Int. J. Adv. Res. Biol.Sci. 2(4): (2015): 279–290

International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Characterization of *Bacillus* species producing Bacteriocin-like inhibitory substances (BLIS) isolated from fermented food in Burkina Faso

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Abstract

We characterized thirteen strains isolated from indigenous fermented foods (Bikalga and fermented milk) by microbiological and molecular methods. The use of the primer pairs Bsub5F and Bsub3R, Ba1F and Ba1R , allowed to classify strains into two groups: the group of *Bacillus subtilis* (strains Bik4, Lf6 and Bik8) and the group of *Bacillus cereus* (strains Bik1 , Bik2, Bik3 , Bik5, Lf2 , Lf3 , Lf4 , Lf5 , Bik6 , Bik7). The use of the primer pairs LbF and LbR designed for *Lactobacillus* sp. confirm our results because no results were obtained after electrophoresis gel of PCR products. The inhibitory activity of the *Bacillus* strains against *Micrococcus luteus* has been demonstrated by the agar diffusion technique through the wells. The inhibition diameters range from 6.00 mm (lower activity observed for *Bacillus subtilis* Bik8) to 22.00mm (highest activity observed for *Bacillus cereus* Lf4). The antimicrobial substances produced by the thirteen strains from both *Bacillus subtilis* and *Bacillus cereus* group can be assimilate to BLIS while their peptide nature has not been confirmed yet. Thus, the indigenous fermented food from Burkina Faso contain *Bacillus* strains able to produced BLIS active against *Micrococcus luteus*, which can contribute to secure products more.

Keywords: Bikalga, Fermented milk, BLIS, Burkina Faso, *Bacillus subtilis*, *Bacillus cereus*.

Introduction

Bacteria belonging to the genus *Bacillus* have a long and distinguished history in the realms of biotechnology. They were probably first used by the Japanese in the preparation of a traditional fermented food from rice straw and soybean (Claus and Berkeley, 1986; Priest, 1993). They are aerobic, endospore forming, gram-positive, rod-shaped organisms (Combet-Blancet al., 1995; Jenson, 2014), grow aerobically, and usually produce catalase. *Bacillus* are found in diverse environments such as soil and clays, rocks, dusts, aquatic environment, vegetation, food and the gastrointestinal tracts of various insects and animals(Nicholson, 2002).Phylogenetically, bacteria from the genus

Bacillus belong to class I of the phylum *Firmicutes* i.e. bacilli. The genus *Bacillus* includes many species of Gram-positive, rod-shaped bacteria which are able to grow under aerobic and anaerobic conditions (i.e. they are facultative) and thus differ from *Clostridium* spp., which are strictly anaerobic. Several species of *Bacillus* are recognized as human foodborne pathogen. *Bacillus cereus* and *Bacillus anthracis* are well recognized as foodborne, whereas the evidence for the pathogenicity of *Bacillus subtilis* and *Bacillus licheniformis* is less well developed. Illness has been reported due to other species, including *Bacillus pumilus, Bacillus thuringiensis* and *Brevibacillus brevis* (Jenson, 2014). *Bacillus* species are phenotypically and genotypically heterogeneous (Priest, 1993; Slepecky and Hemphill, 2006) and consequently, they exhibit quite diverse physiological properties such as the ability to degrade many different substrate derived from plant and animal sources, including cellulose, starch, proteins, agar, hydrocarbons and also biofuels (Lutz et al., 2006). This diversity in physiological properties, which is reflected by the considerable diversity of *Bacillus* strains, thus allowed these bacteria to colonize a wide variety of ecological habitats (Crielly et al., 1994; Scheldeman et al., 2005; Abriouel et al., 2011; Alvarez-Ordóñez et al., 2014).

Bacillus species are known to produce a wide arsenal of antimicrobial substances, including peptide and lipopeptide antibiotics, and bacteriocins (Stein, 2005; Abriouel et al., 2011).The production of antimicrobial substances and sporulation capacity confer *Bacillus* strains with a double advantage in terms of their survival in different habitats. The presence of *Bacillus* species on food does not always imply spoilage or food poising, because some species or strains are even used as for example, *Bacillus subtilis* strains used in Natto production(East Asian fermented food)(Hosoi and Kiuchi, 2003). Furthermore, specific *Bacillus subtilis* strains are also used as a starter culture for fermenting soybeans into a traditional West African condiment dawadawa (Terlabie et al., 2006) or for fermenting African mesquite seeds in the production of the Nigeria food condiment okpehe (Oguntoyinbo et al., 2007). This ability to survive and grow in such different ecosystems (soil, clays, rocks, dusts, aquatic environment, vegetation, food, gastrointestinal tracts of various insects and animals) is based on the production of their robust endospores, their diversity in physiological properties and their growth requirements(Abriouel et al., 2011).

During the last decade, several works were dedicated to the characterization of the micro flora of bikalga (Dakwa et al., 2005; Azokpota et al., 2006; Jeyaram et al., 2008;Ouoba et al., 2008; Parkouda et al., 2009; Mo et al., 2010; Oguntoyinbo et al., 2010). These studies demonstrated the prevalence of *Bacillus* species (*B.subtilis, B. coagulans, B. amyloliquefaciens, B. faciens, B. pumilis, B. cereus, B. thuringiensis, B. brevis* and *B. licheniformis*) in the fermentation process and their role in the bioconversion of the products. Bikalga is condiment produced by traditional uncontrolled alkaline fermentation of *Hibiscus sabdariffa*. This food additive is used as major condiments in many African countries(Savadogo et al., 2011).Bikalga is an excellent source of protein with essential amino acids and also contain lipids, carbohydrates, essential fatty acids and vitamins(Ouoba et al., 2003; Yagoub et al., 2004). Many families often use Bikalga as a meat substitute. These type of condiments improve nutritional values of foods as well as sensory properties and as taste enhancer (Savadogo et al., 2011).The traditional process of preparation of the Bikalga consists in boiling the seeds of *H. sabdariffa* for about 10 hours before washing and placing them in a container tightly closed for spontaneous fermentation of 72 hours(Ouoba et al., 2008). In this study we identified and characterized *Bacillus* strains that produce BLIS for furthermore applications using microbiological and molecular methods.

Materials and Methods

Biological material and study area

Thirteen strains were isolated from Bikalga (Bik1, Bik2, Bik3, Bik4, Bik5, Bik6, Bik7 and Bik8), and fermented milk (Lf2, Lf3, Lf4, Lf5 and Lf6). The strains were stored at +4°C in brain heart infusion broth supplemented with 15% of glycerol. This study were conducted from May 2013 to July 2014 at Laboratoire de Biotechnologie et de Microbiologie of Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles in Unité de Formation et de Recherche en Sciences de la Vie et de la Terre of Université de Ouagadougou (CRSBAN/UFR-SVT) and Laboratoire de Biologie Moléculaire of Centre

National de Recherche et de Formation sur le Paludisme (CNRFP)/Ouagadougou.

Strains isolation and purification

Thirteen strains were inoculated by streaking tight on Nutrient Agar (Liofilchem, Italy) and incubated at 37°C for 24 hours. Each isolated colony was reinoculated in Trypticase Soya Broth (TSB) and incubated for 24 hours at 37°C.Pure cultures were obtained by repeated streaking of isolated colonies on Trypticase Soya Agar (TSA) medium. One to five colonies of each strain were cultured in Brain Heart Infusion supplemented with 15% of Glycerol for 24 hours at 37°C and conserved at +4°C.

Physiological and biochemical characterization

Gram staining, oxidase activity(Kovacs, 1956), catalase activity, production of acetoin (VP) (Clark and Lubs, 1915), sporulation, nitrite reduction, urea, gelatin and esculin reactions were carried out according to the routine procedure, and cell morphology was examined by light microscopy.
Gas and H_2S production from glucose was performed using Kliger Iron Agar (Liofilchem, Italy). Carbohydrate (glucose, lactose, sucrose, and mannitol) fermentation patterns were determined using API20E gallery (API BioMerieux, France) following the manufacturer's instructions.

Antimicrobial activity test

The antimicrobial activity was determined by agar well diffusion assay (AWDA)(Khay et al., 2011) using cell-free culture supernatants (CFS). Bacteria's strains were grown in Trypticase Soya Broth (TSB) at 37°C for 16 hours, and the cultures were centrifuged at 6,000 rpm for 15 min at room temperature. pH of each CFS was adjusted to pH6.5 with 10N NaOH. 50µl of CFS was placed into well (6mm) of agar plates that were previously seeded with 5ml of indicator microorganism, *Micrococcus luteus* (10^6 UFC/ml). The plates were let on the bench for one hour for absorption, and then were incubated anaerobically at 37°C. After 24 hours, each plate were subsequently examined for inhibition zones (presence of halo or clear zone around well). Inhibition was recorded as negative if no halo or clear zone was observed around the well. The diameter of each halo displayed was measured using a ruler.

Genomic DNA extraction

Genomic DNAs from each isolates were prepared by using the QiAmp DNA Mini Kit (QiAgen, France) according to the manufacturer's instructions.

Identification at genus level

PCR mixture consisted of 8 µl of H_2O, 12.5µl of Master Mix (Fermentas GmbH, St Leon, Rot, Germany), 1.5µl of primer B-K1/F (5'-TCACCAAGGCRACGATGCG – 3'), 1.5 µl of primer B-K1/R (5'-CGTATTCACCGCGGCATG-3') at a final concentration of 20µM and 1.5µl of extracted DNA of each strain. The primers pairs B-K1/F and B-K1/R are designed for *Bacillus* sp. (Wu et al., 2006; Savadogo et al., 2011). *Bacillus subtilis* ATCC6633 and *Bacillus licheniformis* ATCC14850 were used as positive control. The primer pairs LbF (5'-GGAATCTTCCACAATGGACG-3') and LbR (5'-CGCTTTACGCCCAATAAATCCGG-3') were used to see if our strains belong to *Lactobacillus* genus(Bakar et al., 2010).

Amplification consisted of 30 PCR cycles in a Thermocycler (DNA thermal cycle TC-412, United Kingdom). The cycling program was: initial denaturation at 94°C for 5min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C (for primers B-K1/F and B-K1/R)/60°C (for primers LbF and LbR) for 45 seconds and elongation at 72°C. The PCR was ended with a final extension at 72°C for 10 min and the amplified product cooled at 4°C.

Identification at species level

Two (2) different primers pairs were used to classify our strains to *Bacillus subtilis* group (primer pair Bsub5F and Bsub3R) or *Bacillus cereus* group(Ba1F and Ba1R) according respectively to(Wattiau et al., 2001; Savadogo et al., 2011) and(Chang et al., 2003; Savadogo et al., 2011). PCR mixture consisted of 8 µl of H_2O, 12.5µl of Master Mix (Fermentas GmbH, St Leon, Rot, Germany), 1.5µl of each primer (20µM)(Bsub5F: 5'-AAGTCGAGCGGACAGATGG-3', Bsub3R: 5'- CCAGTTTCCAATGACCCTCCCC-3', Ba1F: 5'- TGCAACTGTATTAGCACAAGCT-3', Ba1R: 5'- TACCACGAAGTTTGTTCACTACT-3') and 1.5µl of extracted DNA of each strains. Amplification consisted of 30 PCR cycles in a Thermocycler (DNA thermal cycle TC-412, United

Kingdom). The cycling program was: initial denaturation at 94°C for 5min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 45 seconds and elongation at 72°C. The PCR was ended with a final extension at 72°C for 10 min and the amplified product cooled at 4°C.

Screening for Bacteriocins genes

DNA amplification was conducted in a DNA thermal cycler TC-412 (Serial No.: 137370-2, United Kingdom). The primers pairs BacF (5'-AAGAGTTTGATCCTGGCTCAG-3') and BacR (5'-CTACGGCTACCTTGTTACGA-3')(Diop et al., 2008) were used. PCR mixture consisted of 8 µl of H_2O, 12.5µl of Master Mix (Fermentas GmbH, St Leon, Rot, Germany), 1.5µl of each primer (20µM) and 1.5µl of DNA template. The cycling program was: initial denaturation at 94°C for 5min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 45 seconds and elongation at 72°C. The PCR was ended with a final extension at 72°C for 10 min and the amplified product cooled at 4°C.

Electrophoresis

The DNA fragments were separated by loading 10 µl of each PCR product with 2 µl of loading buffer to 1.5% Agarose gel (Eurogenetec) containing 2% of RedSafe (Intron Biotechnology). Hypperladder 50 bp and Hypperladder100bp (Bioline, USA) were DNA molecular markers used as standard for the determination of fragment length. The Agarose gel was run in Tris Borate EDTA (TBE 0.5X) buffer for 60 min under 120V, 50mA and photographed under ultraviolet light illumination.

Results and Discussion

Strains characterization

All the 13 isolates were found to be Gram-positive, and gave positive test for catalase and nitrate reduction whereas the strains Bik4, Bik5, Lf2 and Lf5 respond positively for urease (Table1). The cells form are rod-shaped, with pairs (strains Lf4) and chains arrangement. No strains produced gas or hydrogen sulfide (H_2S) from glucose (Table1). The 13 strains were motile and are spore forming bacterium. The feature that distinguishes bacilli taxonomically is the formation of dormant structures, formed from within the bacterial cell, called endospores. These dormant structures are significant in food microbiology because they are resistant to heat and to desiccation (Jenson, 2014). Under suitable conditions, endospores will germinate and the resultant vegetative cells will grow and reproduce by binary fission. The life cycle and survival of bacilli are dependent on their gram-positive cell wall structure and ability to form endospores (Jenson, 2014). Spore formation in *Bacillus* takes place when the cell culture reaches the stationary growth phase. Sporulation may be induced by nutritional deprivation, or cell density and is affected by numerous factors, such as temperature, pH, aeration, and availability of various nutrients(Jenson, 2014).

Only two (2) strains (Bik1and Bik2) are not able to produce acetoin (Table1). Acetoin is a product of fermented metabolism in many prokaryotic and eukaryotic microorganisms and also in *Bacillus* spp.(Nakano et al., 1997; Huang et al., 1999). Acetoin is one of intermediate compound produced by bacteria, responsible of flavor and aroma found in fermented food as Bikalga.

The color of Kliger Iron Agar slant was turned from red to yellow, which indicated that all strains were able to ferment the sugar glucose. The strains Bik1 and Lf4 were not able to use the sugar sucrose.

Antibacterial activities

All strains were checked about their antibacterial activities against *Micrococcus luteus*. All strains inhibited the growth of *Micrococcus luteus*. The diameter of inhibition of antibacterial substances produced by *strains* against *Micrococcus luteus* is listed in Table 2.

Bacillus species are known to produce a wide arsenal of antimicrobial substances, including peptide and lipopeptide antibiotics, and bacteriocins (Abriouel et al., 2011). Clear zone was found around each well filled with CFS (Table2). The diameter of clear zone ranged from 6.00 ±0.00mm (lower activity observed for *Bacillus cereus* strains Bik8) to 22.00 ± 4.82 mm (higher activity observed for *Bacillu scereus* strain Lf4). The production of antibacterial substances and sporulation capacity confer *Bacillus* strains with a double advantage in terms of their survival in different habitats.

Table 1:Biochemical, morphology and physiological characters of selected strains

Tests performed	Strains												
	Bik1	**Bik2**	**Bik3**	**Bik4**	**Bik5**	**Bik6**	**Bik7**	**Bik8**	**Lf2**	**Lf3**	**Lf4**	**Lf5**	**Lf6**
Cell form	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cellular arrangement	Chains	Chains	Chains	Chains	Chains	Chains	Chains	Chains	Chains	Chains	Pairs	Chains	Chains
Motile	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-
Spore-forming	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram	+	+	+	+	+	+	+	+	+	+	+	+	+
Gas and H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-
NO_2	+	+	+	+	+	+	+	+	+	+	+	+	+
Urea	-	-	-	+	+	-	-	-	+	-	-	+	-
Acetoin (VP)	-	-	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	-	-	+	+	+	+	+	+
Esculin	+	+	+	+	+	-	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	+	+	+	+	+	+	+	+	+	-	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	-
Presumptive identification	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.

+: Positive reaction -: Negative reaction

Table 2: Diameter of inhibition (mm) of the *Bacillus* strains.

Strains	diameter (mm)
Bik1	12.00 ± 2.65
Bik2	12.33 ± 2.52
Bik3	11.50 ± 0.00
Bik4	10.00 ± 1.80
Bik5	13.28 ± 0.78
Bik6	8. 33 ± 0.58
Bik7	9.33 ± 2.31
Bik8	6.00 ± 0.00
Lf2	11.00 ± 1.41
Lf3	14.17 ± 2.12
Lf4	22.00 ± 4.82
Lf5	11.50 ± 1.41
Lf6	12.67 ± 0.58

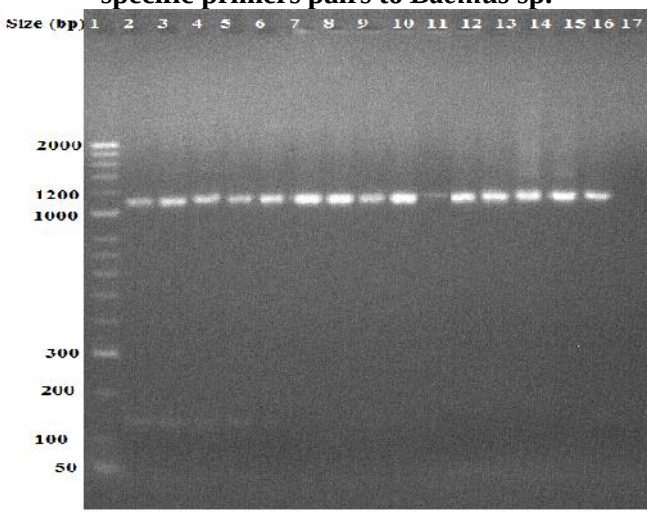
The well diameter (6mm) is included

The addition of 10N NaOH to adjust the pH of the CFS to value of 6.5 had allowed us to work with a supernatant of proteinaceous nature, such as bacteriocins, lipopeptide antibiotics. Antibacterial peptides produced by ribosomal synthesis are commonly referred to as bacteriocins, and these are a heterologous groups of proteinaceous antibacterial substances that are produced by bacteria from every major lineage (Riley and Wertz, 2002a, b;Abriouel et al., 2011). They display a high degree of target specificity against related bacteria, although many have a large or wider spectrum of activity (Jack et al., 1995; Cotter et al., 2005). Many other antibacterial substances that were not well characterized are known as bacteriocin-like inhibitory substances (BLIS). This is often used when the peptide nature of the antimicrobial compound has not been confirmed(Abriouel et al., 2011). Thus the antimicrobial substances produced by the thirteen strains characterized as *Bacillus subtilis* or *Bacillus cereus* (Table1-2; Figure 1-4) can be assimilate as BLIS. Bacteriocins and BLIS produced by the *Bacillus* genus sensu lato may be considered as the second most important after bacteriocins produced by the lactic acid bacteria (LAB). Several bacteriocins or BLIS produced by *Bacillus* species fall within class II of LAB which includes the pediocin-like bacteriocins (class IIa) and the two-peptide bacteriocins (class IIb) (Klaenhammer, 1993; Drider et al., 2006; Nes et al., 2007). Some bacteriocins produced by *Bacillus* sp. belong to the group of Lantibiotics(Willey and van der Donk, 2007; Asaduzzaman and Sonomoto, 2009; Bierbaum and Sahl, 2009). Lantibiotics are ribosomally synthesized as precursor peptides. They are post-translationally modified by the degradation of serine and threonine residues and subsequent intramolecular addition to cysteine, resulting in the formation of (β-methyl) lanthioninethioether bridges, the characteristics structural elements for lantibiotics(Chatterjee et al., 2005; Willey and van der Donk, 2007; Bierbaum and Sahl, 2009). In the case of *Bacillus*, it is also important to corroborate the ribosomal synthesis of true bacteriocins, because this group of bacteria is prolific in the production of antimicrobial peptide (or lipopeptides) by nonribosomal synthesis (such as the utirins and others). Several works have been reported that *Bacillus* sp. can produce bacteriocins/BLIS active against *Micrococcus luteus* (Lee et al., 2001; Saleem et al., 2009; Savadogo et al., 2011)

Strains Identification

Genomic DNA of the thirteen strains were isolated and tested by PCR using specific *Bacillus* sp. primer pairs for their identification. The PCR allowed typing mainly at genus level by using primer pairs B-K1/5F and B-K1/5R and one (1) group of bacteria was obtained (Figure1). The group was characterized by one constant DNA band and comprised the reference isolates *Bacillus subtilis* ATC6633 and *Bacillus licheniformis* ATC14580 (Figure1). The bands

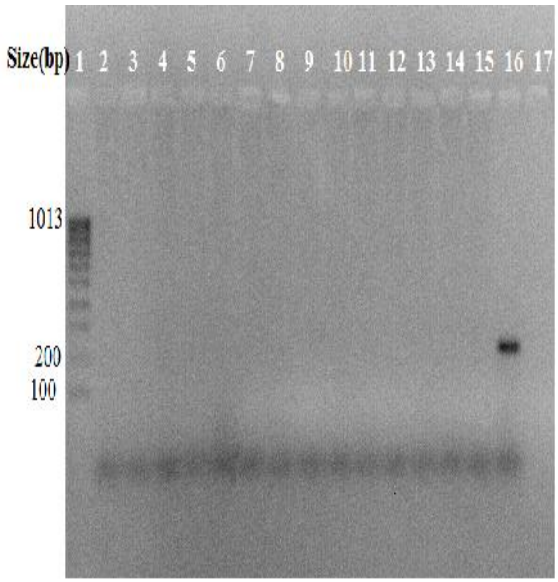
Figure 1:Identification of all strains by PCR at genus level using B-K1/5F and B-K1/3R specific primers pairs to *Bacillus* sp.



Lane 1: Molecular weight marker; Lane 2: Strain Bik1 ; Lane 3: Strain Bik2; Lane 4: Strain Bik3; Lane 5: Strain Bik4; Lane 6: Strain Bik5; Lane 7: Strain Lf2; Lane 8: Strain Lf3; Lane 9: Strain Lf4; Lane 10: Strain Lf5; Lane 11: Strain Lf6; Lane 12: Strain Bik6; Lane 13: Strain Bik7; Lane 14 : Strain Bik8; Lane 15 : *Bacillus subtilis* ATCC6633; Lane 16 : *Bacillus licheniformis* ATCC14850; Lane 17 : Negative control; bp: bases pairs

observed were 1000 to 1200 bp of length. Those primer pairs have been used by (Wu et al., 2006; Savadogoet al., 2011) to characterize their isolates at *Bacillus* genus level. They obtained bands of 1114 bp. No positive results were obtained when using specific primer LbF and LbR designed for *Lactobacillus* sp. No bands were obtained in gel agarose except for positive control (Figure2). Those resultats indicate that all the strains used in this study with bacillary form are not belonging to *Lactobacillus* genus.Thus those results allow us to classify our strains into *Bacillus* sp.

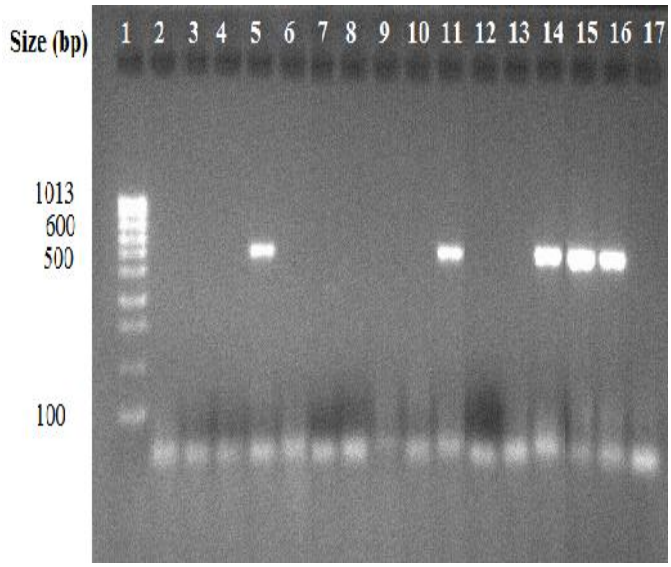
Figure 2: PCR profiles of *Bacillus* strains using specific primer pairs (LbF and LbR) to *Lactobacillus* sp.



Lane 1: Molecular weight marker; Lane 2: Strain Bik1; Lane 3: Strain Bik2; Lane 4: Strain Bik3; Lane 5: Strain Bik4; Lane 6: Strain Bik5; Lane 7: Strain Lf2; Lane 8: Strain Lf3; Lane 9: Strain Lf4; Lane 10: Strain Lf5; Lane 11: Strain Lf6; Lane 12: Strain Bik6; Lane 13: Strain Bik7; Lane 14 : Strain Bik8; Lane 16: *Lactobacillus* sp. as positive control; Lane 17: Negative control; bp: bases pairs

Using the Bsub5F and Bsub3R primer pairs, the strains Bik4, Lf6 and Bik8 could be classified in the *Bacillus subtilis* group (*Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. pumilus*) according to results found (Figure3). The primer pairs Bsub5F and Bsub3R are designed for *Bacillus subtilis* group. Only three (3) bands (corresponding to three strains) were observed after migration on agarose gel (Figure 3). The size of bands obtained was almost 600 bp. The same size was obtained by (Wattiau et al., 2001; Savadogo et al., 2011). These results confirmed that the strains Bik4, Lf6 and Bik8 belong to *Bacillus* genus, especially to the group of *Bacillus subtilis* (*Bacillus subtilis* or *Bacillus licheniformis* or *Bacillus amyloliquefaciens* or *Bacillus pumilus)* (Wattiau et al., 2001; Savadogo et al., 2011). The intensity of bands indicate that strains Bik8 are *Bacillus licheniformis* compared to positive control (*Bacillus licheniformis* ATCC14580), and strains Bik4 and Lf6 are *Bacillus subtilis* compare with positive control (*Bacillus subtilis* ATCC6633).

Figure 3: PCR profiles of *Bacillus* strains using specific primer pairs (Bsub5F and Bsub3R) to *Bacillus subtilis* group.



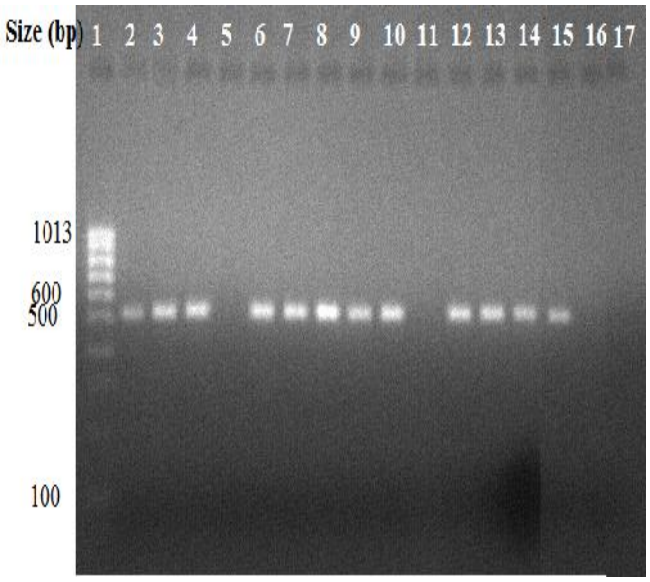
Lane 1: Molecular weight marker; Lane 2: Strain Bik1 ; Lane 3: Strain Bik2; Lane 4: Strain Bik3; Lane 5: Strain Bik4; Lane 6: Strain Bik5; Lane 7: Strain Lf2; Lane 8: Strain Lf3; Lane 9: Strain Lf4; Lane 10: Strain Lf5; Lane 11: Strain Lf6; Lane 12: Strain Bik6; Lane 13: Strain Bik7; Lane 14 : Strain Bik8; Lane 15 : *Bacillus subtilis* ATCC6633; Lane 16 : *Bacillus licheniformis* ATCC14850; Lane 17 : Negative control; bp: bases pairs

The result obtained by using primer pairs Ba1F and Ba1R showed that the strains Bik1, Bik2, Bik3, Bik5, Bik6, Bik7, Lf2, Lf3, Lf4 and Lf5 are*Bacillus cereus* (Figure 4). Ba1F and Ba1R primer pairs were designed to target groEL gene from *Bacillus cereus*. The tested strains respond positively by giving one band which was almost 500 bp as obtained by(Chang et al., 2003; Savadogo et al., 2011).

Detection of bacteriocins genes

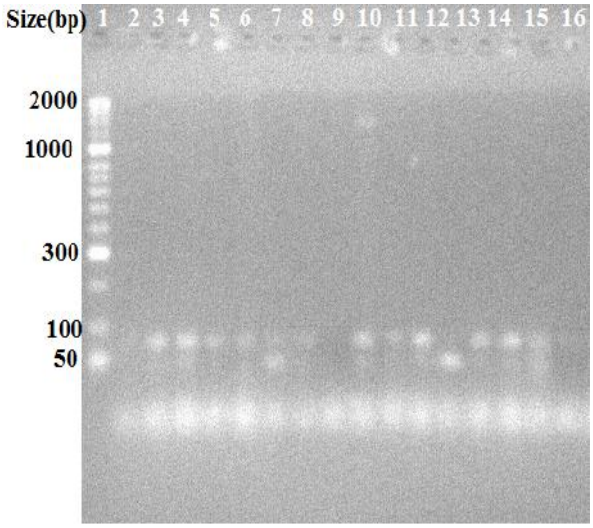
All the thirteen strains were amplified with specific primer pairs BacF and BacR designed to target 16S rDNA gene of bacteriocins(Diop et al., 2008)**.** The running electrophoresis revelated that only *Bacillus subtilis* strain Bik1 and *Bacillus cereus* strain Lf4gave a negative result (Figure5). The eleven bands obtained have size ranging from 50 to 100 bp.Those results indicate that the eleven strains can produce antimicrobial compounds which can be assimilated to bacteriocins/BLIS because they have bacteriocins genes.

Figure 4:PCR profiles of *Bacillus* strains using specific primer pairs (Ba1F and Ba1R) to *Bacillus cereus*.



Lane 1: Molecular weight marker; Lane 2: Strain Bik1 ; Lane 3: Strain Bik2; Lane 4: Strain Bik3; Lane 5: Strain Bik4; Lane 6: Strain Bik5; Lane 7: Strain Lf2; Lane 8: Strain Lf3; Lane 9: Strain Lf4; Lane 10: Strain Lf5; Lane 11: Strain Lf6; Lane 12: Strain Bik6; Lane 13: Strain Bik7; Lane 14 - 15: *Bacillus cereus* LMG 168 as positive control; Lane 16: Strain Bik8; Lane 17: Negative control; bp: bases pairs

Figure 5 :Detection of bacteriocins genes from all *Bacillus* strains using primers pairs Bac1 and Bac2



Lane 1: Molecular weight marker; Lane 2: Strain Bik1; Lane 3: Strain Bik2; Lane 4: Strain Bik3; Lane 5: Strain Bik4; Lane 6: Strain Bik5; Lane 7: Strain Lf2; Lane 8: Strain Lf3; Lane 9: Strain Lf4; Lane 10: Strain Lf5; Lane 11: Strain Lf6; Lane 12: Strain Bik6; Lane 13: Strain Bik7; Lane 14 : Strain Bik8; Lane 15: *Lactobacillus* sp. as positive control; Lane 16: Negative control; bp: bases pairs

Conclusion

In this study we characterize thirteen strains with bacillary form. Three (3) strains (Bik4, Lf6 and Bik8) are *Bacillus subtilis* and ten are *Bacillus cereus* according to characteristics tests. All strains can produce substances with antibacterial activities and can be assimilate as BLIS. These antibacterial substances are active against *Micrococcus luteus*. It would be interesting to continue the study seeking to determine the nucleotide sequence of the BLIS producing strains and also determine the nature of bioactive substances / BLIS produced by these strains of *Bacillus* (Bacteriocins or NRPS) using molecular methods.

Acknowledgments

The authors wish to thank Bakary TARNAGDA for his excellent assistance when performing biochemical and physiological characterization and antimicrobial activity of the isolates.

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