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Identification of culturable bacteria associated with the rhizosphere of *Lablab purpureus* growing in Namibia.

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Abstract

Dolichos lablab (*Lablab purpureus* (L.) sweet)) is a multipurpose drought tolerant protein-rich legume crop native to Africa and grown in warm temperate to tropical climates for its edible seeds and manure. *Lablab purpureus* holds significant benefits to subsistence farmers and offers a great potential for sustainable crop productivity, especially in marginalised areas. Its uses range from human consumption as a vegetable to improving soil fertility, and as forage. Notwithstanding *Lablab purpureus* important potential functions in Namibia, there is currently limited information regarding the plant's rhizosphere bacteria. The study aimed at identifying *Lablab purpureus* natural rhizosphere bacteria. Isolation of rhizosphere bacteria involved the use of general media (Luria Bertani agar and tryptic soy agar); selective media such as Rhizobium and Yeast Extract Mannitol (YEM) Congo red from soil sample extracts. Eighty-five strains of bacteria were isolated and were subsequently identified by 16S rRNA gene sequencing analysis. The results showed that they belonged to the following genera, *Bacillus, Streptomyces, Exiguobacterium, Stutzerimonas, Rhizobium, Acidovorax, Agrobacterium, Psychrobacter, Priestia, Planococcus, Bhargavaea, Stenotrophomonas, Caulobacter, Peribacillus, Niallia, Athrobacter, Sphingobium, Enterobacter, Sphingobacterium, Sinorhizobium, Flavobacterium, Microbacterium, Metabacillus, Neobacillus,* and Pseudomonas which are reported to have growth promoting substances. The study highlighted the potential use of these plant growth promoting rhizobacteria for inocula production or biofertilisers for enhancing growth and nutrient content of beans and other crops under field conditions. The study was the first report of *Lablab purpureus*'s rhizosphere associated bacteria in Namibia.

Keywords: Rhizosphere bacteria, *Lablab purpureus*, Dolichos lablab, 16s rRNA, Namibia.

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Introduction

Dolichos lablab (*Lablab purpureus* (L) sweet) also known as Dolichos bean, Indian bean or Hyacinth bean is a perennial herb from the family Fabaceae endowed with high protein content and nutritional value [1]. This drought-tolerant under-utilised crop has a global social recognition that stretches from human food (vegetable and pulse) to soil fertility improvement to high-quality animal fodder [2-4]. This crop is indigenous to India and Africa and is mostly grown by small-scale farmers in the semi-arid, dry poor soils of Africa, Southeast Asia and America [2,3,5,6]. Agricultural methods involving inappropriate use of chemical pesticides and fertilisers which are usually costly, not readily available and have caused environmental problems need to be addressed. Alternative ways of sustainably meeting agricultural demands involve using rhizobacteria or other microbial inoculants for plant growth and development [7].

According to Pervin *et al.*, [8] plants have the unusual ability to form symbioses with soil bacteria. The exchange of resources between roots and the soil environment takes place in the rhizosphere, which is a biologically active interface around plant roots [9]. Sugars, carbohydrates, and secondary metabolites released by plant roots shape the microbial community structures and have an impact on rhizosphere zone activities [10]. Different plant species have different amounts and qualities of these root exudates, and they can select particular microbial communities, including fungi and bacteria that have different roles in the soil [11].

Rhizobacteria directly promote plant growth by controlling levels of plant hormones or by helping in procurement of essential minerals, phosphorus, and nitrogen; or indirectly

as biocontrol agents that decrease the repressive effects that different pathogens have on plant growth [12]. They improve plant yield and growth when applied to seeds or crops and are usually mediated by root exudates such as siderophores, enzymes, sugars, and amino acids [7,10,13]. Rhizosphere bacteria are of great importance because they promote plant productivity by regulating nutrient mineralization, acting as environmental buffers, permitting decomposition, and by enhancing water relations [14].

Identifying the rhizosphere bacteria growing in association with *Lablab purpureus* will explain on whether they have any role in plant growth promotion. It could elucidate how they adapt to the Namibian environment and can be useful in other agricultural practices. Moreover, information on the bacteria that are promoting its growth have the potential to increase other crops' drought stress tolerance and thus be beneficial agricultural bioresources. The aim of this study was to isolate and identify the bacteria associated with *Lablab purpureus* rhizosphere.

Materials and Methods

Sample collection and process

Lablab purpureus root samples with bulk rhizosphere soil were collected from 2 varieties in Windhoek (Whk1 and Whk2) at Olympia suburb (Coordinates: -22.5652880, 17.1103930) and were placed in sterile individually labelled zip lock bags. These were then transported on ice to the laboratory for further analysis. Seeds from 5 bean accessions (IC-0623096, IC-0623072, HA 4, IC-0623005 and IC-0623043) were planted in pots containing soil from Bagani Research Centre and were taken to the lab for processing after they had shown the first 3 leaves.

The two (2) Windhoek varieties were purposefully sampled, whereas the other 5 varieties grown in the pots were randomly chosen from the other 24 varieties.

The plants in the pots were uprooted and shaken to get rid of the bulk soil (Figure 1). For the 2 Windhoek varieties, samples were aseptically removed from the zip lock bags and shaken off to get rid of bulk soil. Using a sterile blade, gloves and weighing boat, 0.3 grams of rhizosphere roots from each variety were weighed.

Isolation of bacteria

By carefully placing 0.3 g of rhizosphere roots from each sample in 25 ml of sterile Phosphate Buffered Saline (PBS) in a 50 ml falcon tube, rhizosphere bacteria were isolated from soil. The tube was then vortexed, and the suspension serial diluted to 10^{-10}. Thereafter, each dilution (100 µl) was spread on Luria Bertani (LB) agar, Tryptic Soy Agar (TSA), Rhizobium media agar and Yeast Extract Mannitol (YEM) Congo red agar plates substituted with the fungicide nystatin (100 µg/ml) and growth was observed until bacterial colonies appeared. Selected colonies were subcultured on the appropriate media until pure colonies were obtained (Figure 2).

Figure 1. *Roots from* Lablab purpureus *after uprooting the plant and shaking off excess soil from roots.*

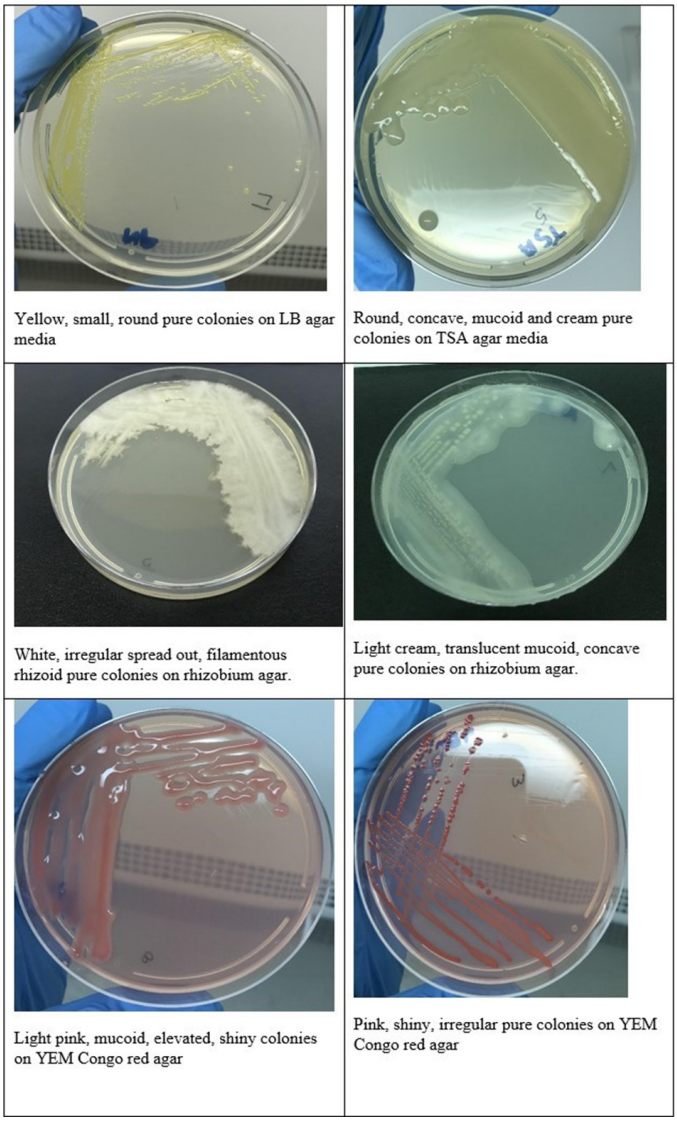

Figure 2. *Picture of some of the pure cultures of the rhizosphere bacteria isolated.*

Catalase test

Using a sterile inoculation loop, a little bit of bacteria was spread onto a dry microscope slide. A drop of 3% hydrogen peroxide was applied to the bacteria using a pipette. The presence or absence of bubbles immediately gave an indication whether that bacterium is catalase negative or catalase positive [15].

DNA extraction

The pure cultures of the strains were each inoculated in 100 ml of fresh LB or tryptic soy broth and incubated for 48 hours to increase the mass of cells [16]. The DNA from each isolate (strains 1-39) were extracted from the broth using the protocol from the Zymo bacteria DNA isolation Kit. The other strains that were not extracted from the kit, were used directly in colony PCR.

Conventional PCR of bacterial genomic DNA

The primers were purchased in lyophilized form from Inqaba Biotech (South Africa) and reconstituted in accordance with the manufacturer's instructions. From a stock solution of 100 µM, a working solution (10 µM) was made for each primer. They were kept until use at -20°C.

Primers for 16S rRNA gene were 27F (5-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5-CGGTTACCTTGTTACGACTT-3) with amplicon length of approximately 1500 bp as described by Zahid *et al.*, [16].

The amplification conditions: Qualitative PCR was carried out following DNA extraction from the samples. A 25 µl overall reaction made up of 12.5 µl of VWR Red Taq DNA Polymerase (2× MM) with buffer (2 mM $MgCl_2$) from life sciences, 3 µl of template DNA from the samples, 7.5 µl of nuclease-free water, and 1 µl of both reverse and forward primer were used. Additionally, a negative control was included, consisting of all the components but with 3 µl of nuclease-free water in place of the template DNA. This was carried out for all PCR processes. Conventional PCR was performed in the molecular biology laboratory at UNAM on a MULTIGENE OPTIMAX Cycler from Labnet International Inc. The conditions used were

denaturation at 95°C for 4 minutes, final denaturation at 95°C for 1 minute; annealing at 55°C for 30 seconds; extension at 72°C for 1 minute; final extension at 72°C for 1 minute and final hold at 4°C.

The PCR products were examined using 2% gel electrophoresis. Using agarose gel with a 1 kb ladder and 4 µl of PCR products loaded onto it and the gel had been stained with ethidium bromide. An image of the agarose gel was captured using a digital camera after it had been run for 45 minutes at 120V in a 1× TBE buffer.

The PCR bands that were visualised were those of about 1500 bp in size and those amplicons were then sent to Inqaba Biotech for sequencing.

Colony PCR

Bacterial strains whose DNA was not extracted using the kit were used directly in colony PCR as described by Pesce *et al.*, [17] using the same 16S primer sequences. A single pure colony was aseptically picked and added to an Eppendorf tube that had 80 µl of sterile distilled water. It was then vortexed for 15 minutes and thereafter incubated at 96°C for 10 minutes. It was let to cool at 4°C for ten minutes. PCR was performed using the supernatant.

For a 25 µl colony PCR reaction, 11.5 µl supernatant from the tubes was added to a PCR tube together with 12.5 µl of the master mix and 1 µl each of reverse and forward primers. The conditions used in the thermocycler were denaturation at 95°C for 4 minutes, final denaturation at 95°C for 1 minute; annealing at 55°C for 30 seconds; extension at 72°C for 1 minute; final extension at 72°C for 1 minute and final hold at 4°C.

The PCR products were examined using 2% gel electrophoresis. The agarose gel had a 1 kb ladder and 4 ul of PCR products loaded onto it and the gel had been stained with ethidium bromide. An image of the agarose gel was captured using a digital camera after it had been run for 45 minutes at 120V in a 1× TBE buffer.

The PCR bands that were visualised were those of about 1500bp in size and those amplicons were then sent to Inqaba Biotech for sequencing (Figure 3).

Isolate identification and phylogenetic analysis

The sequences were cleaned and edited using BioEdit software version 7.2.5 [18] and Chromas software version 2.6.6 (http://www.technelysium.com.au/chromas.html). The Basic Local Alignment Search Tool (BLAST) program was used to align and analyse the DNA sequences to determine the closest matches (Table 1). The MEGA version 11.0.13 was used to create the phylogenetic tree and inferred with neighbour joining in 1000 replicates of bootstrap. The bootstrap consensus tree (Figure 4) was obtained.

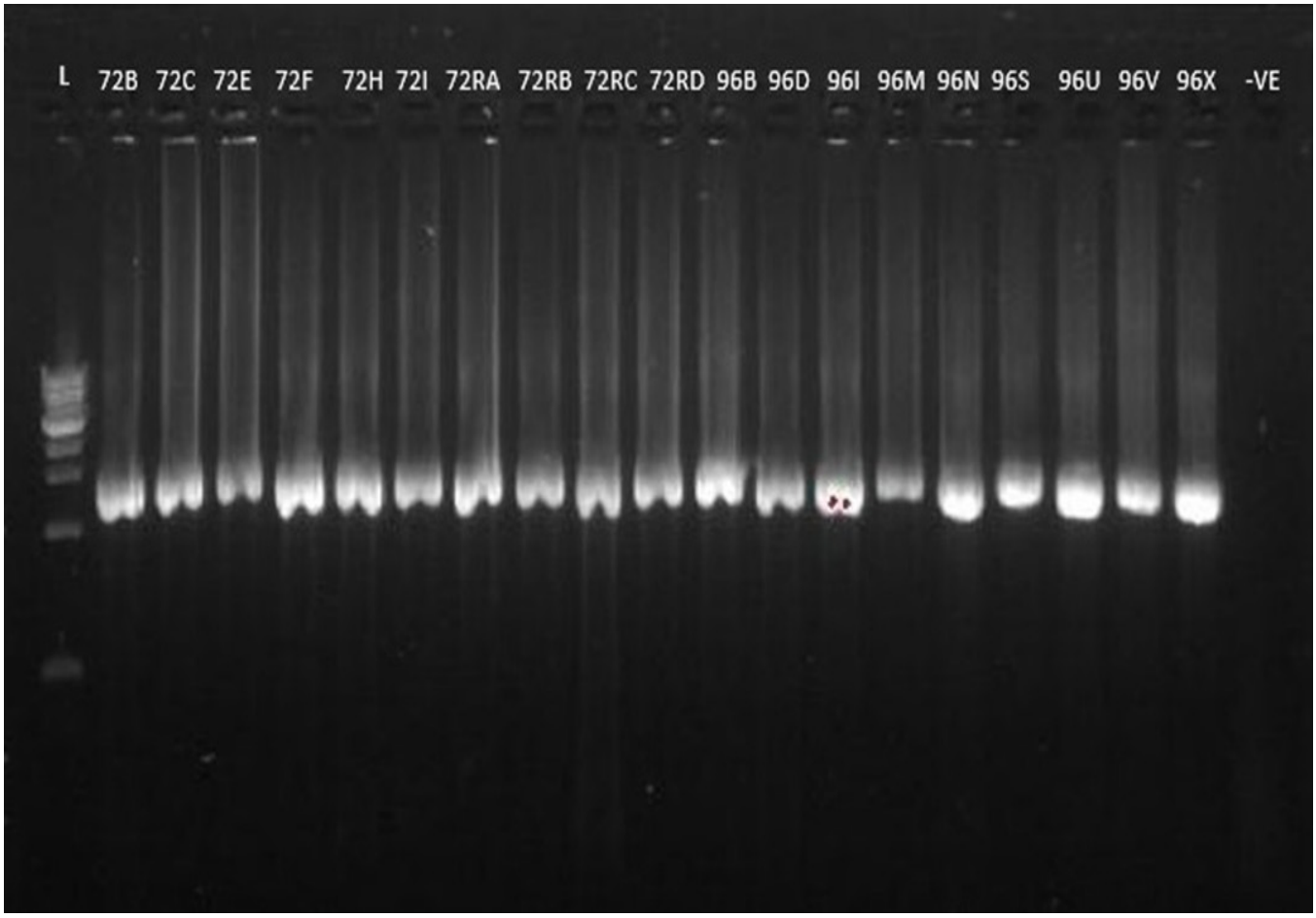

Figure 3. *Agarose gel of some of the 16s bacteria strains from colony PCR: L=1 kb DNA ladder and -VE=negative control.*

Table 1. *Molecular identification of isolated bacterial strains based on 16S rDNA sequences.*

Bacteria strain code	Homology to the reference strains	% Percent identity	Accession numbers from GenBank
1	*Exiguobacterium aurantiacum*	99.44	OR921826
2	*Stutzerimonas kunmingensis*	99.13	OR921827
4	*Peribacillus simplex*	99.72	OR921828
5	*Bacillus wiedmannii*	99.59	OR921829
5C	*Peribacillus acanthi*	98.77	OR921830
5D	*Acidovorax delafieldii*	99.14	OR921831
5E	*Agrobacterium tumefaciens*	99.71	OR921832
5F	*Priestia aryabhattai*	100	OR921833
6	*Microbacterium testaceum*	99.87	OR921834
7	*Priestia aryabhattai*	99.86	OR921835
8	*Planococcus ruber*	99.27	OR921836
9	*Bacillus wiedmannii*	100	OR921837
10	*Streptomyces caviscabies*	98.61	OR921838
11	*Rhizobium rosettiformans*	99.6	OR921839
12	*Bacillus albus*	100	OR921840
13	*Priestia megaterium*	99.86	OR921841
14	*Agrobacterium tumefaciens*	100	OR921842
15	*Agrobacterium tumefaciens*	98.7	OR921843
16	*Exiguobacterium mexicanum*	99.87	OR921844
16B	*Exiguobacterium mexicanum*	100	OR921845
17	*Stenotrophomonas maltophilia*	99.34	OR921846
18	*Agrobacterium fabrum*	97.98	OR921847
19	*Pseudomonas japonica*	99.76	OR921848
20	*Agrobacterium pusense*	99.62	OR921849
21	*Bacillus licheniformis*	99.88	OR921850
22	*Bhargavaea cecembensis*	95.77	OR921851
23	*Bacillus fungorum*	99.76	OR921852
24	*Bacillus cereus*	99.77	OR921853
25	*Priestia flexa*	96.68	OR921854
26	*Bacillus cereus*	99.76	OR921855
27	*Bacillus cereus*	99.65	OR921856
28	*Bacillus amyloliquefaciens*	100	OR921857
29	*Bacillus licheniformis*	98.2	OR921858
30	*Peribacillus frigoritolerans*	99.88	OR921859
31	*Stutzerimonas chloritidismutans*	98.92	OR921860
32	*Niallia taxi*	99.65	OR921861
33	*Planococcus plakortidis*	95.59	OR921862

34	*Caulobacteraceae bacterium*	100	OR921863
35	*Bacillus subtilis*	99.77	OR921864
36	*Bacillus wiedmannii*	99.87	OR921865
37	*Arthrobacter sedimenti*	93.6	OR921866
38	*Bacillus cereus*	100	OR921867
39	*Priestia megaterium*	99.34	OR921868
72B	*Agrobacterium tumefaciens*	100	OR921869
72C	*Bacillus cereus*	99.17	OR921870
72E	*Agrobacterium tumefaciens*	100	OR921871
72F	*Pseudomonas knackmussii*	100	OR921872
72H	*Agrobacterium tumefaciens*	100	OR921873
72I	*Agrobacterium tumefaciens*	99.74	OR921875
72RA	*Agrobacterium tumefaciens*	99.86	OR921876
72RB	*Sphingobium mellinum*	98.2	OR921877
72RC	*Priestia megaterium*	98.27	OR921878
72RD	*Priestia flexa*	83.53	OR921879
96B	*Bacillus wiedmannii*	99.29	OR921880
96D	*Bacillus thuringiensis*	100	OR921881
96L	*Bacillus toyonensis*	99.43	OR921882
96M	*Neobacillus niacini*	99.29	OR921883
96N	*Neobacillus niacini*	98.43	OR921884
96S	*Agrobacterium pusense*	98.97	OR921885
96U	*Streptomyces gardneri*	99.72	OR921886
96V	*Agrobacterium tumefaciens*	99.86	OR921887
96X	*Flavobacterium panacis*	84.81	OR921888
96Z	*Bacillus subtilis*	99.71	OR921889
FB1	*Exiguobacterium mexicanum*	99.31	OR921890
HA2	*Priestia megaterium*	99.18	OR921891
HA8	*Flavobacterium anhuiense*	99.25	OR921892
HA11	*Rhizobium herbae*	98.64	OR921893
HAC	*Enterobacter cloacaes*	99.87	OR921894
HAD	*Psychrobacter nivimaris*	99.42	OR921895
HAE	*Psychrobacter namhaensis*	96.73	OR921896
HAF	*Bacillus thuringiensis*	99.43	OR921897
HAG	*Stutzerimonas stutzeri*	99.3	OR921898
HAH	*Sinorhizobium meliloti*	100	OR921899
PG1	*Sphingobacterium zeae*	89.38	OR921900
PG4	*Pseudomonas koreensis*	98.35	OR921901
PG10	*Pseudomonas koreensis*	99.86	OR921902

PG14	*Pseudomonas koreensis*	99.86	OR921903
PG15	*Stenotrophomonas lacuserhaii*	99.61	OR921904
PG18	*Metabacillus niabensis*	100	OR921905
PG19	*Pseudomonas poae*	98.87	OR921906
PP1	*Bacillus cereus*	100	OR921907
PP2	*Stenotrophomonas maltophilia*	99.86	OR921908
PPA	*Agrobacterium tumefaciens*	100	OR921909
PPB	*Pseudomonas canavaninivorans*	99.55	OR921910
PPF	*Priestia aryabhattai*	100	OR921911

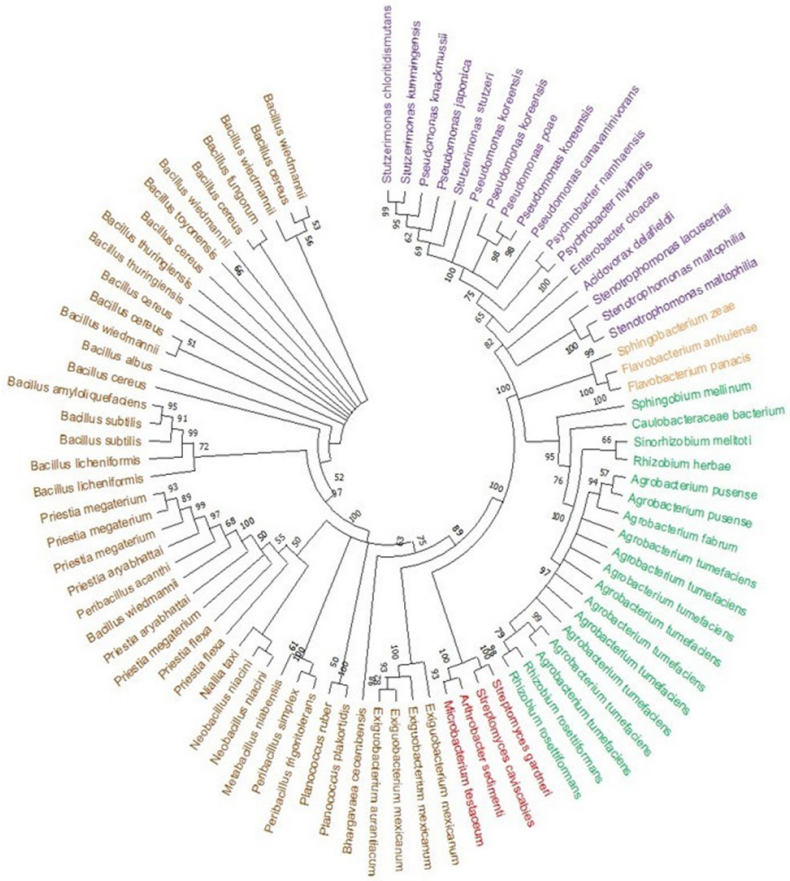

Figure 4. *Circular consensus tree showing the phylogenetic relatedness of rhizosphere bacteria associated with* Lablab purpureus *based on 16S rRNA gene sequences. The percentage of replicate trees (≥ 50%) in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown below the branches. The evolutionary history was inferred* via *the Neighbour-joining method using MEGA 11.*

Results

A total of 85 bacterial strains were isolated from *Lablab purpureus* rhizosphere. 16S rRNA gene sequencing was used to identify the isolated bacteria.

Catalase reaction

Most isolates were found to be catalase positive except strains: 6, 13, 72E, 72I and 72H representing 5.9% of all isolates.

Molecular Identification of bacteria strains

From the *Lablab purpureus* rhizosphere soil samples, a total of 85 bacterial strains were isolated and identified using 16S rRNA gene sequencing (Table 1). The sequences were also submitted to GenBank where accession numbers were assigned to them. Among the identified genera were *Bacillus, Streptomyces, Exiguobacterium, Stutzerimonas, Rhizobium, Acidovorax, Agrobacterium, Psychrobacter, Priestia, Planococcus, Bhargavaea, Stenotrophomonas, Caulobacter, Peribacillus, Niallia, Athrobacter, Sphingobium* and *Pseudomonas.*

Phylogenetic analysis

Clade one (purple colour) in Figure 4 consists of 17

sequences representing 20% of the rhizobacteria isolated. This clade consisted of six different genera, five of which belong to the *Gamma proteobacteria. Stutzerimonas* had a percentage similarity of 98-99% to the closest strain from the NCBI database and was supported by strong bootstrap values >95%, *Pseudomonas* (percentage similarity of 98-99%) with a bootstrap of 98%, Psychrobacter (percentage similarity 96-99%) with a bootstrap of 100%, *Stenotrophomonas* (99% similarity) supported by a 100% bootstrap value and *Enterobacter* (99% similarity) also with a bootstrap of 100%. This suggests that they belong to those genera. One sequence in this clade was not in a cluster and belongs to the class *Betaproteobacteria*. It has a 99% percentage similarity to the genus *Acidovorax* from NCBI Database but a weak bootstrap value that suggests lower confidence of it being grouped into that genus.

Three sequences resulting from clade 2 (orange) are all from the phylum *Bacteroidetes*. The sequences are representing only 3.5% of all the rhizobacteria isolated. Two belong to the genus *Flavobacterium* (percentage similarity 84-99%) and supported by a bootstrap of 100%. The other sequence had an 89% percentage similarity to *Sphingobacterium* from the NCBI database and a bootstrap of 100%. Due to their low sequence similarity, the sequences in this clade are most likely from new genera.

Clade 3 (green) consisted of 18 sequences accounting for 21.1% of the sequences isolated. They all belong to the class Alpha proteobacteria. The genus *Agrobacterium* (percentage similarity 97-100%) and bootstrap of 97% formed the majority (66.7%) and polytomic section in this clade. Two sequences with percentage similarity of 99% and bootstrap of 98% clustered with significant support to indicate that they belong to the genus *Rhizobium*. *Sinorhizobium* (100% similarity) clustered with Rhizobium with a moderate bootstrap of 66%. The genus *Sphingobium* (98% similarity) with a bootstrap of 96% and the genus *Caulobacter* (100% similarity) supported by a strong bootstrap also belonged to this clade.

Clade 4 (red) accounted for only 4.7% of rhizosphere bacteria isolated from *Lablab purpureus*. The sequences were of three genera that belong to *Actinomycetes*. The genus *Microbacterium* (99% similarity), *Athrobacter* (93% similarity), *Streptomyces* (percentage similarity 98-99%) with respective bootstrap values of 93%, 93% and 100% to support that these sequences are indeed related to those genera.

The 43 sequences derived from clade 5 (brown) represented 50.6% of all isolated sequences. This deeply branched clades' sequences were all from the phylum *Baciollota* and from 9 genera. The genera *Bacillus* (percentage similarity 98-100%) with bootstrap values ranging from 51-99%, *Exiguobacterium* (percentage similarity 99-100%) with bootstrap range of 82-100%, *Planococcus* (percentage similarity 95-99%) with 100% bootstrap, *Bhargavaea* (percentage similarity of 95%) with 75% bootstrap and *Peribacillus* (percentage similarity 98-99%) with 100% bootstrap are all well supported to suggest they belong to those genera. The genera *Niallia* (99% similarity), *Metabacillus* (100% similarity), *Neobacillus* (98 to 99% similarity) and *Priestia* (percentage similarity 83-100%) had moderate bootstrap values of 50%, 61%, 50% and 50-100% respectively to support their groupings.

Discussion

The catalase activity found in 94.1% of the isolated bacteria has evolutionary and advantageous implications for the *Lablab purpureus* bacteria association. Catalase-producing bacteria are extremely resilient to physical, chemical, and environmental stress [15]. *Lablab purpureus* thrives in challenging drought conditions with high daytime temperatures above 37°C, acidic soils, and low nutrient soils.

In this study, culturable bacterial analysis of *Lablab purpureus* rhizosphere revealed a considerable bacteria diversity. Using colony morphology and 16S rRNA gene sequencing, 85 strains were identified. The identified genera were *Bacillus, Streptomyces, Exiguobacterium, Stutzerimonas, Rhizobium, Acidovorax, Agrobacterium, Psychrobacter, Priestia, Planococcus, Bhargavaea, Stenotrophomonas, Caulobacter, Peribacillus, Niallia, Athrobacter, Sphingobium, Enterobacter, Sphingobacterium, Sinorhizobium, Flavobacterium, Microbacterium, Neobacillus, Metabacillus,* and *Pseudomonas*. Bacteria in these genera have been found in literature to have PGP traits and can thus be useful in establishment of biofertilisers.

In the study done by Di Benedetto *et al.*, [19] *Pseudomonas* produces siderophores and ammonium at different levels (high, moderate, or weak). *Pseudomonas* species produces auxins, and profoundly increases crop production when grown in artificially dry environments, directly influencing the promotion of plant growth under drought stress. Bacteria that are under an iron stress produce and secrete siderophores to bind iron [19]. The same authors' *Bacillus* isolates produced a lot of ammonium and showed a high level of nitrification. Their findings supported the ability of *Bacillus* and related genera to promote plant growth.

Bacteria genera associated with potassium metabolism are *Bacillus*, *Pseudomonas* and *Rhizobium* and they achieved this by making potassium more available for plant use [20]. The same authors reported that rhizobia are involved in nitrogen fixation and nitrogen metabolism. Their findings also showed that *Streptomyces* species alleviated metal contamination stress in plants by producing siderophores.

Priestia megaterium was described to increase plant disease resistance and exhibits potential as a future biocontrol agent [21]. Since they were able to demonstrate that this bacterium produces ACC deaminase, has nitrogen-fixing capabilities, secretes Indole Acetic Acid (IAA), and can solubilize both potassium and phosphate, the same authors

claimed that it is bacteria that promotes plant growth.

In the study by Suharjono and Yuliatin [22] *Bacillus wiedmannii* was found to release IAA hormone, had nitrogen-fixing properties as well as being able to solubilize phosphate in order to enhance plant growth. The same authors also reported that *Pseudomonas putida* quantified IAA and siderophores and enhanced phosphate solubilization.

The genera *Exiguobacterium*, and *Arthrobacter* are capable of synthesising siderophores, nitrogen fixation, and phosphate solubilization; *Arthrobacter* is also capable of synthesising the hormone IAA [23]. These authors also report that bacteria belonging to the genera *Pseudomonas*, *Agrobacterium* and *Stenotrophomonas* through endophytic associations can give plants nitrogen. The ethylene-producing precursor 1-Amino-Cyclopropane-1-Carboxylate (ACC) is also used by these three bacteria genera, which reduces the production of plant hormones produced in response to stressful conditions [23]. *Exiguobacterium* bacteria are thought to be able to tolerate salinity stress and regulate secondary metabolites in plants [14]. Moreover, Shen *et al.*, [24] state that *Exiguobacterium* is also of importance in microplastic degradation in the environment.

The bacteria of the genus *Niallia* were found to be catalase positive and having optimum growth temperature of 30°C. In nutrient agar, it seemed to also be able to grow in temperature range of 5-40°C. *Peribacillus* bacteria produce siderophores, IAA, ACC deaminase, they are also phosphate solubilizing and are nitrogen fixing bacteria [25].

Romanenko *et al.*, [26] described *Psychrobacter* as being catalase positive and have an ability to grow at high temperature of 37°C. Bacteria in this genus also have ACC deaminase activity, phosphate solubilizing activities and can produce indole and siderophores [27].

Bacteria in the genus *Acidovorax* promote shoot and root growth and are also beneficial by reducing insect growth such as in the suppression of pests like aphids [28]. These bacteria also compete with pathogens, and they produce hormones and secondary metabolites which promote plant growth [29]. According to Manorama *et al.*, [30] the genus *Bhargavaea* is catalase positive and has an ideal growth temperature of 37°C although the cells can also grow from 15-55°C.

Planococcus genus of bacteria has ACC deaminase, IAA production and is also phosphate solubilizing [31]. Boss *et al.*, [32] found *Sphingobium* to produce IAA and siderophores. The genus *Stutzerimonas* has bacteria with very useful biodegradation capabilities [33].

Caulobacter bacteria produce cytokinin, auxins and have ACC deaminase activity all of which help improve plant growth [34].

According to Weyenberg and Yoshida [35] phylogenetic trees can be used to organise various types of biological data and to make inferences about possible occurrences in an organism's evolutionary past. The consensus phylogenetic tree constructed (Figure 4) shows how this study's isolates are related to the ones in the NCBI database.

Five clades were observed (Figure 4) in this study with various clusters and tight sub-clusters with strong bootstrap support. Berta *et al.*, [36] state that branches with high bootstrap values such as those in the range of 70-100% indicate greater confidence in those groupings. Genera that had groupings with bootstrap values of 100% were *Exiguobacterium, Streptomyces*, *Peribacillus*, *Neobacillus* and *Flavobacterium*. Some of these bacteria have been isolated before. *Streptomyces* have been isolated from rhizospheres and bulk soils of plants [37]. Santoyo *et al.*, [38] state that *Neobacillus* is a common inhabitant of the rhizosphere. *Exiguobacterium* has been found in root nodules of fenugreek and the rhizosphere of cowpea [39,40].

Flavobacterium was isolated from wild legume nodules [41]. Furthermore, *Peribacillus* has been isolated from soybean roots and the rhizosphere of pepper [42,43]. The clusters with bootstrap values between 70-100% were found to be of the genera *Agrobacterium, Pseudomonas, Sphingobacterium, Stutzerimonas, Planococcus, Bhargavaeae, Rhizobium, Caulobacter, Sphingobium, Psychrobacter, Enterobacter, Acidovorax, Stenotrophomonas, Sinorhizobium, Microbacterium, Athrobacter,* and *Priestia*. This suggests that these clusters have close phylogenetic relatedness to their nearest matches in NCBI database and that the groupings that were observed are likely to be accurate and reflect the true evolutionary relationship among the bacteria [36].

Conclusion

Analysis of *Lablab purpureus* rhizosphere revealed a great diversity of bacterial communities. The bacteria were found to belong to the genera *Bacillus, Streptomyces, Exiguobacterium, Stutzerimonas, Rhizobium, Acidovorax, Agrobacterium, Psychrobacter, Priestia, Planococcus, Bhargavaea, Stenotrophomonas, Caulobacter, Peribacillus, Sphingobacterium, Niallia, Athrobacter, Sphingobium, Enterobacter, Sinorhizobium, Flavobacterium, Microbacterium, Metabacillus, Neobacillus,* and *Pseudomonas*. These bacteria play a role in promoting plant growth and are very useful in the establishment of inoculums and biofertilisers to help improve plant growth and increase crop yields. This in turn will lead to the reduction in the use and over dependence of harmful chemical fertilisers on agricultural land. The bootstrap values seen in the phylogenetic trees of this study mostly fall within high values range. Indicating that the groupings that were observed are likely to be accurate and reflect the true evolutionary relationship among the bacteria. This study provides a foundation and a point

of reference for potential future studies in understanding plant-microbe interactions in this drought tolerant legume, *Lablab purpureus*.

Declarations

Conflict of interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Author Contributions

Jean Damascène Uzabakiriho: Lead the research design of the isolation and identification of rhizosphere bacteria. Discussed the results and contributed to the final manuscript. Percy Chimwamurombe: Project conception, and article writing. Jeya Kennedy: Lead the research design and implementation. Collaborated in sample collection and contributed to the discussion and interpretation of the results.

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