International Journal of Plant & Soil Science

32(2): 35-46, 2020; Article no.IJPSS.54865
ISSN: 2320-7035

Characterization of Indigenous Rhizobia Strains Associated to Soybean [*Glycine max* (L.) Merrill] in Benin

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Authors' contributions

This work was carried out in collaboration among all authors. Author MCCZ designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author FB managed the literature searches. Authors PH and FT designed and supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2020/v32i230244
Editor(s):
(1) L. S. Ayeni, Adeyemi College of Education, Nigeria.
Reviewers:
(1) Raghvendra Pratap Singh, India.
(2) S. Martyniuk, Institute of Soil Science and Plant Cultivation, Poland.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54865

Original Research Article

Received 21 December 2019
Accepted 28 February 2020
Published 06 March 2020

ABSTRACT

Legumes such as soybean establish symbiotic relation with nitrogen fixing bacteria such as Rhizobia. Nitrogen fixation via legume-rhizobium symbiosis is the most important source of Nitrogen in agro-ecosystems. But environmental stresses are important limiting factors for this process. Hence, the aim of this study is to evaluate the physiological characteristics and Plant Growth Promoting (PGP) properties of soybean rhizobia. A total of 28 Rhizobia strains obtained from soybean root nodules collected in from three Agro-Ecological Zones (zones 3, 4 and 5) producers of soybean in Benin were used. The physiological characteristics include utilization of carbon source, tolerance to temperature, salinity and pH, resistance to antibiotics and heavy metals. The PGP properties were relative to production of indole, hydrogen cyanide and ammonia

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and catalase test. The results revealed that, irrespective of their geographical regions, the 28 isolates were grouped into five Clusters. Most of them tolerated neutral to alkaline pH and high salt stress and 17% of them could grow at 40°C. Most of them showed resistance to heavy metal and antibiotics. These isolates tested were able to use a broad range of carbohydrates as sole source of carbon. Production of indole, hydrogen cyanide and ammoniac were respectively found on 56%, 41% and 44% of isolates but all isolates gave positive reaction to catalase test. These rhizobial isolates showing best physiological and PGP properties could be good candidates to establish a successful symbiosis with soybean under the variation of environmental conditions that prevail in Benin.

Keywords: Soybean; rhizobium; plant growth promoting properties; environmental stress; Benin.

1. INTRODUCTION

Rhizobia are a community of soil bacteria that have ability to establish symbiotic relations with many legumes such as soybean (*Glycine max)*, form root nodules, place in which the biological nitrogen fixation is carried out [1,2]. Nitrogen-fixing rhizobial symbiosis can play a particularly important role for growth and productivity of plants, their adaptation to the habitat and competition for environmental space [3]. Reliable classification and identification of specific rhizobial strains is important for the study of their symbiotic relationship with plants [4]. Most recent taxonomic studies used a polyphasic approach with phenotypic, genetic, chemotaxonomic and phylogenetic data for establishing a comprehensive picture of the relationships of the bacteria and to propose a suitable classification [5,6,2,7]. Wolde-Meskel et al. [8] showed that the classical phenotypic characterization of Rhizobia strains is very helpful for primary classification of Rhizobia. This phenotypic study is necessary for identification and separation of new rhizobial strains adapted to marginal edapho-climatic conditions [6] and to provide preliminary information about their genetic diversity [7].

Generally, stable characteristics used to define isolated strains are the Gram staining, color, shape, size, and texture of colonies and the ability to alter the pH of the media [4]. Tolerance to salinity, tolerance to acid or alkali, tolerance to temperature and resistance to antibiotics and heavy metal were used by many researchers to determine a wide physiological diversity of many tested isolates [9]. Recently, some species of rhizobia strains were found to exhibit plant growth promoting (PGP) properties as they improved the growth of some crops through mechanisms (such as phytohormone production, phosphate solubilisation, siderophore production and biocontrol activities) that are independent of biological nitrogen fixation [10,11].

In Benin, studies evaluating the diversity of rhizobial bacteria remain scarce in spite of the great diversity of legume crops in the region. The aim of this study was to evaluate the physiological diversity and some PGP properties of Rhizobia isolated from soybean root nodules from field-growing plants in Benin.

2. MATERIALS AND METHODS

2.1 Materials

This study used 28 effective Rhizobium strains associated to soybean roots and four references strains (IRAT FA3, STM3043, STM3045 and USDA 110). These 28 Rhizobium strains were selected after a screening test (nodulation test and effectiveness test) of 102 Rhizobia strains isolated from soybean roots nodules collected from three Agro Ecological Zones of Benin [12]. The origins and the morphological characterization of these strains are presented in Table 1 [12]. All strains were Gram- negative and did not absorb red color when cultured in Yeast Extract Mannitol Agar (YEMA) medium containing Congo red dye. 72% of them were fast growers (colonies visible after three days of incubation) and 28% were slow growers (longer than five days). Reaction on YEMA with Bromothymol Blue (BTB) distinguished 13% alkaline producers and 87% acid producers.

2.2 Physiological Characterization of the Isolates

The ability of Rhizobial isolates to grow at different pH was tested in YEM broth by adjusting the pH to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 with NaOH (1N) and HCl (1N). After 7 days of incubation at 30°C, bacterial growth was compared with the controls [13].

Temperature Tolerance was investigated by incubating bacterial cultures in YEM agar at 4, 30, 35 and 40°C [13].

All isolates were examined for their tolerance to salt on YEMA supplemented with 1, 2, 3, 4, 5, 7, 8 and 9% of NaCl (w/v) [14,1]). The medium YEMA was used as control.

The use of different carbohydrate as carbon source was tested on YEMA medium by replacing the mannitol with the carbohydrate to be tested [13]. The carbohydrates tested were: sucrose, lactose, galactose, maltose and glucose.

Intrinsic antibiotic resistance was tested on YEM agar medium supplemented with one of the following antibiotics spectinomycin, rifampicin, ampicillin and gentamycin at the concentrations of 50 µg ml-1. The medium was supplemented with different amounts of antibiotics according to the method described by Somasegaran and Hoben [15]. Antibiotic solutions were sterilized by filtration (0.22 µm) and added to the sterile YEM agar at 55°C.

The resistance of strains to heavy metals was also determined on solid YEM medium. The stock solutions of metals were filter-sterilized and added to sterile YEM medium as following concentrations: 500 µg/ml for $MnCl_2$, 100 µg/ml for $CuSO_4$, $ZnCl_2$ and $HgCl_2$ [14].

2.3 Determination of Plant Growth Promoting (PGP) Properties of the Isolates

Catalase test was performed to study the presence of enzyme catalase which hydrolyzes H_2O_2 into H_2O and O_2 in bacterial strains. Hydrogen peroxide is often used as a topical disinfectant in wounds and the bubbling that is seen is due to the evolution of O_2 gas [16]. H_2O_2

Table 1. Origins and morphological characterization of Rhizobia strains used in the study

Isolate identities	Agro-ecological zone	Site location	Type or growth	Gram staining	BTB test
LMSEM50	AEZ 3	Bembèrèkè	Fast	Negative	Blue
LMSEM77	AEZ 3	Bembèrèkè	Fast	Negative	Blue
LMSEM20	AEZ 3	N'dali	Fast	Negative	Yellow
LMSEM81	AEZ 3	N'dali	Fast	Negative	Yellow
LMSEM101	AEZ 3	N'dali	Slow	Negative	Blue
LMSEM37	AEZ 3	Nikki	Fast	Negative	Yellow
LMSEM99	AEZ 3	Nikki	Slow	Negative	Yellow
LMSEM53	AEZ 3	Pèrèrè	Fast	Negative	Blue
LMSEM98	AEZ 3	Pèrèrè	Fast	Negative	Blue
LMSEM22	AEZ 4	Copargo	Slow	Negative	Blue
LMSEM24	AEZ 4	Copargo	Slow	Negative	Blue
LMSEM25	AEZ 4	Copargo	Fast	Negative	Yellow
LMSEM32	AEZ 4	Copargo	Fast	Negative	Yellow
LMSEM2	AEZ 4	Djougou	Fast	Negative	Yellow
LMSEM86	AEZ 4	Djougou	Fast	Negative	Yellow
LMSEM7	AEZ 4	Ouaké	Fast	Negative	Yellow
LMSEM46	AEZ 4	Ouaké	Fast	Negative	Yellow
LMSEM85	AEZ 4	Ouaké	Fast	Negative	Yellow
LMSEM47	AEZ 4	Kouandé	Fast	Negative	Yellow
LMSEM93	AEZ 4	Kouandé	Fast	Negative	Yellow
LMSEM97	AEZ 5	Bantè	Slow	Negative	Blue
LMSEM48	AEZ 5	Dassa	Fast	Negative	Yellow
LMSEM96	AEZ 5	Dassa	Slow	Negative	Blue
LMSEM1	AEZ 5	Glazoué	Fast	Negative	Yellow
LMSEM3	AEZ 5	Glazoué	Fast	Negative	Yellow
LMSEM78	AEZ 5	Glazoué	Fast	Negative	Yellow
LMSEM17	AEZ 5	Savè	Fast	Negative	Yellow
LMSEM44	AEZ 5	Savè	Fast	Negative	Yellow
IRAT FA3	France	-	Slow	Negative	Blue
STM3043	France	-	Slow	Negative	Blue
STM3045	France	-	Slow	Negative	Blue
USDA110	Kenya	-	Slow	Negative	Blue

is a potent oxidizing agent that can wreak havoc in a cell; because of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide. A drop of 3% hydrogen peroxide was added to the colony on sterile glass slide and mixed well using a sterile loop [17]. Appearance of effervescence indicated catalase activity.

Indole production test was investigated by inoculating bacteria in peptone water (indole free). The suspension was incubated at 37°C for 24 h. Upon reading, five drops of KOVACKS reagent were added to the bacterial suspension. The appearance of a red ring at the meniscus of the suspension show indole production [18].

The test of Hydrogen Cyanide (HCN) production was performed as follow. The bacterial cell cultures were streaked on nutrient agar medium, which contained 4.4 g per liter of glycine. A Whatman filter paper was soaked in 0.5% picric acid solution and it was placed inside the lid of the plate. The plates were sealed with parafilm and incubated at 30°C for four days. The appearance of a light brown to dark brown color of the filter paper indicated HCN production [17].

For Ammonia production test, fresh bacterial colonies are cultured in 10 ml of peptone water and incubated at 28°C for four days [19]. After incubation, 0.5 ml of Nessler's reagent was added to each tube [17]. The development of faint yellow to dark brown color indicated the production of ammonia.

2.4 Data Analysis

A numerical analysis of Phenotypic and PGP properties variables was done. The results of all phenotypic tests PGP properties have been indicated by 1 to 3 for every positive result and by 0 for all negative results. A computer cluster analysis was carried out using a similarity coefficient and a dendrogram was constructed. Principal component analysis of the strains communities was performed using multivariate cluster analysis in Minitab software version 14.1.

3. RESULTS

3.1 pH and Salt (NaCl) Tolerance

The soybean nodulating Rhizobia isolated from different Agro-Ecological Zones of Benin had a wide diversity in their different pH tolerance. All tested isolates grew in moderately acidic pH (5) to neutral pH (7) and slightly alkaline pH (8) but the growth is optimal at pH 7 (Table 2). Some isolates (LMSEM 3, LMSEM 7, LMSEM 20, LMSEM 32, LMSEM 37, LMSEM 78, LMSEM 81, LMSEM 85 and LMSEM 99) showed an acid tolerant character since they grew at pH 4. Similarly, most of strains showed alkaline tolerant character since 87.5% and 84.5% of the isolates grew at pH 9 and 10, respectively.

Tolerance to sodium chloride (NaCl) varied among the strains. A part from strains LMSEM 24 (which didn't grow with 3% to 9% NaCl), LMSEM 78 and LMSEM 97 (no growth with 5% to 9% NaCl), all the strains tolerated NaCl concentration ranged from 1% to 5% NaCl. Some strains exhibited high tolerance to sodium chloride since 62.5% and 31% grew with 7% and 9% NaCl.

3.2 Temperature Tolerance and Carbon Sources Utilization

As shown in Table 3, all isolates exhibited generally good growth at 30°C and most of them also showed good growth at 35°C. None of the tested isolates could be able to tolerate and grow at 4°C while 37% of them could grow at 40°C.

All soybean Rhizobia strains were able to use the five sources of carbon tested. Maltose and glucose were the best sources of carbon assimilated by all isolates. However, sucrose was the least metabolized.

3.3 Intrinsic Antibiotic Resistance and Heavy Metals Resistance

Results presented in Table 4 indicated different antibiotic resistance patterns among the rhizobial strains tested. Respectively, 53%, 56% and 69% of strains were tolerant to spectinomicin, gentamicin and rifampicin. All isolates (100%) were resistant to ampicillin.

The soybean associated bacteria showed a wide resistance to heavy metals tested. Except for a few numbers (25%), all the strains were sensitive to HgCl2 but 100% of them tolerated $CuSO_4$, $ZnSO_4$ and $MnCl_2$.

3.4 Plant Growth Promoting (PGP) Properties of the Isolates

Some soybean nodulating Rhizobia showed PGP characteristics such as catalase, indole,

ammoniac and hydrogen cyanide production (Table 5). The results revealed that 56%, 41% and 44% of isolates produced respectively indole, hydrogen cyanide and ammoniac. All isolates gave positive reaction to catalase test.

3.5 Strains Community

The thirty-eight isolates and the four reference strains formed five clusters at similarity level of 67% according to their phenotypic characteristics and their PGP properties (Fig. 1). The first cluster (C1) included strains LMSEM 1, LMSEM 2, LMSEM 3 and LMSEM 7. The second cluster (C2) gathered six strains (LMSEM 16, LMSEM 53, LMSEM 50, LMSEM 81 and IRAT FA3). The third cluster (C3) assembled strains LMSEM 17, LMSEM 48, LMSEM 22, LMSEM 48, LMSEM 44, LMSEM 47, LMSEM 25, LMSEM 98, USDA 110 and STM3043. The fourth cluster (C4) was composed of strains LMSEM 20, LMSEM 32, LMSEM 37, LMSEM 85, LMSEM 96 and STM3045. The fifth cluster (C5) was consisted of strains LMSEM 24, LMSEM 78, LMSEM93, LMSEM 97, LMSEM 99 and LMSEM 101.

Table 2. pH and salt (NaCl) tolerance of the isolates

Strains	pH							NaCl						
	4	5	6	7	8	9	10	1%	2%	3%	4%	5%	7%	9%
LMSEM1	-	1+	2+	3+	1+	1+	-	3+	2+	3+	3+	1+	1+	1+
LMSEM2	-	1+	2+	3+	1+	-	-	3+	2+	3+	3+	2+	2+	1+
LMSEM3	1+	1+	2+	3+	1+	-	-	3+	2+	2+	2+	2+	2+	1+
LMSEM 7	1+	1+	2+	3+	1+	-	-	3+	2+	2+	2+	2+	2+	1+
LMSEM16	-	1+	2+	3+	1+	1+	1+	3+	3+	3+	3+	2+	1+	-
LMSEM17	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	-	-
LMSEM20	1+	1+	2+	3+	2+	1+	1+	3+	2+	2+	2+	3+	1+	1+
LMSEM22	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	-	-
LMSEM24	-	1+	2+	3+	1+	-	-	3+	1+	-	-	-	-	-
LMSEM25	-	1+	2+	3+	2+	1+	1+	3+	3+	1+	1+	1+	1+	-
LMSEM32	1+	1+	2+	3+	2+	1+	1+	3+	3+	2+	2+	2+	-	-
LMSEM37	1+	1+	2+	3+	1+	1+	1+	3+	2+	2+	2+	2+	1+	-
LMSEM44	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	-	-
LMSEM46	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	-	-
LMSEM47	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	-	-
LMSEM48	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	-	-
LMSEM50	-	1+	2+	3+	1+	1+	1+	3+	2+	3+	3+	2+	1+	-
LMSEM53	-	1+	2+	3+	1+	1+	1+	3+	3+	3+	3+	2+	-	-
LMSEM77	-	1+	2+	3+	1+	1+	1+	3+	3+	3+	3+	2+	1+	-
LMSEM78	1+	1+	2+	3+	2+	1+	1+	3+	2+	1+	1+	-	-	-
LMSEM81	1+	1+	2+	3+	2+	1+	1+	3+	2+	3+	3+	3+	1+	-
LMSEM85	1+	1+	2+	3+	2+	1+	1+	3+	3+	2+	2+	3+	1+	-
LMSEM93	-	1+	2+	3+	2+	1+	1+	3+	1+	1+	1+	1+	-	-
LMSEM96	-	1+	2+	3+	2+	1+	1+	3+	3+	2+	2+	2+	1+	-
LMSEM97	-	1+	2+	3+	2+	1+	1+	3+	1+	1+	1+	-	-	-
LMSEM98	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	1+	1+
LMSEM99	1+	1+	2+	3+	2+	1+	1+	3+	1+	1+	1+	1+	1+	1+
LMSEM101	-	1+	2+	3+	2+	1+	1+	3+	1+	1+	1+	1+	1+	1+
IRAT FA3	-	1+	2+	3+	1+	1+	1+	3+	3+	3+	3+	2+	1+	-
STM3043	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	1+	1+
STM3045	-	1+	2+	3+	1+	1+	1+	3+	2+	2+	2+	2+	1+	-
USDA110	-	1+	2+	3+	1+	1+	1+	3+	2+	1+	1+	1+	1+	1+

-: no growth; 1+: low growth; 2+: moderate growth; 3+: Good growth

Table 3. Temperature tolerance and carbon sources utilization of the isolates

Strains	Carbon sources utilization					Temperature tolerance			
	Lactose	Galactose	Maltose	Glucose	Sucrose	4°C	30°C	35°C	40°C
LMSEM1	2+	2+	2+	3+	1+	1+	3+	-	-
LMSEM2	2+	2+	2+	3+	1+	1+	3+	-	-
LMSEM3	2+	2+	2+	2+	2+	1+	3+	-	-
LMSEM 7	2+	2+	3+	3+	2+	1+	3+	-	-
LMSEM16	2+	2+	3+	3+	1+	1+	3+	3+	1+
LMSEM17	2+	3+	3+	3+	1+	1+	3+	3+	1+
LMSEM20	1+	2+	3+	3+	2+	1+	3+	3+	1+
LMSEM22	2+	2+	3+	3+	1+	1+	3+	3+	1+
LMSEM24	2+	2+	2+	2+	1+	1+	3+	3+	-
LMSEM25	1+	2+	3+	3+	1+	1+	3+	3+	-
LMSEM32	2+	2+	3+	2+	2+	1+	3+	2+	1+
LMSEM37	2+	1+	3+	3+	2+	1+	3+	2+	-
LMSEM44	2+	1+	3+	3+	2+	1+	3+	-	-
LMSEM46	2+	2+	3+	3+	1+	1+	3+	3+	-
LMSEM47	2+	1+	3+	2+	1+	1+	3+	3+	-
LMSEM48	2+	2+	3+	3+	1+	1+	3+	3+	-
LMSEM50	2+	2+	3+	3+	2+	1+	3+	-	-
LMSEM53	2+	2+	3+	3+	1+	1+	3+	2+	-
LMSEM77	2+	3+	3+	2+	2+	1+	3+	2+	1+
LMSEM78	2+	2+	3+	3+	1+	1+	3+	2+	1+
LMSEM81	2+	2+	3+	3+	1+	1+	3+	3+	1+
LMSEM85	2+	2+	3+	3+	2+	1+	3+	3+	1+
LMSEM93	2+	2+	3+	3+	2+	1+	3+	3+	-
LMSEM96	2+	2+	3+	3+	2+	1+	3+	2+	-
LMSEM97	2+	2+	3+	3+	1+	1+	3+	2+	-
LMSEM98	2+	2+	3+	2+	1+	1+	3+	2+	-
LMSEM99	2+	2+	3+	3+	1+	1+	3+	2+	-
LMSEM101	2+	2+	3+	3+	1+	1+	3+	2+	-
IRAT FA3	2+	2+	3+	3+	2+	1+	3+	3+	1+
STM3043	1+	2+	3+	3+	2+	1+	3+	2+	-
STM3045	2+	3+	3+	3+	2+	1+	3+	2+	1+
USDA110	2+	2+	3+	3+	1+	1+	3+	2+	-

-: no growth; 1+: low growth; 2+: moderate growth; 3+: Good growth

Principal component analysis applied on physiological characteristic and PGP properties of Rhizobia strains revealed that the first two axes explained 86% of the initial information (Fig. 2). The first principal component is positively correlated with neutral pH (pH 6 and pH 7), Nacl (1%), temperature (30°C) and Ampicillin but negatively with glucose and HgCl. The second principal component is negatively correlated with NaCl (2%, 3%, 4% and 5%), Gentamycin and Indole. Projection of strains communities in system axes (Fig. 2) revealed that community of strains of cluster 1 (C1), cluster 3 (C3) and cluster 5 (C5) were opposed to community of strains of cluster 2 (C2) and cluster 4 (C4) when the first component was considered. The community of strains of cluster 1 was opposed to community of strains of cluster 2, cluster 3, cluster 4 and cluster 5 when the second component was considered.

4. DISCUSSION

Strains exhibited a great tolerance to salinity since some of them were able to grow in 9% NaCl. This NaCl tolerance range agreed with previous reports [7,14]. According to Konate et al. [14], salt tolerance strains can give to rhizobial strains advantages to survive, multiply in saline soil and efficiency infect their host plants.

A wide variation was found among soybean rhizobial isolates with regard to their tolerance to pH of the medium. All tested isolates tolerate

moderately acidic pH (5) to neutral and slightly alkaline pH (8) but some isolates showed an acid tolerant character since 28% of them grew at pH 4. These results are in concordance with those of Jida and Assefa [20]. However, alkaliphilic range is very low in this study compared to other studies. For example, high alkalotolerant rhizobia strains were reported in chickpea in the North-West Indo Gangetic Plains [21].

Our results showed that most of isolates from soybean plants exhibited tolerance to antibiotic such as gentamycin and rifampicin and spectinomycin. But they were all resistant to ampicillin. This result indicated that strains exhibited a multiple antibiotic resistance. The same result was described for many rhizobial strains [4,22]. The antibiotic resistance of the strains could partially explain a possible mechanism to overcome antagonism exerted by other organisms in the soil under field condition.
The optimum temperature for growth of all isolates was between 30 and 35°C. Beyond this range, the isolates showed variations in their growth. Only 37% of strains grew at 40°C. Rao [23] showed that the slow and fast-growing Rhizobia that nodulate soybean could grow equally well at 35°C; but a few tolerated 40-45°C among which the fast growers formed a higher proportion. However, these Rhizobia could tolerate high soil temperature since Rhizobia are more resistant to high temperatures in soil than in laboratory medium [24]. Strains were highly resistant to $CuSO_4$, $MnCl_2$ and $ZnCl_2$ but they were mostly sensitive to $HgCl_2$. Their resistance to metals is very similar to what it knows in general on Rhizobia. Tolerance to heavy metals could be exploited in the bioremediation trials aimed at improving the quality of soil contaminated with heavy metals sites [14,25].

The nutritional requirements of Rhizobia are quite variable and one of the nutritional needs of Rhizobia that can be used as a phenotypic character is the utilization of carbohydrates as a sole carbon source. Our results showed that the majority of tested soybean Rhizobia was able to use a large range of carbohydrates such as galactose, lactose, glucose, maltose and sucrose. But sucrose was the least carbon source utilized by isolates. This is in line with the result of other studies [20,7,26]. According to Singh et al. [27], rhizobial isolates may not grow on lactose, however this is one of the best carbon sources for all isolates including the reference strain. The ability to use a wide range of carbon sources is beneficial for the bacteria living in the soil and may be related to their high competitiveness in a natural environment [28].

Our results showed that some Rhizobia had PGP properties such as production of indole, ammonia and HCN and catalase activities. Several studies showed that different strains of Rhizobium species are endowed with PGP characteristics [9,29,30,31]. Indeed, rhizobial strains enjoy saprophytic life when there are no legume hosts

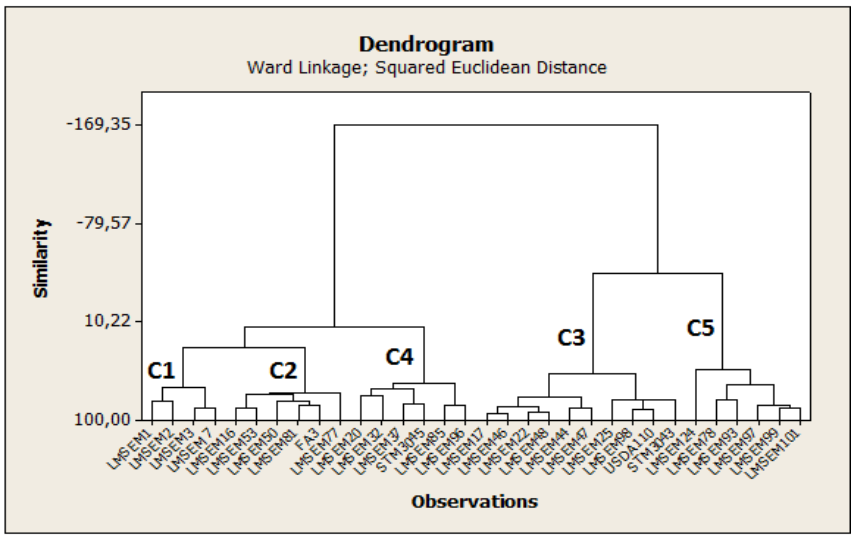

Fig. 1. Strains community's composition based on phenotypic and PGP properties of rhizobial strains

Table 4. Intrinsic antibiotic resistance and heavy metals resistance of the isolates

Strains	Heavy metals				Intrinsic antibiotic resistance			
	$CuSO_4$	$ZnSO_4$	$MnCl_2$	$HgCl_2$	Spectino	Genta	Rifam	Ampi
LMSEM1	1+	2+	2+	1+	-	-	-	1+
LMSEM2	2+	2+	1+	-	-	1+	1+	1+
LMSEM3	2+	3+	3+	-	-	1+	1+	1+
LMSEM 7	1+	2+	2+	-	-	1+	1+	1+
LMSEM16	2+	1+	3+	1+	1+	-	1+	1+
LMSEM17	1+	2+	2+	1+	1+	-	-	1+
LMSEM20	1+	3+	2+	1+	1+	1+	1+	1+
LMSEM22	2+	2+	1+	1+	1+	-	1+	1+
LMSEM24	2+	3+	3+	-	3+	-	-	1+
LMSEM25	2+	2+	2+	1+	1+	1+	1+	1+
LMSEM32	2+	3+	3+	-	1+	-	1+	1+
LMSEM37	3+	3+	3+	1+	-	1+	1+	1+
LMSEM44	1+	2+	3+	1+	-	-	-	1+
LMSEM46	1+	2+	2+	1+	-	-	-	1+
LMSEM47	1+	1+	2+	1+	-	-	-	1+
LMSEM48	1+	1+	1+	1+	-	-	1+	1+
LMSEM50	1+	3+	3+	1+	-	1+	-	1+
LMSEM53	1+	2+	2+	1+	1+	-	1+	1+
LMSEM77	1+	3+	2+	1+	1+	1+	2+	1+
LMSEM78	3+	3+	3+	1+	3+	-	2+	1+
LMSEM81	1+	2+	2+	1+	-	1+	1+	1+
LMSEM85	3+	3+	3+	1+	2+	1+	3+	1+
LMSEM93	3+	3+	3+	1+	2+	-	1+	1+
LMSEM96	3+	3+	3+	1+	1+	1+	2+	1+
LMSEM97	3+	3+	3+	1+	1+	-	-	1+
LMSEM98	2+	2+	2+	1+	1+	1+	1+	1+
LMSEM99	3+	3+	3+	1+	-	1+	-	1+
LMSEM101	3+	3+	3+	1+	1+	1+	1+	1+
IRAT FA3	1+	2+	1+	-	-	1+	1+	1+
STM3043	1+	1+	2+	-	-	1+	-	1+
STM3045	3+	2+	2+	1+	-	1+	1+	1+
USDA110	1+	2+	1+	-	1+	1+	1+	1+

-: no growth; 1+: low growth; 2+: moderate growth; 3+: Good growth; Spectino: Spectinomycin ; Genta : Gentamycin ; Rifam : Rifampicin; Ampi : Ampicillin

Rhizobia, become attracted to the roots of non-legume crops and nourish root exudates in the rhizosphere [20]. Therefore, Rhizobia with PGP characteristics would increase the yield of non-legume crops in rotation or mixed cropping with legumes [9,29]. Presence and intensity of HCN production can play a significant role in antagonistic potential of bacteria against phytopathogens; therefore, serve as bio-control agent [19]. Ammonia is one of the most important inorganic chemical important in pharmaceutical and agricultural industries and has broad applications [19]. It is a volatile substance produced by many rhizobacteria that is toxic to fungi. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and abiotic stress [31]. Plant growth promotion properties, resistance to environmental stress support the rhizobia in their saprophytic survival (free-living state), effective nodulation with legumes, and consequently their symbiotic efficiency as stated by Singh et al [32].

Based on the data of the above phenotypic characteristics and PGP properties, dendrograms were constructed. Five groups of Rhizobia were formed, but the dendrogram revealed that majority of the isolates could not form a cluster according to their geographical regions. These results are similar to those of [33,34,35]. Nevertheless, Rai et al. [36] showed that majority of the rhizobial isolates could form a cluster according to their geographical regions apart from some exceptions.

Table 5. Plant Growth Promoting (PGP) properties of the isolates

Strains	Catalase	Indole	Ammoniac	Hydrogen cyanide
LMSEM1	+	-	+	-
LMSEM2	+	-	-	-
LMSEM3	+	-	-	-
LMSEM 7	+	-	-	+
LMSEM16	+	+	+	-
LMSEM17	+	-	-	-
LMSEM20	+	+	-	+
LMSEM22	+	+	+	-
LMSEM24	+	-	-	+
LMSEM25	+	+	+	-
LMSEM32	+	-	-	+
LMSEM37	+	+	-	-
LMSEM44	+	-	-	-
LMSEM46	+	-	+	+
LMSEM47	+	+	-	-
LMSEM48	+	-	-	-
LMSEM50	+	+	-	-
LMSEM53	-	+	+	-
LMSEM77	-	+	-	+
LMSEM78	-	-	+	+
LMSEM81	+	+	-	-
LMSEM85	-	-	-	-
LMSEM93	+	+	+	-
LMSEM96	-	+	+	+
LMSEM97	-	-	-	+
LMSEM98	-	+	+	+
LMSEM99	-	-	-	-
LMSEM101	+	+	+	+
IRAT FA3	+	+	+	-
STM3043	+	+	+	-
STM3045	+	+	-	+
USDA110	+	+	+	+

+: indicates positive result; -: indicates negative result

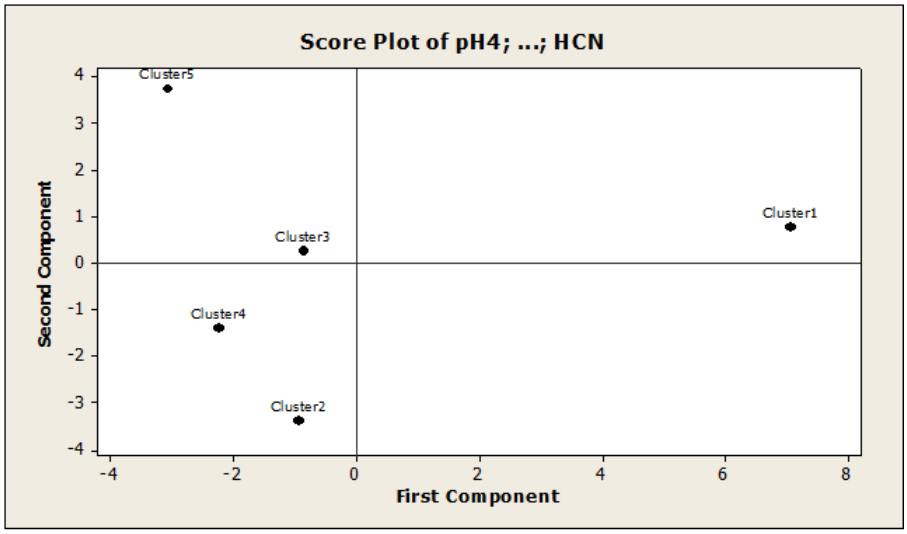

Fig. 2. Projection of strains communities in a system of axes

5. CONCLUSION

Rhizobia strains isolated from soybean root nodule in Benin are phenotypically diverse. Some isolates have shown tolerance to different limiting factors, such as acidic and alkaline pH, salinity, temperature, metal toxicity and antibiotic. In addition, they are endowed with PGP features such as indole, catalase, ammoniac and hydrogen cyanide production. However, the methods used for distinguishing rhizobial strains were morphological, physiological and biochemical. Unfortunately, these traditional methods of Rhizobium characterization frequently fail in the identification of strains to a species level. So, molecular methods should be used for isolates identification do to the necessity to obtain a better understanding of genetic diversity Rhizobia.

ACKNOWLEDGEMENTS

This work was funded by the University of Abomey-Calavi through the project « Productivité des Systèmes Agriculture-Elevage Intégrés » (PROSAEI) of competitive Research Fund Program. The Technical Cooperation Project BEN/5/007 funded by the International Atomic Energy Agency (IAEA) provided equipments and consumables that support the laboratory analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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