported to the Microbiology laboratory at Masinde Muliro University of Science and Technology, Kenya for analysis.

Isolation of nodule associated bacteria

Nodule associated bacteria were isolated from surface-sterilized nodules according to the method described by Rincon et al. [30]. The nodule surfaces were first sterilized with 75% ethanol, followed by 0.1% mercuric chloride for about 3 min and then extensively rinsed six times with sterile distilled water. The water from the sixth rinse was streaked on yeast extract mannitol agar (YMA) to confirm the complete removal of nodule epiphytes before the nodules were crushed with a flame-sterilized blunt-tipped pair of forceps. The exudates of the crushed nodules were cultured on yeast-mannitol agar (YMA) medium at 28°C for 3 days, and a single colony was selected for further culture. The validation of the culture purity was performed by repeated streaking on Yeast extract mannitol agar medium and cellular examination in the microscope. The isolates were then stored in 20% glycerol at -70°C.

DNA extraction, PCR amplification, and sequencing of 16SrRNA gene

Genomic DNA was isolated using QIAamp® genomic DNA kit following the manufacturer's instructions and 16SrRNA gene was amplified using the universal primers, 27f (5'AGAGTTTGATCCTGGCTCAG 3') and 1492r (5' TACGGCTACCTTGTTACGACTT 3') which are complementary to conserved regions of the bacterial 16SrRNA gene. Amplification was carried out in 25 µL reaction volumes containing the following: 2.5 µL 10X PCR reaction buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl) and 1.5 µL 25 mM MgCl2 solution, 4.0 µL 1.25 mM, dNTPs, 0.5 µL of 27f primer (200 ng/µL), 0.5 µL of 1492r primer (200 ng/µl), 0.1 µL AmpliTaq Gold DNA polymerase and 1 µL of DNA as template. The reaction volume was adjusted up to 25 µL with sterile ultrapure water. The PCR thermal cycling conditions consisted of an initial denaturation step at 94°C for 3 min, followed by 30 cycles of denaturation (1 min at 94°C), annealing for 1 min at 57°C and extension for 2 min at 72°C, followed by a final extension at 72°C for 8 min. Double distilled water was used as negative control to check for false positive as a result of contamination of the reagents. PCR amplified products were separated on 1.0% agarose gels in 1X TBE buffer at 10 V cm^{-1} for 30 minutes.

After the gel was photographed, the bands were located by using UV lamp, cut out and placed in a 2 mL Eppendorf tube. The PCR fragments were then extracted from the gel using Qiagen Gel purification kit following the manufacturer's instruction. Sequencing reactions were performed at Bioneer, South Korea using the BigDye Terminator v3.1 sequencing Kit (Applied Biosystems, USA) with the primers 27f, and 1492r and sequenced products were analyzed using an automatic sequencer, ABI3730XL (Applied Biosystems).

Phylogenetic data analysis

Consensus sequences of the forward and reverse primers were generated in BioEdit ver. 7 [31] and then nucleotide alignment was generated by CLUSTAL W [32] implemented in BioEdit ver. 7. The alignment file was then loaded in MEGA 6 where the evolutionary history was inferred using the Neighbor-Joining method [33]. The bootstrap consensus tree inferred from 1000 replicates [34] was taken to represent the evolutionary history of the taxa analyzed [34]. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates were collapsed. The evolutionary distances were computed using the Jukes-Cantor method [35] and are in the units of the number of base substitutions per site. The analysis involved 24 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd and noncoding. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5 [36].

Results

Genetic diversity and distribution of nodule associated bacteria

This study reports a total of 24 strains of rhizobacteria isolated from common bean nodules, including *Delfitia*, *Rhizobia*, *Acinetobacter*, *Pseudomonas*, *Providencia*, *Enterobacter*, and *Klebsiella*. The 24 sequences submitted to the NCBI GenBank were assigned accession numbers as shown in Table 1.

Organism name	Strain/sample	NCBI Accession number
Enterobacter hormaechei	E1	KX856071.1
Pseudomonas koreensis	E2	KX856072.1
Providencia rettgeri	E3	KX856073.1
Providencia rettgeri	E4	KX856074.1
Pseudomonas koreensis	E5	KX856075.1
Providencia rettgeri	E6	KX856076.1
Enterobacter cloacae	E8	KX856077.1
Pseudomonas sp.	E9	KX856078.1
Pseudomonas sp.	E10	KX856079.1
Enterobacter sp.	K1	KX856080.1
Klebsiella pneumoniae	K2	KX856081.1
Providencia sp.	K3	KX856082.1
Pseudomonas koreensis	K5	KX856083.1
Enterobacter hormaechei	K6	KX856084.1
Delftia sp.	K7	KX856085.1
Rhizobium sp.	S2	KX856086.1
Delftia sp.	S3	KX856087.1
Rhizobium sp.	S4	KX856088.1
Delftia sp.	S5	KX856089.1
Rhizobium sp.	S6	KX856090.1
Delftia lacustris	S7	KX856091.1
Delftia lacustris	S8	KX856092.1
Enterobacter asburiae	S9	KX856093.1
Acinetobacter calcoaceticus	S10	KX856094.1

Table 1: NCBI identity of nodule associated bacteria (NAB) obtained from nodules of common beans.

Phylogenetic analysis

The isolates clustered into five clades on the phylogenetic tree shown in Figure 1, Clade A contained *Delfitia spp.* With GenBank accession numbers KX856092.1, KX856091.1, KX856085.1, KX856089.1, and KX856067.1. Clade B contained *Rhizobia spp.*, KX856088.1, KX856090.1, and KX856086.1. Both of these clustered were supported by 100% bootstrap confidence. Clade C contained members of *Pseudomonas spp.* with accession numbers KX856083.1, KX856075.1, KX856079.1, KX856078.1 and KX856078.1 whose branch was supported by 99% bootstrap confidence. Members of *Providencia spp.* formed Clade D with a branch supported by 100% bootstrap confidence and they included strains with accession numbers KX856082.1, KX856073.1, KX856076.1, and KX856074.1. Finally, *Enterobacter spp.* formed Clade E which contained strains with accession numbers KX856093.1, KX856080.1, KX856084.1, KX856071.1, and KX856077.1.

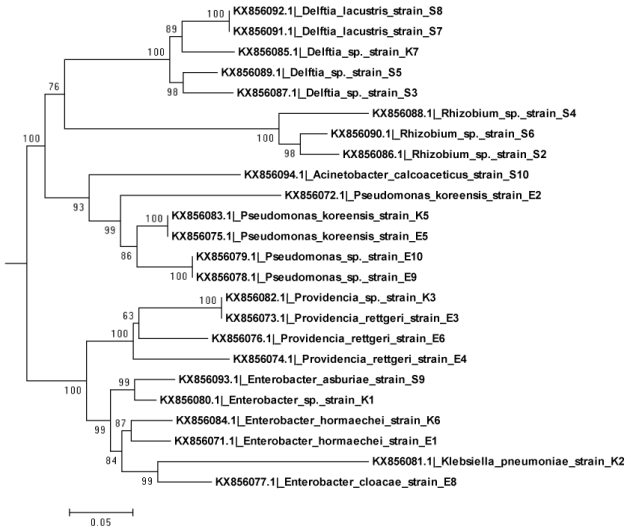

Figure 1: Phylogenetic tree. Organisms in the same clusters are genetically similar, while those in different clusters are genetically different.

Most of the isolates in each genus were genetically diverse with the exception of only a few members (Table 2). Among the genera *Enterobacter*, all the species were genetically diverse, the maximum [max] distance was 0.1831 ± 0.0125 between *Enterobacter asburiae* strain S9 and *Enterobacter* cloacae strain E8 while the minimum evolutionary distance was 0.0761 ± 0.0077 between *Enterobacter*

hormaechei strain K6 and *Enterobacter hormaechei* strain E1. The overall evolutionary distance among members of *Enterobacter* was 0.1416. Among members of *Delfitia*, the maximum evolutionary distance was 0.1183 ± 0.009 between *Delftia sp.* strain S3 and *Delftia lacustris* strain S7 and *Delftia lacustris* strain S8. *Delfitia lacustris* strains S7 and S8 had exactly 0.00 evolutionary distances meaning they were genetically identical. The overall evolutionary distance of *Delfitia* was 0.0988. Maximum evolutionary distance among members of Pseudomonas was 0.2553 ± 0.0152 between *Pseudomonas koreensis* strain E2 and *Pseudomonas sp.* strain E9 and *Pseudomonas sp.* strain E10. Genetic distance between *Pseudomonas sp.* strains E9 and E19 and *Pseudomonas koreensis* strain K5 and *Pseudomonas koreensis* strain E5 was 0.00. The overall mean evolutionary distance among members of *Pseudomonas* was 0.1344. The minimum evolutionary distance among members of *Providencia* was 0.1707 ± 0.0129 between *Providencia rettgeri* strain E4 and *Providencia sp.* strain K3 and *Providencia rettgeri* strain E3. Again, *Providencia sp.* strain K3 and *Providencia rettgeri* strain E3 had an evolutionary distance of 0.00 meaning that they were genetically identical. The overall genetic distance among members of these genera was 0.1269. In *rhizobia genera*, *Rhizobium_sp.* Strain S6 and *Rhizobium sp.* Strain S4 had the highest evolutionary distance of 0.1375 ± 0.0115. The overall genetic distance among members of Rhizobia was 0.1088. It follows that members of the genera *Enterobacter* were more diverse and members of *Delfitia* were less genetically diverse as shown in Figure 2.

Species 1	Species 2	Dist.	Err
KX856093.1|*Enterobacter asburiae* strain S9	KX856084.1|*Enterobacter hormaechei* strain K6	0.1485	0.0117
KX856093.1|*Enterobacter asburiae* strain S9	KX856080.1|*Enterobacter sp.* strain K1	0.0916	0.0093
KX856084.1|*Enterobacter hormaechei* strain K6	KX856080.1|*Enterobacter sp.* strain K1	0.1373	0.0105
KX856093.1|*Enterobacter asburiae* strain S9	KX856077.1|*Enterobacter cloacae* strain E8	0.1831	0.0125
KX856084.1|*Enterobacter hormaechei* strain K6	KX856077.1|*Enterobacter cloacae* strain E8	0.1608	0.012
KX856080.1|*Enterobacter sp.* strain K1	KX856077.1|*Enterobacter cloacae* strain E8	0.1714	0.0116
KX856093.1|*Enterobacter asburiae* strain S9	KX856071.1|*Enterobacter hormaechei* strain E1	0.1457	0.0122
KX856084.1|*Enterobacter hormaechei* strain K6	KX856071.1|*Enterobacter hormaechei* strain E1	0.0761	0.0077
KX856080.1|*Enterobacter sp.* strain K1	KX856071.1|*Enterobacter hormaechei* strain E1	0.1373	0.0107
KX856077.1|*Enterobacter cloacae* strain E8	KX856071.1|*Enterobacter hormaechei* strain E1	0.1646	0.012
KX856092.1|*Delftia lacustris* strain S8	KX856091.1|*Delftia lacustris* strain S7	0	0
KX856092.1|*Delftia lacustris* strain S8	KX856089.1|*Delftia sp.* strain S5	0.113	0.0089
KX856091.1|*Delftia lacustris* strain S7	KX856089.1|*Delftia sp.* strain S5	0.113	0.0089
KX856092.1|*Delftia lacustris* strain S8	KX856087.1|*Delftia sp.* strain S3	0.1183	0.009
KX856091.1|*Delftia lacustris* strain S7	KX856087.1|*Delftia sp.* strain S3	0.1183	0.009
KX856089.1|*Delftia sp.* strain S5	KX856087.1|*Delftia sp.* strain S3	0.1034	0.0083
KX856092.1|*Delftia lacustris* strain S8	KX856085.1|*Delftia sp.* strain K7	0.0965	0.0083
KX856091.1| *Delftia lacustris* strain S7	KX856085.1|*Delftia sp.* strain K7	0.0965	0.0083
KX856089.1|*Delftia sp.* strain S5	KX856085.1|*Delftia sp.* strain K7	0.1121	0.009
KX856087.1|*Delftia sp.* strain S3	KX856085.1|*Delftia sp. Strain* K7	0.1165	0.0088
KX856083.1|*Pseudomonas koreensis* strain K5	KX856079.1|*Pseudomonas sp.* Strain E10	0.093	0.0083
KX856083.1|*Pseudomonas koreensis* strain K5	KX856078.1|*Pseudomonas sp.* Strain E9	0.093	0.0083
KX856079.1|*Pseudomonas sp.* Strain E10	KX856078.1|*Pseudomonas sp.* Strain E9	0	0
KX856083.1|*Pseudomonas koreensis* strain K5	KX856075.1|*Pseudomonas koreensis* strain E5	0	0
KX856079.1|*Pseudomonas sp.* strain E10	KX856075.1|*Pseudomonas koreensis* strain E5	0.093	0.0083
KX856078.1|*Pseudomonas sp.* strain E9	KX856075.1|*Pseudomonas koreensis* strain E5	0.093	0.0083
KX856083.1|*Pseudomonas koreensis* strain K5	KX856072.1|*Pseudomonas koreensis* strain E2	0.2305	0.0147

KX856079.1|*Pseudomonas sp.* strain E10	KX856072.1|*Pseudomonas koreensis* strain E2	0.2553	0.0152
KX856078.1|*Pseudomonas sp.* Strain E9	KX856072.1|*Pseudomonas koreensis* strain E2	0.2553	0.0152
KX856075.1|*Pseudomonas koreensis* strain E5	KX856072.1|*Pseudomonas koreensis* strain E2	0.2305	0.0147
KX856082.1|*Providencia sp.* Strain K3	KX856076.1|*Providencia rettgeri* strain E6	0.1301	0.0108
KX856082.1|*Providencia sp.* strain K3	KX856074.1|*Providencia rettgeri* strain E4	0.1707	0.0129
KX856076.1|*Providencia rettgeri* strain E6	KX856074.1|*Providencia rettgeri* strain E4	0.1599	0.012
KX856082.1|*Providencia sp.* Strain K3	KX856073.1|*Providencia rettgeri* strain E3	0	0
KX856076.1|*Providencia rettgeri* strain E6	KX856073.1|*Providencia rettgeri* strain E3	0.1301	0.0108
KX856074.1|*Providencia rettgeri* strain E4	KX856073.1|*Providencia rettgeri* strain E3	0.1707	0.0129
KX856090.1|*Rhizobium sp.* strain S6	KX856088.1|*Rhizobium sp.* Strain S4	0.1375	0.0115
KX856090.1|*Rhizobium sp.* strain S6	KX856086.1|*Rhizobium sp.* Strain S2	0.0609	0.0074
KX856088.1|*Rhizobium sp.* strain S4	KX856086.1|*Rhizobium sp.* Strain S2	0.128	0.0108

Table 2: Estimates of evolutionary distance among members of the same genus.

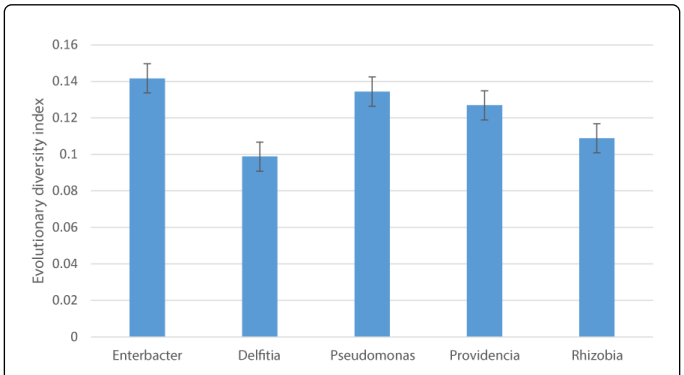

Figure 2: Overall evolutionary distances among members of the same genus.

Discussion

Genetic diversity and distribution of nodule associated bacteria

This result supports other studies that found more than one species of rhizobacteria in the nodules of various leguminous plants. Stajković et al. [37] reported the isolation of 115 bacterial strains from 15 nodules, of which almost 60% were rhizobia while the rest belonged to several other genera. According to the results reported by Rajendran et al. [19] about 10% of the surface sterilized nodules tested showed the presence of endophytic nonrhizobial flora and some nodules showed more than one morphologically distinct nonrhizobial colonies. Kuklinsky-Sobral et al. [38] who reported the isolation of nodule endophytes belonged to the genera *Phyllobacterium*, *Sphingomonas, Rhodopseudomonas, Pseudomonas, Microbacterium, Mycobacterium,* and *Bacillus* from soya bean nodules. Costa et al. [39] isolated the genera *Agromyces, Bacillus, Brevibacillus, Delfitia, Dietzia, Enterobacter, Methylobacterium, Microbacterium, Micrococcus, Paenibacillus, Pseudomonas, Rhizobium, Rhodococcus, Sphingobacterium*, and *Stenotrophomonas* from *Phaseolus vulgaris*. Probably all the organisms whose presence has a beneficial relation might get associated with the plant nodules.

Pseudomonas sp. was distributed in the whole of Western Kenya region because it was isolated from all the nodule samples of common beans collected from the slopes of Mt. Elgon, shores of Lake Victoria at Kisumu and Kakamega, Its population was high in nodules from the common beans grown in Kakamega. This is an indication that it's the best-adapted nodule associated bacteria in this region. Owing to its importance as plant growth promoting bacteria [40], more sensitive characterization techniques are required to determine the type of species found in Western Kenya. *Rhizobia sp.* was isolated from the common beans grown on the slopes of Mt. Elgon and shores of Lake Victoria at Kisumu, but it was not isolated from those from Kakamega. Although there were very few plants with nodules from this region, nodulation has always been believed to be the reserve of rhizobia [41,42] this, therefore, calls for further studies on all other nodule associated bacteria with the aim of finding out if other bacteria apart from rhizobia are also capable of inducing nodulation. The population of rhizobia was high in Kisumu soils; in fact, it was the most abundant species of rhizobacteria in Kisumu soils. *Enterobacter sp.* was isolated in Kakamega and Mt. Elgon soils but not Kisumu soils and its population was highest and most abundant in Kakamega soils. *Providencia sp.* was isolated in Kakamega and Mt. Elgon soils and abundantly in Mt. Elgon soils. *Klebsiella sp.* was isolated in Mt. Elgon and Kakamega soil with similar abundance. *Delfitia sp.* was isolated from Kisumu and Kakamega soils and abundantly in Kisumu soils. *Sphingobacterium sp.* and *Acinetobacter sp.* was isolated only from Kisumu soils with similar abundance.

Phylogenetic analysis on the basis of 16SrRNA gene sequences provided better understanding in the evaluation of genetic diversity of NAB isolated in this study. The neighbor-joining tree constructed put the isolates into two main clusters, the second cluster was further subdivided into six other sub-clusters. Even most isolates in the same sub-cluster differed in their genetic distances showing that most of the

nodule associated bacteria in the soils of Western Kenya are genetically different.

16SrRNA gene of the isolates was highly conserved but with variable regions which make it a good marker in studying evolutionary diversity. This is in tandem with other studies which have shown that the 16SrRNA gene is efficient in defining the genera because it is conserved but have variable regions, just enough to determine genetic diversity in organisms [43]. However, it has limitations in identifying species, due to the possible occurrence of genetic recombination and horizontal gene transfer resulting in sequence mosaicism [44,45], and perhaps this might be the reason why members of different genera clustered together on the phylogenetic tree. Another limitation of identifying bacteria based on the analysis of 16SrRNA genes is that species that are closely related may not always be differentiated because of the sequence conservation of 16SrRNA gene [46]. To overcome these difficulties, the use of other genes including protein-coding genes with greater sequence divergence than 16SrRNA genes, are recommended as alternative genetic markers for identification of the nodule associated bacteria [46].

Conclusion

Common bean nodule associated bacteria in Western Kenya soils are genetically diverse as shown by 16SrRNA phylogenetic analysis. This might be due to different climatic conditions experienced in the region. More studies are therefore recommended to determine their growth promotion ability in order to develop inoculants that are adapted to this region.

Declarations

The authors declare that they have no competing interests.

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