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MOLECULAR CHARACTERIZATION OF RHIZOBIA STRAINS ASSOCIATED WITH MUNGBEAN (*VIGNA RADIATA*), IN SOILS OF SUDANO-GUINEAN AND SAHELIAN AGROECOLOGICAL ZONES OF MALI

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ABSTRACT

In the Sudano-Guinean and Sahelian zones of Mali, the decline in soil fertility is a key challenge for a sustainable enhancement of agricultural productivity. In addition, studies on the diversity of bacteria associated with Mungbeans are rare, with very few studies conducted on their identification and molecular characterization in the soils of the major agricultural production basins in the Sudano-Guinean and Sahelian Guinean zones of Mali. This study was therefore motivated not only by these different challenges of productivity and soil fertility, but also by the numerous nutritional and agronomic interests of mung beans and the possibility of using its associated bacteria as bio-fertilizers. It aims at contributing to the improvement of mung bean (Vigna radiata L) productivity and the promotion of its cultivation using inocula based on indigenous and efficient bacteria. To achieve this objective, soil samples were taken from various farmers' fields in the two studied areas. The Physico-chemical characteristics of the soils were determined, and trapping trials were conducted in the greenhouse on these soils with Mung bean as the trap plant. The nodules formed on the plants were harvested, the rhizobia contained in these nodules were isolated, the IGS of the 16S-23S rDNA of the isolates was analyzed using PCR-RFLP, and an inoculation test was carried out in a growth chamber to verify the infectivity of the isolates. Eighty-three (83) isolates divided into two groups according to their color (whitish, yellowish) and three (03) IGS types (I, II, III) were encountered with a large dominance of IGS type I in the Guinean zone and that of IGS types I and II in the Sahel zone. Two (02)

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isolates of type IGS I showed a higher infectivity. These results show a low diversity of rhizobium strains associated with Mung bean in the studied soils. It would be interesting to extend this study to other areas of Mali for strains that associate with Mung bean.

Keywords: Mung bean, Guinean zone, Sahelian zone, rhizobia, molecular charactérization, Mali.

1. INTRODUCTION

The Malian economy is essentially based on the rural sector, which employs around 80% of the active population. The contribution of the agricultural sub-sector to GDP is estimated at 46% (DNSI, 2013). Unfortunately, there has been a decline in the fertility of most agricultural soils and consequently in crop yields. This decline fertility is due to acidification and nutrient deficiency of soils and is a threat to the sustainability of agriculture. Despite many efforts, this phenomenon persists and is becoming increasingly worrying and is a major concern for both agricultural producers and the authorities (ELD, (2020) BLANCHARD Melanie et al (2010) SG/MDR, 2002). This decline soil fertility seriously compromises the achievement of food self-sufficiency. In addition, studies on the diversity of bacteria associated with Mungbeans are rare, with very few studies conducted on their identification and molecular characterization in the soils of the major agricultural production basins in the Sudano-Guinean and Sahelian Guinean zones of Mali. In these areas, the decline in soil fertility is explained by the impossibility of achieving better harvests without the use of chemical fertilisers, which are unfortunately costly and have harmful effects on the environment, animal and human health, and promote the formation of nitrous oxide (N2O), recognised as a greenhouse gas (GHG) (Bernard and Jean-François, 2008) A study by Vital et al (2012) demonstrated the presence of chemical contaminants in the soils of the two major agricultural zones (CMDT/Sikasso, Office du Niger/Ségou) of Mali.

In view of all the above, it is imperative to resort to other more environmentally friendly fertilization techniques. In this context, particular emphasis should be placed on the use of certain crops, including legumes, which are able to associate with certain microorganisms (bacteria) in the soil. This association improves plant growth and yield, as well as soil productivity.

Our study focused on the southern Sudanese zone, which covers about 6% of the national territory, in the extreme south of the country. Rainfall in this zone ranges from 1300 to 1500 mm per year, and the Sahelian zone: rainfall ranges from 200 to 700 mm per year. This zone includes the regions of Mopti, the northern part of the regions of Ségou, and the southern part of the country.

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The introduction of mungbean cultivation in different agro-ecological zones in some African countries (Kenya and Senegal (Cissé et al., 2011)) in order to contribute to the fight against hunger has given encouraging results. The mung bean is indeed an annual short-cycle (60-65 days) seed legume of the Fabaceae family and widely cultivated in tropical and subtropical regions of the world (Ramanujam, 1981; Trung et al., 1985). Mung bean also represents an opportunity to produce protein-rich food for rural populations under changing climatic conditions (Arsenault et al., 2015; Foyer et al., 2016; Keatinge et al., 2011). It is also known for its anti-hypertensive, anti-diabetic and anti-carcinogenic properties. Like many other legumes, Mungbean can fix atmospheric nitrogen in symbiosis with soil bacteria called "rhizobia". Rhizobia are Gram-negative, strict aerobic, non-spore-forming soil bacteria capable of fixing atmospheric nitrogen in symbiosis with plants of the large family Leguminosae and plants belonging to the genus Parasponia of the family Ulmaceae. First described by Frank (1889), the rhizobium-legume symbiosis presents a model for studying the association between eukaryotes and prokaryotes. When functioning well, the nitrogen-fixing symbiosis can provide adequate nitrogen nutrition to plants and thus ensure good production and save farmers the cost of chemical fertilizers that are considered polluting (Faghire et al., 2011; Woyessa and Assefa, 2011). The symbiosis allows the natural enrichment of the soil in nitrogen and the reduction of fertilizer inputs. Moreover, this Rhizobium-legume symbiosis is very specific, a given Rhizobium is only able to perform a nitrogen-fixing symbiosis if the other partner belongs to its host spectrum, and the opposite is true. It is within this framework that Rhizobium strains that associate with Mung beans were isolated and characterized.

The objective of this work was to

- Determine the Physico-chemical characteristics of the soils studied;

- Isolate the rhizobium strains contained in the soils of the two climatic zones;

- To characterize the isolates obtained by molecular techniques;

- Select the most infectious isolates.

2. MATERIALS AND METHODS

2.1. Sites

The study was conducted in two zones (Southern Sudanian zone and Northern Sudanian zone). Two sites were chosen in each zone: M'Pégnesso and Kléla at 110 41' 15"N 50 39' 50"W in the Sikasso region in the southern Sudanian zone and two in Ségou / Cinzana: Kondia (13.29320N 5.9371370W and Sorobougou (13.3000220N 5.9055950 W) in the northern Sudanian zone.

2.2 Materials

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2.2.1. Plant material Seeds of mung bean (Vigna radiata). Variety Mungo vert, Cycle 60 to 70 days

2.2.2. Culture media

YMA +RC [Composition/L: Mannitol: 10g; K2HPO4: 0.5g; NaCl: 1mL; MgSO4.7H2O: 10 mL; CaCl2: 1mL; Fe3SO4:10mL; Yeast Extract: 2g; 10mL Congo Red, Agar: 15g] of pH: 6.8 YM[Composition/L: Mannitol: 10g; K2HPO4: 0.5g; NaCl: 1mL; MgSO4.7H2O: 10 mL; CaCl2: 1mL; Fe3SO4:10mL; Yeast Extract: 2g; 10mL, YMA [Composition/L: Mannitol: 10g; K2HPO4: 0.5g; NaCl: 1mL; MgSO4.7H2O: 10 mL; CaCl2: 1mL; Fe3SO4:10mL; Yeast Extract: 2g, Agar: 15g] pH: 6.8, Jensen's medium K2HPO4 (20g/l) 10ml, NaCl (20g/l) + MgSO4 + 7h2O (20g/10ml), CaHPO4 (50g/l) 20ml, FeCL3 + 6H2O (4g/l) OR 11.1ml FeCL3 + 6H2O in soluion10ml, JENSEN'S OLIGO ELEMENT 1ml H2O.qsq1000mlPH adjusted to 6.7

2.3 METHODS

2.3.1. Soil sampling

Twenty-five soil samples were taken from four farmers' fields in two sites in each of the two zones, two fields per site. These sites were distributed between different communes. An experimental plot of 16.50 m x 15.25 m was defined in each field. Samples were taken by daba and auger at 25 points distributed over the two diagonals, the two medians, the centre of the plot and eight other places in the plot. 04 points were selected per diagonal and per median, one in the centre and one per place. Samples were taken with a daba to a depth of 30 cm and used for the greenhouse test. A 16 kg composite sample was taken from each plot, placed in a new sterile sample bag and labelled. The samples were augered and used for physico-chemical analysis. The fields and sampling points were geo-referenced using a GPS.

2.3.2. Determination of the physico-chemical characteristics of the experimental soils

This work was carried out in the HEINZ IMHOF soil laboratory of the IPR/IFRA in Katibougou. Analyses were carried out on water pH, KCl pH, organic matter content, total nitrogen, potassium and assimilable phosphorus. The following methods were used:

- Potentiometric method for the activity of hydrogen ions in suspension in soil/water and/or KCl for the measurement of pH. The soil/solution (water and/or KCl) ratio is 1g/2.5 ml;

- Anne method for the determination of carbon in organic matter
- International method with application of the Robinson pipette for particle size analysis;

- Kjeldahl method for the determination of total nitrogen; Water extraction method for the analysis of phosphorus and potassium.

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2.3.3. Trapping of rhizobium strains

A trapping trial was conducted in the greenhouse on soils from both areas. The soils were contained in 3 kg plastic pots, each filled 3/4 full (2.25 kg per pot). Soils were collected from four fields, two in the Sudano-Guinean zone (Sikasso) and two in the Sahelian zone (Ségou). The Mung bean (Vigna radiata) was used as a trap plant. The different soils were studied separately and five pots were considered per soil. The Mung bean seeds were sorted, sanitised and seeded in the pots (3 per pot). These pots were placed on an iron cultivation table (figure1). The plants were regularly watered with tap water. The trial lasted 45 days. All this work was carried out at the LMS-FST/USTTB.

Figure 1: Pots containing Mung bean plants

2.3.4. Harvesting, sanitising and crushing of nodules

After 45 days of cultivation, the plants were removed from the pots, the nodules formed on the plants were carefully harvested and then placed in tubes.

2.2.5. Isolation of rhizobia strains

The harvested nodules were asepticised one by one with calcium hypochlorite (3.3%) and alcohol (96°) and crushed each in 1mL of sterile distilled water in an Eppendorf tube. Spreading of milled material and purification of isolates

The different macerates were plated on YMA medium in Petri dishes. Two plates were considered per broth and 50µl of broth was used per plate (i.e. 120 plates in total). These plates were sealed with Parafilm and then placed in a regulated oven at 37°C for 48 hours for

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incubation. After 48 hours, the plates were removed from the oven. Two plates showed signs of contamination and were discarded. Bacterial colonies formed in the remaining 118 dishes were observed and those showing the aspects of rhizobia (colour, consistency, odour) were selected and purified by successive subculturing and incubation on YMA medium contained in other Petri dishes. After purification, the colonies were separately subcultured according to their appearance on YMA medium contained in Petri dishes. They were then separately subculturedonto YMA+RC medium and incubated as before in order to identify rhizobium colonies according to their affinity for the dye (Congo Red). Only colonies that had not absorbed Congo Red were considered and purified as before on YMA medium (figure 2).

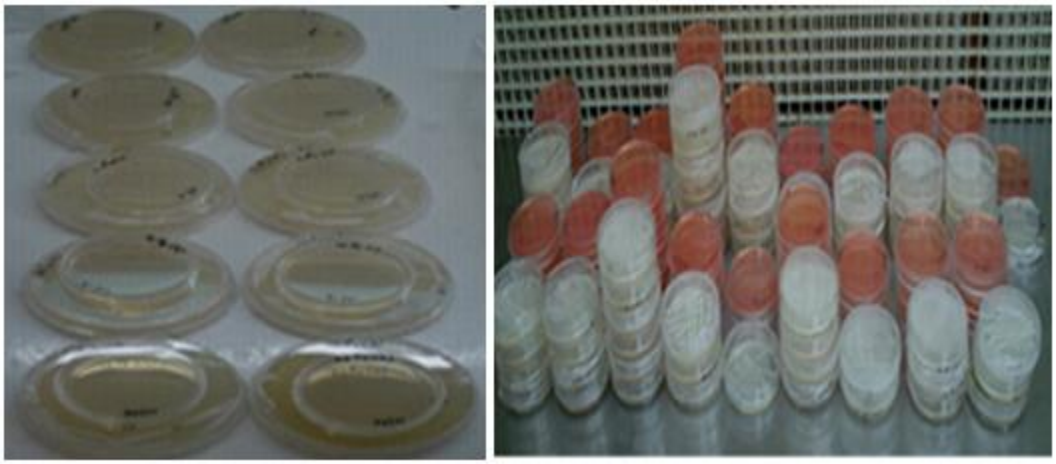

Figure 2: Petri dishes containing YMA medium (white) and YMA+RC medium (red) used for the isolation of rhizobia strains.

2.2.6. Molecular characterisation of isolates

This characterisation was performed on the suspension (liquid culture) of the 79 isolates. The isolates were studied separately.

Preparation of the suspensions

One bacterial colony from each isolate was inoculated into YM medium in small 20 mL test tubes each containing 4 mL of the medium. These tubes were placed in metal racks and shaken at 100 rpm on an orbital shaker for 48 hours.

Heat shock

2 mL of each suspension was taken and placed in an Eppendorf tube. These tubes were carried on floats. These floats were then placed in an aluminium cup containing boiling water (100°C)

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for 10 minutes (Figure 4) to release the bacterial DNA. The samples were then flash-cooled in a freezer at -20°C for 10 minutes. They were then heated at 100°C for 10 minutes.

Amplification by PCR (Polymerase Chain Reaction)

The region studied was the intergenic space (IGS), which is a variable area of DNA located between the genes coding for the 16S and 23S ribosomal RNAs (Figure 3). The sequences of the primers used to amplify the IGS of the 16S-23S rDNA.

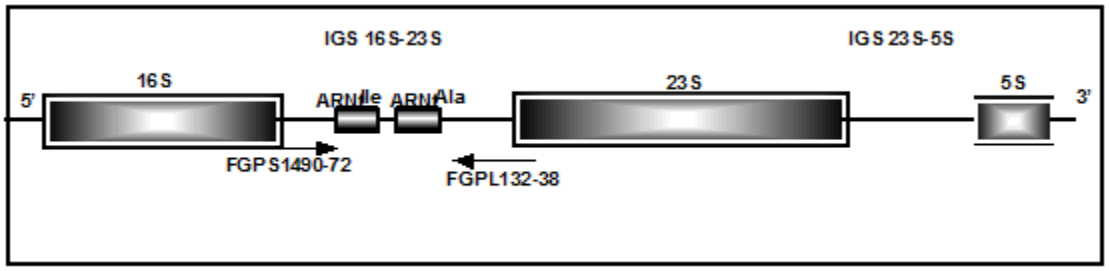

Figure 3: Schematic of anrrn operon of DNA coding for ribosomal genes and the position of the primers used to amplify the IGS region of the 16S-23S rDNA (Krasova-Wade, 2003)

Amplification was performed in a thermal cycler. A rational volume of 25 µl was considered and consisted of the reaction mix (Taq polymerase: 0.2 µl; Mgcl2: 2.5 µl, DNTP: 1.25 µl; two primers (1.25 µl per primer; buffer: 11 µl; ultrapure water: 4.55 µl) and the DNA (3 µl). The reaction mix was first placed in a PCR tube and then the DNA was added. The tubes were then placed in a GeneAmp PCR System 2400 thermal cycler (Perkin Elmer).

The amplification program was the IGS 16S-23S program and the number of cycles was 35.

Table 1: Name, sequence and reference of primers used for PCR

Nom	Séquence (5' → 3')	Références
FGPS 1490- 72	TGCGGCTGGATCCCCTCCTT	Normand *etal*., 1996
FGPL 132- 38	CCGGGTTTCCCCATTCGG	Ponsonnet*et al*., 1994

Each cycle consisted of three phases: Denaturation (94°C for 5 minutes and 30 seconds), Hybridisation or Pairing (57°C for 30 seconds) and finally Elongation (72°C for 1 minute). These three phases are followed by a cooling phase (20°C).

The PCR products (amplifiats) were checked by agarose gel electrophoresis (1%) in Tris-Borate buffer. 3 µl of amplification product was placed in each well and allowed to migrate in an

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electrophoresis tank for 45 minutes at 100 volts. 3 µl of 1 kb molecular weight marker (Pharmacia Biotech) was used as a size reference for the PCR products. After migration, the gel was stained by immersion for 30 min in Ethidium Bromide solution (Appendix I), rinsed with distilled water for 15 min and visualised with the Gel Doc for the observation of PCR bands.

Restriction Fragment Length Polymorphism (RFLP) analysis

The analysis consists of cutting the amplified DNA with restriction endonucleases (enzymes) to obtain a typical restriction profile. The fragment will be characterised by a combination of typical profiles obtained for the two endonucleases. A PCR-RFLP group includes strains whose amplified fragments have the same combination of standard profiles. The enzymes HaeIII and MspI (Pharmacia) were chosen for their discriminatory capacity, which was demonstrated in previous rhizobium characterisation studies (Neyra, personal communication). These restriction enzymes recognise specific cleavage sites: 5'-GG / CC-3' for Hae III and 5'-C / CGG-3' for Msp I.

The 63 PCR amplifiers were studied separately and 8 µl were considered per amplifier. The RFLP mix was composed of 11 µl of each of the two enzymes and 22 µl of each of the buffers (Hae III buffer, Msp I buffer). After preparation, the mixture was divided into Eppendorf tubes at a rate of 12 µl per tube and 8 µl of amplified material was added to each tube, giving a reaction volume of 20 µl. The tubes and their contents were placed in the oven regulated at 37° C for digestion (restriction). After three hours they were removed from the oven and stored at minus 20°C.

The restriction fragments were separated by 2.5% Metaphor agarose gel electrophoresis (FMC Bio-Products, Rockland, Marine USA) in TBE buffer (Appendix I) at 100 volts/cm-1 for 3 h -3 h 30 min. The 100 bp molecular marker (Pharmacia Biotech) was used as a molecular weight scale. The gels were stained, and the DNA fragments were visualized as before. Band sizes were measured with Launch Doc-ItLS software. Identical profiles were grouped into different "IGS types".

2.2.6. Ineffectiveness test isolates

This ineffectiveness test was performed with representatives of the different ISG groups or types from the isolates obtained from the four soils during the PCR-RFLP. This test was carried out in order to ensure the nodulation capacity of the isolates studied. The number of isolates to be tested for the different IGS groups was chosen according to the number of profiles obtained. The isolates were grown separately in YM medium in 20 mL test tubes. The test was conducted in a culture chamber in tubes containing Jensen's medium. After preparation, the medium was divided into Gibson tubes (Vincent, 1970) at a rate of 30 ml per

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tube (Figure 8). These tubes were closed with aluminium foil and tape and autoclaved at 120°C for 20 minutes. After sterilisation, two holes were made in the foil of each tube, one for subculturing and the other for watering. Four tubes were retained per isolate. The mung bean seeds were treated as before and then germinated in Petri dishes containing agar water. After germination, the tubes were filled with sterile distilled water by introduction through the watering hole. The gemmules were transplanted into the tubes containing Jensen's medium, one per tube, by passing the radicle through the transplant hole. The cotyledons of the gemmules were covered with Kleenex paper moistened with sterile distilled water. To maintain aseptic conditions, the watering hole was sealed with a sterile rubber stopper. The tubes were kept in racks. These racks were enclosed in trays for five days. On day 6, the seedlings were inoculated with the liquid culture (suspension) of the isolates. Four replicates (4 tubes) were considered per isolate. After inoculation, the tubes were transferred to culture boxes (Figure 4). The boxes and their contents were then subjected to a photoperiod of eight hours at night and sixteen hours during the day in a culture chamber at constant temperature (28° C).

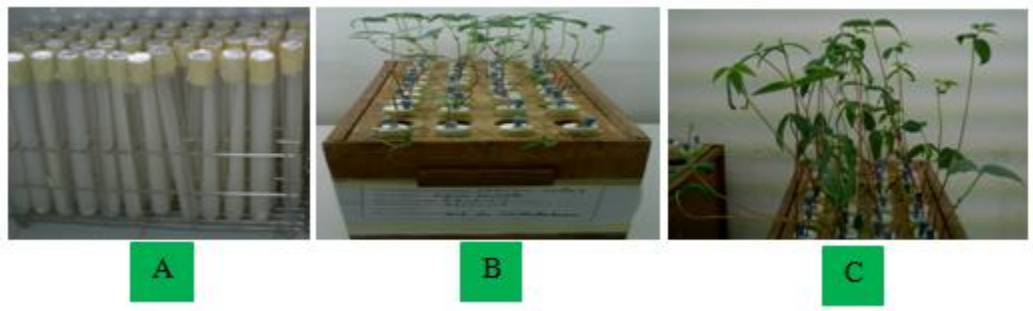

Figure 4: Gibson tubes containing Jensen medium (A) and culture boxes containing young plants of different ages (B, C).

3. RESULTS

3.1. Determination of the physico-chemical characteristics of the experimental soils

The results showed four textural classes: clayey-silty, silty-silty-sandy, silty-sandy and clayey-silty-sandy, respectively in Kléla, M'Pégnesso, Kondia and Sorobougou. The organic matter content is extremely low in the Kondia field soil, very low in the Sorobougou, M'Pégnesso soil and low in the Kléla soil. All the study soils are characterised by a very low level of nutrients, notably nitrogen and assimilable phosphorus, and by a strong acid reaction, as attested by their water pH and KCl pH, which are 4.24 and 4.13; 4.27 and 4.05; 4.35 and 4.15; 4.64 and 4.59, respectively for the Sorobougou, Kondia, M'Pégnesso and Klela field soils (Table 2).

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Table 2: Average value obtained for the physico-chemical parameters of the study soils

Sites	Granulométrie				pH eau	pHKcl	MO %	N %	P assim (ppm)	K (ppm)
	% A	% LT	% ST	Classe Texture						
M'Pégnesso	27,68	19,15	53,16	LAS	4,35	4,15	0,9	0,03	0,35	4,92
Kléla	41,42	43,71	14,87	AL	4,64	4,59	1,4	0,05	0,61	6,68
Kondia	11,21	34,49	54,30	LS	4,27	4,05	0,1	0,02	0,44	4,44
Sorobougou	8,60	18,45	72,95	LSA	4,24	4,13	0,2	0,02	0,15	3,74

MO: organic matter, N: nitrogen, P: assimilable, LAS: silty clay, AL: silty clay, LS: silty sand, K: potassium

From these results, it appears that the soils studied are all acidic and silty. Also, in addition to the silt, the soil of the Sorobougou and M'Pégnesso field is clayey-sandy.

3.2. Isolation of rhizobia strains

After several purifications, 83 isolates were obtained, the Mpégnesso soil gave the highest number of isolates followed by the Kléla soil, after the Kondia soil and the Sorobougou soil gave the lowest. Their distribution by soil is shown in Table 3.

Table 3: Names of soils and number of isolates obtained

Sols	Nombred'isolats
Sorobougou : S1	17
Klela: S2	25
M'Pégnesso: S3	28
Kondia: S4	23

These isolates can be divided into two groups according to their color (whitish, yellowish). These two groups are shown in Figure 5.

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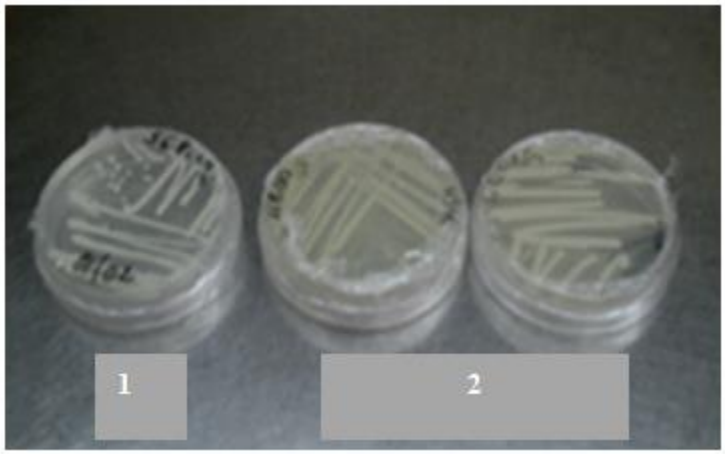

Figure 5: whitish (1) and yellowish (2) isolates.

3.3 Molecular characterization of isolates

3.3.1. PCR analysis

Of 83 isolates tested, 63 amplified and gave the corresponding rhizobium profile.

3.3.2. RFLP analysis

After RFLP, the 63 amplifications analysed gave profiles. These profiles are divided into three (03) IGS groups (G1, G2, and G3).

The number of isolates profiling the different groups within each soil is presented in table 3.

Table 4: Number of isolates profiling the different groups per soil.

Goupes IGS	Sols et nombre d'isolats / sol			
	Sol 1	Sol 2	Sol 3	Sol 4
G1	7	11	5	7
G2	4	3	6	6
G3	1	6	2	5

After digestion with the two restriction enzymes HaeIII and MspI; 3 types (I, II, III,) were identified with 30 samples for group I, 19 samples for groups II and 14 samples for group III.

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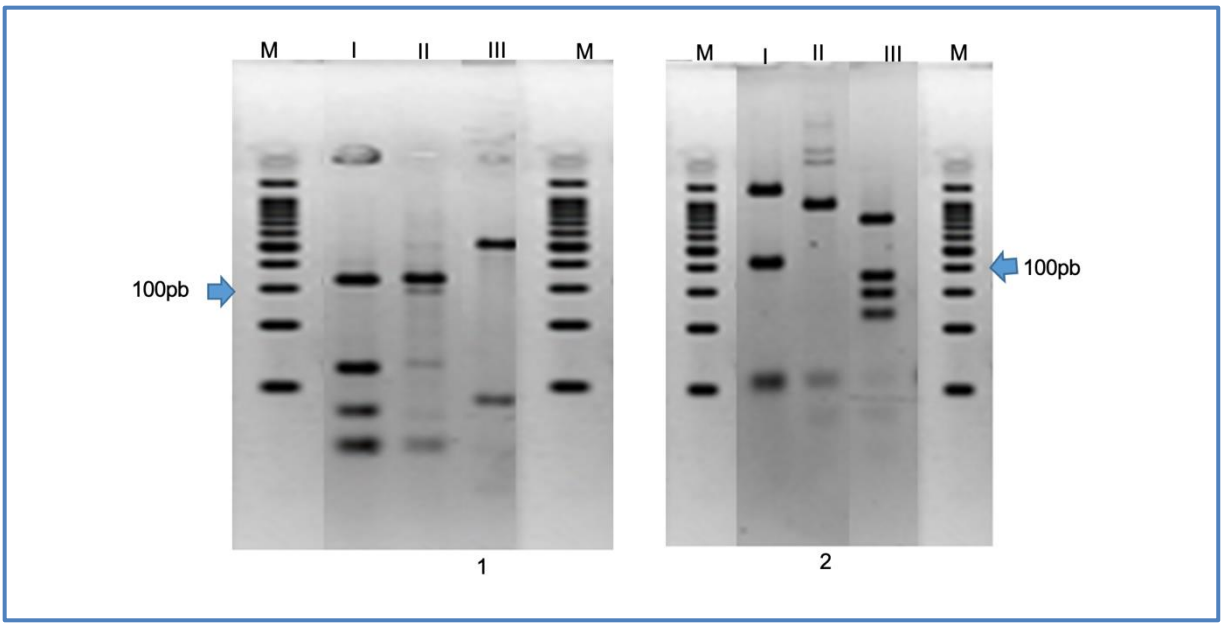

M: marker, I: group I isolate, II: group II isolate, III: group III isolate

Figure 6: Images of some IGS profiles obtained with Hae III (1) and Msp I (2) in mung bean nodules.

3.4. Inffectiveness test

The number of isolates tested was for G1, G2 and G3 respectively. Group I isolates gave more tubes with nodulated plants with 4 nodulated tubes for isolate S4P1-2B2 followed by S2P4-1B2 which had 3 nodulated tubes.

The results obtained are presented in Table 4.

Table 5: IGS groups, tubes and nodulation

Noms de l'isolat	Groupes IGS	Tubes et nodulation			
		Tube 1	Tube 2	Tube 3	Tube 4
S8P162B	G1	0	0	0	0
S8P5-1A	G1	0	0	0	0
S2P4-1B2	G1	*	*	*	0
S6P1-A	G1	0	*	0	*
S6P1-1B	G1	*	m	0	*
S4P1-2B2	G1	*	*	*	*
S4P3-2BS	G1	0	0	0	0
S4P1-1A	G1	*	0	m	*

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S4P5-1B	G2	0	0	0	0
S5P2-1B	G2	0	0	0	0
S5P3-1B	G2	*	0	m	0
S8P2-2B2	G2	0	0	0	*
S5P4-1B	G3	m	0	0	0
S6P5-2B	G3	*	0	*	0

m: dead plant; 0: no nodulation; *: nodulation

4. DISCUSSION

In this study we isolated and characterized the rhizobia that associate with Mungo bean on four different soils. Examination of the root system of the plants collected from the four soils after trapping allowed us to verify that they had nodulated. Indeed, the plants collected from each soil showed nodules on their root system that are characteristic of the Rhizobium-legume symbiosis. This behaviour, which has already been reported for many legumes introduced in several regions of the world, is described as symbiotic promiscuity (Perret, 2000). It is important to point out at this level that the shape of the root nodules of legumes is determined by the host plant (Hirsch et al., 2001). In general, legumes of tropical origin such as beans (Phaseolus vulgaris) all produce nodules of a definite type, generally unbranched, whereas legumes from temperate regions such as chickpeas produce nodules of an indeterminate type, generally branched (Corby, 1988, Allen and Allen, 1981). This is thought to result from the presence in the soil of rhizobium strains capable of reacting specifically to a range of flavonoids and thus able to nodulate a range of host plants. After spreading the nodule crushes on YMA medium, we obtained colonies that differed in colour and appearance, and some isolates were found to be slow strains. The traditional phenotypic characterisation is still accepted as the most important step for the identification and separation of newly isolated bacteria (N sebbane- 2007). In rhizobia, it forms the basis for formal description. It has also been extensively used in associative endophytic bacteria for species separation (Baldani and Baldani, 2005).

After RFLP the isolates could be grouped into three types. This shows that there are very few species that associate with mung bean. Indeed the rhizobium-legume symbiosis is very specific (Esylla, et al 1997), a given Rhizobium is only able to perform a nitrogen-fixing symbiosis if the other partner belongs to its host spectrum, while the same legume has other bacterial partners (Nicoud et al 2021). The amplitudes of the spectra of legumes and rhizobia are very variable.

Other bacterial symbionts show a moderately specific host spectrum, such as Sinorhizobium meliloti which associates with species of the genera Medicago, Meliloti and Trigonella (De lajudie et al., 1994). In addition, host spectrum studies have revealed that Rhizobium associates with only one legume species (Zahran 2001, Perret et al., 2000).

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5. CONCLUSION

At the end of our activities, we can say that mung bean is very selective in establishing symbiosis with rhizobia. Therefore, there are not many rhizobium strains in the soils of the studied areas that associate with mung bean.

REFERENCES

[1] Zahran (2001) Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology journal of biotechnologiy 91(2-3), 143-153, 2001

[2] **(**Etude sectorielle Eau et Assainissement en Afrique de l'Ouest francophone Rapport Mali WATER AID Décembre 1998)

[3] (Baldani et Baldani, 2005) Histoiry on the biological nitrogrn fixating research ingraminaceous plants: special emphasis on the Brazilian exparience

[4] Quentin Nicoud (2021). Study of terminal bacteroid differentiation features during the legume-rhizobium symbiosis. Plant breeding. Université Paris-Saclay, 2021. English. ffNNT : 2021UPASB007ff. fftel03208429

[5] Jean-Marc Sanchez février 2017 Légumineuse / Bactérie Rhizobium = Symbiose

[6] (DNSI, 2013).

[7] Dreyfus B, Garcia JL, Gillis M (1988) Caracterization of *Azorhizobiumcaulinodans* gen. nov., sp. Nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata. Int. J. Syst. Bacteriol., 1988, 38, 89-98*.

[8] Faghire M, Bargaz A, Farissi M, Palma F, Mandri B, Lluch C, Tejera García NA, Herrera-CerveraJA, Oufdou K, Ghoulam (2011). Effect of salinity on nodulation, nitrogen fixation and growth ofcommon bean (*Phaseolus vulgaris*) inoculated with rhizobial strains isolated from the Haouzregion of Morocco. *Symbiosis*, 55:69-75.

[9] Cisse, M., Diouf, M., Gueye, T., Fall, A. (2011) Linking policy, research, agribusiness and processing enterprise to develop mungbean (*Vigna radiata)* production as an export crop from Senegal River Valley *In*: Innovations as key to the Green Revolution in Africa. Bationo A, Waswa B. Okeyo, J.M.; Maina, F. Kihara, J.M.(Eds).

[10] Dudeja, S.S., Sheokand, S., Kumari, S. (2012) Legume Root Nodule Development and Functioning under tropics and subtropics: Perspectives and Challenges. LegumeRes, 35, 2, 85-103.

[11] E Sylla , I Ndoye, AT BA, B Dreyfus-Bulletin de IFAN, SerieA, 1997 – Document.ird.FR : Specificté de la symbiose Rhizobium-Légumineuse (Ptérocarpus) au Sénégal.

[12] EM/NDCN. (2008). Yearly report of activities 2007, Bamako, January 2008, appendix 1.

[13] ERA/USAID Project (2016). Mungbean: Une Légumineuse Alimentaire Adaptée aux conditions chaudes et sèches. Guide de production : de la production à la récolte, 2p.

[14] FAO. (2005). FAO information system on water and agriculture. Mali. FAO - Water Earths and Water WFO Welcome. aquastat, 7p.

[15] Hussain, I., Burhanuddinand, M., Bhuiyan, M.K.J. (2010) Evaluation of Physiochemical properties of Wheat and Mung bean from Bangladesh. *Internet J. Food Saf, 12, 104-108.*

[16] Jayarambabu, N., Siva, K.B., Venkateswara, R.K., Prabhu, Y.T. (2014) Germination and Growth Characteristics of Mungbean Seeds (*Vigna radiata L.*) affected by Synthesized Zinc Oxide Nanoparticles, International *Journal of Current Engineering and Technology*,4, 3416-3416.

[17] Johnson JM, Houngnandan P, Kane A, Sanon K, Neyra M. 2013. Diversity patterns of indigenous arbuscular mycorrhizal fungi associated with rhizosphere of cowpea Vigna unguiculata L. Walp. in Benin, West Africa. Pedobiologia, 56(3): 121– 128.

[18] Karamany, E.L. (2006) Double purpose (forage and seed) of mung bean production 1-effect of plant density and forage cutting date on forage and seed yields of mung bean (*Vigna radiata* (L.) Wilczck). *Res. J. Agric. Biol. Sci*, 2, 162-165.

[19] Kulsum, M.U., Baque, M.A., Karium, M.A. (2007) Effects of different nitrogen levels on the morphology and yield of blackgram, *J. Agron* 6, 125-130.

[20] Kumar, V.L., Singhal, A. (2009) Germinating seeds of the mung bean, *Vigna radiata* (Fabaceae), as a model for the preliminary evaluation of cytotoxic effects of drugs. *Biocell* ,33, 1, 19-24.

[21] NIS (2010). Temporary results of the general Census of population and habitations.

[22] Pandiyan. M., Senthil, N., Ramamoorthi, N., Muthiah, A.R., Tomooka, N., Duncan, V., Jayaraj, T. (2010) Interspecific hybridization of Vigna radiata x 13 wild Vigna species for developing MYMV donar. *Electronic Journal of Plant Breeding*, 1, 4, 600-610.

[23] Perret et al 2000: Molécular Bas is of Symbiotic Promiscuity : April 2000. Microbiology and Molecular Biologiy Reviws 64(1) :180-201

[24] Rehman, A.U., Muhammad, A.A., Babar, M.A., Muhammad, S., Amjad, A., Ahsan, R.M. *(2009)* Mungbean/ greengram (*V. radiata*), Genetic studies of yield related traits in mungbean (*Vigna radiata* L. Wilczek). *Australian Journal of Crop Science, 3, 6, 352-360*.

[25] Shabnum, S., Nidaa, H., Farah, K., Rana, A.H., Sehrish, R., Sum aira, R., Zaryab, K., Mushtaq, A., Muhammad, Z. (2012)Comparative nutritional analysis between *Vigna radiata*and *Vigna mungo* of Pakistan. *African Journal of Biotechnology*, 11, 25, 6694-6702.

[26] Somasegaran and Hoben, 2012

[27] Suneja, Y., Kaur, S., Gupta, A.K., Kaur, N. (2011) Levels of nutritional constituents and anti-nutritional factors in black gram (*Vigna mungo* L.). *Food Res. Int*, 44, 2, 621-628.

ISSN: 2455-6939

Volume: 08, Issue: 06 "November-December 2022"

[28] Vijay, LK., Abhishek, S. (2009) Germinating seeds of the mung bean, *Vigna radiate* (Fabaceae), as a model for the preliminary evaluation of cytotoxic effects of drugs. *BIOCELL*, 33, 1, 19-24.

[29] Vincent JM (1970). A manual for the practical study of root nodule bacteria. International Biological Programme Handbook N°15 Blackwell Scientific Publications. Oxford and Edinburgh. 164 p.

[30] Yang, J.K., Yuan T.Y., Zhang W.T., Zhou, J.C., Li, Y.G. (2008) Polyphasic characterization of mung bean (*Vigna radiata* L.,) from different geographical regions of china. *Soil Biol. Biochem*, 40, 1681-1688.

[31] N sebbane (2007) Béjaia, Université Abderhamane Mira. Faculté des Sciences de la Nature et de la vie, 2007

