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Full Length Research Paper

Genotypic and phenotypic diversity among *Bacillus* species isolated from Mbuja, a Cameroonian traditional fermented condiment

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Dichotomous keys based on morphological, cultural and biochemical tests have long been used to identify *Bacillus* species. The analysis of 16S rDNA is suggested to be used for identification that is more exact. The present study was carried out to compare a conventional phenotypic method with the analysis of the 16S rRNA and *gyr*B gene for better identification, to determine their phylogenetic relationships and to contribute to selecting starter cultures for Mbuja production. Twenty-six (26) *Bacillus* strains isolated from 12 samples of Mbuja formerly identified by phenotyping as representatives of eight species (*licheniformis*, *polymyxa*, *laterosporus*, *cereus*, *circulans*, *subtilis*, *pumilus* and *brevis*) were studied. Results of genotypic analyses were not concurrent with previous phenotypic identification. *Bacillus* from different species were able to cluster together to form phylogenetic groups. An insight of these groups revealed important genetic diversity between strains from the same species. *Bacillus subtilis* and close relatives were the most abundant and presented appreciable biochemical traits. This group could therefore be considered for starter selection.

Key words: Mbuja, *Bacillus* sp., phenotyping, genotyping, starter.

INTRODUCTION

Mbuja is a traditional condiment produced in Cameroon by fermenting 10 day-old cooked seeds of *Hibiscus sabdariffa* in earthen ware pots. We have earlier showed that Mbuja is of good anti-oxidant quality and could potentially help in maintaining consumer health (Mohamadou et al., 2007). The main bacteria associated with the fermentation process belong to the *Bacillus* genera (Mohamadou et al., 2009). The role of *Bacillus* species in other African condiments has been studied, including the production of Soumbala in West Africa (Ouoba et al., 2003a, b). The ability of *Bacillus* sp. to develop a variety of biochemical activities in the substrate contribute to the development of desirable sensory characteristics of the condiments. Given that biochemical and sensory characteristics of fermented products are a direct consequence of the metabolic activity of the fermenting microflora, the characterization of the most important microorganisms appears to be not only a key for understanding these changes in the seeds but also identify and possibly select optimal starter cultures.

Both phenotypic and molecular typing methods are used to this effect. Phenotypic methods include the use of API 50CHB (Ouoba et al., 2004) and the dichotomic key of Gordon (Cavalcanti et al., 2007; Guerra-Cantera et al., 2005) based on a set of morphological characteristics

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and biochemical tests, including carbohydrates fermentation and enzyme detection of species. However, due to the variety of ecosystems in which the species grow, conventional methods often result to ambiguous identification.

Recently, different techniques have been applied for the typing and identification of bacteria, among which are: amplification of the transcribed intergenic region 16S-23S (ITS-PCR) for differentiating species (Johnson et al., 2000; Ouoba et al., 2010), use of restriction fragment-linked polymorphism analysis of the intergenic transcribed region to differentiate between species and strains (Joung and Coté, 2001), pulse field gel electrophoresis (PFGE) to differentiate strains (Mendo et al., 2000) and 16S rDNA sequencing to describe phylogenic relationship among species (El-Helow, 2001).

In previous studies (Mohamadou et al., 2009), we reported the isolation and identification of 26 *Bacillus* strains using the dichotomous key of Gordon et al. (1973). Eight species were identified: *Bacillus subtilis, Bacillus pumilus, Bacillus brevis, Bacillus polymyxa, Bacillus licheniformis, Bacillus laterosporus, Bacillus cereus* and *Bacillus circulans.* The aim of the present work was to compare identification of *Bacillus* sp. using phenotypic tests and 16S rDNA analysis in order to achieve a complete identification and phylogenetic relationship between isolates and to screen their technological properties for use as starter cultures in controlled fermentations.

MATERIALS AND METHODS

Bacteria

Bacillus strains studied in this work were isolated from 12 samples of Mbuja (fermented *Hibiscus sabdariffa* seeds), purified and identified phenotypically in a previous work by Mohamadou et al. (2009). These strains were stored in liquid nitrogen in the University Laboratory of Biodiversity and Microbial Ecology (EA 3882, IFR 148, Brest, France) before use. They were identified by the dichotomous key of Gordon et al. (1973) as *B. laterosporus* (S8, S9, S11, S14, S15, S17 and S22), *B. pumilus* (S2, S12, S16, S21, and S23), *B. licheniformis* (S4, S6, S7, and S20), *B. brevis* (S3, S19, SX, SY), *B. subtilis* (S1, S13), *B. cereus* (S10 and SAc), *B. circulans* (S18) and *B. polymyxa* (S5).

Genotyping

Extraction of genomic DNA

The total DNA was extracted from pure cultures of cells. Cell lysis was realised by heat shocks (Cavalcanti et al., 2007) with some modifications and ultrasounds. Young cultures were suspended in 300 µl of sterile physiological water (9 g/l of NaCl) and frozen at –22°C for 24 h before being heated to 80°C for 10 min in dry conditions using a ThermoStat plus (Eppendorf). The suspensions obtained were subsequently treated by ultrasounds for 10 min. DNA extracts were checked for purity and concentration using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA).

PCR amplification of the 16S rDNA

Bacterial 16S rDNA were amplified by PCR using bacterial universal primers. Two couples of primers (Table 1) were used: w18/po2 (used for S3, S7, S9, S10, S11, S12, S14, S16, S19, S20, S21, S22, S23, SY and SAc) and PA/PH (S1, S2, S4, S5, S6, S8, S13, S15, S17, S18 and SX). Reactions were carried out in a total volume of 27 µl consisting of 0.5 µM of each primer, 1X *Taq* Titanium™ DNA (BD Biosciences, Clontech Laboratories, Mountain View, USA), 200 µM of each dNTP, 1× of *Taq* Titanium PCR buffer, sterile distilled water and 3 µl of DNA extract (80 ng/µl). After an initial denaturation for 2 min at 94°C, 25 cycles were performed using a PTC-100 Programmable Thermal Controller (MJ Research Inc, Waltham, USA) with the following parameters: 1 min denaturation at 94°C, 1 min elongation at 72°C and 1 min annealing at 55°C. The final elongation was 10 min at 72°C. The amplification products were checked on agarose gels (1% w/v, Promega, Madison, USA) stained with ethidium bromide (0.5 mg/l).

PCR-amplification of gyrB

Specific amplification was performed for presumable *B. cereus* species revealed by phenotypic methods. PCR assays were performed as described above using PCR primer sets designed from the gyrase subunit B gene (*gyr*B) to differentiate between *B. cereus* (BC1 and BC2r), *B. thuringiensis* (BT1 and BT2r) and *B. anthracis* (BA1 and BA2r) (Yamada et al., 1999) (Table 1). Reactions were carried out in a total volume of 20 µl consisting of 1 µl (80 ng/µl) of each *Bacillus* template DNA, 1 µM of each primer, 1× BD TitaniumTM Taq DNA Polymerase (BD Biosciences, Clontech Laboratories, Mountain View, USA), 1× BD Titanium Taq PCR Buffer, and sterile water (7.7 µl). An initial denaturation for 5 min at 95°C was followed by 30 cycles with the following parameters: 1 min denaturation at 94°C, 2.5 min elongation at 72°C and 1.5 min annealing at 58°C. The final elongation was 7 min at 72°C. The amplification was checked on agarose gels (1%, Promega, Madison, USA) stained with ethidium bromide (0.5 mg/l) and bands were visualised under UV (Gel Doc 2000, BIORAD, Hercules, USA).

Sequencing of the 16S rDNA

16S rDNA sequencing was performed on 10 µl of PCR product (40 ng/µl) using the Montage µPCR (EMD Millipore Corporation, Billerica, MA, USA) kit as described by Renault et al. (2007). The reaction of sequence was carried out by a GeneAmp® PCR System 9700 thermocycler (Applied Biosystem, Carlsbad, CA, USA) according to the following conditions: an initial denaturation for 5 min at 96°C; 50 cycles of amplification including a denaturation for 30 s at 96°C, a hybridization for 30 s at 57°C and an extension for 4 min at 60°C; a final extension of 10 min at 72°C. Two couples of primers were used: w18/po2 and PA/PH. Each 5 µl of the reaction volume contained 1 µl of DNA; 1 µl of primer (12 ng/µl); 0.5 µl of Big Dye Terminator V 3.1; 0.75 µl of 5× buffer and 1.75 µl of distilled water. Sequencing was completed on an automatic sequencer, Applied Biosystems 3130xl Genetic Analyser (GIS Ouest Génopole-GENOMER, Roscoff, France).

Sequence analysis and phylogenetic relationships between the strains

Sequence analysis was performed with the sequences in the NCBI database (www.ncbi.nlm.nih.gov/blast) using the Basic Local Alignment Search Tool, BLAST (Camacho et al., 2009). Closest related 16S rDNA sequences, including sequences from type strains for each species, were downloaded from NCBI

Table 1. Sequences and target positions of the primers.

Primer designation	Sequence (5' → 3')	Target (position[1])
w18	GAGTTTGATCMTGGCTCAG	Gene rRNA16S (F9)
po2	GCGTGTGTACAAGACCC	Gene rRNA 16S (R1401)
PA	AGAGTTTGATCCTGGCTCAG	16S rRNAgene
PH	AAGGAGGTCATCCAGCCGCA	16S rRNAgene
BC1	ATTGGTGACACCGATCAAACA	*gyr*B gene
BC2r	TCATACGTATGGATGTTATTC	*gyr*B gene
BT1	ATCGGTGATACAGATAAGACT	*gyr*B gene
BT2r	CCTTCATACGTATGAATATTATTT	*gyr*B gene
BA1	AATCGTAATATTAAACTGACG	*gyr*B gene
BA2r	CCTTCATACGTGTGAATGTTG	*gyr*B gene

[1]The position corresponds to the extremity 5' of the primer in reference to 16S rRNA (Brosius et al., 1981); *PA* and *PH* are designed by Edwards et al. (1989) for 16S rRNA gene. F and R designate respectively forward primer and reverse primer. *BC1* and *BC2r* are specific primers for gyrase B gene amplification in *Bacillus cereus*; *BT1* and *BT2r* are specific for *Bacillus thuringiensis*; *BA1* and *BA2r* are specific for *Bacillus anthracis* (Yamada et al., 1999).

(http://www.ncbi.nlm.nih.gov/) and RDP (http://rdp.cme.msu.edu/hierarchy/hierarchy_browser.jsp)databases to perform phylogenetic analyses. The following strains and the accession numbers of their 16S rDNA were used: *B. subtilis* DSM 15029^T (AF074970), *B. amyloliquefaciens* HR62 (AY055225), *B. megaterium* ATCC 14581^T (JF749282), *B. pumilus* ATCC 7061^T (AY876289), *B. cereus* ATCC 14579^T (AE016877), *B. horikoshii* DSM 8719^T (AB043865), *B. flexus* 86664 (AB021185), *B. altitudinis* 41KF2b^T (AJ831842), *B. safensis* FO-036B^T (AF234854), *B. aryabhattai* 412384 (JN411292), *B. thuringiensis* ATCC 10792^T (GQ911556), *B. weihenstephanensis* DSM 11821^T (AJ841876), *B. mycoides* ATCC 6462^T (AB021192) and *Brevibacillus brevis* 1393 (AB271756). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was calculated according to Felsenstein (1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). Evolutionary analyses were conducted with MEGA5 (Tamura et al., 2011).

Analysis of phenotypic profiles of bacteria

Cultural, morphological and biochemical properties were analyzed for the identification of the 26 *Bacillus* strains by the dichotomous key in the previous work (Mohamadou et al., 2009). Data analyzed included cell size and Gram staining, catalase test, growth in anaerobic conditions, growth at 50 and 65°C and in the presence of 7% NaCl, Voges-Proskauer (V-P) test, fermentation of mannitol, utilisation of citrate, production of acid and gas from glucose, reduction of nitrates, hydrolysis of starch, gelatine, casein and lecithin. These physico-chemical properties were computed in order to determine the major phenotypic clusters and to relate these clusters with phylogenetic clusters obtained by 16S rDNA sequencing.

Statistical analysis

Bacillus strains were profiled based on morphological and physiological characters by Principal Component Analysis (PCA) using StatBox 6.6 software (StatBox logiciels, Grimmersoft, Issy-les-Moulineaux, France).

RESULTS AND DISCUSSION

Identification of *Bacillus* sp. by 16S rDNA sequencing

Total DNA extracted from bacteria culture had acceptable purity (A_{260}/A_{280} comprised between 1.87 and 2.08) with concentrations varying from 80 to 240 ng/µl. Partial 16S rDNA sequences of variable sizes were obtained with the two couples of primers: for sequences amplified with PA/PH (S1, S2, S4, S5, S6, S8, S13, S15, S17, S18 and SX) the size was 332 to 569 bp against 791 to 1351 bp for those obtained with w18 and po2 (S3, S7, S9, S10, S11, S12, S14, S16, S19, S20, S21, S22, S23, SY and SAc).

All 26 partial 16S rDNA sequences were deposited in GenBank database under accession numbers JQ410767 to JQ410792 (Table 2). The BLAST analysis showed good coverage to reference sequences in databases, between 98 and 100%. Five species were identified: *B. subtilis* (S2, S7, S12, S15, S16, S17, S18, S19, S20, S21 and SY), *B. megaterium* (S3, S8, S9, S11 and S14), *B. amyloliquefaciens* (S1, S5, S13 and SX), *B. pumilus* (S4, S6, S22 and S23) and *B. cereus* (S10 and SAc). Compared to the previous identification, a new species profile is drawn from these results (Figure 1).

Differentiation between the species of the *B. cereus* group

Specific amplification of the *gyr*B gene using three sets of primers designed for *B. cereus* group helped in identifying *B. cereus* group. S10 and SAc were amplified by BT1 and BT2r primers and were identified as *B. thuringiensis* (Table 2).

Table 2. Comparison between phenotypic and genotypic identifications of the 26 *Bacillus* strains.

Strain		Identification by 16S rDNA sequencing (similarity)		Phenotypic identification (Mohamadou et al., 2009)
GenBank accession numbers	Sequences lengths	Closest related bacteria accession number	Similarity (%)	
JQ410767	S1 (513 bp)	*B. amyloliquefaciens* 16S ribosomal gene (CP002627)	98	*B. subtilis*
JQ410768	S2 (531 bp)	*B. subtilis* 16S ribosomal RNA gene (HQ848276)	99	*B. pumilus*
JQ410769	S3 (823 bp)	*B. megaterium* 16S ribosomal RNA gene (HQ336301)	99	*B. brevis*
JQ410770	S4 (332 bp)	*B. pumilus* 16S ribosomal RNA gene (EU373436)	99	*B. licheniformis*
JQ410771	S5 (471 bp)	*B. amyloliquefaciens* 16S ribosomal RNA gene (FN386705)	100	*B. polymyxa*
JQ410772	S6 (516 bp)	*B. pumilus* 16S ribosomal RNA gene (JF431426)	99	*B. licheniformis*
JQ410773	S7 (873 bp)	*B. subtilis* 16S ribosomal RNA gene (FN393910)	100	*B. licheniformis*
JQ410774	S8 (568 bp)	*B. megaterium* 16S ribosomal RNA gene (HQ874436)	100	*B. laterosporus*
JQ410775	S9 (915 bp)	*B. megaterium* 16S ribosomal RNA gene (FJ613535)	99	*B. laterosporus*
JQ410776	S10 (1083 bp)	*B. cereus* 16S ribosomal RNA gene (DQ521606)	98	*B. cereus**
JQ410777	S11 (934 bp)	*B. megaterium* 16S ribosomal RNA gene (EU239098)	99	*B. laterosporus*
JQ410778	S12 (791 bp)	*B. subtilis* 16S ribosomal RNA gene (FJ573171)	98	*B. pumilus*
JQ410779	S13 (566 bp)	*B. amyloliquefaciens* 16S ribosomal RNA gene (CP002634)	100	*B. subtilis*
JQ410780	S14 (1351 bp)	*B. megaterium* 16S ribosomal RNA gene (DQ485416)	98	*B. laterosporus*
JQ410781	S15 (567 bp)	*B. subtilis* 16S ribosomal RNA gene (FJ392729)	100	*B. laterosporus*
JQ410782	S16 (1346 bp)	*B. subtilis* 16S ribosomal RNA gene (AY030330)	99	*B. pumilus*
JQ410783	S17 (478 bp)	*B. subtilis* 16S ribosomal RNA gene (HQ848276)	100	*B. laterosporus*
JQ410784	S18 (563 bp)	*B. subtilis* 16S ribosomal RNA gene (HQ848276)	100	*B. circulans*
JQ410785	S19 (849 bp)	*B. subtilis* 16S ribosomal RNA gene (AY030330)	99	*B. brevis*
JQ410786	S20 (1344 bp)	*B. subtilis* 16S ribosomal RNA gene (DQ057582)	99	*B. licheniformis*
JQ410787	S21 (1341 bp)	*B. subtilis* 16S ribosomal RNA gene (AY917141)	99	*B. pumilus*
JQ410788	S22 (1342 bp)	*B. pumilus*16S ribosomal RNA gene (DQ209209)	97	*B. laterosporus*
JQ410789	S23 (1015 bp)	*B. pumilus* 16S ribosomal RNA gene (EU874880)	98	*B. pumilus*
JQ410790	SX (569 bp)	*B. amyloliquefaciens* ribosomal RNA gene (JF346868)	99	*B. brevis*
JQ410791	SY (1345 bp)	*B. subtilis* 16S ribosomal RNA gene (DQ400916)	99	*B. brevis*
JQ410792	SAc (1345 bp) bp)	*B. cereus* 16S ribosomal RNA gene (DQ523499)	99	*B. cereus**

*The DNA was amplified by *Bacillus thuringiensis* gyrB primers.

Phylogenetic diversity among strains

A phylogenetic tree was constructed by the method of Neighbor-Joining with the MEGA 5 program using the alignment of the 229 sequences of the 26 *Bacillus* sequenced in this study and the 14 closest related 16S rDNA sequences available in RDP and NCBI databases (Figure 2).

The resulting tree revealed four groups containing 39 *Bacillus* and 01 *Brevibacillus* species while a single species, *B. horikoshii* remained ungrouped. The largest group (GI) consisted of species identified as *B. subtilis, B. amyloliquefaciens* and *B. pumilus.* This group included the genus type species of *B. pumilus*, *B. subtilis* and *B. safensis.* Group II contained *B. megaterium*, *B. flexus* and *B. aryabhattai.* All the *B. cereus* strains are found in the same cluster (Group III) which included also *B. thuringiensis, B. weihenstephanensis* and *B. mycoides.* The last group relates closely to a strain identified as *B. pumilus* to a reference and sole *Brevibacillus brevis*.

Assessment of phenotypic profiles of the strains

Depending on their morphological, cultural and biochemical properties, the 26 *Bacillus* strains showed three different profiles by PCA analysis. Figure 3A represents the two principal components

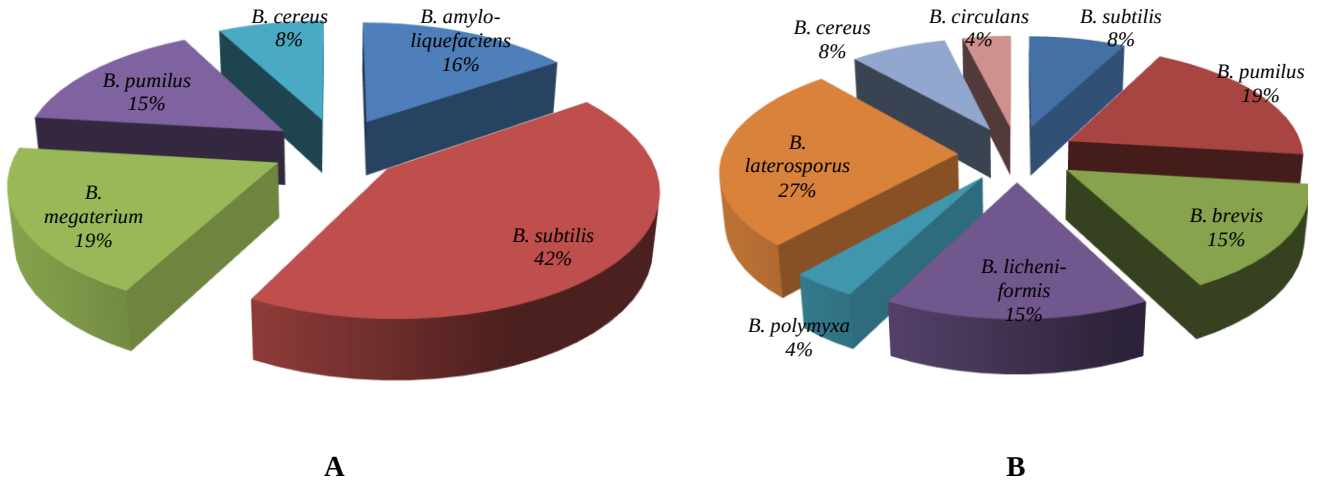

Figure 1. Distribution of the *Bacillus* species identified by 16S rDNA analysis (A) and by Gordon et al. (1973) (B).

components (PC) where PC1 and PC2 accounted for 62% of the total variation. The first axis (34.46%) was positively correlated with aerobic growth, hydrolysis of starch, gelatine, casein, fermentation of glucose, reduction of nitrates and salt tolerance; the second (27.53%) was positively correlated with cell size, use of citrate and pH in V-P medium. PC1 was negatively correlated with fermentation of mannitol while PC2 was negatively correlated with growth in V-P medium and at 50°C. Both axes were negatively correlated with lecithinase production. PC1 reflected biochemical properties (enzymatic activities) while PC2 traduced morphology and growth conditions.

In this study, we reported a new identification using the 16S rDNA and *gyr*B gene analysis, for 26 *Bacillus* strains isolated in *Mbuja* and formerly identified by the dichotomous key of Gordon et al. (1973). The results of the microbial profile described in this paper and previously (Mohamadou et al., 2009) are not fully coincident since genotypic identification resulted in taxa often different from those described by the phenotypic method (Table 2). Hence, *B. subtilis* is the most dominant species isolated in the final products of *Hibiscus sabdariffa* fermented seeds followed by *B. megaterium, B. amyloliquefaciens* and *B. pumilus*. However, the 16S rDNA and the phenotyping fully agreed in *B. cereus* identification. Further, *gyrB* gene analysis revealed that these two strains were more specifically *B. thuringiensis.* An insight of these species genetic relationship through a phylogenetic tree that included closest relatives revealed 4 heterogeneous clusters (Figure 2). Groups I, II, III and IV contain five, three, four and two species, respectively.

Group I clustered *B. subtilis* and four other species. The relatedness of *B. subtilis* with *B. amyloliquefaciens* and *B. pumilus* has been earlier reported by other authors (Priest et al., 1987). These species are known as members of the *subtilis*-group. Recently, phylogenetic studies based on the 16S rDNA gene showed that *B. safensis* clusters with other *Bacillus* species, the nearest neighbour being *B. pumilus* (Satomi et al., 2006). However, the internal branches within this cluster are not supported by a strong bootstrap value (<40%). This result confirms the low ability of 16S rDNA sequencing to resolve between the *subtilis*-group species indicated by Rooney et al. (2009). The PCA analysis of the strains, based on phenotypic properties (Figure 3B), gave quite the same profiles as 16S rDNA. The Group I members are therefore closer to each other by common characteristics such as fermentation of glucose, salt (Figure 3B), suggesting that reliance on these characteristics alone should not be sufficient to differentiate between *B. subtilis* and its close relatives.

The next group (GII) of species was represented by *B. megaterium, B. flexus* and *B. aryabhattai.* Although the strains clustered with *B. megaterium* type strain ATCC 14581^T, the internal branches are also supported by weak bootstrap. Moreover, S3 forms a significant cluster with *B. aryabhattai.* The members of this group share common phenotypic traits: big cells (>10 µm in size) fermenting mannitol, using citrate as carbon source and yielding a pH >6.5 in V-P medium (Cluster A) (Figure 3B).

The third and important group was formed by *B. cereus* which clustered with types strains of *B. cereus, B. thuringiensis, B. weihenstephanensis* and *B. mycoides.* These species are known to be genetically closer to each other and very hard to distinguish on the basis of the 16S rRNA sequences; their genome show high level of similarities and share almost identical 16S rDNA sequences (Ash et al., 1991; Rasko et al., 2005). Further gene analysis to discriminate between the species of *B. cereus* group using specific primers revealed that S10 and SAc were *B. thuringiensis.* However, for some authors, use of *gyr*B in some cases does not absolutely distinguish *B. cereus* from its close tolerance, enzymatic activities (hydrolysis of starch and proteins). As for genotypic grouping, the phenotypic properties clustered

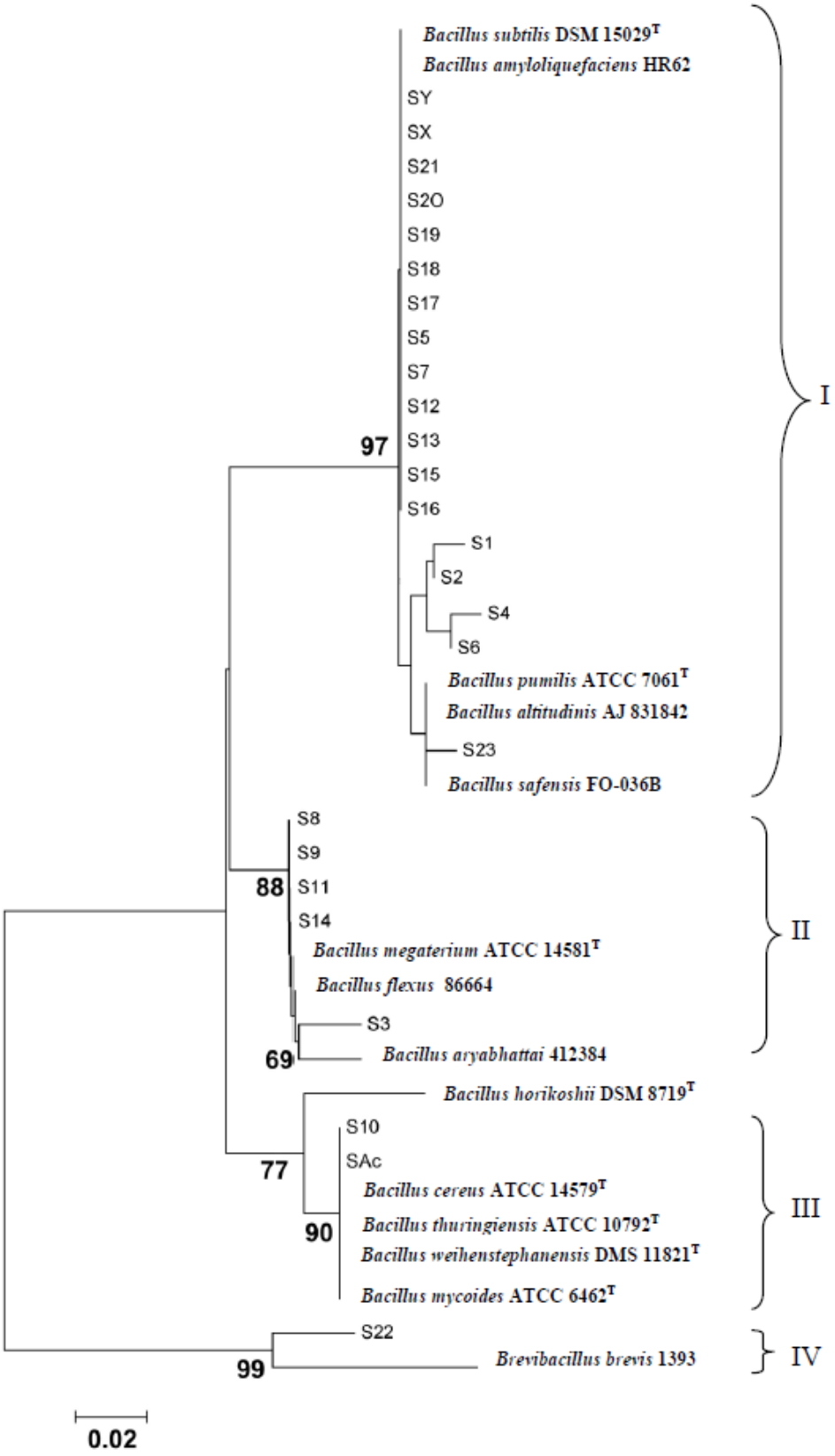

Figure 2. Phylogenetic tree showing distance between sequences of 16S rDNA of *Bacillus* sp. constructed by the method of Neighbor-joining. Values at the main branching points represent the percentage of bootstrap. Only bootstrap values higher than 70 are shown. Taxa followed by a “T” are type strains.

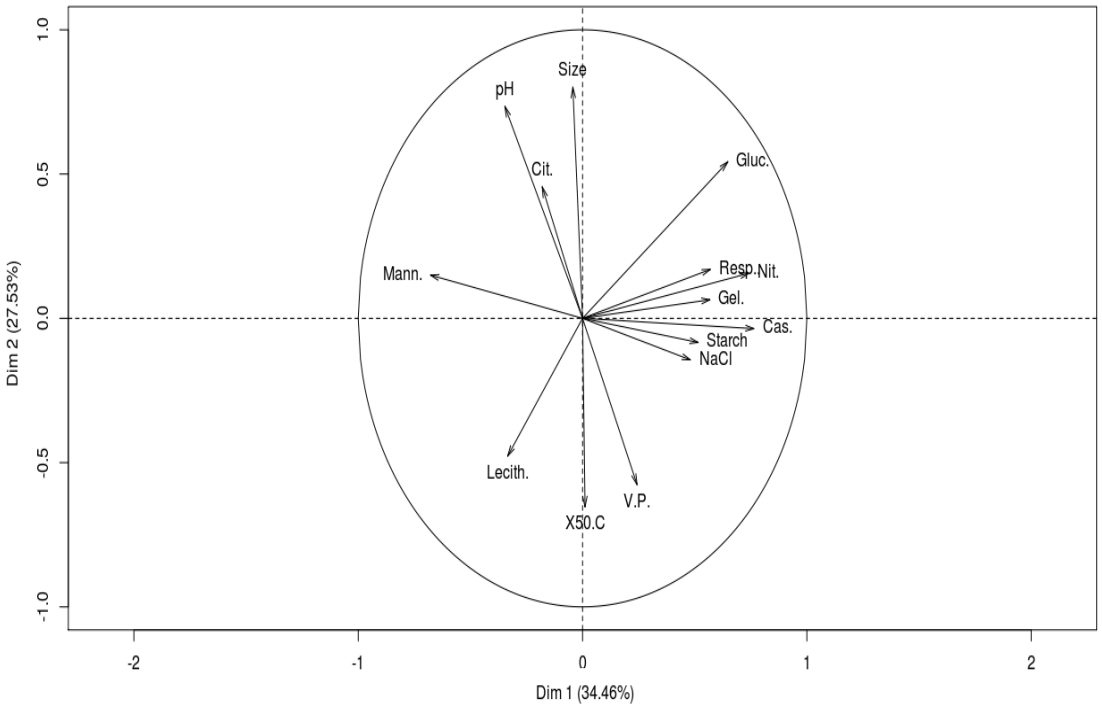

Figure 3A. Variables' representation in the correlation circle. These variables are: cell size (size), growth in V-P medium (V.P.), growth at 7% of NaCl (NaCl), growth at 50°C (X50C), aerobic growth (Resp), fermentation of mannitol (Mann), fermentation of glucose (Gluc), use of citrate (Cit), reduction of nitrates (Nit), hydrolysis of starch (Starch), hydrolysis of casein (Cas) and hydrolysis of gelatine (Gel).

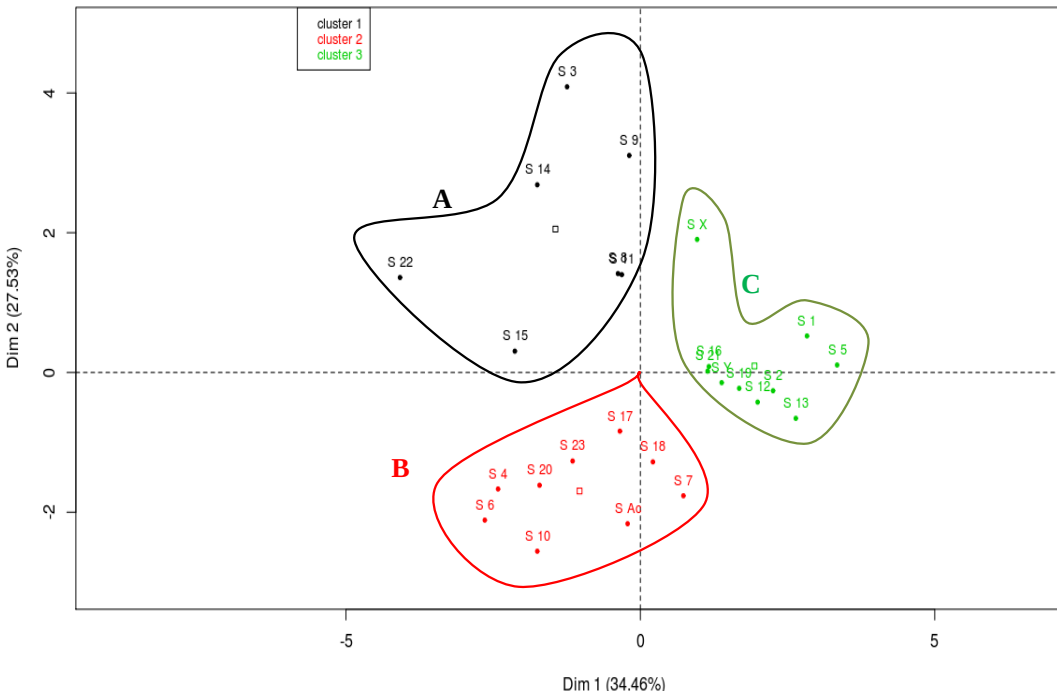

Figure 3B. Principal component analysis profiles based on morphological (cell size), cultural (growth in V-P medium, at 7% of NaCl, at 50°C, aerobic growth) and biochemical (fermentation of mannitol, glucose, use of citrate, reduction of nitrates, and hydrolysis of starch, casein and gelatine) parameters of the strains. Percentage of variation accounted for by each axis is indicated in parentheses. A, B and C are the 3 PCA clusters.

together different species (Cluster C) relatives (Chen and Tsen, 2002) neither do biochemical traits: lecithinase and hemolysis detected in these species.

Most *Bacillus* species identified in this work have also been isolated in other African traditional condiments, including Soumbala and Dawadawa produced by fermentation of *Parkia biglobosa* seeds and Okpehe (Ikenebomeh, 1989; Odunfa, 1985; Ouoba et al., 2004; Amoa-Awua et al., 2006; Oguntoyinbo et al., 2007). Their biochemical properties were mostly proteolysis, starch and lipid hydrolysis, characters associated with the development of desirable flavours (Ouoba et al., 2003a, b). However, the detection of *B. cereus,* a food poisoning, is associated with two toxin mediated types of food poisoning: emetic and diarrheal syndromes (Yamada et al., 1999; Batt, 2004; Acheson, 2009). It is therefore an indicator of products contamination during processing or storage.

The present study has, on the basis of 16S rRNA and *gyr*B gene analysis, provided more accurate identification to *Bacillus* species isolated from Mbuja and identified phenotypically. Phylogenetic analyses clearly indicated the diversity of these species and a certain genetic variability within species representatives. Nevertheless, *B. subtilis* and close relatives were the most important group isolated in Mbuja. Interestingly, members of *B. subtilis* group (including *B. pumilus* and *B. amyloliquefaciens*) share interesting biochemical traits. These technological properties could be positively used in a controlled fermentation process of *Hibiscus sabdariffa* proteinaceous seeds to produce a condiment with high nutritional and organoleptic characteristics. Therefore, starter should be developed through combination of members of the strains from this group.

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