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Diversity and Phylogenetic Relationships of Proteolytic Bacteria Isolated from Fermented Pepper and Soil in Brazzaville, Republic of Congo

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Abstract: The diversity of bacteria was explored in two different environments in Brazzaville: soil and fermented peppers. The count showed that the total flora is greater in the fermented pepper (9.4. 104 CFU/g) than in the soil of 3.7. CFU/g, bacteria of the *Bacillus* genus are numerically more important in soil (9.3.104 CFU/g) than in fermented pepper (7.7.104 CFU/g). The morphotypes of all isolates presented small, medium and pink and whitish colored colonies, are catalases positive, protease producers, Gram positive, and spore forming, including one Gram negative isolate. 16S rDNA PCR of the isolates, Agarose Gel electrophoresis, sequencing and in silico analysis of the sequences were performed. The amplicons showed sizes closed to 1500bp, BLASTn analysis of the sequences made it possible to identify Twenty (20) strains, with a predominance *Bacillus cereus sensu stricto* strains *(40%).* Taking by environment, six (6) in the soil, namely *Bacillus cereus sensu stricto* (16.66*%), Bacillus thuringiensis* (16.66%), *Bacillus anthracis* (16.66%), *Bacillus welmani* (16.66%) and a *Bacillus albus (*16.66%), fourteen (14) strains in fermented peppers namely: *Bacillus cereus sensu stricto* (21.42%), *Bacillus thuringiensis* (7.14%), *two Pseudomonas sp*, *Bacillus thuringiensis* (3.57%), *Bacillus sp (3.57%*), *Bacillus albus* (1), *Lysinibacillus sp* (3.57%), *uncultured (3.57%*). All strains produce the proteolytic enzyme with diameters ranging of more than two cm. The phylogenetic inference using 16S rDNA analysis shows that these bacteria are very closed, excepted the *Pseudomonas sp* and *uncultured bacteria*, all belong to *Bacillus cereus* group, forming a coherent taxonomic group.

Keywords: Soil, Fermented Peppers, PCR, Sequencing, In Silico Analysis

1. Introduction

Chili is a perennial shrub under favorable climatic conditions, it is considered as vegetable and belongs to the genus *Capsicum*, the fruit of the chili pepper has a small box with the placenta on which the seeds are arranged. Chilli can be consumed fresh directly, fried, in sauce or canned. It is often mixed with various other vegetables. In the dehydrated state, the pepper is used in the form of powder and is used in the seasonings of dishes, braised meats etc. [1].

By its various natural properties, it produces a molecule called: "capsaicin alkaloid", From the biological point of view, this compound allows the fruits and seeds of the plant which produces them to be less exposed to predation

and would have a positive effect on the excretion mechanism [2].

The different types of *Capsicum* are commonly classified according to the characteristics of the fruit: flavor, color, shape, taste, size [3]. The number of species recorded varies greatly according to the authors. Today, taxonomists agree on 25 species, including around twenty wild species and 5 domesticated species: *Capsicum annuum L., Capsicum frutescens, Capsicum chinense, Capsicum pubescens* and *Capsicum baccatum var pendulum* [4]. Pepper contains microorganisms. Among the *Bacillus* identified in peppers, we can mention: *Paenibacillus sp., Bacillus marisflavi, Bacillus pseudomycoides, Bacillus pumilus, Bacillus megaterium* and*, Bacillus cereus* [5].

The soil is a heterogeneous medium comprising a wide variety of microorganisms including bacteria, the bacterial communities of the soil can be counted both by conventional techniques, molecular biology techniques linked to cultivation, and by metagenomics techniques. Among the soil bacteria there are those of the genus *Bacillus* including *Bacillus cereus*. Bacteria can be identified by molecular analysis of the gene encoding rRNA16S, this gene is often highly conserved between species and also species of the same genus, so it is an important molecular tool for bacterial classification.

The omnipresence of *Bacillus cereus* in the soil implies that a transfer can take place to a host in contact with this same soil [6], inevitably this also contributes to the fact that food contamination is frequent. and can arrive either directly at the place of production of the raw material or at the time of processing due to the persistence of the spores on the equipment because they have a strong capacity for adherence.

Bacillus cereus is often in certain foods and is responsible for poisoning, Bacterial proteases are of great industrial importance, with numerous applications in pharmacy, waste treatment, agri-food [7-9]. The bacteria of the genus *Bacillus* listed, produce proteases of interest, these are: *B. sterothermophilus, B. cereus, B. megaterium, B. mojavensis, and B. subtilis* [10].

In this work, the count of bacteria is made in two samples, one of pepper and the other of soil, a phenotypic characterization of the bacterial isolates is carried out with the search for bacteria of the genus *Bacillus* and particularly *Bacillus cereus*. Molecular identification of isolates whose colonies are pink on Mossel medium was made by PCR amplification and sequencing of the 16S rRNA gene. A Bioinformatics analysis and the construction of phylogenetic trees closed this study.

2. Materials and Methods

2.1. Collection of Samples and Counting

Red peppers close to the characteristics of the species "*Capscicum annum*", were purchased at the Total market in Brazzaville. These peppers were crushed in a sterilized mortar and placed in a pot. Six (6) days of fermentation.

The soil was taken from the garden of the Faculty of Science and Technology of Marien Ngouabi University.

For both pepper and soil samples, decimal dilutions were made using sterile distilled water in the same proportions 1g of pepper/ in 10ml of distilled water for the stock solution and also 1g of soil in 10ml of water distillated. Two culture media were used PCA to assess the total aerobic mesophilic flora (FMAT) and mossel medium for bacteria of the genus *Bacillus* [11, 12].

After inoculation, the Petri dishes are then placed in the oven at 37°C. The reading is done after 24 hours. The count of bacteria in the samples was determined in CFU/g by the following formula [13, 14].

2.2. Phenotypic Characterization of Isolates

2.2.1. Macroscopic and Microscopic Characteristics of Isolates

This study consists of the direct observation with the naked eye of the morphological aspect of the colonies obtained on the purification medium, based on the shape, size, pigmentation, consistency and contour of the colonies called by morphotype, according to Bergey's Manual of Systematic Bacteriology [15]. An optical microscope (OPTIKA, Italy) with objective 40 (G×400) was used.

The microscopic observation made with an optical microscope (OPTIKA, Italy) with the objective 40 (G×400) made it possible to specify the appearance of the cells, the arrangement and sometimes the mobility.

2.2.2. Biochemical Characteristics of Isolates

Gram staining and KOH test have been used to characterize bacteria and distinguish between gram positive and gram negative.

Isolates were tested for catalase [16]. The isolates were tested for their ability to sporulate, briefly, the isolates are placed in suspension in peptone water then subjected to a thermal shock at 80°C for 10 min in a water bath, then placed back in the oven. Growth is monitored for 24 hours. This allowed to distinguish sporogenic from non-sporogenic isolates, this being followed by microscopic observation [17, 18].

2.3. Screening of Protease-Producing Isolates

The production of protease from the isolates was carried out in a medium containing glucose, 0.5% (w/vol); peptone, 0.75% (w/v); and NaCl solution, 5% (vol/vol); $MgSO_4 \cdot 7H_2O$, 0.5% (w/v); KH_2PO_4, 0.5% (w/v); and $FeSO_4 \cdot 7H_2O$, 0.01% (w/v)] and maintained at 37°C for 48 h in a shaking incubator (60 rpm) [8, 19]. At the end of the fermentation, the optical density was measured with a zuzi model 4211/5 type spectrophotometer, the culture medium was then centrifuged at 7000 rpm for 5 min to obtain a clear supernatant, which was used as enzyme source. A Petri dish containing 1% agarose agar and containing 0.2g of casein (10% skimmed milk) was prepared and 50 microliters of the supernatant was placed in the wells on the gel. The Petri dish was then incubated at 37°C for 12 hours, the observation of a

clear halo testifies to the production of protease. The measurement of the diameter of the halo made it possible to evaluate the production of protease [19-21].

2.4. Molecular Identification by 16S rRNA Gene of Isolates

2.4.1. Extraction of Genomic DNA

The DNA extraction method was based on the protocol of the DNA extraction kit: ZR DNA CARD EXTRACTION KIT, respecting the manufacturer's instructions.

2.4.2. PCR Amplification of the 16S rRNA Gene of Isolates

(i). Designation and Synthesis of Primers

Universal primers for amplifying genes encoding 16S ribosomal rRNA are used [11, 12, 21]. The sequences of these primers are: fD 1 5'-AGAGTTTGATCCTGGCTCAG-3' and rP 2 5'-ACGGCTACCTTGTTACGACTT-3'. This pair of primers was synthesized by Macrogène France.

(ii). Mix Composition and PCR Conditions

An optimization of the different PCR protocols is carried out in order to obtain the optimal concentrations of each reagent used, necessary for the reaction mixture. The PCR reaction is carried out in a final volume of 50 µL containing: 39.5 µL of sterile distilled water, 1 µL of each primer (sense and antisense), 1 µl of dNTP, 5 µL of the enzyme buffer (OneTaq standard Buffer) and 0.5 µL of the enzyme (OneTaq polymerase) and subsequently 2 µL of DNA are added to the reaction. A negative control is included in the sample to validate the reaction. For each isolate, three PCR reactions are performed using universal 16S rRNA gene primers. After preparing the PCR reactions, the 0.5 ml microtubes are then placed in a thermocycler (Biometrica), according to the following steps: first initial denaturation at 95°C. for 5 min. 30 cycles, having for each: denaturation at 95°C. for 30 seconds, hybridization at 55°C. for 30 seconds, elongation at 72°C. for 1 minute 30 seconds, and a final elongation at 72°C. for 5 minutes. Storage of the PCR fragments (amplicons) is carried out at 4°C.

(iii). 1% Agarose Gel Electrophoresis of DNA

1g of agarose is weighed and mixed with 100 ml of TBE buffer (1X). The mixture is heated in the microwave oven until a homogeneous mixture is obtained (gel solution). After cooling to room temperature, the lukewarm gel solution is poured into a mold containing the previously placed comb, used to make wells. Once solidified, the comb and supports from the molds are carefully removed. The mussel is then placed in the electrophoresis tank containing the TBE migration buffer. 5 µL of Syber green are mixed with 5 µL of PCR products (amplicons) or genomic DNA samples and homogenized using a micropipette. The DNA samples and the size marker are then carefully placed in the wells of the gel. The migration is carried out at 80 volts and 400 mA for approximately 45 min with a generator (BIORAD). The PCR fragments are then visualized from a computer combined with a Gel Doc EZ imager UV device.

2.4.3. Sequencing of PCR Fragments

The technique used by was used. Classical PCR products positive for 16S genes were purified using the NucleoFast 96 PCR plate (Macherey-Nagel EURL, France) and sequenced using BigDye terminator chemistry on an ABI3730 sequencer (Applied Biosystems, Foster City, CA, United States) [12, 22]. Sequencing was performed by electrophoresis on a 3730xl-Titania DNA analyzer (Applied Biosystems) using the same primers as those used for PCR amplification of genes encoding 16S rRNA [22].

2.5. Analysis of Results

Microsoft Excel was used for statistical analyzes of data related to graphs and other charts. For bioinformatic sequence analysis, assembly was done by DNA Baser assembler. The in-silico analysis was carried out from the NCBI (National Center for Biotechnology Information, Los Alamos, USA) using BLAST (Basic Local Alignment Search Tool) which is an algorithm used by a family of five programs that allow the alignment of a new sequence against a database [23]. The search for homologs was carried out by BLASTn, the homologous sequences between a query sequence (or "query" sequence) and all the sequences present in the nucleic bank was launched and the parameters such as the "score" which depends on the homology with the query sequence, the size of the bank and the value of the "E-Value". The smaller this value is, the greater the homology between the query sequence and that of the bank [24, 25].

The multiple sequence alignment was done by Bio Edith and the phylogenetic tree showing the evolution between sequences and the different relationships between different bacteria was built by MEGA. The phylogenetic reconstruction is inferred by the UPGMA method [26]. The distances during evolution were evaluated by the maximum likelihood method [27].

3. Results

3.1. Enumeration

Table 1 shows the counting results on PCA and on Mossel for the two samples used. For the Total Aerobic Mesophilic Flora on PCA, there are more microorganisms in the pepper than in the soil. For Mossel, more bacteria of the *Bacillus* genus are recorded in the soil than in peppers.

Table 1. *Enumeration of microorganisms on PCA and mossel Mediums for two samples (fermented peppers and soil).*

Mediums	Sol	Fermented peppers
PCA (1)	3,7. 10^4 UFC/g	9,4. 10^4 UFC/g
MOSSEL (2)	9,3. 10^4 UFC/g	7,7. 10^4 UFC/g

3.2. Phenotypic Characterization of Mesophilic Isolates

From our 2 samples, 45 (forty-five) isolates were obtained, including 20 (twenty) from the soil sample and 25 (twenty-five) from the pepper sample, 2 kinds of pigmentations observed: *Bacillus spp*: Macroscopic observation gave

isolated colonies on Mossel with typical characteristics of *B*. sp, that is to say flat and yellow colonies, pink (red) coloration. In general, the pink or red color is characteristic of *B. cereus*.

The detailed results of the phenotypic study are presented in the form of a table describing the variable size of the colonies (from 0.5 x 1.2 µm up to 2.5 x 10 µm), variable shape (rhizoid, fusiform, circular), the surface (smooth, rough or sticky) with a regular or irregular outline, the greasy or even dry consistency. Microscopic examination revealed that most of the isolates are in the form of straight rods with variable mobility, often arranged in isolated cells, grouped in pairs thanks to a peritrichous ciliature.

Mostly Gram positive, all our isolates showed a type of aerobic respiration as demonstrated by the 100% positivity of the catalase test and their sporogenic characteristic was confirmed thanks to their ability to produce spores after a heat treatment of 10min at 80°C. Allowing us to presumptively deduce the presence of sporulated aerobic *Bacillus* [28]. The 45 isolates are divided in several morphotypes, the most important were about colonies as listed: small and punctiform, irregular, middle and punctiform, circular.

3.3. Proteolytic Activity of Isolates

3.3.1. Proteolytic Enzyme Production Assay

Figure 1 shows that all our isolates have proteolytic activity. This hydrolysis carried out after 24 hours' incubation of the petri dishes at 37°C, resulted in the formation of a clear halo around the well on the gel of each dish. The results were evaluated at 100% of the rate of casein hydrolysis by the enzymes produced by our isolates. The latter was more pronounced at the isolate level (RI19, RI5 and JI8, JI16).

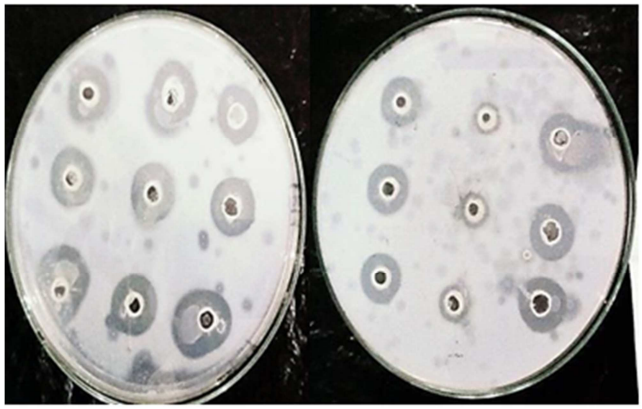
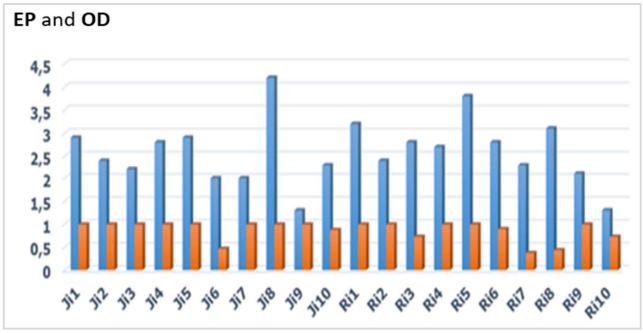


Figure 1. *Profile of clear Halos for the casein digestion by isolates.*

3.3.2. Evaluation of OD and Enzyme Production of Isolates from Soil

Figure 2 shows variability in growth and protease production in bacterial isolates from soil, isolates Ji1, Ji8, Ri1, Ri5 and Ri8 show numerically greater growth than the others. All these isolates show good growth.

Isolates Ri1 and Ri8 present a numerically equal enzymatic production, for a numerically different growth, this poses the problem of the distinction of the growth and the enzymatic production and also of the specificity of each isolate.

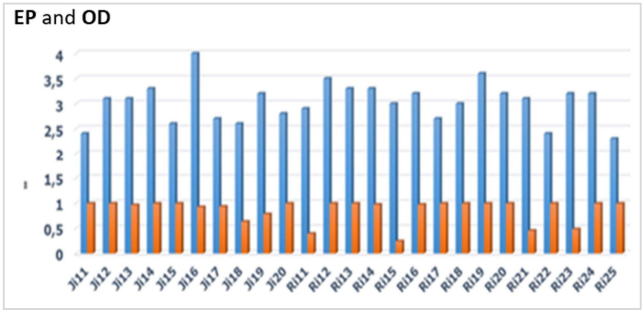


EP = enzyme production in bleu, OD = Optical density in red (growth)

Figure 2. *Profiles of Ep and OD for isolates from soil.*

3.3.3. Evaluation of OD and Enzyme Production of Isolates from Pepper

Figure 3 shows that the growth is variable from one isolate to another, most of the isolates except Ji18, Ri11, Ri15, Ri21, Ri23 show growth evaluated at 0.9 in terms of optical density. All the isolates studied present an interesting enzymatic production with higher peaks for the isolates: Ji16, Ri12, Ri19. The Ji15, Ri17, Ri22 and Ri25 isolates present a high optical density, but a moderate enzyme production, this suggests that growth and enzyme production are indeed two distinct phenomena. However, the fact that all the growing isolates show enzyme production suggests that these two distinct phenomena are related. Finally, the variability of enzyme production from one isolate to another reflects the specificity of each isolate.



EP = enzyme production in bleu, OD= Optical density in red (growth)

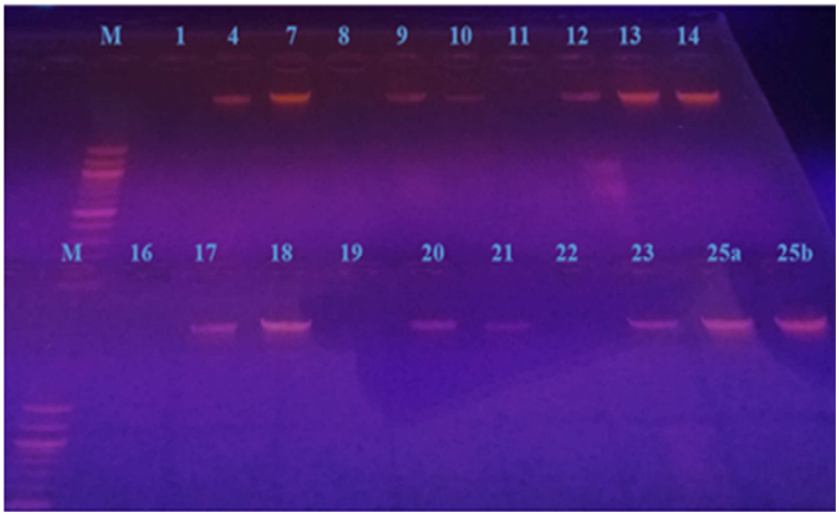
Figure 3. *Profiles of EP and OD for isolates from fermented pepper.*

3.4. Molecular Identification

3.4.1. Agarose Gel Electrophoresis

(i). 8% Agarose Gel Electrophoresis of Genomic DNA

Figure 4 shows the results of genomic DNA extraction on gel electrophoresis with clearly visible bands. This is an electrophoretic profile. The bands of genomic DNA are all placed far from the last band of the marker, indicating their large size. Indeed, the largest band of the marker is at 10Kb.

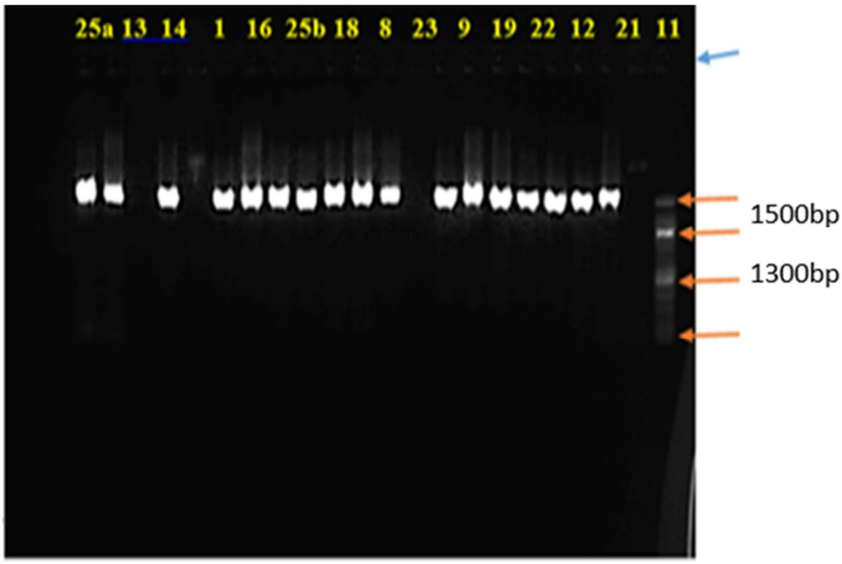


M = Marker of Molécular weight, 1-25 DNA of isolates

Figure 4. *0,8% Agarose Gel Electrophoresis of grnomic DNA fragments of isolates of this study.*

(ii). 1% of Agarose Gel Electrophoresis of Amplified PCR Fragments of 16S rRNA Gene

Figure 5 shows the electrophoretic profile on Agarose Gel of the fragments resulting from PCR amplification of the 16S rRNA gene of twenty (20) isolates. All the PCR fragments are aligned on the same position from the point of view of the molecular weight, their size is close to 1500bp.



M = Marker of Molecular weight

Figure 5. *1% Agarose Gel Electrophoresis of 16 S rDNA PCR amplified fragments of iolates in this study.*

3.4.2. In Silico Analysis of 16S rDNA Sequences of Isolates

(i). BLASTn and Identification of Strains by Their Sequences

According to the parameters of BLASTn (score, percentage of similarity, E value), the results which present the characteristics of each sequence and the sequence homolog of each identified isolate, thus tables 2 and 3 show the different species and strains identified for all of the two soil and chilli samples and also their representativeness in percentage.

Table 2. *Bacterial strains isolated from soil.*

species	Number	Percentage
Bacillus cereus	2	33,33%
Bacillus thuringiensis	1	16,66%
Bacillus anthracis	1	16,66%
Bacillus albus	1	16,66%
Bacillus weldamanii	1	16,66%

In table 2, It is shown that the different bacterial strains isolated from the soil are all from the group of *Bacillus cereus*, a total of six strains divided into five species

including two of *Bacillus cereus sensu stricto*, therefore predominant.

Table 3. *Bacterial strains isolated from fermented pepper.*

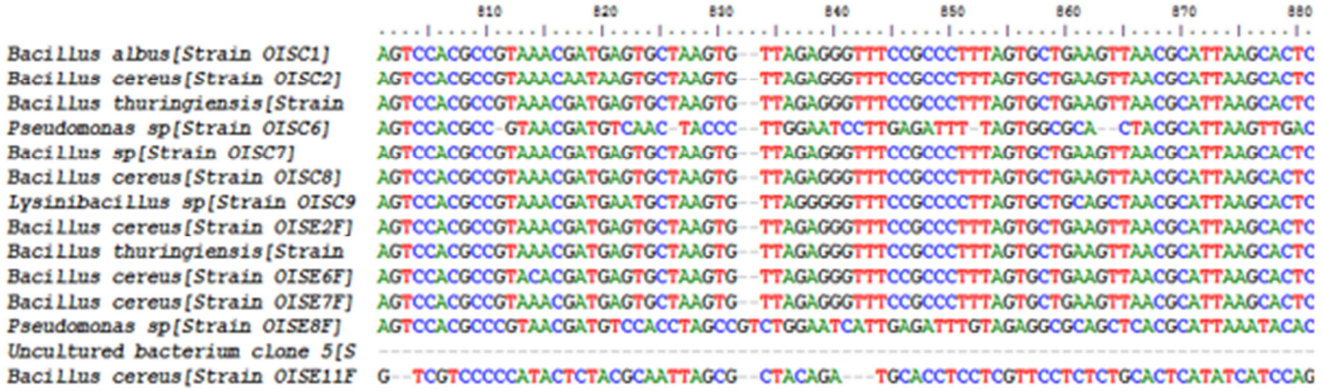
species	Number	Percentage
Bacillus cereus	6	42,85%
Bacillus thuringiensis	2	14,28%
Bacillus sp	1	7,14%
Bacillus albus	1	7,14%
Pseudomonas sp	2	14,28
Lysinibacillus sp	1	7,14%
Uncultured bacterium	1	7,14%

In Table 3, these are the strains isolated from the pepper sample, a total of fourteen bacterial strains divided into seven species, with a predominance of *Bacillus cereus sensu stricto* 6 strains, apart from the group of *Bacillus cereus* we find two strains of Pseudomonas, one strain of *Lysinibacillus sp* and one not *cultivable bacterium.*

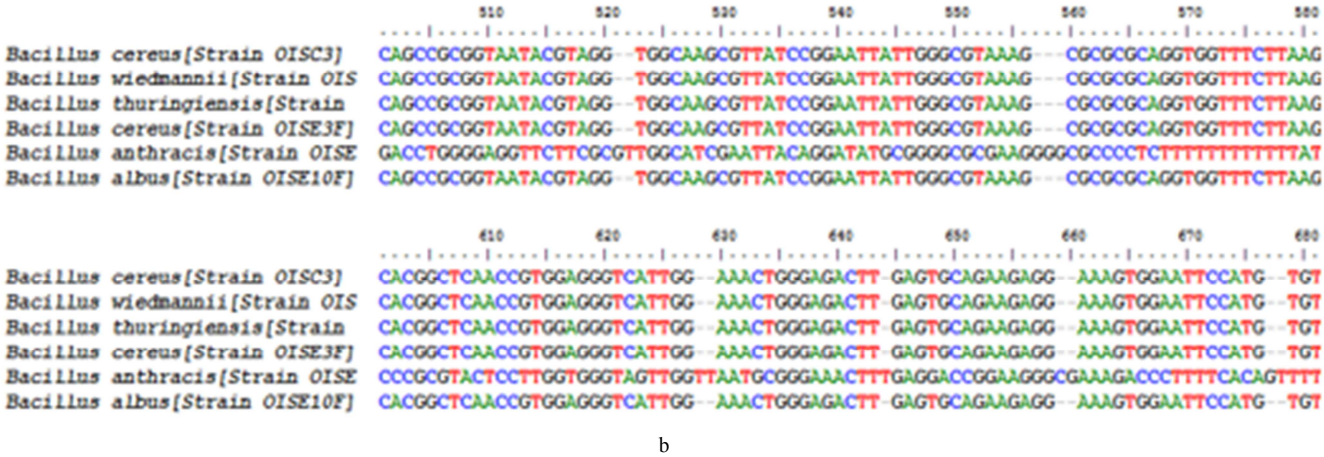
(ii). Multiple Alignment of Isolate Sequences

The multiple alignment of the sequences of the identified strains at the soil level (Figure 6a) and at the pepper level (Figure 6b) shows a high similarity characteristic of the *Bacillus cereus* group. At the level of Figure 6a one can note differences between the sequences of this group and *Pseudomonas sp* particularly at the level of positions 832-833 where the deletion is visible on all the sequences of the group of *Bacillus cereus*, while *Pseudomonas sp* presents a couple GT, this deletion was verified against the reference sequence on GenBank of the two species *Pseudomonas sp* and *Bacillus cereus sensu sticto*.

In Figure 6b, we can observe 521 and 522, a deletion for all the other strains of the *Bacillus cereus* group, while *Bacillus anthracis* carries at this level a couple, GT The same is true for positions 628 and 629, the deletion is observed for all other species of the *Bacillus cereus* group, while *Bacillus anthracis* presents a TT pair.



a



b

Figure 6. *Multiple séquences Alignement of 16S rADN for bacteria belonged to group of Bacillus cereus. Figure 6a for the strains isolated from fermented pepper and Figure 6b for strains isolated from soil.*

(iii). Phylogenetic Inference Test of the Different Isolates

Figure 7 presents the phylogenetic classification of bacteria of the *Bacillus cereus* group isolated from soil and pepper. Strains of bacteria isolated from soil and strains isolated from pepper. Forty sequences in total are presented in the phylogenetic tree, a bacterial diversity with a predominance of bacteria from the *Bacillus cereus* group. The sequences are very close to each other. *Bacillus cereus* group bacteria always form a consistent group with their database counterparts.

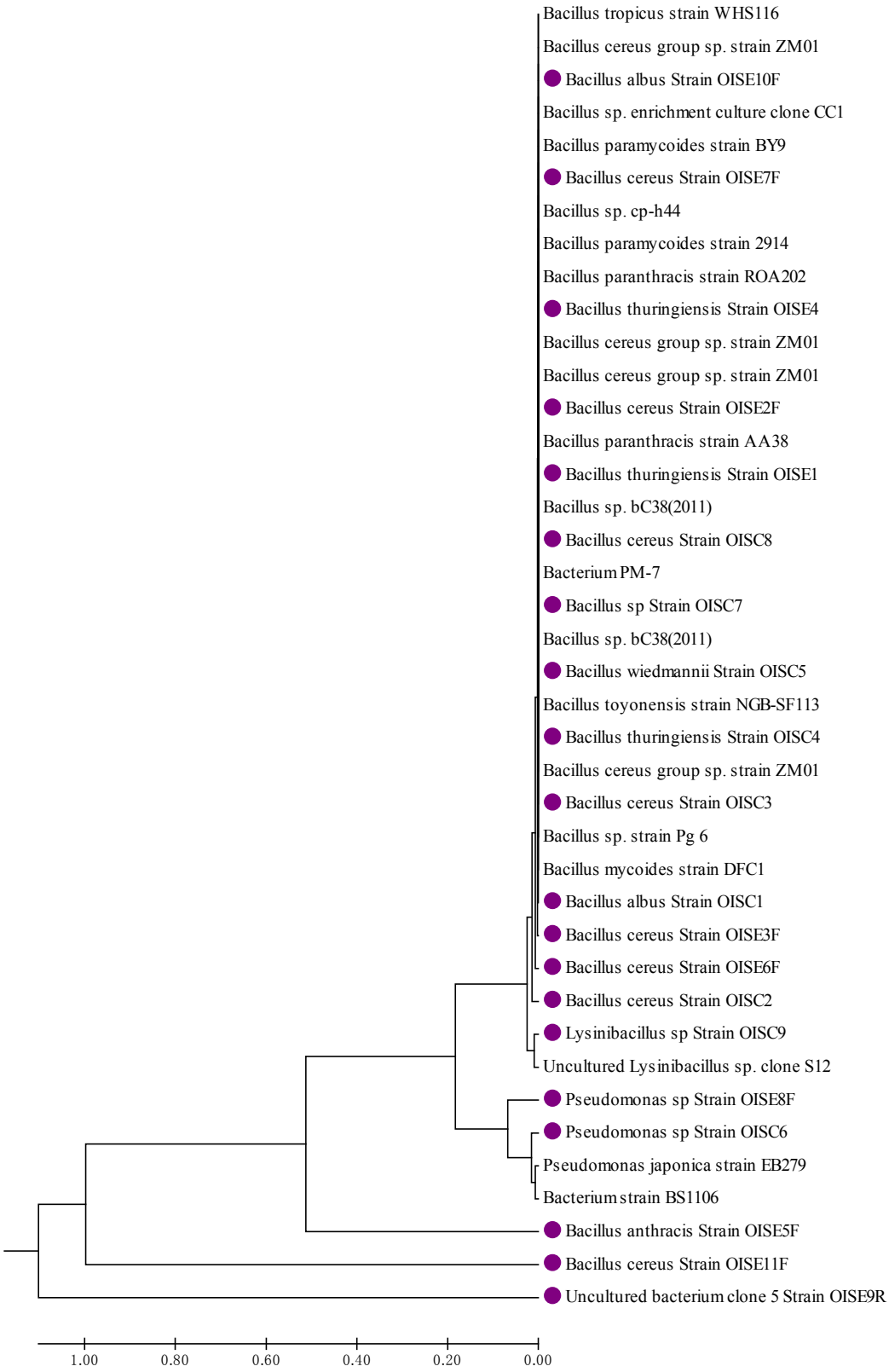


Figure 7. *Phylogenetic tree of isolates in the present study and their homologs. Phylogenetic relationships between all strains for the two environnements soil and fermented pepper are shown. Forty sequences are used, among them twenty sequences are from this study, they have a red round before on the tree. Others (twenty) are homologs from the collection nt in which bélongs GenBank.*

3.4.3. Percentages of Different Isolates Identified by the Analysis of 16S rRNA Gene

Figure 8 shows the diversity of strains isolated from pepper and soil in percentage, this diversity is predominated by bacteria from the *Bacillus cereus* group.

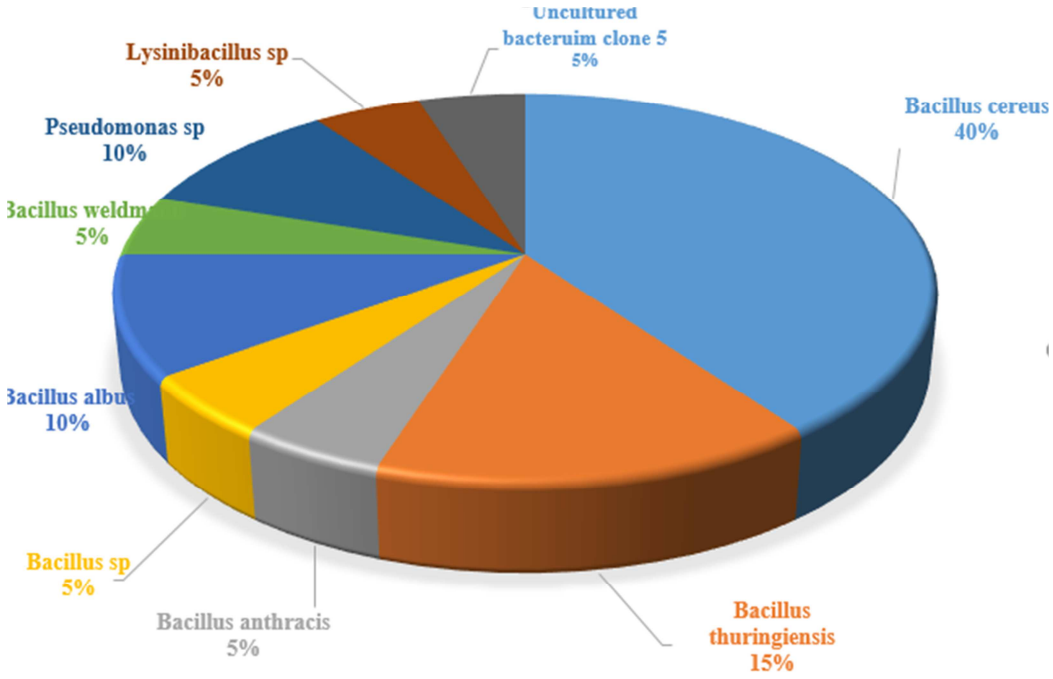


Figure 8. *Representability of different strains from the total identified in this study, including the two environments: soil and fermented peppers.*

4. Discussion

This study aimed to explore the diversity of *Bacillus* group bacteria in two different environments soil and fermented peppers. Conventional microbiology techniques have made it possible to count, isolate and characterize the bacterial isolates. Molecular level of identification and phylogenetic classification were also performed.

The enumeration of microorganisms showed that the soil and the fermented peppers both contain a total aerobic mesophilic flora and also bacteria of the genus Bacillus and many others. If the total flora is greater in the fermented pepper (9.4. CFU/g) than in the soil of 3.7. UFC/g, bacteria of the genus Bacillus are numerically more important in the soil (9.3. 10^4 UFC/g) than in fermented pepper (7.7. UFC/g). Due to the presence of bacteria in the two samples, these results are comparable with those obtained in other works [5, 11, 21]. These three authors respectively worked on fermented peppers, on the soils in Brazzaville and crushed and cooked pumpkin seeds. These authors were also able to isolate bacteria in their samples.

Some works showed that in various soils, the concentration of *B. cereus* reached 6. CFU/g [28, 29].

In the same vein and in an aspect other than the soil. A study has shown *Bacillus cereus* in the rate varying between 1.1. CFU/g and 3.9. CFU/g [30].

Therefore, this bacterial load highlighted in our work, specifically in pepper, using the selective medium of *Bacillus cereus* (Mossel), is likely to promote the occurrence of food poisoning. Some authors state that the emetic and diarrheal syndrome due to Bacillus cereus can occur when the bacterial load in a food reaches 5. to 8.10^4 CFU/g respectively [31]. ingested bacterial load greater than 5.10^4 CFU/g is considered dangerous for the population [32]. But also depending on the conditions of handling, storage of food and cleaning of equipment, the bacterial load can quickly increase and reach a level dangerous for consumption (up to 4. CFU/g).

Then the isolation of the pure colonies carried out at 37°C for 24 hours of incubation on a MYP medium (Mossel), medium supposedly selective for *B. cereus*. 45 mesophilic isolates were obtained, of which 25 were pink and 20 yellow. The presence of polymyxin in the Mossel medium therefore served to inhibit part of the secondary microflora and confer its selectivity on the medium [33].

The pink coloring of the colonies served as an indicator for the identification of *Bacillus cereus* because it was found that the bacteria of the *Bacillus cereus* group do not ferment mannitol, being mannitol negative. Thus favoring the basic character of the phenol red present in the medium with a pH of 8.4. On the other hand, the yellow coloration of the isolates characterizes the fermentation of mannitol, thus the adaptation of the colored indicator to yellow with a pH of 6.6 acidic characters of the colored indicator. Result confirmed by those highlighted during the identification of *Bacillus cereus* in pasteurized milk after incubation of culture media at 30°C for 24 hours [34].

The colonies obtained also presented different morphotypes both for our results and for the aforementioned authors. On the soil sample, our results showed differences both morphological and in terms of molecular identification, because our results do not contain staphylococci as shown in other works [11].

The genomic DNA fragments obtained after DNA extraction have a size greater than 10Kb, these results are also in accordance with those obtained during DNA extraction obtained fragments of identical size [21]. The 16S

rDNA PCR fragments obtained showed a size close to 1500 Kb on 1% Agarose Gel electrophoresis, these results are consistent with those obtained in other works, who working respectively on fermented foods, pumpkin seeds and soils in Brazzaville have during rDNA PCR amplification of bacterial isolates all obtained fragments PCR whose size is close to 1500 Kb by electrophoresis [35, 11, 12]. These results clearly reflect the standard size of the gene coding for the 16S rRNA.

The sequencing of the 16S rDNA amplicons confirmed the size of the fragments at 1500Kb, a bioinformatics analysis by BlastN of our sequences allowed, according to the percentage of Similarity and E. value, to identify all the bacterial isolates, these isolates were assimilated for the soil with bacterial strains all belonging to the *Bacillus cereus group*, a total of six strains divided into five species including two of *Bacillus cereus sensu stricto*, therefore predominant.

A way for us to confirm on the one hand the hypothesis according to which bacteria of the genus Bacillus can be isolated not only from very varied media such as rocks, soils, aquatic environments and the intestines of various insects and animals but also from different food consumption [36]. Starting from this aspect, therefore, make a relief between the phenotypic attractions in relation to the Molecular identification resulting from the amplification of the gene of 16S rRNA.

We had also demonstrated that the genus *Bacillus* is also known for its enzymatic potential. More particularly the production of proteolytic enzymes. The latter was evaluated at 100% for all of our isolates. Demonstrate by the result of the grouping of the diameters of the enzymatic degradation halos of casein generally varying in our work between 1.3 cm and 4.3 cm. Highly expressed enzyme production attributed to isolates Ri15, Ri19, Ri12, JI16 all from pepper and JI8 from soil. We also note a broad expression of the proteolytic potential attributed mostly to isolates from pepper, i.e. 57.75% with diameters greater than 2cm for all isolates, including the average of clear halos evaluated at 3.3 cm higher than those from soil or 42.25% with an average of 3.4cm and having diameters of clear halos less than 2cm for some identified isolates.

We therefore issue a hypothesis explaining the membership of our isolates to endo-peptidases, in accordance with the methodology of casein hydrolysis used in this work, corresponding to the direct use of pure overnight culture without going through a centrifugation, with the aim of highlighting the endo-proteases characterized by a good hydrolysis potential and involved in numerous functionalities in the organism [34]. Result much higher than those obtained in most work [35]. From a slightly more explicit point of view, each species has a temperature at which it degrades a given type of macromolecule [37, 20, 8].

As can be clearly seen in our work, a greater phenotypic specificity of the isolates identified is attributed to isolates from pepper than to those identified using soil. As a result, we can therefore hypothesize that most of the species of the *B. cereus* group have a highly resistant sporiferous character which is the basis of food contamination, in particular pepper contamination and resistance to capsaicin mainly presents in the used pepper.

In the soil, *B. cereus* can potentially contaminated the raw material of the food and be found in all the stages of the manufacture of food more particularly in the production as well as in the setting on sale jars of chilli to market, with a high probability of contaminating it, modifying its taste, thus causing food poisoning by the secretion of enterotoxins and proteolytic enzymes. Result which seems far different because that the diversity of the group of *Bacillus cereus* is very high in the natural isolates which come from the soil [38].

The inferred phylogenetic classification with the strains obtained identified is in harmony with the reference phylogeny, in fact the different strains of the *Bacillus cereus* group form a coherent group with their GenBank counterparts. These strains are very close to each other, it is important to use another housekeeping gene than that of rRNA16S to clearly distinguish at the level of molecular phylogeny the distances that are created between the different strains of the *Bacillus cereus group*. proteolytic enzymes with a fibrinolytic character have been recognized as interesting molecular markers for the phylogenetic classification of bacteria of the genus *Bacillus* [39, 40]. In this work, the proteolytic bacteria of the *Bacillus cereus* group certainly obey this rule and this also helps to explain their affinity.

5. Conclusion

This work has been allowed our group to isolate, characterize and identify molecularly bacteria from to different environments soil and fermented pepper, most of isolates were gram positive, spore forming, proteolytic enzyme producers. Using Mossel medium, we normally found *Bacillus cereus* group bacteria as the predominant group. Based 16S rDNA phylogenetic classification confirmed the coherent monophyletic group of *Bacillus cereus* with many strains of the group. These bacteria will be among the most important contaminants of the peppers; we think that the contamination may come from soil.

Conflict of Interest

Authors declare no conflict of interest.

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