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Research Article

ISOLATION AND PHENOTYPIC CHARACTERIZATION OF NATIVE ENDOPHYTIC COFFEE BACTERIA (*Coffea canephora* P. var robusta) IN DALOA, CÔTE D'IVOIRE

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Abstract- Endophytic bacteria could boost coffee production in Côte d'Ivoire. This study evaluated the endophytic potentialities of coffee to be integrated into ecological engineering. Endophytes of Robusta have been isolated. Fifteen endophytes including 10 roots and 5 leaves were isolated. *Bacillus, Actinomycetes* and *Pseudomonas* have been identified. Ten isolates were tolerant to NaCl up to 10% and 3 isolates to KCl for the same concentrations. Thirteen isolates tolerated potassium nitrate up to 8% while sodium nitrate inhibited even 1%. These bacteria were able to grow in a pH range (4 to 12). With the exception of ciprofloxacin, other antibiotics have been tolerated up to at least 75 μgmL^{-1}. Good tolerance to heavy metals up to 50 μgmL^{-1} was noted for at least 70% of strains. This study confirms the coffee tree as a natural host of endophytic bacteria, which would be well controlled to help the sustainable production of Robusta.

Keywords- *Endophytic bacteria, Coffea canephora, Daloa*

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Introduction

The development of Côte d'Ivoire has long been based on several industrial crops and more specifically on the cultivation of cocoa, coffee, oil palm, rubber and cashew. In fact, Ivorian agriculture is dominated by the cocoa-coffee pairing, which accounts for 40 % of export earnings, 20 % of GDP and supports more than six million ivorians, with 600,000 farms in operation [1]. However, after being ranked third world coffee producer (*Coffea canephora*) for nearly thirty years since the independence years, production has fallen sharply. From a production of 285 164 tons in 1990, the production of coffee increased in 2016 to 105 601 tons [2, 3]. Similarly, since 2016, Côte d'Ivoire has become the 7th largest producer of *Coffea canephora* Robusta variety, covering only 12 % of world production [4]. Daloa, the flagship town of the west-central forest of Côte d'Ivoire with its many administrative changes (commune, sub-prefecture, department and region and university town) associated with neighboring localities, constitute the second production area coffee with an estimated production of more than 50 thousand tons per year. Coffee production remains the activity of nearly 23.2 % of households [5, 6]. The main causes of this fall would be, the aging and the extensive driving of the orchards, the disinterestedness of the coffee producers in favor of the cocoa, the climatic conditions less and less satisfactory, the fluctuation of the price but also the attacks of the orchards by the diseases, weeds and pests [6, 7]. Coffee growing in Côte d'Ivoire faces several ecological and phytosanitary constraints. To date, against the persistence of the ravages due to pests, the most used means of struggle remain the use of chemical pesticides [8, 9]. However, crop production through the uncontrolled use of these synthetic products has adverse economic, ecological and health consequences [10]. Thus, with a view to curbing the misuse of synthetic pesticides, the concept of integrated protection has been introduced in plant protection programs through biological control [11, 12]. This struggle can be summed up as the control of a problematic organism by natural enemies [13, 14]. The endophytic microflora of the coffee plant could play a fundamental role in the restoration and maintenance of soil fertility, in the protection against certain soil-borne pathogens and in the mineral nutrition of coffee plants. Also, one of the current challenges of research is to design sustainable farming systems while preserving ecological balances. The mastery of this issue requires a better knowledge of the resources of the ecosystems and the development of ecological technologies based on these resources, especially native coffee endophytes. Traditionally, endophytic bacteria were once assimilated to latent pathogens that do not cause damage or beneficial effects for host plants [15, 16]. In recent years, many researchers have reported the involvement of these endophytes in improving agricultural production and defending host plants through resistance to pathogens. This is called biofertilization, bioremediation by fixing and reducing molecular nitrogen to ammonia, solubilizing phosphate, producing phytohormones, or even producing antibiotics, siderophores, stress-induced systemic resistance. abiotic and xenobiotic degradation [17-20]. A large amount of literature exists on all aspects of coffee production and use. However, very little exists on the native microorganisms of this plant and more specifically its endophytic bacteria, particularly in West Africa and more precisely in Côte d'Ivoire and singularly in the Daloa region, which is a zone of high production of Robusta coffee. In this study, the objective was to evaluate the ability of native endophytic strains isolated from coffee organs to respond positively to criteria for integrating them into a biotechnology leading to the production of effective biological inocula. Specifically, the ability to withstand salt stress, high concentrations of heavy metals and common antibiotics.

Materials and Methods

Presentation of the study area

The study area is the Department of Daloa, located in the Upper Sassandra region in west-central Côte d'Ivoire between 6° and 7° north latitude and 7° and 8° west longitude. The sampling sites are two Robusta producing villages: Mimia (6° 39'24.46"N; 6° 29'27.88"W) and Zokoguhe (7° 4'15.55"N; 6° 26'50.65 "W). The Daloa Department and sampling sites are illustrated in [Fig-1].

Table-1 *Summary of morphological and biochemical characters of endophytic bacteria isolated from coffee seedlings (Coffea canephora P var robusta)*

Isolates	Origin	Organs		Breathing	Catalase	Form	Grouping mode	Gram	Genera
		Roots	Leaves						
BEAC 1	Mimia	+	-	AS	+	bacilli	indiv.	+	ND
BEAC 2	Zokogue	-	+	AS	+	bacilli	chain	-	*Pseudomonas*
BEAC 3	Mimia	+	-	AS	+	bacilli	diploid	+	*Bacillus*
BEAC 4	Zokogue	+	-	AS	+	bacilli	indiv.	+	*Bacillus*
BEAC 5	Zokogue	+	-	AAF	-	bacilli	indiv.	+	*Actinomyces*
BEAC 6	Mimia	+	-	AS	+	bacilli	indiv.	+	*Bacillus*
BEAC 7	Zokogue	-	+	ANS	+	bacilli	triploid	+	*ND*
BEAC 8	Zokogue	+	-	ANS	-	bacilli	diploid	+	*ND*
BEAC 9	Zokogue	+	-	AAF	+	bacilli	indiv.	+	*ND*
BEAC 10	Zokogue	+	-	AS	+	bacilli	chain	+	*Bacillus*
BEAC 11	Zokogue	+	-	AS	+	bacilli	indiv.	+	*Bacillus*
BEAC 12	Zokogue	-	+	AS	+	bacilli	diploid	+	*Bacillus*
BEAC 13	Mimia	-	+	AS	+	bacilli	indiv.	+	*Bacillus*
BEAC 14	Mimia	+	-	AS	+	bacilli	diploid	+	*Bacillus*
BEAC 15	Zokogue	-	+	AS	+	bacilli	indiv.	+	*Bacillus*

ANS : strict anaerobic; AS : strict aerobic; AAF : aero-anaerobic optional; Catalase : (+), (-) absence of catalase, ND : not determined, indiv : individual

Table-2 *Viability of endophytic coffee bacteria (Coffea canephora var robusta) in an acidic and basic culture medium*

Diameter of colonies in different culture media (mm)				
Isolates	Dac (±sd)	Dbas (±sd)	*t- value*	*p- value*
BEAC 1	5.9 ± 3.9	10.5 ± 1.5	3.19	0.011 *
BEAC 2	6.25 ± 4.06	9.50 ± 1.6	2.13	0.062 ns
BEAC 3	8 ± 5.3	14 ± 1.6	3.09	0.015*
BEAC 4	8.25 ± 4.5	12 ± 2.3	1.81	0.103 ns
BEAC 5	6 ± 3.8	9.7 ± 1.3	2.64	0.028*
BEAC 6	8.25 ± 5.6	10.5 ± 1.5	1.08	0.314ns
BEAC 7	6.5 ± 4.1	9.5 ± 0.7	2.02	0.081 ns
BEAC 8	6.25 ± 3.9	9.5 ± 0.7	2.27	0.055 ns
BEAC 9	6 ± 3.8	9.2±1.3	2.25	0.054 ns
BEAC 10	8.5 ± 6	11.3 ± 2	1.26	0.240 ns
BEAC 11	6.63 ± 4.3	9.7 ± 1.8	1.89	0.090 ns
BEAC 12	7.13 ± 4.7	10.5 ± 1.3	1.96	0.086 ns
BEAC 13	8.25 ± 5.8	11.6 ± 2	1.57	0.154 ns
BEAC 14	6.75 ± 4.6	9.6 ± 1.6	1.67	0.131 ns
BEAC 15	7.13 ± 4.8	10 ± 1.6	1.63	0.141 ns

Dac : diameter in acid medium; Dbas : diameter in basic medium; sd : standard deviation;
** : significant difference; ns : not significant difference, threshold of significance p= 0.05.*

Plant material

The study material consists of young 2-month-old coffee nurseries from different nurseries in the two Robusta producing villages. These nurseries are at the same time producers who use these seedlings either to expand their farms or to replace other dead plants in the fields. In Mimia, three nursery growers were visited. The first had 110 seedlings, the second of 90 and the third of 115 seedlings. Five apparently healthy seedlings of Robusta were collected from each producer, a composite sample of 15 seedlings in Mimia. The same provisions were adopted at Zokogue. However, the three nursery growers had 85, 100 and 105 seedlings, respectively. A composite sample of 15 seedlings was obtained at Zokogue. A total of 30 Robusta seedlings were analyzed. These harvested samples are packaged in a stomacher sachet and sent directly to the Laboratory for Host-Microorganism and Evolution Interactions (LIHME) of the Jean Lorougnon Guédé University for microbiological analyzes.

Sample preparation for endophyte research in robusta

Endophytic bacteria of Robusta have been sought from both roots and leaves. For each sample, the roots are separated from the leaves. These organs were cut separately and rinsed thoroughly with sterile distilled water. The surface of each type of sample was then sterilized according to the authors of the references [21]. On gram of root from each plant was cut and surface-sterilized successively with ethanol (70 %) for 5 min, sodium hypochlorite (25 %) for 5 min and sodium hydroxyl (4 %) for 10 min, followed by thorough washing in sterile distilled water and then ground with 1mL of pure water. A volume of 0.1mL of the mixed of each tissue was directly spotted on YEM medium and other volume was diluted (20x) and used for bacterial enumeration in the leaves or roots of *Coffea canephora* robusta according to the authors of the references [22].

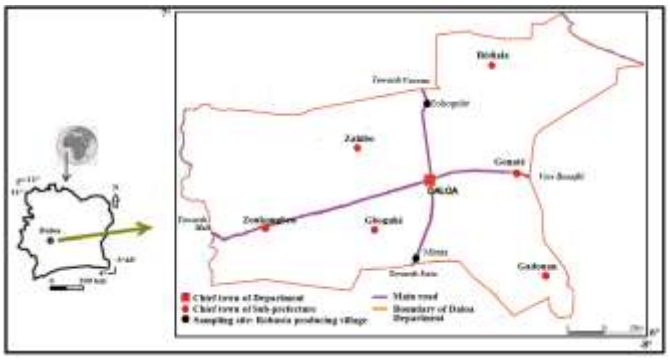

Fig-1 Map of Daloa Department with Sampling Sites

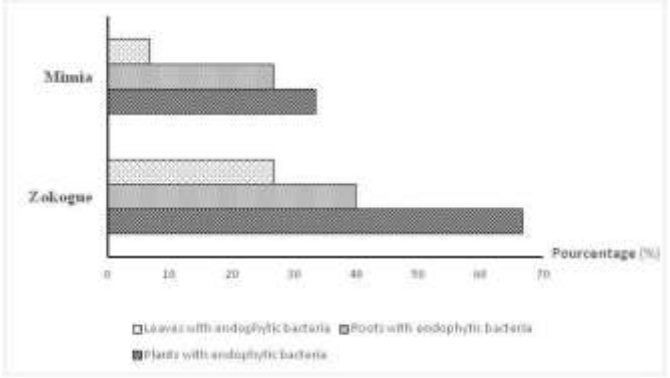

Fig-2 The distribution of native endophytes of Robusta according to the sites and the different organs of the coffee seedlings (*Coffea canephora* P var robusta)

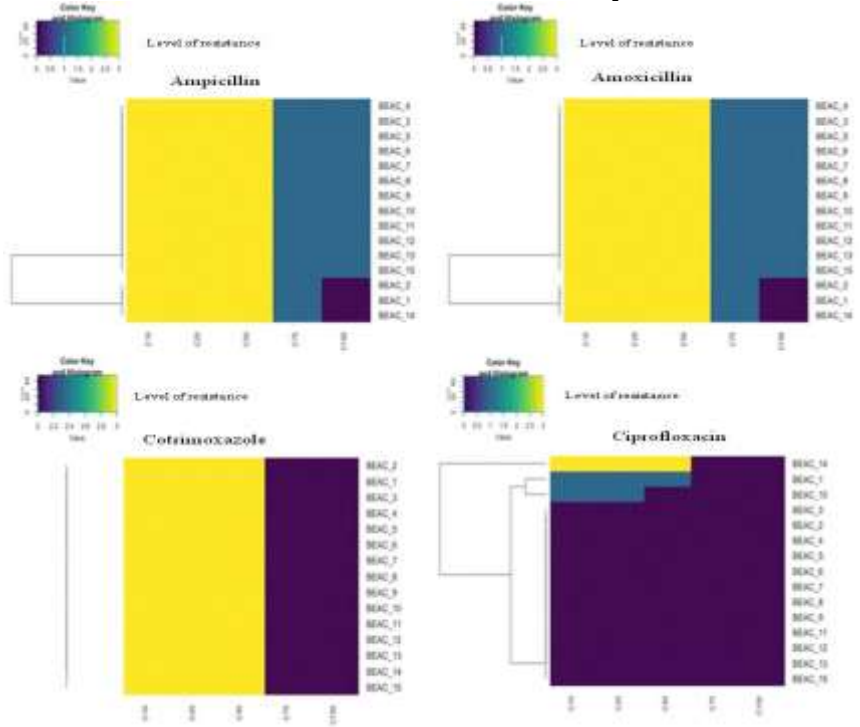

Fig-3 Heat Map showing the resistance or susceptibility profile of the isolates to the different antibiotics tested

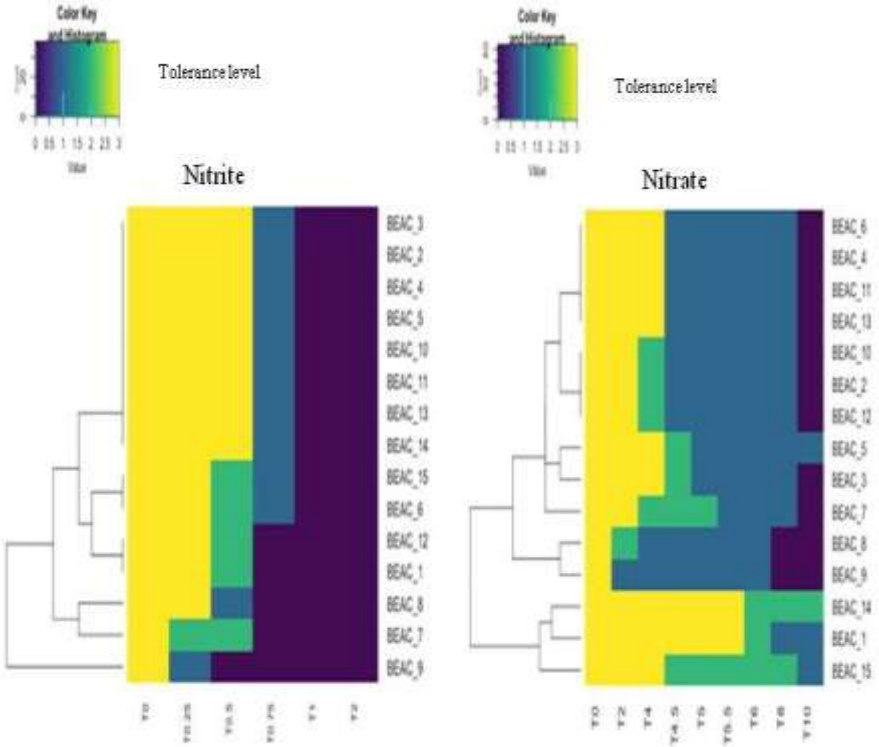

Fig-4 Heat Map showing the tolerance of isolates to nitrite and nitrate

The stems and roots of young Robusta seedlings are cut into small pieces of about 5 mm with a sterile blade. These stems and roots are thus deposited separately on the surface of the previously prepared and sterilized YEM agar. The different cultures are then incubated at 30°C. for 5 days.

Phenotypic characterization

All tests for phenotypic characterization were performed isolates were made on YEM agar. For the evaluation of the growth of the isolates, the Petri dish was subdivided into small sectors according to the number of strains to be tested (15 sectors) each of which was inoculated with 10 µl of a freshly prepared pre-culture, of density close to 1 corresponding to about 108cells / mL. After 3 days of incubation at 30°C, bacterial growth was compared to the controls. Two replicates were done for each treatment.

Biochemical characterizations

Various standard tests have been performed on the different colonies or strains for a first classification. These are Gram stain, catalase search, oxidase and respiratory type.

Isolation of *Bacillus* spp, *Pseudomonas* spp and *Actinomycetes*

Following the various standard biochemical tests, specific media including Mossel agar, cetrimide agar base and Glycerol Bacto Agar medium (GBA) were used for isolation of *Bacillus* spp, *Pseudomonas* spp and *Actinomycetes*. For the former, a quantity of 10 µL of a freshly prepared pre-culture (previously identified Gram positive and catalase positive) is spread on the surface of a Petri dish, containing 20 mL of previously prepared Mossel agar, and then incubated at 30°C for 24 hours.

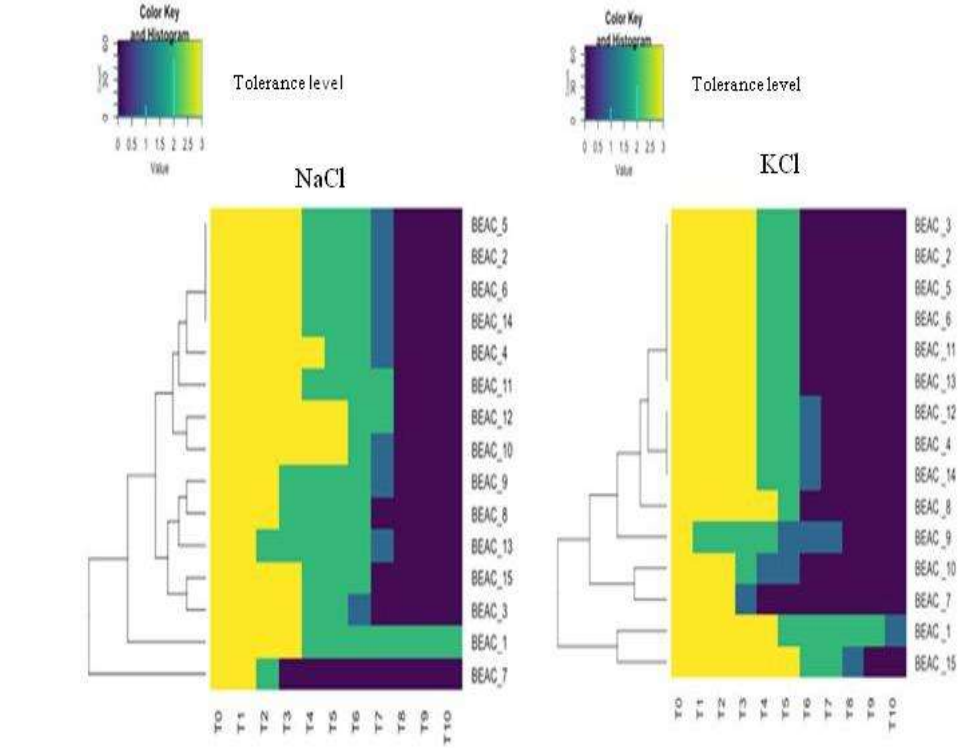

Fig-5 Heat Map showing the tolerance of isolates to different salts

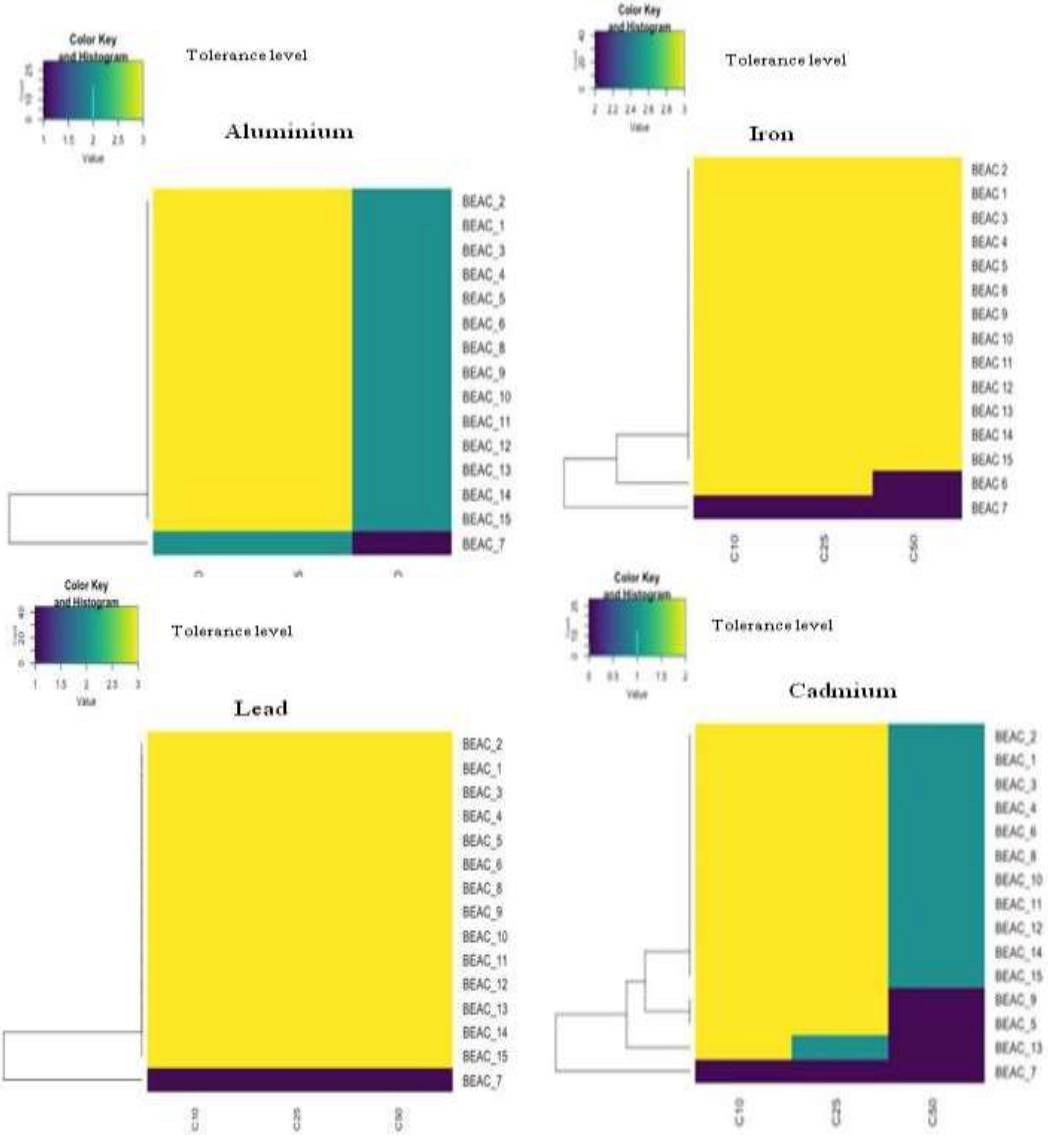

Fig-6 Heat Map montrant la tolérance des isolats vis-à-vis des différents des métaux lourds

Colonies characteristic of the *B. cereus* group are rough, dry, pink (negative mannitol), surrounded by a pink precipitation halo and a transparent zone indicating the production of lecithinase [23]. For the latter, the Gram-negative bacilli strains, equipped with oxidase and catalase, were streak-seeded with a sterile loop on the surface of the cetrimide agar and then incubated at 30°C. the oven for 24 to 48 hours. After 48 h of incubation, blue to green and fluorescent colonies under UV lamp (360 nm) are considered to be *Pseudomonas* spp. *Actinomycetes* were carried out according to the authors of the references [24]. Research and isolation of *Actinomycetes* was performed on Glycerol Bacto Agar. Presumptive colonies are subcultured on the said agar then the cultures incubated at 25°C. for 21 days. *Actinomycetes* were first confirmed on the basis of their characteristic morphology of their colonies. Then, a second confirmation was made by an optical microscope observation of mycelium filaments in the air.

Physiological characterizations and resistance to abiotics stresses

The physiological characterization and the study of the resistance to the various abiotic stresses were carried out according to the authors of the references [22].

Sodium and potassium chloride tolerance

It was conducted on agar plates at variable concentrations ranging from 1 to 10 % (w/v) of NaCl or KCl concentrations.

Reduction of sodium nitrite and potassium nitrate

It was evaluated by growth determination on solid YEM medium free of salt and supplemented with sodium nitrite ($NaNO_2$) and potassium nitrate (KNO_3) at different concentrations.

pHs tolerance

Tolerance to pH was tested on YEM plates at different pH values using the buffers HI 7007 (pH 7.01) buffer and HI 70004P (pH4.01) buffer solution for calibration. YEM agar was made acidic with HCl for pH values below 7.13. NaOH was used for basic preparations when pH should be above 7.13.

Antibiotics tolerance

The intrinsic resistance of strains was determined on solid YEM medium containing the following sterilized antibiotics: ampicillin, amoxicillin, cotrimoxazole and ciprofloxacin, at different concentrations 10, 50, 75 and 100 gmL^{-1}.

Heavy metal resistance

The resistance to heavy metals was carried out according to the authors of the references [25]. The heavy metals studied are: lead acetate ($Pb._2$ (CH_3COO)), aluminum chloride ($AlCl_2$), iron (II) sulfate 7 hydrate ($FeSO_4.7(H_2O)$) and cadmium ($CdCl_2$) at concentrations of: 10; 25 and 50 $\mu g.mL^{-1}$. The various heavy metals were added to the TY medium and sterilized as follows.

Digital analysis of data

Statistical analyzes were conducted with the R Studio version 3.3.1 statistical software (2016). The Student's test with Welhs correction to a degree of significance ($\alpha = 0.05$) was used with the realization of a heat map showing the degree of resistance or sensitivity coded by the color variability ranging from violet (sensitive) to yellow (resistant).

Results

Isolates of native endophytic bacteria of the coffee tree

Fifteen (15) bacteria could be isolated from *Coffea canephora* P. var robusta organs, including 10 roots and 5 leaves. Ten of the 15 strains came from the Zokogue site. The BEAC code was chosen to refer to associative endophytic bacteria of the coffee tree. BEAC (1 ; 3 ; 4 ; 5 ; 6 ; 8 ; 9 ; 10 ; 11 ; 14) come from the roots and BEAC (2 ; 7 ; 12 ; 13 ; 15) come from leaves. Colonial morphological observation showed three major types of colonies. There were flat or raised circular colonies with a lobed or regular outline with a transparent, translucent or opaque smooth surface, whitish or orange in color and between 2 and 5 mm in diameter (BEAC 1 ; 2 ; 5 ; 7 ; 8 ; 9). Other colonies had flat rhizoidal shapes with a lobed or regular contour with a smooth surface or a faint opaque, whitish-colored filament, and between 12 and 39 mm in diameter (BEAC 3 ; 4 ; 6 ; 10 ; 11 ; 12 ; 15). A colony in the form of a convex point with a regular outline; opaque smooth surface of yellow color and with a size of 1 mm in diameter (BEAC 14) has also been noted. The isolates were composed of approximately 93% Gram + bacteria or 14 out of 15 bacteria. They were all straight bacilli of variable size with rounded ends, isolated or grouped or often in chains. The majority were catalase (87 %) with different respiratory types. The identification of these bacilli required the use of other complementary tests. The different selective media have made it possible to highlight certain genera and species. The summary of the morphological and biochemical characters as well as the main genera identified are presented in [Table-1].

Distribution of isolates by plant site sites and organs

The Robusta seedlings of Zokogue harbored more associative endophytic bacteria than those of Mimia. In fact, nearly 67 % of Zokogue seedlings harbored endophytic bacteria compared to 33% of those of Mimia. The roots were the organs that harbored more endophytic bacteria, regardless of the site. In Zokogué, 40 % of the roots of endophyte seedlings (67 %) also harbored endophytic bacteria. In Mimia, this proportion was 26 % for 33 % of bacterial endophyte seedlings. The distribution of isolates according to the sites and the different organs of Robusta seedlings studied are summarized in [Fig-2].

Physiological characteristics

Resistance of isolates to antibiotics

The pattern of resistance or tolerance of strains to ampicillin showed two groups of individuals. The first group consisted of 12 or 80% isolates that resisted up to 100 $\mu g.mL^{-1}$. The second group consisted of isolates BEAC 1, 2 and 14, and were sensitive from 75 $\mu g.mL^{-1}$. For amoxicillin, almost all strains resisted up to 100 $\mu g.mL^{-1}$ except for BEAC 5 which was sensitive at concentrations below 100 $\mu g.mL^{-1}$. As for cotrimoxazole, all isolates showed an identical profile. They resisted for concentrations ranging from 10 to 100 $\mu g.mL^{-1}$. Ciprofloxacin was found to be more active. Indeed, only these isolates BEAC (1, 10 and 14) resisted concentrations ranging from 10 to 50 $\mu g.mL^{-1}$. Growth of other isolates was inhibited even at the lowest dose (10 $\mu g.mL^{-1}$). The profile of isolates for different antibiotics is shown in [Fig-3].

Reduction of potassium nitrate and sodium nitrite

Isolates showed good tolerance to nitrate. 87 % of the isolates tolerated up to 8% potassium nitrate and 4 strains or 26 % were able to grow to more than 10 %. As for sodium nitrite, it inhibited the growth of isolates even at 1 %. The reduction of potassium nitrate and sodium nitrite isolates is illustrated in [Fig-4].

Tolerance of isolates to salts

The isolates tolerated even high salt concentrations: NaCl and KCl. The tolerance limit ranged from 3 % with the BEAC 7 isolates up to 10% with the BEAC 1 strain for NaCl and from 4 to 10% with the same strains for KCl. However, the isolates were found to be more sensitive to KCl than to NaCl. Indeed, at 7% KCl, only 20 % was viable while for NaCl, about 67 % of strains were viable at the same concentration. The tolerance of the isolates to the different salts is shown in [Fig-5].

Tolerance of isolates at pHs

Statistical tests have shown that the pH of the medium has a significant influence on the growth of 3 bacterial strains, *i.e.*, 20 %. In fact, for the BEAC 1, 3 and 5 isolates, the test showed a significant difference ($p < 0.05$) between the average diameters in acidic and basic medium with a predominance of the average diameter of the colonies in basic medium. These bacteria would tolerate the alkaline medium better than the acid medium, they could be basophilic. While for the 12 other isolates, no significant difference ($p > 0.05$) was recorded between mean diameter in basic medium and in acid medium.

These bacteria would therefore tolerate both acidic and basic media. However, the growth of all strains was inhibited at very acidic pHs (pH 3 and pH 3.5). The acid and alkaline pH tolerance of the isolates is summarized in [Table-2].

Tolerance of heavy metal isolates

The tolerance profile of bacteria with respect to aluminum and lead has 2 groups of individuals. Indeed, all the bacteria expressed a good tolerance up to 50 µg.mL^{-1} with the exception of the BEAC 7 strain which was the least tolerant at the same concentrations. For iron, all isolates showed good resistance of 10 to 50 µg.mL^{-1}. The response of bacteria to cadmium classified them into 3 major groups: a first group containing the most tolerant isolates, about 73 % of the strains are viable even at a concentration of 50 µg.mL^{-1} a second group (BEAC 5, 9 and 13) less tolerant developed at concentrations below 50 µg.mL^{-1} and a third (BEAC 7), the most sensitive, was inhibited at a concentration of 10 µg.mL^{-1}. Apart from cadmium, the isolates had good tolerance to the other heavy metals tested. The tolerance of the isolates to the different heavy metals is shown in [Fig-6].

Discussion

The present study was conducted with the objective of isolating endophytic bacteria from the organs of the coffin and carrying out tests to evaluate their ability to withstand different stresses. Fifteen isolates were found from leaves and roots of apparently healthy coffee seedlings of the Robusta variety. Previous studies on the diversity of endophytic bacteria in coffee have also highlighted the presence of endophytic bacteria in the roots, stems, leaves and fruits of coffee [26, 27, 28]. These results confirm that endophytic bacteria reside in various living plant tissues and are ubiquitous in monocotyledonous and dicotyledonous plants, ranging from woody tree species. [22, 29], with herbaceous plants [30, 31]. In addition, more root isolates than foliar isolates were obtained. This difference in charge can be explained by the fact that most of the endophytic bacteria are of telluric origin. Thus, the root system in the soil is the first organ colonized by endophytic bacteria. According to the work of reference [32], a substantial number of endophytic associative bacteria (103 to 107 cells) can colonize the vascular system (phloem and xylem) of the host plant with a high density in the roots and which gradually decreases as to the leaves. Morphological and biochemical characterizations highlighted the coexistence in the seedlings of Robusta, of three endophytic bacterial genera including 60% of *Bacillus*, 6.66 % of *Actinomyces*, and 6.66 % of *Pseudomonas*. The remaining isolates (26.66 %) of gram-positive bacteria are being identified. These results are close to those obtained by the authors of the reference [27], which isolated 63 endophytic strains of the coffee fruits of which 76 % belonged to the *Bacillus* genera. The predominance of the genus *Bacillus* is thought to be due to the developmental stages or age of the coffee plants according to previous work [27, 33] but also to the diversity of microorganisms present in the soils. The presence of *B. subtilis* and that of *B. megaterium* in fruits as well as *B. cereus* in leaves of *C. arabica* had been reported in previous study [33]. All of these isolated endophytic bacteria, *Bacillus*, *Pseudomonas*, *Actinomyces* and others, would play an important role in improving growth and protecting coffee plants against disease. The agronomic interest of these isolates lies primarily in their intrinsic characteristics. In particular, their ability to withstand the biotic and abiotic stresses of their environment in the context of ecosystem degradation and climate change. The tests carried out showed that all the isolates had good resistance to almost all the usual antibiotics tested. Many studies conducted on the action of certain antibiotics on the viability of endophytic bacteria already reported this resistance. In fact, *B. endophyticus* isolated from the interior of the cotton tissues (*Goussypiums* sp.) resisted and showed normal growth in the presence of 100 µgmL^{-1} of ampicillin according to the work of the reference [34]. In addition, *Pseudomonas fluorescens* was isolated from the rhizosphere of an olive tree and resisted 25 µgmL^{-1} of amoxicillin according to the study with the reference [35]. However, growth of all isolates was inhibited by a quinolone antibiotic (ciprofloxacin). This strong activity of ciprofloxacin against isolates may be due to the novelty of this antibiotic. Quinolones (ciprofloxacin) are among the new classes of antibiotics used. One of the causes of resistance is the repeated contact of microorganisms with antibiotics. The sensitivity of endophytic bacteria to quinolones had already been demonstrated in the work of the reference [22]. In addition, the resistance of several strains of coffee to several antibiotics has been reported by other authors on other endophytic bacteria that are symbiotic [36] and associative [37]. The resistance of endophytic bacteria to a wide range of antibiotics gave them a greater ability to resist biotic pressures imposed by other microorganisms in their living environment. Thus, these bacteria would adapt better to their environment. The reduction of sodium nitrite and/or potassium nitrate is an important feature of endophytes. In this study, the growth of endophytic bacteria was inhibited by sodium nitrite even at concentrations below 1 %. In general, nitrite is considered toxic because it alters cellular functioning by generating inhibition of active transport and the functioning of certain enzymes such as aldolase or hexokinase as pointed out by the researchers of the reference [38]. However, endophytic bacteria isolated from cocoa in work with reference [22] could have increased in the presence of sodium nitrite up to 6 %. The inhibitory action of sodium nitrite on coffee isolates may be due to the inability of these to achieve nitration. They would not make enzymes capable of reducing nitrite to nitrate such as nitrite reductase for example. In addition, most isolates have the ability to reduce potassium nitrate (KNO_3). Similar results have been obtained by other authors [36], with symbiotic bacteria. The ability of endophytic bacteria to reduce nitrate is an ecologically important feature that must be considered when selecting an endophytic strain for ecological engineering aimed at remedying eutrophic media. It allows these rhizobacteria to have a nitrogen source for their survival and to reduce the nitrate level accumulated in their immediate environment, hence their major role in the bioremediation and bioremediation of degraded ecosystems, according to the authors of reference [39]. In addition, the isolates showed a high tolerance to salt concentrations (NaCl). This halotolerance is identical to that of endophytic bacteria isolated from cocoa (*Theobroma cacao* L.) which tolerated concentrations up to 9 % according to the authors of the reference [22]. Other authors [40] have also shown this halotolerance in symbiotic lupine bacteria (*Lupinus luteus* L.) that tolerated up to 10 % NaCl. Indeed, most microorganisms have limited salt requirements and are inhibited by NaCl contents greater than 2 %, except for halophilic species that grow in saline environments and withstand 15 % NaCl levels. However, other bacteria do not need salt for their growth, but can grow in their presence and support up to 10% salt according to the author of the reference [41], as is the case here of coffee endophytes. Endophytic coffee bacteria had good pH tolerance. Indeed, these bacteria could grow in a wide pH range (4 to 12). These results are consistent with those reported by reference researchers [31], who found that endophytic maize bacteria were perfectly tolerant of pHs ranging from 4 to 11. Other more recent studies have shown that some *Actinomycetes* bacteria in desert soils have pHs ranging from 3.8 to 11 [42]. Resistance to abiotic stresses of endophyte isolates was also assessed for heavy metals. All isolates showed good tolerance to the high concentrations of the four heavy metals tested (aluminum, iron, lead and cadmium). These results confirm the data of the authors of references [29] and [43] that already revealed the tolerance of endophytic bacteria to heavy metals. In addition, the tolerance of isolates to several heavy metals (multi-tolerance), presages their good potential to be competitive even under difficult conditions and thus to be used as a biological basis for the production of effective inocula. for a sustainable coffee crop. Endophytic isolates of coffee due to their ability to tolerate high concentrations of heavy metals can be used for the bioremediation and bioremediation of soils polluted by heavy metals. This bioremediation is based on the ability of microorganisms to tolerate high concentrations of heavy metals [44]. Indeed, research is increasingly focusing on the use of plant growth promoting rhizobacteria as an effective means of "bio-remediation" of soils contaminated by heavy metals. [45, 46]. Then, these native coffee endophytic bacteria (*Coffea canephora* var robusta), isolated in the present study, would be good candidates.

Conclusion

Coffee production (Robusta) is down sharply in Côte d'Ivoire because of the many difficulties encountered in its cultivation. The solutions used further degrade soil quality and growing conditions. This study provides a glimpse of a sustainable alternative to coffee growing. This work revealed the presence of endophytic bacteria with high potential in different organs of Robusta seedlings in the nursery.

Efficient exploitation of the potential of these native bacteria through ecological engineering could help improve the production and sustainable cultivation of Robusta coffee in Côte d'Ivoire.

Application of research: These isolates could be used to develop ecological engineering for sustainable coffee production (*Coffea canephora* var robusta) in Côte d'Ivoire.

Research Category: Endophytic bacteria, *Coffea canephora*

Abbreviations: analysis of variance (ANOVA), plant growth promoting rhizobacteria (PGPR), Glycerol Bacto Agar medium (GBA), Yest extract mannitol (YEM).

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Research project name or number: Improvement of the production and sustainable cultivation of coffee in Côte d'Ivoire by the potentialities of endophytic bacteria (no number).

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Study area / Sample Collection: Department of Daloa, Upper Sassandra region in west-central Côte d'Ivoire. The sampling sites are two Robusta producing villages: Mimia and Zokoguhe

Cultivar / Variety name: *Coffea canephora* var robusta

Conflict of Interest: None declared

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Ethical Committee Approval Number: Nil

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