

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: https://journal.hibiscuspublisher.com/index.php/JOBIMB



Molecular Identification, Phylogenetic Classification and Proteolytic Capacity of Cultivable Bacteria Isolated from Soils in Brazzaville, Republic of Congo

Ngo-Itsouhou^{1,3}, Nguimbi Etienne^{1,2,3}*, Kayath Aimé Christian^{1,2,3}and Ampa Raoul¹

¹Laboratoire de Biologie Cellulaire et Moléculaire (BCM), Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville, République du Congo. ²Institut de Recherches en Sciences Exactes et Naturelles (IRSEN), Brazzaville, Republic of the Congo. ³Unité de Microbiologie Moléculaire et Bioinformatique, Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville, Republic of the Congo.

> *Corresponding author: Dr. Nguimbi Etienne, Molecular Microbiology and Bioinformatics Unit, Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville, Republic of the Congo. Tel 242. 055476155 Email: etienne.ng1612@gmail.com

HISTORY

Received: 24thSeptember 2019 Received in revised form: 25th of October 2019 Accepted: 18th of November2019

KEYWORDS

molecular identification phylogenetic classification proteolytic activity bacteria soils

ABSTRACT

Soils present a biodiversity of bacteria, part of this microflora is cultivable, it can be estimated, characterized and identified. Four zones each comprising three sites were sampled in Brazzaville in the Republic of Congo. Phenotypic characterization, molecular identification and proteolytic capacity of soil bacteria were conducted. One of the factors, the pH was measured, the pH values were between 6 and 7. From the classical microbiology techniques, we counted the total flora of the microorganisms in (CFU / g.103), it is between 47 ± 11.2 and 214 ± 58.2 and that of bacteria of the genus *Bacillus* from 51.3 ± 5.0 to 74 ± 30.7 . Cocci and bacilli were obtained. Gram- and Gram + bacteria distinguished, all bacteria were catalase +, some had sporulation, others not. Thirty-one (31) isolates were phenotypically characterized and 16S rDNA PCR was performed. Ten (10) strains were sequenced and the phylogenetic classification of the identified strains presented. Blastn's research on the 16S rDNA sequences of the different strains shows similarity rates (96.77% -100%) and E. value (9.00E-93 -0.0), these two indices allowed the identification of the strains studied. The percentages of the identified strains are as follows: Bacillus cereus (20%) = MN6 and MN14, Bacillus pumilus (10%) = MN7, Bacillus thuringiensis (10%) =MN12, B sp. (10%) = MN17, Bacillus subtilis (10%) = MN26, Staphylococcus haemolyticus (10%) = MN8, Staphylococcus saprophyticus (10%) = MN21, Staphylococcus sp. (10%) = MN21MN22, Staphylococcus gallinarum (10%) = MN24. The phylogenetic tree shows that the bacteria belong to two main monophyletic genera, the genus Bacillus and the genus Staphylococcus. The proteolytic capacity of Bacillus bacteria was assessed in parallel with growth. The optical density was between (0.8- 0.93) and proteolytic enzyme production between (9-20mm). The parallelism between growth and enzymatic production shows that the two phenomena are distinct.

INTRODUCTION

Soil is a complex heterogeneous environment that contains a wide variety of communities and bacterial species [1]. As a result, access to bacterial diversity in an exhaustive manner is one of the major challenges of the last decades in soil microbiology and the techniques dedicated to it have been constantly evolving [2]. Before the development of molecular techniques, the identification and taxonomic classification of bacteria required their isolation, cultivation and characterization on the basis of morphological, physiological, metabolic, biochemical and ecological criteria. These criteria of classification quickly found their limits because of the phenotypic and physiological diversity of the bacteria but also by the simplicity of the classification engendered [3]. Pliant this problem, the development of approaches Molecular revolution has revolutionized the world of soil microbiology by enabling the refinement of bacterial classification and by significantly increasing the number of species considered, compared to methods based on only culturable bacteria [4]. Among these bacteria are bacteria of the genus *Bacillus* which, like other bacteria, can be characterized on a molecular level by 16S rDNA. Comparison of genomic sequences of bacterial species shows that the 16S rRNA gene is highly conserved within the species and between species of the same genus and can be used as a tool for the specification of bacteria [4,5]. This method has been used successfully on a large number of bacterial species [4,6].

Bacteria of the genus *Bacillus* have several subspecies, the species have several properties, including the production of proteolytic enzymes) [7,8]. *Bacillus* bacteria produce proteolytic enzymes depending on culture conditions, and each strain has optimal growth and enzyme production conditions [9-12]. In this work, four soil samples taken from different locations in Brazzaville in republic of Congo were used to characterize bacterial biodiversity. After enumeration, isolates were identified by PCR of their 16S rDNA. Dix (10) isolates of which the PCR products were sequenced correspond to six bacteria of the genus *Bacillus* and four of the genus Staphylococcus. The phylogenetic classification based on the gene encoding 16S rRNA was developed, and the property to produce proteolytic enzymes evaluated for bacteria of the genus *Bacillus*.

MATERIALS AND METHODS

Soil sampling and pH measurement.

From November 2016 to September 2017, 12 soil samples were collected in clean, dry, sterile glass tubes with a sterile spatula. Collection took place in four areas of Brazzaville and for each zone three samples were taken. All samples were transferred to the laboratory; under sterile conditions for analysis. The map in **Table 1** shows the different sampling sites in the city of Brazzaville (Republic of Congo).

Table I. Distributi	ion of sampling	areas of soil	samples in	Brazzaville.
---------------------	-----------------	---------------	------------	--------------

Areas	Stations (GPS)	Sites
Town center	194	Beach Market
	195	Bassin of Congo Terminal
		(TBC)
	196	Mamiwata
Boulevard	197	Puma Service Station
	198	Foreign Affairs Strangers
	199	Tennis Club
Marien Ngouabi	200	School years
University	201	Campus I
	202	University library
Sciences Faculty	203	gardin
and Technologies	204	High School Pierre
-		Savorgnan de Brazza
	205	Saint Exupery School

A pH meter was used to measure the pH values of the different soil samples .

Culture conditions and enumeration

One gram of each of the twelve samples of the sols was diluted in a tube containing 10 ml of distilled water and seeded by plating on nutrient medium (PCA), to count the total flora.To obtain the representative isolates of bacteria of the genus *Bacillus*, the 3 samples from each zone were mixed forming composites, then diluted and seeded by plating on Mossel nutrient medium, the dishes were incubated at 37 ° C for about 12 hours. The colonies obtained were counted by counting. The total viable count was performed using the casting method described by [13]. The counting unit is then expressed as colony forming (CFU) per gram of soil.

CFU / g = N / v * dN: colony number, V: dilution volume, d: dilution

Isolation and phenotypic characterization of isolates

The colonies obtained on PCA medium were purified on LB and those obtained on Mossel purified on Mossel. The isolates obtained were prepared for the tests [14]. The purification was done by three successive episodes. From the bacterial culture, isolate colonies were identified according to Bergey's manual on identification of *Bacillus* species modified by [15]. a classification manual of the genus *Bacillus* [15], and also according to [16]. The dishes were incubated at 37 ° C for about 12 hours.

The isolates were stored in liquid LB broth, a 1% glycerol supplement and placed at $+ 4^{\circ}$ C after a 24-hour incubation at 37°C.The phenotypic characteristics of the colonies were determined with a culture of 12 h incubated at 37°C in nutrient agar. The morphology of the colonies (shape, size, color, appearance, contour) was supplemented by a microscopic examination carried out between slide and coverslip from the cultures used to study the cultivation characteristics. The microscopic examination (the shape, clustering mode and cell mobility), Gram stain, catalase assay [17], and Sporulation [18] were performed. have been identified morphologically and physiologically up to the genus level, according to the (Bergey's) manual method of bacteriology and [14].

Molecular identification

Extraction of genomic DNA

Extraction of the bacterial genomic DNA was performed using a commercial DNA microorganism (Nucleospin Microbial DNA) genomic extraction kit. Proteinase K and RNase have been added to eliminate protein and RNA contaminations on DNA, which allows the production of good quality DNA according to the manufacturer's instructions described in the manual. Using 31 isolates, the DNA of each bacterium was extracted by adding in a microtube some colonies of bacteria and 1000 ml of liquid LB. The mixture was vortexed for 1 minute. We centrifuged the tubes at 1000 x g for 3 minutes, then discarded the supernatant, and then took 100 μ l of BE that was deposited in the tubes that contained the pellet.

The mixture was pipetted into Nucleospin Bead Tube Type B, and 40 μ l MG Buffer, 10 μ l Proteinase K was added, then stirred for 6 min, centrifuged at 11000 x g for 30 sec. Then 600 μ l of Buffer MG was added, vortexed for 3 s and centrifuged at 11000 x g for 30s. The Nucleospin Microbial DNA Colum Load tubes 500-600 μ l sample was changed and centrifuged at 11000 x g for 30 sec. The first wash consists of adding 500 μ l of BW and centrifuging at 11,000 × g for 30 sec. For the second wash the columns were changed and added with 500 μ l of BW and centrifuged at 11000 x g for 30 s. 100 μ l of BE was added and let stand for 1 min, centrifuged again at 11000 x g for 30 s. DNA in the volume of 100 μ l was eluted and were kept at - 20° C for later use.

Primers design and PCR reaction

Universal primers to amplify 16S rDNA were (fD1; 5'-AGAGTTTGATCCTGGCTCAG -3 'and rP2; 5' ACGGCTACCTTGTTACGACTT -3 ') [19]. In a total volume

of 50 microliters, the PCR reaction mixture was prepared as follows: 1 μ l deoxynucleotide triphosphate (dNTP), 10 μ l buffer, 32.75 μ l of distilled water and 0.25 μ l of enzyme 2 μ l primer fD1, 2 μ l rP2 primer and 2 μ l of DNA. The amplification is carried out in a thermocycler (BIO-RADT100Thermal Cycler) The PCR amplification conditions were as follows: an initial denaturation step at 95 ° C. for 5 min, followed by 30 cycles each comprising a denaturation step at 95 ° C. for 30 s, hybridization at 55 ° C for 30 s, initial elongation 72 ° C for 1 minute 30 s and finally a final elongation step 72 ° C for 10 minutes

Agarose gel electrophoresis of PCR products

The PCR products were analyzed by electrophoresis on 0.8% agarose gel at 100 volts for 45 minutes with TBE buffer. The coloration was made with a solution of 1 μ g/ml of ethidium bromide. The gel was visualized by Transilluminator UV type BIOBASE and recorded by the digital camera.

Sequencing of amplicons and assembly

The PCR products resulting from the amplification of the gene coding for the 16S rRNA were purified using the NucleoFast 96 PCR plate (Macherey-Nagel EURL, France) and sequenced by the company Masrogene using the BigDye terminator chemistry on an ABI sequencer. 3730 (Applied Biosystems, Foster City, California, USA). DNA Baser sequence Assembler was used for sequence assembly. The resulting sequences were processed with Bioedith Aligner software for alignments.

Proteolytic enzyme assay

To test the production of the proteolytic enzyme we used the modified techniques [20]. The bacteria were cultured in LB medium with stirring at 37 ° C, for 24 hours after culture the medium was centrifuged for 5 minutes at 5000rpm and the supernatant recovered. In a 250 ml Erlenmeyer flask containing 100 ml of 0.1 N PBS, we dissolved 1 g of agarose, heated to complete dissolution, after cooling to 55-60 ° C, we added 10 ml of skimmed milk, homogenized the mixture. We then sank in the Petri dishes; after solidification, we prepared wells in the gel and place in each well 50 ml of supernatant resulting from the centrifugation of the culture. The boxes are placed in an oven at 37 ° C for about 12 hours. Observation of a clear translucent zone indicates that the strain produces a caseinolytic proteolytic enzyme (caseinolytic protease) [24, 22, 23, 12].

Analysis and treatment of results

The texts were entered with world, the tables and graphs of different Fig. 6 were made by Excel. The optical density was measured by a Zuzi spectrophotometer, model 4211/50. Sequence analysis was performed by Blastn (Basic Local Alignment Search Tool) available on the National Biotechnology Information Center database (http://www.ncbi.nlm.nih.gov)Blastn has, with its parameters, in particular the similarity rate, identified the different isolates according to equivalent strains on the basis of data used. The phylogenetic tree was constructed by MEGA 7 using the neighbor joining method with 1000 bootstrap replicates. The scale bar indicates the number of differences in nucleotide substitutions per sequence.

The study of evolutionary divergence was performed as a measure of the notation similarity matrix; the lower score meant a close neighbor with a similar evolutionary origin. The total variability of the sequence was calculated as the mean distance [36 -38].

RESULTS

pH values of the samples

Table 2. pH values of different soils for each GPS station.

GPS	
Station	pН
194	6.5
195	6.5
196	6.6
197	6.6
198	6.7
199	7.0
200	6.8
201	6.5
202	6.4
203	6.7
204	6.5
205	7.0
Note pH value	is between 6.5 and 7

Enumeration

Enumeration of total flora

Table 3 shows the results of enumeration of the total flora of the different soil samples taken from different areas in Brazzaville.

Table 3. Enumeration of the total flora of bacteria on PCA.

Tubes	T1	T2	Т3	T4	T5	T6
UFC/g.10 ³	60±4	55.6±4.5	30.8±4.7	47±11.2	214±58.2	130±9
Tubes	T7	T8	T9	T10	T11	T12
$UFC/g.10^3$	186±17.0	210±26.4	87.3±11.05	76±31.7	113.3±30.5	153.33±3

This table shows that the total flora is variable from one sample to another, it is, the low bacterial biomass is tube 3 while the strongest is in tube 5.

Enumeration of bacteria of the genus Bacillus on Mossel

Table 4 shows the count of bacteria of the genus *Bacillus* on mossel medium, C1, C2, C3 and C4 represent the 4 sampling zones, each of which comprises 3 sites:

Table 4. Enumeration of bacteria of the genus Bacillus on Mossel.

Composite	C1	C2	C3	C4
UFC/g.103	51.3±5.0	54.6±11.2	62.2±36.2	74±30.7

This table shows a variation of the number of bacteria of the genus *Bacillus* according to the zone. In zone C4 these bacteria are more represented and in C1 they are less represented

Isolation and Phenotypic Characterization of Isolates The different characteristics of bacteria is shown in **Table 5**.

 Table 5. shows the phenotypic characteristics of the 31 isolates, the characterization procedures are described in material and methods.

Isolates	Colon	yColour	Aspect	Form of cell	Arrang-	Gram	Catal-	Sporul-	Mobi-
	form	colony	colony		ement		ase	ation	lity
MN1	Rod	Yellow	Round	Mobile Bacillus	Isolated	+	+	+	+
MN2	Rod	Red	Bomb	Mobile Bacillus	Isolated	+	+	+	+
MN4	Rod	Yellow	Plat	Mobile Bacillus	Isolated	+	+	+	+
MN5	Rod	Yellow	Round	Mobile Bacillus	Isolated	+	+	+	+
MN6	Rod	Yellow	Round	Mobile Bacillus	Isolated	+	+	+	+
MN7	Rod	yellow	Round	Chain Bacillus	Isolated	+	+	+	+
MN8	Shell	yellow	Round	Cocci mobile	heap	+	+	+	-
MN9	Rod	Yellow	Bomb	Chain Bacillus	Isolated	+	+	+	+
MN10	Rod	Yellow	Round	Mobile	Isolated	+	+	+	+
MN12	Rod	Yellow	Plat	Cocci chain	Isolated	+	+	+	+
MN13	Rod	Red	Round	Mobile Bacillus	Isolated	+	+	+	+
MN14	Rod	Yellow	Round	Mobile Bacillus	Isolated	+	+	+	+
MN15	Rod	Yellow	Round	Mobile Bacillus	Isolated	+	+	+	+
MN16	Rod	Yellow	Bomb	Bacillus chain	Isolated	+	+	+	+
MN17	Rod	Red	Plat	Mobile Bacillus	Isolated	+	+	+	+
MN18	Rod	Red	Plat	Mobile Bacillus	Isolated	+	+	+	+
MN19	Rod	Yellow	Plat	Mobile Bacilllus	Isolated	+	+	+	+
MN20	Rod	Red	Round	Bacille chain	Isolated	+	+	+	+
MN21	Shell	white	Plat	Cocci mobile	heap	+	+	+	-
MN22	Shell	Yellow	Bomb	Cocci mobile	heap	+	+	+	-
MN23	Rod	Yellow	Plat	Cocci mobile	Isolated	+	+	++	+
MN24	Shell	Yellow	Round	Cocci mobile	Isolated	+	+	+	-
MN25	Rod	Red	Round	Mobile Bacillus	Isolated	+	+	+	+
MN26	Rod	Yellow	Round	Mobile Baclillus	Isolated	+	+	+	+
MN27	Rod	Yellow	Plat	Mobile Bacillus	Isolated	+	+	+	+
MN28	Rod	Yellow	Bombe	Mobile Bacillus	Isolated	+	+	+	+
MN29	Rod	Red	Round	Mobile Bacillus	Isolated	+	+	+	+
MN30	Rod	Yellow	Round	Mobile Bacillus	Isolated	+	+	+	+
MN31	Rod	Red	Round	Mobile Bacillus	Isolated	+	+	+	+
MN32	Rod	Red	Round	Mobile Bacillus	Isolated	+	+	+	+
MN33	Rod	Yellow	Round	Mobile Bacillus	Isolated	+	+	+	+

Molecular identification

Agarose gel electrophoresis of the PCR amplification products of the gene encoding 16S rRNA from the universal primers is shown in **Fig.** 1.





Fig. 1. 0.8% agarose gel electrophoresis of PCR products of the 16S rRNA gene from 31 isolates.M = molecular weight marker, MN1, MN2, MN4, MN5, MN6, MN7, MN8, MN9, MN10, MN12, MN13, MN14, MN15, MN16, MN17, MN18, MN19, MN20, MN21, MN22, MN23, MN24, MN25, MN26, MN27, MN28, MN29, MN30, MN31, MN32, MN33 different isolates.

Sequence analysis

The ten sequenced PCR products were analyzed by Blastn which is a local similarity search program that uses a heuristic method [36,37]. The significance of the alignments is evaluated statistically according to the length and composition of the sequence, the size of the bank and the scoring matrix used. Blast presents its results with a list of sequences with significant alignment, each associated with a score and an E. Value. The lower the E. Value, the higher the alignment score is significant. The search for the homologues for each sequence entry makes it possible, by the similarity percentage, to specify the equivalent strain on the basis of data consulted, Table VI presents the results obtained for the identification of each isolate.

Table VI. Blastn Results for Strain Identification by Similarity of 16S rDNA.

	Max	% de	Accession	
code	Score E. Value	similarity	Number	Equivalent Strain
MN7	353 9,00E-93	3 100.00%	MK601669.1	Bacillus pumilus
MN6	18140.0	97.90%	CP018931.1	Bacillus cereus
				Staphylococcus
MN8	398 4,00E-10	699.10%	KX009746.1	haemolyticus
				Bacillus
MN12	296 1,00E-73	597.14%	HQ917121.1	thuringiensis
MN14	418 3,00E-112	299.56%	HQ694071.1	Bacillus cereus
MN17	7120.0	98.04%	JN874746.1	Bacillus sp
				Staphylococcus
MN21	737 0.0	97.03%	KT003250.1	saprophyticus
MN22	102 3,00E-1	796.77%	AB740361.1	Staphylococcus sp
				Staphylococcus
MN24	1162 0.0	96.84%	MK408479.1	gallinarum
MN26	411 5,00E-110	099.12%	JX683721.1	Bacillus subtilis

This table shows that: the NCBI BLAST homology had a similarity of 100% with MN7 *Bacillus* pumilus (MK601669.1), MN26 99.12% *Bacillus* subtilis (JX683721.1), MN14 99.56% *Bacillus* cereus (HQ694071.1), MN17 98.4% *Bacillus* sp (JN874746.1) MN12 97.14 *Bacillusthuringiensis* (HQ917121.1), MN6 97.90% *Bacillus* cereus (CP018931.1) and MN8 99.10% *Staphylococcus haemolyticus* (KX009746.1), MN2 97.3% *Staphylococcus saprophyticus* (KT003250.1).

Representativity of strains identified by 16S rDNA

Fig. 2 shows the different percentages of the strains identified, we can note the presence of two genera bacteria of the genus *Bacillus* and *Staphylococci*.



Fig. 2. Representability of strains identified by 16S rDNA.

Phylogenetic classification of strains identified by the gene encoding 16S rRNA

The multiple alignment of the sequences of the bacterial isolates is shown in **Fig. 3**. This alignment makes it possible to observe that the gene encoding the 16S rRNA exhibits both variable regions and non-variable regions, but the conserved areas are larger than the non-conserved regions. There is the presence of indels (insertions deletions.

	10 20 30 40 50 60 7
MN/ B. PUMIIUS 165 ARN FIDOSOM	-CARATTICTOTCACTTAGG-CGGCTGGCTCCARARAGGTTACCCCACCGACTTCGGGTGTTACAARCTC
MN6 B. cereus 165 ARN ribosoma	TCTTTACGATCTTCTTGGCGGCGGCTGGCTCCATAAAGGTTACCTCACCGACTTCGGGTGTGCAAACTC
MN8 S. haemolyticus 16S ARN ri	ATGCATTGCCGGGTGCTATACATGCA-GTCGAGCGAACAGATAAGGAGCTTGCT C
MN12 B. thuringiensis 16S ARN	CGACTTCTGTCACTTAGGCGGCTGGC-TCCAAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTC
MN14 B. cereus 165 ARN ribosom	
MN17 B. sp 16S ARN ribosomal	CCCAATCGGTCATCCTA-GTCTTACCTGCGAGTCTAGCGAATGGATTAGTAGCTTGCTC
MN21 S. saprophyticus 165 ARN	
MN22 S. sp 165 ARN ribosomal	CATGA AATGGCGGGGTGCTATACATGCAAGTCGAGCGAACAGATAAGGAGCTTGCTC
MCL D. Sp 105 Add 110050MLL	
MN24 S. galillarum 105 ARN 11D	
MN26 B. SUDTILIS 165 ARN FIDOS	CGACTTCTGTCACTTAGGCGGCTGGC-TCCAAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTC
MK601669.1 B. pumilus 16S ARN	GCA-GTCGACCGACAGA-AGGGAGCTTGCTC
HQ694071.1 B. cereus 16S ARN	GTGCGCGTGCTATACATGCA-GTCGAGCGAATGGATTAAGAGCTTGCTC
KX009746.1 S. haemolyticus 16S	GCCCGTGGGGGGTGCTATACATGCA-GTCGAGCGAACAGATAAGGAGCTTGCTC
	210 220 230 240 250 260 27
MN7 B. pumilus 16S ARN ribosom	GGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCT-TTGTACCGTCCATTGTAG
MN6 B. cereus 16S ARN ribosoma	AGATTTGTGGGATTGCCGCGCACTAGCGAGTTGGTGAGGCAACGGCTCCTGCCGGTTAAGATGCGTAG
MN8 S. haemolyticus 16S ARN ri	GGTTTTG-CTATCACTTATATATGGACCCGCGCCGTATTAGCTATGTTGGTAACAGCCCGTTA
MN12 B thuringieneis 165 APN	
ANTE D. CHUITAGICHDID 100 ANT	
MN14 B. Cereus 165 ARN FIDOSOM	GGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTA-GCTGGTGAGGTTACTGCC
MN17 B. sp 16S ARN ribosomal	GGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTA-GTTGGTGAGGTAA
MN21 S. saprophyticus 16S ARN	GGTTTTG-CTATCACTTATAGATGGACCCGCGCCGTATTAGCTA-GTTGGTAAGGTAA
MN22 S. sp 16S ARN ribosomal	GGTTTTG-CTATCACTTATAGATGGACCCGCGCCGTATTAGCTA-GTTGGTAAGGTAA
MN24 S. gallinarum 16S ARN rib	AGATTTGTGGGATTGGCTTAACCTCGCGGGTTCGCTACCCT-GCGGCTCGTGCAGGGAAG
MN26 B. subtilis 165 ARN ribos	AATTTTT-TTATAAAT-ATCCACCCCTCTCCCTTACACCACTTT-CCTAATTATATAA
MK601669 1 B mumiling 169 ADM	GGTTTCGGCTGTCBCTTBCBGBTGGBCCCGCGCGCGCGCG
HO694071 1 B cereus 165 APN	
HEORETAC I & Decedar Internation	
KX009/46.1 S. naemolyticus 16S	GGTTTTG-CTATCACTTATAGATGGACCCGCCGTATTAGCTA-GTTGGTAAGGTAA
	310 320 330 340 350 360 37
	310 320 330 340 350 360 37
MN7 B. pumilus 165 ARN ribosom	310 320 330 340 350 360 37 ATGA TGALTGALOG CALCCLARCT COCOLOGICAL COCOLARCT ALCONG CALCALA
MN7 B. pumilus 165 ARN ribosom MN6 B. cereus 165 ARN ribosoma	310 320 330 340 350 360 37 ATGA-TGALTTGALGFCALCCCCACCTFCCCCGGCTFCCCCGGCTGACGACGACGACGACGACGACGACGACGACGACGACGACG
MN7 B. pumilus 165 ARN ribosom MN6 B. cereus 165 ARN ribosoma MN8 S. haemolyticus 165 ARN ri	310 320 330 340 350 360 37 ATGA TOTATTGACOTATCCCCACCTTCCTCCCOOTTGCCACCOGCAOTCACCTTAGACOTCACCTA GOGATORABCACCOGCACCTATTACCTCCTATOGCAGCOGCACCTACATTAGCAATCACATACCGOCAATCAC CCC-CTCCTACTACATCACTACCTACCTCCATTTACCTATTAGCAATAACCACCTAGCCCCTAACCCC
MN7 B. pumilus 165 ARN ribosom MN6 B. cereus 165 ARN ribosoma MN8 S. haemolyticus 165 ARN ri MN12 B. thuringionsis 165 ARN	310 320 340 350 360 371 ATMA-TOMOTOR ACCOLLAGE TOMOTOGOL CARGONAL AT ACADEMIC CLAUDE SCALE OF ACCOLLAGE TOMOTOGOL CARGONAL AT ACADEMIC CLAUDE SCALE OF ACCOLLAGE TOMOTOGOL CARGONAL AT ACADEMIC CLAUDE GOGLAR CARGA CARGONAL AT ACADEMIC CLAUDE SCALE OF ACCOLLAGE TOMOTOGOL CARGONAL AT ACADEMIC CLAUDE SCALE OF ACCOLLAGE TOMOTOGOL CARGONAL AT ACADEMIC CLAUDE COMUNICATION AND AND AND AND AND AND AND AND AND AN
MN7 B. pumilus 165 ARN ribosom MN6 B. cereus 165 ARN ribosoma MN8 S. haemolyticus 165 ARN ri MN12 B. thuringiensis 165 ARN MN14 B. cereus 165 ARN ribosom	310 320 330 340 350 360 37 ATGA TOTALTGACOTATCCCCACCTTCCCCCCGOTTGCCACCGGCACTACACTTAGACGCCACACT GOGATOMAGACACCGGGACCACTACACTCCCTACOGAGGGCAGCGGAATATCGAATAGGAA
MN7 B. pumilus 165 ARN ribosom MN6 B. cereus 165 ARN ribosoma MN8 S. haemolyticus 165 ARN ri MN12 B. thuringionsis 165 ARN MN14 B. cereus 165 ARN ribosom MN17 B. so 165 ARN ribosomal	310 320 330 340 350 360 37 Artis - Subartización Altrica Calculatoria 310 100 300 370
MN7 B. pumilus 165 ARN ribosom NM76 B. cereus 165 ARN ribosoma NM78 S. haenolyticus 165 ARN ri NM12 B. thuringiensis 165 ARN NM14 B. cereus 165 ARN ribosomal NM17 B. sp 165 ARN ribosomal	310 320 310 340 300 371 ATGA 700 701 700 701 700 701 701 701 701 701 701 701 701 701 701 701 700 701
MN7 B. pumilus 165 ARN ribosom MN6 B. cereus 165 ARN ribosom ARN 5. haenolyticus 165 ARN ri MR12 B. thuringiensis 165 ARN MR15 B. thuringiensis 165 ARN MR16 J. S. sprophyticus 165 ARN MR21 G. saprophyticus 165 ARN	310 320 330 340 350 360 37 ATSA - TOLT TOLOGOT A SECONDARY OF CONSIGNATION CONSIGNATION CONTRACTOR CONT
MN7 B. pumilus 165 ARN ribosom NM76 B. cereus 165 ARN ribosoma NM78 S. haenolyticus 165 ARN NM14 B. cereus 165 ARN rib NM14 B. cereus 165 ARN ribosomal NM17 B. sp 165 ARN ribosomal NM21 S. sp 165 ARN ribosomal	310 320 340 350 360 370 ATGA ASTA STATUS
MN7 B. pumilus 165 ABN ribosom MN6 B. corrus 165 ABN ribosom MN8 B. hancipticus 165 ABN ri MN12 D. thuringionsis 165 ABN MN14 B. corresus 165 ABN ribosom MN17 D. sp 165 ABN ribosom MN122 S. sp165 ABN ribosom MN22 S. sp165 ABN ribosom	310 320 330 340 350 360 37 ATGA FORTINGLOFICATECOCCACTTOR COCCODE ANT CACTTAMARTINGCALTS 360 37 37 360 37 ATGA FORTINGLOFICATECOCCACTTOR COCCODE ANT CACTTAMARTINGCALTS 360 37 37 36 360 37 ATGA FORTINGLOFICATECOCCACTTOR COCCODE ANT CACTTAMARTINGCALTS 360 37 37 36 <t< th=""></t<>
MM7 B. pumilus 165 ABW ribosom MM8 B. corrus 165 ABW ribosom MM8 B. haemolyticus 166 ABW ribosom NM14 B. thuringionsis 168 ABW ribosom NM14 B. cercus 168 ABW ribosomal NM22 B. saprophyticus 168 ABW NM22 B. sapito ABW ribosomal NM24 B. gallinarum 165 ABW ribo	310 320 340 350 360 371 ATUA - TOURT CONCERNETT CONCERNETT CONCERNENT AND TALE CONCERNETT AND TALE CONCERNET AND TALE AND TALE CONCERNETT AND TALE CONCERNETT AND
NN7 B. pumilus 165 ABN ribosom NN8 B. cercus 165 ABN ribosom NN8 B. haemolyticus 165 ABN ribosom NN1 B. hurigiansis 165 ABN NN14 B. cercus 165 ABN ribosom NN15 S. polytak ribosom NN25 S. sp 165 ABN ribosom NN24 S. sp115 ABN ribosom NN24 S. sp111arum 165 ABN ribo	310 320 310 310 300 311 ATGA. TORATTGLOOTCATECCCALCOTTCOTCOGGITTGCALCOGOLAGTCALCTTALAUTGCCALATA 300 301<
MM7 B. pumilus 165 ABW riborom KM7 B. corous 166 ABW riborom MK7 B. thuringionnis 166 ABW MK14 A. thuringionnis 168 ABW MK14 A. corous 165 ABW riborom MK21 B. saprophyticus 168 ABW MK25 B. subtilis 165 ABW riborom MK26 S. subtilis 165 ABW ribor MK651650:1 B. pumilus 168 ABW 1654071.1 B. corous 168 ABW	310 320 330 340 350 360 37 ATGA SMALT REACTOR AND COLLECT TO COLLECT TO COLLECT AND AND COLLECT AND
MM7 B. pumilus 165 ABM ribosom MM6 B. cereus 165 ABM ribosoma NM8 6. haemolyticus 165 ABM ribosom NM1 8. h. cereus 165 ABM ribosom MM17 B. spits ABM ribosomal MM22 B. spits ABM ribosomal MM22 B. spits ABM ribosomal MM22 B. spits ABM ribosomal MM25 B. spits ABM ribosomal MM25 B. spits ABM ribosomal MM26071.1 B. cereus 165 ABM R609071.1 B. cereus 165 ABM	310 320 340 350 360 371 ATGA 300 340 360 370 371 ATGA 300 370
MM7 B. pumilus 165 ABM ribosom MM6 B. corrus 165 ABM ribosom MM8 S. heendyticus 165 ABM ri MM1 B. hurringiess 165 ABM MM1 B. hurringiess 165 ABM M17 B. eg 165 ABM ribosom MM21 S. eg 165 ABM ribosomal MM24 S. subtilis 165 ABM ribosomal MM24 S. subtilis 165 ABM ribosomal MM26 S. subtilis 165 ABM ribosomal MM26407.1 B. cereus 165 ABM ribosomal	310 320 330 340 350 360 37 310 320 330 340 350 360 37 310 320 320 360 360 37 310 320 320 360 350 360 37 310 320 320 360 360 37 360 37 310 320 320 360 360 37 360 37 310 320 360 360 37 360 37 360 37 310 320 360 37 37 37 360 360 37 360 37 360 360 37 360 360 360 360 360 360 37 360 360 360 37 360 360 37 360 360 360 360 360 360 360 360 360 360 360 <td< th=""></td<>
MM7 B. pumilus 165 ABM ribosom MM7 B. correus 165 ABM ribosoma MM7 B. haemolyticus 165 ABM MM7 B. b. correus 165 ABM ribosom MM7 B. spices 165 ABM ribosomal MM72 B. spices 165 ABM ribosomal MM72 B. spices 165 ABM rib MM72 B. spices 1165 ABM Rib040716.1 S. haemolyticus 165	310 320 340 350 360 371 ATGA. TOBATTGRAFT ATCCCCACCTTCCCCCQGTTGCCACCGGCAGTCACTCACTTAGATGCCCACTG 370<
MN7 B. pumilus 165 ABN ribosom MN6 B. cercus 165 ABN ribosom NN8 S. haemolyticus 165 ABN ribosom NN1 B. b. thringionsis 165 ABN NN1 B. g. cercus 165 ABN ribosom NN1 B. g. b. SADN ribosom NN2 S. g. sp. 165 ABN ribosom NN3 S. g. sp. 165 ABN ribosom	310 320 340 340 360 371 ATGA 700 100
MM7 B. pumilus 165 ABW ribosom MM8 B. carnus 165 ABW ribosoma MM8 S. hasmolyticus 165 ABW ribosom MM1 A. b. cercus 165 ABW ribosom MM1 B. s. carnus 165 ABW ribosom MM2 B. saprophyticus 168 ABW MM2 S. s. saprophyticus 168 ABW ribosom MM2 S. s. sublis 165 ABW ribosom MM2 S. s. ublis 165 ABW ribosom KX009746.1 S. hasmolyticus 165 ABW ribosom 165 ABW ribosom	310 320 330 340 350 360 37 ATUA - SULTI TRACTIC ANCICCLOCITY CONCOUNTING CLOCOGNUT OF CACCOGNUT OF CACCOGNUT AT MARKED CLACCOGNUT AT MARKED AT MARKED AT MARKED AT MARKED AT MARKED AT M
MM7 B. pumilus 165 ABM ribosom MM6 B. cereus 165 ABM ribosoma NM8 B. haemolyticus 165 ABM ribosoma NM1 B. h. cereus 165 ABM ribosoma NM17 B. spits ABM ribosoma NM22 B. spits ABM ribosoma NM22 B. spits ABM ribosoma NM25 B. spits ABM ribosoma NM26 B. spits ABM ribosom Reg64071.1 B. cereus 165 ABM Reg64071.1 B. cereus 165 ABM Reg64071.1 B. cereus 165 ABM ribosom NM6 B. cereus 165 ABM ribosom NM6 B. cereus 165 ABM ribosom	310 320 340 350 360 371 ATGA 700 370 340 350 370 371 ATGA 700 771 770
MAY B. pumilus 165 ABW riborom MAY S. cerewu 166 ABW riborom MAY S. cerewi 166 ABW riborom MAY A. thurpionnis 168 ABW riborom MAY A. S. asprophyticus 168 ABW MAY S. s. saprophyticus 168 ABW MAY S. S. sublis 165 ABW riborom MAY S. S. S. Sabwills 168 ABW KX009746.1 S. haemolyticus 165 MAY S. S. Sublis 165 ABW riborom MAY S. Sublis 165 ABW ri	310 320 310
MM7 B. pumilus 165 ABM ribosom MM7 B. charul 165 ABM ribosoma MM7 B. haemolyticus 165 ABM MM1 B. haemolyticus 165 ABM MM1 B. crews 165 ABM ribosomal MM21 B. saprophyticus 168 ABM MM22 B. spl65 ABM ribosomal MM24 S. sublisis 165 ABM ribosoma MM25 S. sublis 165 ABM ribosoma MM264 D. haemolyticus 165 ABM XX009746.1 B. haemolyticus 165 ABM XX009746.1 B. haemolyticus 165 ABM XX009746.1 B. haemolyticus 165 ABM MM7 B. pumilus 165 ABM ribosoma MM6 B. crews 165 ABM ribosoma ABM B. haemolyticus 165 ABM	310 320 340 350 360 371 ATDA - TORATTORACTICA CONCERNENCE CONCORTEGE CACOUNCE AND CACUTAL AND CONCERNENCE AND CACUUMATION CONCERNENCE AND CONC
MN7 B. pumilus 165 ABN ribosom MN6 B. cereus 165 ABN ribosom N08 S. haemolyticus 165 ABN M01 B. thuringianis 165 ABN M01 B. cereus 165 ABN ribosom N02 S. thuringianis 165 ABN N02 S. saprophyticus 168 ABN N024 S. sabilis 165 ABN ribosom N0509746.1 B. haemolyticus 165 N077 B. pumilus 165 ABN ribosom N06 B. cereus 165 ABN ribosom N06 S. thuringianis 165 ABN ribosom N06 S. thuringianis 165 ABN ribosom N051 B. thuringianis 165 ABN ribosom	310 320 340 340 360 371 ATMA 700 170 170 170 370
MM7 B. pumilus 165 ABW ribosom MM6 B. cerews 165 ABW ribosoma MM6 B. baemolyticus 165 ABW ribosoma NM71 B. thuringionnis 165 ABW ribosom NM71 B. spicio 165 ABW ribosomal NM22 B. spicio 165 ABW ribosomal NM24 S. subtilis 165 ABW ribosomal NM24 S. subtilis 165 ABW ribosoma NM601669.1 B. pumilus 165 ABW ribosom NM601689.1 B. pumilus 165 ABW ribosom NM60178.1 S. haemolyticus 165 NM7 B. pumilus 165 ABW ribosom NM6 B. cerews 165 ABW ribosoma NM61 B. thuringiansis 165 ABW ribosoma NM18 B. cerews 165 ABW ribosomal NM18 B. cerews 165 ABW ribosoma	310 320 330 340 350 370 ATUA 300 310 340 350 370 371 ATUA SUBATTERACTICA INCICALOUTLOC CONSTRUCTION OF TACOUNTY AND TRADECIMALTY ATUA SUBATTERACTICA INCICALOUTLOC CONSTRUCTION OF TACOUNTY AND TRADECIMALTY CCC CTCATATERACTICA INCICALOUTLOC CONSTRUCTION OF TATATERACTICA INTA ANALANCE CONSTRUCTION OF TACATICA SUBATTERACTICA INCICALOUTLOC CONSTRUCTION OF TACATICA CCC CTCATATERACECICALOUTLOC CONSTRUCTION OF TACATICALULA INCICALING
MM7 B. pumilus 165 ABM ribosom MM6 B. cereus 165 ABM ribosom NM8 B. haemolyticus 165 ABM ribosom NM1 B. b. cereus 165 ABM ribosom NM1 B. spice 165 ABM ribosom NM2 B. spice 165 ABM ribosom NM2 B. spice 165 ABM ribosom NM2 B. spice 165 ABM ribosom NK009746.1 S. haemolyticus 165 NM7 B. pumilus 165 ABM ribosom NM6 B. cereus 165 ABM ribosom NM6 B. cereus 165 ABM ribosom NM6 B. thuringienzis 165 ABM ribosom NM1 B. cereus 165 ABM ribosom	310 320 340 350 360 371 ATGA 320 320 340 350 370 371 ATGA 300 120 120 120 370
MN7 B. pumilus 165 ABN ribosom MN6 B. cercus 165 ABN ribosom NN8 S. haemolyticus 165 ABN ribosom NN8 S. haemolyticus 165 ABN ribosom NN1 B. p. folADN ribosom NN2 S. pumilus 165 ABN ribosom NN2 S. pumilus 165 ABN ribosom NN2 S. pumilus 165 ABN ribosom NN6 B. cercus 165 ABN ribosom NN6 B. cercus 165 ABN ribosom NN6 B. chardyticus 165 ABN ribosom NN6 B. cercus 165 ABN ribosom NN6 B. cercus 165 ABN ribosom NN6 B. chardyticus 165 ABN ribosom NN6 B. cercus 165 ABN ribosom NN1 B. p. thoringiessis 165 ABN N11 B. p. thoringiessis 165 ABN N11 B. p. thoringiessis 165 ABN N11 B. p. thoringiessis 165 ABN ribosom	310 320 310
MM7 B. pumilus 165 ABM ribosom MM7 B. charul 165 ABM ribosoma MM7 B. haemolyticus 165 ABM MM7 B. haemolyticus 165 ABM MM7 B. picto 165 ABM ribosomal MM72 B. thuringianus 165 ABM ribosomal MM72 S. subis 165 ABM ribosomal MM74 S. subilis 165 ABM ribosomal MM765 S. subilis 165 ABM ribosomal MM765 B. ubilis 165 ABM ribosomal MM766 B. cercus 165 ABM ribosomal MM7 B. pumilus 165 ABM ribosomal MM7 B. pumilus 165 ABM ribosomal MM7 B. haemolyticus 165 ABM MM71 B. charus 165 ABM ribosomal MM7 B. cercus 165 ABM ribosomal MM7 B. cercus 165 ABM ribosomal MM71 B. charus 165 ABM ribosomal MM71 B. charus 165 ABM ribosomal MM72 S. sp 165 ABM ribosomal	310 320 340 350 360 371 ATGA 300 340 350 360 371 ATGA 300 360 360 371 370
MM7 B. pumilus 165 ABM ribosom MM6 B. cereus 165 ABM ribosoma NM8 S. hemclyticus 165 ABM ribosoma NM1 S. b. thringiansis 165 ABM NM14 B. cereus 165 ABM ribosom NM7 B. sp 165 ABM ribosoma NM24 S. splitharms 165 ABM ribo NM24 S. subtilis 165 ABM ribosom NM6 B. cereus 165 ABM ribosom NM6 S. hemclyticus 165 ABM ribosom NM6 S. hemclyticus 165 ABM ribosom NM6 S. hemclyticus 165 ABM ribosom NM1 B. cy 165 ABM ribosom	310 320 340 310
MM7 B. pumilus 165 ABW ribosom MM6 B. coreus 165 ABW ribosom MM6 B. haemolyticus 165 ABW ribosom MM7 B. b. thrungionsis 165 ABW MM7 B. coreus 165 ABW ribosom MM7 B. spi65 ABW ribosomal MM22 S. spi65 ABW ribosomal MM24 S. subtilis 165 ABW ribosom MM601669.1 B. pumilus 165 ABW ribosom MM601689.1 B. pumilus 165 ABW ribosom MM601689.1 B. pumilus 165 ABW ribosom MM60168.1 C. cercus 165 ABW ribosom MM61 B. coreus 165 ABW ribosom MM61 B. coreus 165 ABW ribosom MM61 B. coreus 165 ABW ribosom MM18 B. cercus 165 ABW ribosom MM18 B. spi6 ABW ribosom MM18 S. spi6 ABW ribosom MM18 S. spi6 ABW ribosom MM18 S. spi6 ABW ribosom MM22 S. spi6 ABW ribosom	310 320 310
MM7 B. pumilus 165 ABM ribosom MM68 B. careus 166 ABM ribosom NM88 A. haemolyticus 166 ABM NM18. b. throngiensis 168 ABM NM18. b. careus 168 ABM ribosom NM17 B. sp 168 ABM ribosom NM22 B. sp 168 ABM ribosom NM22 B. sp 168 ABM ribosom NM28 B. sp 168 ABM ribosom NM18 B. careus 168 ABM ribosom NM17 B. pumilus 168 ABM ribosom NM17 B. pumilus 168 ABM ribosom NM18 B. careus 168 ABM ribosom NM18 B. thuringiantis 168 ABM NM18 L. thuringiantis 168 ABM NM18 B. thuringiantis 168 ABM NM18 B. sp 168 ABM ribosom NM18 B. careus 168 ABM ribosom NM18 B. sp 168 ABM ribosom NM	310 320 320 340 350 360 371 3734 3730 370 370 360 370 370 3734 3734 370

Fig. 3. Part of the multiple alignment of the 16 S rRNA gene sequences of (13) strains of which there are (10) identified strains (MN7, MN6, MN8, MN12, MN14, MN21, MN22, MN26, MN24, MN17) and (3) counterparts (MK601669.1), taken from the database.

The phylogenetic tree

The evolutionary history has been deduced using the neighboring junction method [31]. The optimal tree (**Fig. 4**) with the sum of the branch length = 3.14669626 is shown. The tree is drawn to scale, with branch lengths in the same units as the evolutionary distances used to derive the phylogenetic tree. Evolutionary distances were calculated using the Poisson correction method [32] and are expressed in units of the number of amino acid substitutions per site. The analysis involved 13 nucleotide sequences. All positions with gaps and missing data have been eliminated. Evolutionary analyzes were conducted in MEGA7 [33].



Fig. 4. The phylogenetic tree of identified strains and their homologous.

Proteolytic activity of bacteria of the *Bacillus* genus isolated and identified by their 16S rDNA.

Proteolytic enzyme production

Fig. 5 shows the demonstration of proteolytic enzyme production by bacteria of the genus *Bacillus* identified by their 16S rDNA. The translucent stain testifies to the digestion of the casein, it is the results of the supernatants resulting from the centrifugation of the cultures of which 50 microliters were deposited in the wells of the Gel, and previously placed in the oven during about 12 h. **Fig. 5** shows that the diameter of the translucent zone is variable from one strain to another, MN6 (19 mm), MN17 (20 mm), MN14 (15 mm) has a larger enzyme production whereas, MN26 (10mm), MN7 (11mm) and MN12 (11.3mm) show low enzyme production.



Fig. 5. Digestion of casein for different strains, clear areas indicate proteolysis.MN6, MN7, MN12, MN14, MN17 and MN26 are the different strains of bacteria of the genus *Bacillus* isolated and identified in this study. E. coli K12 which is the negative control and Bam NM76 positive control.

Simultaneous evaluation of proteolytic enzyme production and growth (OD)

Clear area diameters representing proteolytic enzyme (EP) production were measured in correlation with optical density (expression of OD growth), **Fig. 6** shows the results obtained.



Fig. 6. Variation in OD and proteolytic enzyme production for different strains of *Bacillus* isolated and identified.

Note: OD = optical density EP = enzyme production

The parallelism between OD and EP shows that all the strains used showvariable growth from one strain to another. All the identified *Bacillus* strains produce the enzyme. Enzyme growth and production are two related but distinct phenomena. Strains MN6, MN14, and MN17, which show significant enzyme production, do not have the highest OD, however strains MN26, MN7 and MN12 show less significant enzyme production, strain MN26 has higher OD.

DISCUSSION

In this study, bacterial strains from soil samples collected at 12 sites in Brazzaville, Republic of Congo, were isolated and characterized phenotypically and molecularly. The gene encoding 16S rRNA has been used to characterize Bacterial Biodiversity and the proteolytic capacity of bacteria of the genus *Bacillus* evaluated. The pH values of the samples are between 6 and 7. Determining the pH of the soil is equivalent to measuring its acidity or its basicity, which depends on the proton concentration, that is to say on H3O + ions. the main factors that influence bacterial growth.

Indeed, the survival of microorganisms differs from one group to another depending on the degree of acidity of the medium. [27] and [28] pH is an environmental parameter that determines the development of soil microflora since its fluctuations affect both biotic and abiotic factors of this ecosystem. It particularly influences the heterogeneity of the bacterial communities that remain there [29]. Most bacteria have been shown to grow at near-neutral pH levels, with the optimum growth being between pH 6 and pH 8 [38]. Indeed, the bacterial growth is exponential when the pH values are close to 7 whereas it is slower at lower pH [27]. These results, similar to ours, explain the growth of bacteria, because the pH of the soils of our study are between 6 and 7. Indeed, the soils of Brazzaville are rather acidic in general [31].

The enumeration of the total flora showed a variation of the CFU / g according to each sample these numbers are between 30.8 ± 4.7 CFU / g.103 (for the sample of the tube 3) to 214 ± 58.2 CFU / g 10^3 (for the sample of tube 5). All soils

contain bacteria. A [26] also showed that a soil contaminated by hydrocarbons contains bacteria, these results are similar to ours because of the presence of bacteria in all the soil. For bacteria of the genus Bacillus, the highest bacterial biomass was in the composite (C4) and the lowest in the composite (C1). Here again the variation of CFU / g as a function of the composite. All isolates are catalase +, gram + and all show sporulation. On the other hand, the shape, the color and the appearance of the colonies, the arrangement and the mobility of the cells vary according to the isolates, 27 isolates have the rod shape while 4 present the cocci form. 21 isolates are yellow in color and 12 in red, 18 isolates are of rounded form, 5 isolates are bomb and 8 isolates are flat. These results are similar with de [25] who worked on different food environments. Moutou worked on foods that are also environments, and also found isolates with different morphological characteristics.

Agarose gel electrophoresis at 0.8% PCR products of the 16S rRNA genes of the isolates in this study showed a length of approximately 1500 bp. The study [25] however showed a length of about 1380 bp that are different from our study. In reality, the 16S rRNA is a marker of molecular identification. According to **Fig. 3**, which showed some differences among the nucleotide sequences that were aligned, these results are supported by 16S rRNA, sequencing as a more potent bacterial identification technique, and these results are in agreement with previous studies [34] have successfully used 16S rRNA gene sequencing to identify pathogenic bacteria in the clinical laboratory. [4] Used sequencing of the 16S rRNA gene for bacterial identification and found new bacteria in clinical microbiology laboratories.

Fattahi et al. [35] developed 16S rRNA as a PCR target for the detection of E. coli in rainbow trout. Most sequences showed 99% of identity with the closest sequences already presented in the NCBI databases. MN7 has 100% similarity, MN26 99%, and (MN14, MN17) 98%, (MN6, MN12) 97% for Bacillus and 99% MN8, MN21% and (MN22, MN24) 96% for Staphylococci. Table 4 of Blast shows the correlation between E, score and similarity for strains. Strains MN6, MN24, MMN17 and MN21 show low E. values, and the alignment score is significant. The results are expressed as percent similarity of strains found to identify for our study with closest species (homologs). With regard to the intervals (10-100,110-200, 210-300,310-400,410-500) one observes the Fig. 3 which presents the indel (insertion or deletion) on the first alignment. the second and the third while the fourth does not has almost no indel from which we can talk about the conservation of nucleotides.

The phylogenetic tree represents the phylogenetic relationships between the newly obtained sequences and reference sequences in the databases. It was built by the distance method by Mega 7 software. Fig. 6 shows the parallelism between proteolytic enzyme production and growth, our results show that the growth and production of enzyme vary from one strain to another, isolates MN17 has the largest enzyme production (20mm), while MN26 has the lowest production (10mm), these results are similar to those of [12] which worked on the ntoba mbodi fermented food and also found variation in growth and proteolytic enzyme production as a function of the isolates. Our results suggest that each strain has different growth and enzyme production conditions, as shown by previous work by [39, 40].

CONCLUSION

At the end of our study, we were able to enumerate, isolate, identify and characterize on a phenotypic and molecular level the bacteria present in the soils. The ten of the 31 isolates identified and characterized on the molecular level have allowed after sequencing to distinguish by phylogeny two coherent monophyletic groups bacteria of the genus *Bacillus* and the genus Staphylococcus. The six bacteria of the genus *Bacillus* showed different proteolytic activity and growth showing that growth and proteolytic activity are two distinct phenomena. The 16SrRNA remains a useful tool for the biodiversity of culturable bacteria.

ACKNOWLEDGEMENTS

We thank the members of the Department of Molecular and Cellular Biology of the Faculty of Sciences and Technics of the University Marien Ngouabi, the members of the Molecular and Biology Laboratory of the National Institute of Research in Natural and Exact Sciences (IRSEN)as well as the members of the National Laboratory of Public Health (LNSP) of the Republic of Congo for facilitating our access to their technical platform for our analysis. We express our gratitude to Mrs. Annie Justine Pahou Bilimba for supplying us with reagents.

REFERENCES

- 1. Daniel R. The metagenomics of soil. Nat Rev Microbiol, 2005; 3: 470-8
- 2. Fierer N, Lennon JT. The generation and maintenance of diversity in microbial communities. Am J Bot, 2011; 98: 439-48.
- P. C. Y. Woo, P. K. L. Leung, K. W. Leung, and K. Y. Yuen, Identification by 16s ribosomal RNA gene sequencing of an Enterobacteriaceae species from a bone marrow transplant recipient, J Clin Pathol—Mol Pathol, 2000; 53(4): 211–215.
- Woo, P. C. Y., S. K. P. Lau, J. L. L. Teng, H. Tse, and K-Y. Yuen. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect, 2008; 14(10):: 908-934.
- Suardana, I. Wayan. Analysis of nucleotide sequences of the 16S rRNA gene of novel Escherichia coli strains isolated from feces of human and Bali cattle. J Nucleic Acids, 2014 (2014).
- Boudewijns, Michael, Judith M. Bakkers, Patrick DJ Sturm, and Willem JG Melchers. 16S rRNA gene sequencing and the routine clinical microbiology laboratory: a perfect marriage? J Clin Microbiol., 2006; 44(2): 3469-3470.
- Wang, Y., Zhang, L., Zhang, H., Liu, W., Zhang, Y., Zhang, X, and Sun, T., In vitro assessment of probiotic properties of Bacillus isolated from naturally fermented congee from Inner Mongolia of China. World J Microbiol Biotechnol,2010; 26(8), 1369-1377.
- Miambi, E., J.P. Guyot and F. Ampe, Identification, isolation and quantification of representative bacteria from fermented cassava dough using an integrated approach of culture-dependent and culture-independent methods. Int. J. Food Microbiol. 2003; 82(2): 111-120.
- 9. Zou He-chang. The development of thrombolytic Agents. Chinese Pharma J. 1997; 32(5): 263-267.
- Zheng Yan-bing, Lu Fu-ping, Du Liang-xiang. Optimization of fermentation conditions of fibrinolytic enzyme produced by *Rhizopus chinesis* sp. Ind Microbiol,2000; 30(4): 28-31
- 11. Nguimbi Etienne, Ahombo Gabriel, Moyen Rachel, Ampa Raoul, Alain Vouidibio, Ontsira Esther Nina, Kobawila Simon Charle Louembe Delphin. Optimization of Growth, Fibrinolytic Enzyme Production and PCR Amplification of Encoding Fibrinolytic Enzyme Gene in *Bacillus amyloliquefaciens* Isolated from Ntoba mbodi at Brazzaville. Int J Sci Res 2014; 3(11):2319-7064

- 12. Soloka Mabika Armel Faly, Rachel Moyen, Etienne Nguimbi, Gabriel Ahombo, Raoul Ampa, Aimé Christian Kayath, Alain Vouidibio, Cyr Jonas Morabandza and Simon Charles Kobawila. Production, Partial Purification and Based SDS-PAGE Profiles of Caseinolytic Enzyme in two Bacillus Strains Isolated from Fermented Cassava leaves "Ntoba mbodi" in Congo Brazzaville. J Pure Appl Microbiol. 2017; 11(1);77-86.
- 13. Harrigan, Wilkie F. Laboratory methods in food microbiology. Gulf Professional Publishing, 1998.
- Harrigan, W.F. and M.E. McCance, Laboratory Methods in Food and Dairy Microbiology. 1st Edn., Academic Press, 1976; London, pp: 25-29.
- Wulff E.G., Mguni C.M., Mansfeld-Giese K., Fels J., Lübeck M., Hockenhull J. Biochemical and molecular characterization of *Bacillus amyloliquefaciens*, *B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against Xanthomonas campestris pv. campestris. 2002; Plant Pathol.;51(5):574–584.
- Leyral G., Vierling E., Microbiologie et toxicologie des aliments, 4e ed, Doin Éditions, Leyral G., Joffin C. et J.N, Boursdaise, Larpent 2007
- Carr-Schmid A, et al. Novel G-protein complex whose requirement is linked to the translational status of the cell. Mol Cell Biol 2002; 22(8):2564-74.
- Weisburg W. G., Barns S. M., Pelletier D. A., Lane D. J. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol, 1991;173: 697-703.
- Puri S, Beg QK, Gupta R Optimization of alkaline protease production from *Bacillus* sp. by response surface methodology Curr Microbiol 2002;44:286-290.
- Astrup T., Mullertz S. The fibrin plate method for estimating fibrinolytic activity. Arch. Biochem. Biophys., 1952;40, 346-351.
- Wang S.H., Zhang C., Yang Y.L., Diao M., Bai M.F. Screening of high fibrinolytic enzyme producing strain and characterization of the fibrinolytic enzyme produced from *Bacillus subtilis* LD 8547. World J. Microbiol.Biotechnol., 2008; 24(4) 475-482.
- Wang S.-H., Diao M., Yang Y.-L., Lin W.-Z, Huang B.-F Gene clone and expression of a fibrinolytic enzyme (FE) in Escherichia coli. Ann Microbiol, 2008; 58 (1) 95-98.
- Zhang R.H., Xiao L., Peng Y., Wang H.Y., Bai F., Zhang Y.Z. Gene expression and characteristics of a novel fibrinolytic enzyme (subtilisine DFE) in Escherichia coli.Lett. App. Microbial., 2005; 41: 190-195.
- Didine Priscilla Moutou-Tchitoula1, Etienne Nguimbi1, Stéphanie Giusti-Miller2, Philippe Mora2, Simon Charles Kobawila1, Edouard Miambi2. Assessment of dominant bacterial strains isolated from Ntoba mbodi, an indigenous African alkalinefermented food, and their potential enzyme activities. Afr J Microbiol Res, 2018, 12(32): 779-787.
- Rojo F., Enzymes for aerobic degradation of alkanes. Handbook of Hydrocarbon and Lipid Microbiology, 2010;18p.
- Rousk J., Brookes P. C., Bååth E., Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl. Environ. Microbiol., 2009;75(6): 1589–1596.
- Agamuthu P., Tan Y. S., Fauziah H. S, Bioremediation of hydrocarbon contaminated soil using selected organic wastes. Procedia Environ Sci, 2013;18: 694-702.
- 29. Theodorakopoulos N., Analyse de la biodiversité bactérienne d'un sol contaminé de la zone d'exclusion de Tchernobyl et caractérisation de l'interaction engagée par une souche de Microbacterium avec l'uranium. Aix-Marseille. 2013;198p.
- Nzila J. D., Caractéristiques physiques et socio-économiques de la ville de Brazzaville et ses environs. Synthèse bibliographique, 2005; 8 p.
- Saitou N. and Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and 1987; Evolution 4:406-425.
- Zuckerkandl E. and Pauling L. Evolutionary divergence and convergence in proteins. Edited in Evolving Genes and Proteins by V. Bryson and H.J. Vogel, 1965; pp. 97-166. Academic Press, New York.
- Kumar S., Stecher G., and Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Molecular Biology and Evolution 2016; 33:1870-1874.

- Patel, J. B. "16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory," Mol Diagnos, 2001; vol. 6(4),313–321.
- Fattahi, F., Mirvaghefi, A.H. Farahmand, G. Rafiee, and A. Abdollahi, "Development of 16s rRNAtargeted PCR method for the detection of Escherichia coli in rainbowtrout (*Oncorhynchus mykiss*)," Iran J Pathol, 2013; vol. 8, no. 1, pp. 36–44,
- Altschul S. F., Gish W., Miller W., Myers E. W. & Lipman D. J. Basic local alignment search tool. J Mol Biol, 1990; 215(3), 403– 10.
- Altschul S. F., Madden T. L., Schaffer A. A., Zhang J., Zhang Z., Miller W. & Lipman D. J. Gapped BLAST and PSI-BLAST : a new generation of protein database search programs. Nucleic Acids Res. 1997, 25(17):3389-402
- Bidaud C., Biodégradation des hydrocarbures aromatiques polycycliques. Approche microbiologique et application au traitement d'un sol pollué, in Chemical and Process Engineering. Ecole Nationale Supérieure des Mines de Saint-Etienne.1998; 279p.
- Nihan Sevinc and Elif Demirkan.Production of Protease by Bacillus sp. N-40 Isolated from Soil and Its Enzymatic Properties J. Biol. Environ. Sci. 2011: 5(14), 95-103.
- Adinarayana K, Ellaiah P, and Prasad DS Purification and partial characterization of thermostable serine alkaline protease from a newly isolated Bacillus subtilis PE-11. 2003AAPS Pharm. Sci Tech., 4:1-9