ISSN No:-2456-2165

In Vitro Assessment of IAA Production and Antibiotics Tolerance of Peanut (*Arachis hypogeae* L.) Nodulating Bacteria

NGO NKOT Laurette1*, MBA EDOU Simon Jérémie1,YOUAGANG GOUGUEU Harris Stephane1, NYAKA NGOBISSA Aurelie Irene Laure2, NWAGA Dieudonné3,
1Department of Plant Biology, Faculty of Science, the University of Douala, Douala, Cameroon
2Institute of Agricultural Research for Development; Njombe, Cameroon
3Department of Microbiology, Faculty of Science, the University of Yaounde I, Yaounde, Cameroon

Abstract:- The peanut (*Arachis hypogaea* L.) is an important seed legume in the tropics. In Cameroon, the diversity and the competitivity of rhizobia nodulating this plant are practically unknown while their exploration can allow to further improve its interests in agriculture. The aim of this study was to assess the ability of the rhizobia isolated from two sites soils (Bafoussam and Ebolowa) to synthesize indole-3-acetic acid and to tolerate antibiotics. Rhizobia trapping was realized in plastic pots with peanut as a trap plant. The results were analyzed statistically by ANOVA using R 3.3.1 and GraphPad Prism 5.0. A collection of thirteen isolates was constituted among which seven from Bafoussam and six from Ebolowa. All isolates were fast growing on YEMA-CR medium. In this study, there was a significant production of IAA by the isolates with a maximum of 16.33 µg/mL for AhBf5 followed by AhEb1 (11.58 µg/mL). The isolates in this study were found to be very resistant to amoxicillin, chloramphenicol, tetracyclin and gentamicin but sensitive to rifampicin. The peanut nodulating rhizobia isolates that show the best skills can be recommended for the production of biofertilizers for sustainable agriculture in Cameroon and elsewhere.

Keywords:- *Arachis Hypogaea, Peanut Nodulating Bacteria, Indole-3-Acetic Acid, Antibiotics, Cameroon.*

I. INTRODUCTION

With more than a quarter of a billion people on the brink of hunger, action must be taken swiftly to provide food to areas most at risk. A profound change in the agricultural system is therefore necessary to feed the more than 820 million people who suffer from hunger today. To this end, increasing the capacity of agricultural productivity is one of the main challenges in reducing the problem of hunger [1].

Among legumes, the prominent peanut is cultivated in over 100 countries in the tropics, subtropics and warm temperate regions [2-3]. It is the 13th largest food crop and the 4th most important oilseed crop in the world [4]. World production of unshelled peanut is estimated at 45 million tons, with more than 90 % of this production coming from Asia (67 %) and Africa (26 %) [5]. In much of sub-Saharan Africa, peanut is an important crop for both home consumption and trade [6].

In Cameroon, peanut is a plant of proven nutritional, industrial, health and agricultural interest. However, the production yields of this legume have fallen in recent years, dropping from 1.75 t / ha in 2006 to 1.40 t / ha in 2014, i.e. a drop of 0.34 % [7]. The causes of this drop in peanut productivity are linked, among other things, to the decline in soil fertility due to poor agricultural practices, poor soil condition [8], the excessive use of chemical nitrogen fertilizers and climatic changes; to which is added the competitiveness of microorganisms in the soil for space and nutrients. An alternative to improve soil fertility is to exploit the nitrogen fixing symbiosis by legumes. However, the success of the symbiosis is influenced, among other things, by environmental factors, including the intrinsic resistance to antibiotics produced by various organisms in the rhizosphere ([9-10]. Thus, the selection of strains competitive with PGPR characteristics for the production of biofertilizers is a sustainable solution to soil fertilization and therefore to increasing peanut production yields and limiting pollution from agricultural sources.

II. MATERIAL AND METHODS

- ***Soil sampling and analysis***

In each site, soils were randomly sampled from 4 plots 10 × 10 m plots of recent mixed-crop peanut harvest, sufficiently distant from each other. Samples are taken along the diagonals of each plot, to a depth of 0 to 20 cm, corresponding to the zone of intense microbial activity. In each plot, 10 samples are taken. The samples are sorted and mixed to form a soil sample of approximately 10 kg. Soil samples from 4 plots of the same site are mixed to form a composite sample of approximately 40 kg. Once transported to the laboratory, the composite soil sample is divided into 2 parts:

- a first part is used for trapping BNL (Bacteria Nodulating Legumes);
- a second part is dried in the open air and passed through a sieve with a 5 mm mesh, then a 2 mm mesh. This part is used for physico-chemical analyzes of the soil.

ISSN No:-2456-2165

➢ ***Trapping of peanut nodulating bacteria***

The trapping technique has been used [11] with peanuts as a trap plant. The experimental set-up consisted of 2.5 kg perforated pots filled with soil from the two sampling sites.

➢ ***Isolation and authentication of peanut nodulating bacteria***

The isolation of peanut-nodulating bacteria was carried out according to Vincent's method [11]. The nodules preserved by desiccation are rehydrated by immersion in sterile distilled water for 2 hours. For each site, 30 nodules are used. The nodules are then disinfected by soaking in 95 ˚ ethanol for 10 s, then soaking in an acidified solution of mercuric chloride (HgCl2) at 1% for 30 s. The nodules are rinsed 4 to 5 times in sterile distilled water to remove traces of mercuric chloride. The isolation of peanut-nodulating bacteria was carried out on Yeast Extract Mannitol Agar (YEMA) medium supplemented with Congo Red. The appearance of colonies was observed after 48 h and 72 h. Colonies that did not absorb Congo Red were picked and subcultured regularly in Petri dishes containing YEMA medium and incubated in an inverted position at 28 ° C, until pure and homogeneous colonies were obtained.

The authentication test ensures that isolates obtained from peanut nodules are indeed rhizobia isolates, through their ability to form nodules on the roots of a host legume [11]. For this test, peanut has been used as a host plant. The seedlings inoculated with the purified isolates were watered twice a week with Jensen's nutrient solution, the composition of which per liter of sterile distilled water is as follows: K2HPO4: 0.2 g; MgSO4 ($7H_2O$): 0.2 g; NaCl: 0.2 g; CaHPO4: 1 g; FeCl2: 0.14 g; HBO3: 2.86 mg; $MnSO_4$ ($6H_2O$): 2.03 mg; ZnSO4: 0.22 mg; CuSO4: 0.08 mg; NaMbO4: 0.09 mg. The pH of the medium was adjusted to 6.5 and the medium was then autoclaved at 120 ˚C for 20 min.

➢ ***Morphological characterization of peanut nodulating bacteria***

This was done by describing the colonies aspect on YEMA for the macroscopy, and by the Gram staining for the microscopy (100X).

➢ ***Evaluation of IAA production by peanut nodulating bacteria***

The production of indole-3-acetic acid was tested on liquid culture medium by colorimetric assay [12-13]. Each isolate is seeded in 5 mL of YEM supplemented with 0.1 mg / mL of L-Tryptophan. Three replicates were performed per isolate. The cultures are incubated for 48 h at 28 ± 2 ˚C with shaking of 120 npm [14]. The cultures are then centrifuged at 5000 rev / 10 min. 2mL of the supernatant is mixed with 2 drops of orthophosphoric acid followed by the addition of 4mL of Salkowski's reagent. The appearance of a pink color confirms the production of IAA 30 minutes later. The OD is immediately read at 530 nm after 30 min of incubation [15].

➢ ***Tolerance toward antibiotics of peanut nodulating bacteria***

For this purpose, seven antibiotics were used (amoxicillin, chloramphenicol, gentamicin, penicillin, tetracycline and rifampicin. The method used was the diffusion technique in a solid medium in which the concentration gradient of the antibiotic is achieved by diffusion in agar from a well center [12]. Four concentrations of each antibiotics (10, 20, 50 and 100 µg / mL) were prepared separately. The bacterial inoculum obtained is compared to the Mc Farland scale which is 108 bacteria/ml. In each well formed in the YEMA medium, a 10 µL of a given antibiotic is deposited. Each control well is inoculated with sterile distilled water [16]. Petri dishes are incubated at 28 °C for 7 days. The diameters of the inhibition zones observed around the well was measured using calipers [17].

➢ ***Statistical analysis***

Collected data were analyzed using R (3.3.1), SPSS 16.0 and GraphPad Prism 5.0 at 5% of average probability. ANOVA was made using the Duncan test for repeated measures.

III. RESULTS

➢ ***Soils analysis***

The characteristics of the soils samples are presented in table1. The sample soils were all acid. The soil of Bafoussam was rich in total phosphorus in the contrary of the one of Ebolowa which was very poor.

Table 1: Physical and chemical characteristics of soils

Parameters	Bafoussam	Ebolowa
Sand (%)	65.10	49.03
Clay (%)	9.33	35.40
Loam (%)	25.57	15.57
pH (H_2O)	5.53	4.38
EC (µS/cm)	287	100.30
Organic Carbon (%)	3.81	1.31
Total Nitrogen (%)	0.30	0.10
Assailable Phosphorus (µg/g)	7.13	4.47
Total Phosphorus l (µg/g)	1003.37	58.71
C/N	12.53	12.80
CEC (cmol (+) /Kg)	20.27	6.65

➢ ***Isolation and authentication of peanut nodulating bacteria***

A total of 20 solates were tested for their ability to nodulate *Arachis hypogaea*. The effective nodulation observed with all rhizobial isolates clearly indicated that 13 isolates (07 at Bafoussam and 06 at Ebolowa) were able to nodulate peanut. It was noted that the nodules were pink, indicating the leghemoglobin content, while uninoculated control plants were without nodules. Rhizobial isolates were nomenclatured so as to indicate the name of the legume (Ah-*Arachis hypogaea*), the site of origin (Bf-Bafoussam; Eb-Ebolowa) followed by isolate number.

➢ ***Morphological characterization of peanut nodulating bacteria***

All the isolates were gram negative and rod in shape (Fig.1).

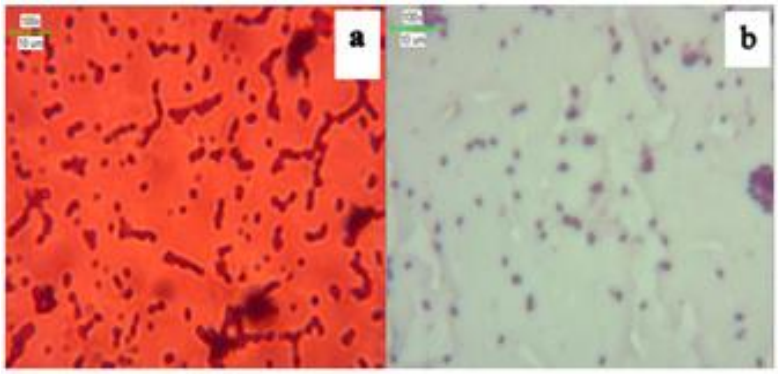

Figure 1: Microscopic aspects of rhizobia isolates nodulating peanut AhBf1 (a) and AhBf4 (b) after Gram staining

The isolates had various morphological characteristics (Table 2). From the 20 isolates obtained, 100% had colonies with convex elevation. All the colonies were round, with diameters between 0.03cm and 0.30cm. AhBf5 formed the largest colonies (3cm) while AhBf10 formed the smallest colonies (0.03cm). The colonies were white or milky in color when grown on YEMA. All the isolates were fast-growing and failed to absorb Congo red in the medium.

Isolates	Diameter (cm)	Shape	Color	Growth	Presence of mucus	Viscosity	Brightness	Elevation	EPS
AhBf1	0.20	Round	Shining white	fast	absent	Viscose	Bright	convex	present
AhBf2	0.05	Round	whitish	slow	absent	Viscose	Bright	convex	present
AhBf3	0.15	Round	White-milky	fast	absent	Viscose	Bright	convex	present
AhBf4	0.10	Round	White-milky	fast	absent	Viscose	Bright	convex	absent
AhBf5	0.30	Round	White-milky	fast	present	Viscose	Bright	convex	present
AhBf8	0.05	Round	White-milky	fast	absent	Viscose	Bright	convex	absent
AhBf10	0.03	Round	whitish	fast	absent	Viscose	Bright	convex	present
AhEb1	0.10	Round	whitish	slow	absent	Viscose	Bright	convex	present
AhEb5	0.10	Round	beige	slow	absent	Viscose	Bright	convex	present
AhEb6	0.10	Round	beige	slow	present	Viscose	Bright	convex	present
AhEb7	0.15	Round	White-milky	fast	absent	Viscose	Bright	convex	absent
AhEb8	0.10	Round	White-milky	fast	absent	Viscose	Bright	convex	present
AhEb9	0.05	Round	beige	fast	absent	Viscose	Not Bright	convex	absent

Table 2: Colony characteristics and cell morphology of peanut nodulating isolates
EPS: Exopolysaccharides

The dendogram (Fig. 2) based on the macroscopic characteristics of the colonies placed isolates into three different groups. Group1 contained 6 isolates (AhEb7, AhBf4, AhBf8, AhBf3, AhEb8 and AhBf5 which were all round in shape, fast growing, bright and convex. The second group was constituted of AhBf10, AhBf1, AhBf2 and AhEb1. The isolates of this group were all bright and homogeneous in aspect. The last group contained AhEb9, AhEb5 and AhEb6.

ISSN No:-2456-2165

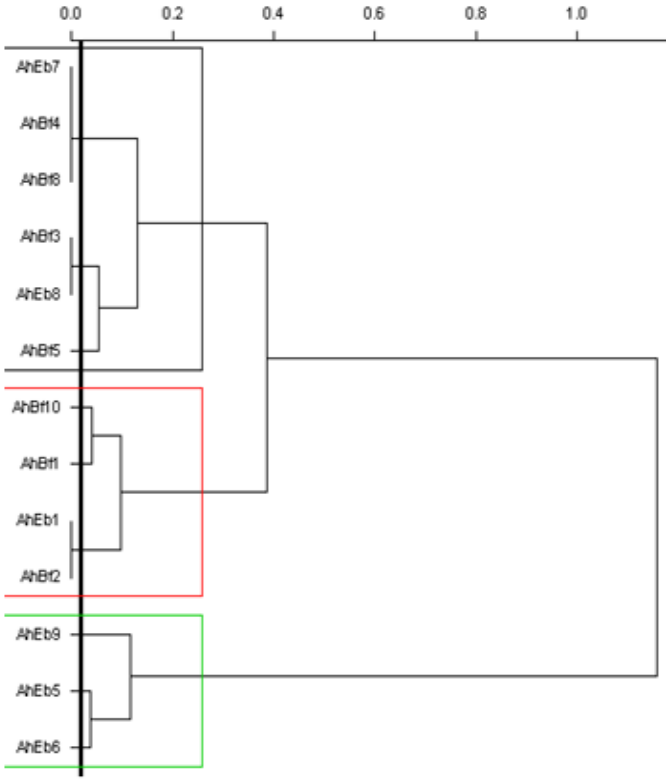

Figure 2: Dendrogram showing the morphological similarities among peanut nodulating rhizobial isolates

➢ ***IAA production by isolates***

The IAA production test were performed on YEM medium supplemented with L-Trp. All the isolates were found to be positive for IAA production. The revelation of this production is translated by the changing of the color of medium to pink-red (Fig. 3) using the Salkowski's reagent.

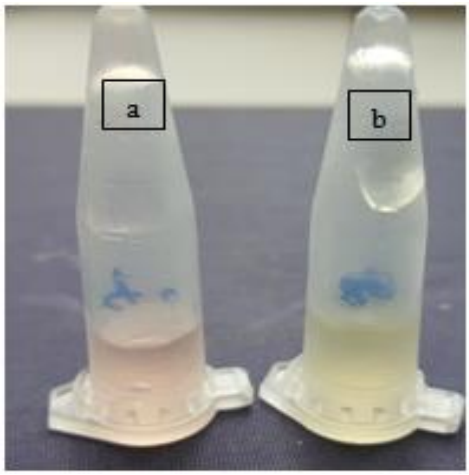

Figure 3: Demonstration of the production of IAA by isolate AhBf5 (a) on YEM medium supplemented with Tryptophan in comparison to the non-inoculated control (b)

Figure 4 shows the amount of IAA produced by peanut nodulating bacteria. The amount of IAA varied with the isolate. Then, AhBf5 produced 16.33 µg/mL with is significantly high than the amount of all the other isolates. The lowest amount of IAA were registered in AhEb7 (1.66µg/mL) and AhBf1 (1.79µg/mL).

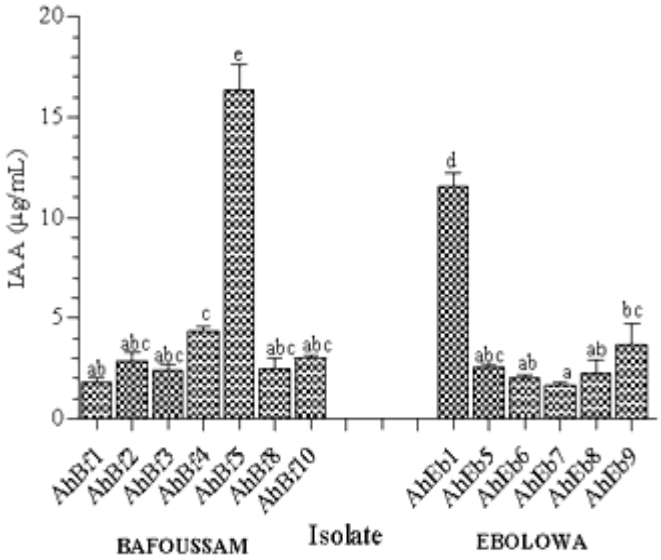

Figure 4: Production of indole-3-acetic acid by rhizobial isolates nodulating peanut. Means with the same letters are not significantly different at 5% of average probability.

➢ ***Tolerance toward antibiotics of isolates***

Antibiotic resistance test of isolates shown that the halo (Fig. 5) diameters increase with an increase in the concentration of antibiotics.

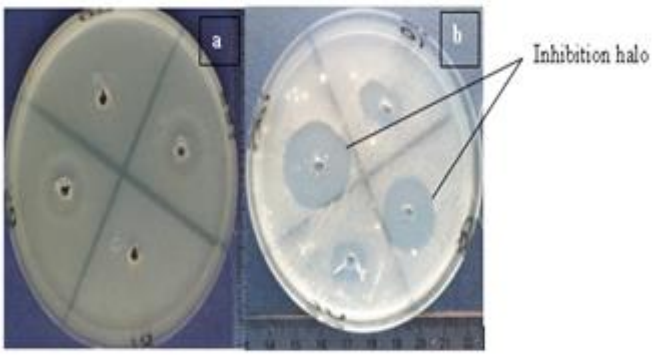

Figure 5: Effect of antibiotics on the growth of peanut-nodulating rhizobia isolates: resistance of isolate AhBf5 to amoxicillin at 10 and 20 µg / mL (a) and sensitivity of isolate AhEb5 to erythromycin at 50 and 100 µg / mL (b)

The minimum inhibitory concentration (MIC) represents the lowest concentration of antibiotic capable of causing inhibition of the growth of a given bacteria. The values of the minimum inhibitory concentrations were measured and the results are shown in Table 3.

Table 3: Minimum inhibitory concentrations of the antibiotics used

Sites	Isolates	MIC (µg/ml) Antibiotics						
		Gen	Chlo	Rif	Amo	Pen	Tet	Ery
Bafoussam	AhBf1	20	10	100	50	10	10	>100
	AhBf2	>100	50	50	>100	100	10	10
	AhBf3	10	50	50	100	50	10	10
	AhBf4	10	>100	10	50	>100	20	10
	AhBf5	10	>100	10	50	10	10	>100
	AhBf8	20	50	20	>100	10	10	100
	AhBf10	50	10	10	10	10	10	10
Ebolowa	AhEb1	50	50	10	>100	10	>100	10
	AhEb5	10	>100	100	>100	10	>100	10
	AhEb6	10	50	10	100	10	10	10
	AhEb7	50	20	10	10	10	>100	10
	AhEb8	>100	10	10	50	10	>100	10
	AhEb9	10	100	10	50	20	10	10

Gen: Gentamycin, Chlo: Chloramphenicol, Rif: Rifampicin, Amo: Amoxicillin, Pen: Penicillin, Tet: Tetracyclin, Ery: Erythromycin

The minimum inhibitory concentration of 10 µg / mL causing inhibition of all isolates except AhBf1, AhBf5 and AhBf8 is obtained in the presence of erythromycin. Minimum inhibitory concentrations of 20 µg / mL are obtained in the presence of gentamicin for isolates AhBf1 and AhBf8, chloramphenicol for isolate AhEb7, rifampicin for isolate AhBf8, penicillin for isolate AhEb7 and tetracycline for isolate AhBf4. Only tetracycline and erythromycin did not produce a minimum inhibitory concentration of 50 µg / mL. Minimum inhibitory concentrations of 100 µg / mL are obtained in the presence of all antibiotics except gentamicin and tetracycline. The MIC > 100 µg / mL are obtained in the presence of gentamicin for the AhBf2 and AhBf8 isolates, in the presence of chloramphenicol for the AhBf4, AhBf5 and AhEb5 isolates, in the presence of amoxicillin for the AhBf2, AhBf8, AhEb1 and AhEb5 isolates, in the presence of penicillin for the AhBf4 isolate, in the presence of tetracycline for the AhEb1, AhEb5, AhEb7 and AhEb8 isolates and in the presence of erythromycin for the AhBf1 and AhBf5 isolates. Only rifampicin did not produce MIC > 100 µg / mL.

Peanut-nodulating rhizobia isolates that have an antibiotic MIC ≥100 µg / mL, therefore high, are the most tolerant. On the other hand, the isolates showing no MIC ≥100 µg / mL are the most sensitive.

Figure 6 shows the percentage resistance of the isolates to the different antibiotics used. It can be seen from this figure that the percentage resistance of the isolates decreases as the concentration of the antibiotic increases. 71.42% of Bafoussam isolates are resistant to concentrations of 10 and 20 µg / mL gentamicin while only 50% of Ebolowa isolates are resistant. Amoxicillin is the antibiotic most tolerated by peanut-nodulating rhizobia isolates; almost half of the isolates tolerate its highest concentration of 100 µg / mL. The most harmful antibiotic for peanut-nodulating rhizobia isolates is rifampicin. No isolate tolerates its highest concentration of 100 µg / mL.

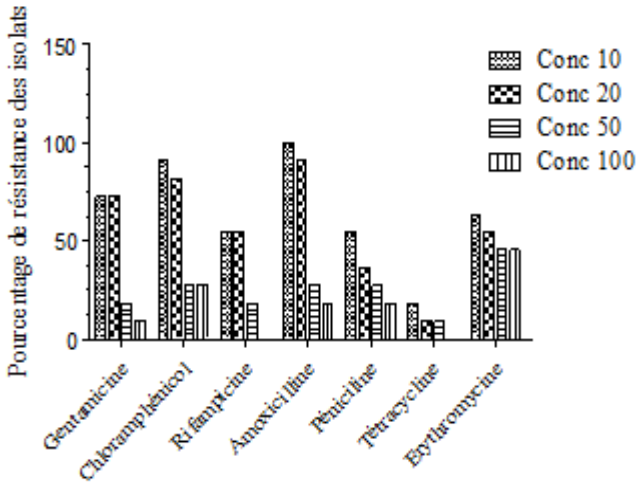

Figure 6: Percentage of resistant isolates based on antibiotic concentrations and sampling site

IV. DISCUSSION

The formation of nodules in the peanut root system on Ebolowa and Bafoussam soils without a history of inoculation testifies to the presence in these soils of peanut nodulating rhizobia. [8] and [12] reported the presence of nodules on peanut and common bean roots respectively on various soils in the forest zone in Cameroon. These results are consistent with those of [18] who reported the appearance of nodules on the roots of five varieties of bean in northern Ethiopia without inoculation. The nodules formed on the 2 soils are of a determined type. This nodule morphology is in agreement

with data from [19] who showed that tropical legumes such as peanuts all form nodules of a specific type.

A collection of 13 peanut nodulating isolates was obtained after authentication. All isolates obtained after trapping do not or very poorly absorb Congo Red. Such results suggest that the isolates obtained are rhizobia [11-20]. The colonies of the PEANUT-nodulating rhizobia isolates obtained are all rounded and form colonies after less than 3 days of incubation on YEMA medium, therefore fast growing. [21] have indicated the existence of strains of rhizobia with rapid growth among the rhizobia nodulating legumes.

After Gram staining, all isolates have a Gram negative cell wall. These results are in agreement with those found by [11], [22], [23], [12] and [24] for the rhizobia isolates. This color is due to the nature of their lipid-rich wall.

The peanut-nodulating rhizobia isolates tested produced IAA. This result is in agreement with that of [25] who observed IAA production by isolates of the nodulating rhizobia *Vigna radiata*. Similarly, [26] reported IAA production by rhizobia strains nodulating bean. [12] noted significant production of IAA by rhizobia isolates from bean in the presence of L-Tryptophan as a precursor. [13] have shown a significant production of IAA by endophytic bacteria isolated from various organs of wheat (*Triticum*) in the presence of 0.1 mg / mL of L-Trp. The peanut-nodulating rhizobia isolates obtained in this study are resistant to amoxicillin, chloramphenicol, gentamycin, penicillin, tetracycline and erythromycin. This result is in agreement with that of [27] who reported the existence of bean nodulating rhizobia isolates resistant to erythromycin and penicillin. [24] reported high resistance of *Rhizobium* strains to penicillin and amoxicillin. Tolerance to antibiotics depends on the bacterial strain [24], the type of antibiotic and its concentration in the medium [28-12]. The difference in the degrees of resistance to antibiotics is linked to the genetic variation of rhizobia strains [24]. This resistance is acquired by horizontal gene transfer [29]. Bacterial species belonging to Rhizobium sp. have been able to show multiple resistance to different types of antibiotics [30].

V. CONCLUSION

The present study focused on the trapping and isolation of peanut-nodulating bacteria in two sites in Cameroon: Bafoussam and Ebolowa and the assessment of the ability of peanut-nodulating rhizobia (*Arachis hypogaea* L.) isolates to resist antibiotics and to produce indole-3-acetic acid.

A collection of thirteen peanut-nodulating rhizobia isolates was assembled. The isolates obtained were chosen because of their ability to form nodules on the roots of peanut (*Arachis hypogaea* L.), and therefore their ineffectiveness. The isolates in the collection enrich the laboratory's strain bank.

All peanut-nodulating rhizobia isolates obtained in this study produced IAA. Bafoussam isolates produce more IAA than those from Ebolowa. The isolate that produces the most is AhBf5 from the Bafoussam site.

This work also made it possible to demonstrate the tolerance of the isolates to antibiotic concentrations of 100 µg/mL. The isolates obtained were resistant to amoxicillin, chloramphenicol, tetracycline and gentamycin but susceptible to rifampicin.

ACKNOWLEDGMENT

The authors sincerely thank all those who contributed to the realization of this work, in particular the soil microbiology research team of the Plant Biology Department of the Faculty of Sciences of the University of Douala-Cameroon.

REFERENCES

[1]. **Anonyme. 2020.** Recession économique mondiale liée à la covid-19: la lute contre la faim doit être au centre de la relance économique.
[2]. **Sharma V., Purohit G. R., Arya R. S. et Harsh M. 2006.** Evaluation of some complete rations in sleep incorporating unconventional feed resources of arid zone in India. Animal Nutrition and Feed Technology-Indian Journals.
[3]. **Guchi E. 2005.** Effect of Storage Time on Occurrence of *Aspergillus* species in Peanut (*Arachis hypogaea* L.) in Easthern Ethiopia. Journal of Applied Environment and Microbiology, 3(1): 1-5.
[4]. **Reddy E. C., Sudhakar C., Reddy N. P. 2011.** Afatoxin contamination in peanut induced by Aspergillus flavus type fungi : A critical review.
[5]. **Anonyme 2013.** Contribution de la recherche à l'amélioration de la production et la consommation des légumineuses alimentaires au Cameroun. IRAD/C2C. 5-57
[6]. **Christie M. E., Kyamureku P., Kaaya A., Devenport A. 2015.** Farmers, peanuts and aflatoxins in Uganda: A gendered approach of Developmental Practices, 25: 4-18.
[7]. **Anonyme. 2015.** Direction Services Agricoles d'el Tarf. Service statistique.
[8]. **Ngo Nkot L., Nwaga D., Ngakou A., Fankem H. et Etoa F. X. 2011.** Variation in nodulation andgrowth of peanut (Arachis hypogaea L.) on oxisoils from land use systems of the humid forest zone in southern Cameroon. African Journal of Biotechnology. 10(20): 3996-4004.
[9]. **Cacciari I., Di Mattia E., Quatrini P., Moscatelli M. C., Grego S., Lippi D. et Paolis M. R. 2013.** Réponses adaptatives des isolats de Rhizobium aux stress.
[10]. **Benselama A. 2015.** Réhabilitation de la culture du *Lablab purpureus* L. ex Sweet et études de son partenaire symbiotique. Thèse de Doctorat 3e cycle LMD en Biotechnologie. Université d'Oran 1. 133 p.
[11]. **Vincent J. M. 1970.** A manual for a practical study of root nodule bacteria. I.B. P, Handbook n° 15, Blackwell (Ed). Oxford. Edingbourgh. p. 104.

[12]. **Youagang G. H. S., Ngo Nkot L., Asseng C. C., Nyaka Ngolisa A. I. C., Ngakou A. et Nwaga D. 2020.** Isolation and characterization of Legume Nodulating Bacteria Isolated from Common bean (*Phaseolus vulgaris* L.) nodules. Global Scientific Journal, 8 (5): 1777-1792.

[13]. **Tehmina K., Shahzad A. K., Nighat N., Tayyaba Y., Muhammad Z., Haroon A., Farrakh M. et Sumaira F. 2019**. Isolation and characterization of culturable endophytic bacterial community of stripe rust–resistant and stripe rust–susceptible Pakistani wheat cultivars. International Microbiology. 22:191–201.

[14]. **Leveau J. H. J. et Lindow S. E. 2005.** Utilization of the plant Hormone Indile-3-Acetic Acid for Growth by *Pseudomonas pudica* Strain 1290. Applied and Environmental Microbiology, 71: 2365-2371.

[15]. **Silini A. 2013.** Effets des molécules osmoprotectrices sur la survie et l'activité d'Azotobacter et sur la croissance du blé dur en milieu salin. Thèse de Doctorat. Université Ferhat Abbas-Sétif. p. 153.

[16]. **Cos P., Arnold J., Vlietinck, Dirk V. B. et Louis M. 2006.** Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof of concept'. Journal of Etnopharmacology, 106: 290-302.

[17]. **Moreira M. R., Ponce A. G., de Valle C. E. et Roura S. I. 2005.** Inhibitory parameters of essential oils to reduce a food borne pathogen. Lebensmittel-Wissenschaft und-Technologie-LWT, 38:565-570.

[18]. **Gebre-E. M., Hadush T. et Fetein A. 2014.** Agronomic performance of some haricot bean varieties (*Phaseolus vulgaris* L.) with and without phosphorus fertilizer under irrigated and rain field conditions in the Tigray and Afar regional states, northern Ethiopia. Momona Ethiopian Journal of Science. 6(2): 95-109.

[19]. **Allen O. N. et Allen E. K. 1981.** The *Leguminosae*, a Source Book of Characteristics, Uses and Nodulation.

[20]. **Shetta N. D., Al-Shaharani T. S. et Abdel-Aral M. 2011.** Identification and characterization of Rhizobium associated with woody legume trees grown under Saoudi Arabia conditions. American-Eurasian Journal of Agricultural and Environmental Sciences. 10: 410-418.

[21]. **Hungria M., Ardley J. O., Hara G. W. et Dilworth M. J. 2016.** Working with rhizobia. ACIAR, Camberra, Australia: 314 pp.

[22]. **Jordan D. 1984.** Family III. Rhizobiaceae, p. 234-242. In N. R. Krieg, and J. G. Holt (éd), Bergey's manual of systematic bacteriology. Williams and Wilkins Co, Baltimore, Md.

[23]. **Ngo Nkot L., Ngo Bisseck M., Fankem H., Adamou S. Kamguia K., Ngakou A. et Etoa F. X. 2015.** Isolation and screening of Indigenous Bambara Peanut (*Vigna subterrenea* L.). Nodulating Bacteria for their Tolerance to Some Environmental Stresses. American Journal of Microbiological Research. 3(2): 65-75.

[24]. **Kanouni L. 2019.** Inhibition des champignons phytopathogènes par Rhizobium. Thèse de Doctorat 3^{e} cycle de l'Université Ferhat Abbas Sétif I, Algérie. 129 p.

[25]. **Muhammad A. A., Zahir A. Z., Muhammad Arshad et Muhammad Arshaf. 2011**. Isolation and screening of rhizobia for auxin biosynthesis and growth promotion of mung bean (*Vigna radiata* L.) seedling under auxinic conditions. Soil Environ. 30(1): 18-26.

[26]. **Stajkovic O., Declic D., Josic D., Kuzmanovic D., Rasulic N. et Knezevic-Vukcevic. 2011.** Improvement of common bean growth by co-inoculation with *Rhizobium* and plant growth promoting bacteria. Roman of Biotecnological Letters, 16: 5915-5926.

[27]. **Rebecca F., Renan A. R., Ernesto O., Marco A. R., Jakeline R., Marcon Diva S. A., Esperanza Martınez-Romero. et Mariangela H. 2013**. *Rhizobium* freirei sp. nov., a symbiont of *Phaseolus vulgaris* that is very effective at fixing nitrogen. International Journal of Systematic and Evolutionary Microbiology. 63: 4167-4173.

[28]. **Graham P. H. 1964.** Studies on the utilization of carbohydrates and Krebs cycle intermediates by rhizobia, using an agar plate method. Antinie van Leeuwenhoek. 30:68-72.

[29]. **Bhargava Y., Marthy J. S. R., Rajesh Kumar T. V. et Narayana Rao M. 2015.** Phenotypic stress tolerance and plant growth promoting characteristics of rhizobial isolates from selected wild legumes of Semi-arid region, Tirupati, India. Advances in Microbiology, 6 : 1-12.

[30]. **Cole M. A. et Elkan G. H. 1979.** Multiple antibiotic resistance in Rhizobium japonicum. Applied and Environmental Microbiology, p: 867-870.