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Full Length Research Paper

Isolation and characterisation of new spore-forming lactic acid bacteria with prospects of use in food fermentations and probiotic preparations

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Five spore-forming bacteria producer of lactic acid were isolated from soils sampled in the vicinity of poultry farms in Burkina Faso. All isolates were Gram-positive, motile, mesophilic, facultative anaerobic, catalase positive rods, and with L(+) lactic acid production. The isolates have been characterized and identified by a polyphasic approach, combining various phenotypic and genetic characteristics. The 16S-rDNA-sequence analyses revealed the membership of two isolates to the genus *Bacillus* and the three other to the genus *Paenibacillus.* The physiological and biochemical analyses showed that the isolates were quite different from known spore forming lactic acid bacteria. Several relevant technological properties were observed, particularly the resistance of the isolates to bile salts and acidic conditions, even the productions of amylolytic and proteolytic enzymes, which could make them good candidates for certain technological applications such as food fermentations and probiotic formulations. Furthermore, the isolation of these microorganisms in the vicinity of farms reinforces the feasibility of their involvement in animal feedstuffs preparations. In conclusion, this work shows an important diversity within the spore-forming lactic acid bacteria and confirms the conclusions of previous works, which have already shown that the SFLAB (Spore Forming Lactic Acid Bacteria) were good candidates for food fermentation and the probiotic formulations.

Key words: Lactic acid, spore-forming bacteria, biodiversity, bacillus, 16S rDNA.

INTRODUCTION

Lactic acid bacteria are the most used microbial groups in fermentation industries (Harlander, 1989). Lactic acid bacteria are involved in several aspects of human life, from food processing to health care (Mombelli and Gismondo, 2000; Coleman et al., 2003). The anti-bacterial properties of lactic acid produced by this bacteria are exploited *in vitro* for food preservation (Mellefont and Ross, 2007) and *in vivo* through probiotic formulations (Saulnier et al., 2009). In the lactic acid bacteria group the genus *Lactobacillus* is the best known and the most used in food fermentations (Collins et al, 1998; Gomez and Malcata, 1999; Kandler and Weiss, 1986). However, the majority of the species belonging to this genus have specific nutritional needs (Elvira et al., 2004) and lower resistance from the environmental conditions using in biomass production at industrial scale, that is, drying and storage processes (Zamora et al., 2006; Kets et al., 1996; Hood and Zoitola, 1988). On the other hand, their use *in vivo* is often affected by the physico-chemical

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Abbreviations: SFLAB, Spore forming lactic acid bacteria.

properties of the digestive tract (Buckenhüskes, 1993). This situation leads regularly the industries to develop in collaboration with the researchers new programmes in order to improve the functional properties and maintenance condition of the strain already used or to find new techniques of production, which can be less restrictive or finally to isolate new bacteria, which could better resist to the conditions of production, storage and formulation without losing their functional properties (Berner and O' Donnell, 1998; Klein et al., 1998; Schillinger, 1999). Within this framework our work aims at isolating new acidifying bacteria producing lactic acid selected from extreme environmental conditions by the means of their ability to sporulate (Sow et al., 2005). The principal spore-forming lactic acid bacteria (SFLAB) are recognized for their ability to produce lactic acid, while they can also produce endospores. The most known SFLAB belong to the genera *Bacillus*, including *B. coagulans, B. racemilacticus* et *B. laevolacticus,* and *Sporolactobacillus,* including *S. inulinus*, *S. racemicus, S. lactosus,* etc (Yanagida et al., 1997; Cavazzoni and Adami, 1993; Nakayama and Yanoshi, 1967a). Beside the *Lactobacillus* species, the SFLAB (*Bacillus* and *Sporolactobacillus*) have the advantage to have lower nutritional requirements (Ohara and Yahata, 1996; Holzapfel and Botha, 1988), a good resistance to different environmental stress (Hyronimus et al., 2000). In addition the SFLAB can provide the major required functional properties wanted among species of the genus *Lactobacillus* (Cavazzoni and Adami, 1993). Hence, the SFLAB are good candidates for industrial using, both in lactic acid production and probiotic preparations. Why it is better to know that certain SFLAB, such as *B. coagulans* and *Sp. inulinus* are already used as probiotic for human and animal nutrition (Cavazzoni and Adami., 1993; Hyronimus et al., 2000). Researches presented here were aimed at one part of the isolation and characterization of new SFLABs and on the other hand to highlight the technological properties of the new isolates with a view on their using as probiotics.

MATERIALS AND METHODS

Samples enrichment and isolation procedures

Soil samples were collected in the vicinity of poultry farm in Burkina Faso with sterile spoons, and kept into clean bags. Six soil samples were collected in three farms i.e., two samples by farm. The microbial enrichment and bacterial isolation were performed on the GYP (Glucose-Yeast-Peptone) medium described by Nakayama and Yanoshi (1967a). The GYP medium consists of 1% (w/v) glucose, 1% (w/v) yeast extract, 1% (w/v) peptone, 0.5% (w/v) $CaCO_3$ and 2,5% (v/v) mineral solution. The medium was adjusted to pH 7.0, for solid GYP 2% (w/v) agar was added to the medium. The mineral solution consists of 5% (w/v) K_2HPO_4, 5% (w/v) KH_2PO_4, 0.1% (w/v) NaCl, 0.1% (w/v) $MnSO_4$, 0.01% (w/v) $CuSO_4$, 0.01% (w/v) $CoCl_2.6H_2O$, 0.1% (w/v) $FeSO_4.7H_2O$ and 2.7 % (w/v) Na_3-Citrate. The enrichment process was carried out with 5 g of soil sample mixed with 100 ml of GYP broth. The sample suspension was heated at 80°C for 10 min to kill all asporogenous bacteria and then incubated under anaerobic condition (BBL™ GasPak™, H_2 + CO_2) at 30°C for two days. After incubation, 100 µl of the turbid broth was spread onto GYP Agar plates prepared with 0,005% (w/v) bromocresol purple. The plates were incubated under aerobic condition at 30°C for two days. Thus, all acid-producing bacteria were recognized by the appearance of yellow zones around colonies. The acidifying bacteria were isolated and purified by repeated isolation and the purity was checked by microscope examination. All positive isolates for the screening criteria were maintained as frozen stocks at -80°C in the presence of 30% glycerol as cryoprotective agent.

Identification and phenotypical characterization

Cell morphology was examined by phase-contrast microscopy. Gram determination was performed using the KOH method, according to Gregersen (1978). All growth tests were performed in a modified GYP medium described by Bayane et al. (2006). All cultures were performed at 30°C according to general methods of bacteriology (Gerhardt, 1994), including the examination of tolerance to different NaCl concentrations. Catalase production test were determined by transferring fresh colonies from Agar to a slide glass and adding 5% H_2O_2 reagent. Cell motility was detected by cultivation in a semi-solid medium. Production of gas from glucose was assayed in roll tubes supplemented with Durham tubes in GYP broth. The quantity and the enantiomeric of lactic acid produced, was determined by enzymatic tests (Roche Molecular Biochemicals, Boehringer Mannheim GmbH, Germany). The ability of the isolates to produce acid from various carbon sources was determined by API 50 CHB test (BioMérieux sa, Lyon, Marcy l'étoile, France) according to manufacturer's instructions.

Determination of the technological properties of the isolates

The ability of the isolates to produce technological enzymes, including lipases, proteases and amylases were examined on modified GYP agar according to Larpent and Larpent (1985). The tolerance to acidic conditions was tested in both sterile phosphate buffer saline and GYP broth according to Hyronimus et al. (2000). The tolerance to bile salts was performed on GYP agar medium containing oxgall bile (0.1 - 1% w/v) (Sigma Chemical Co., St Louis, MO, USA) according to Hyronimus et al. (2000). The minimal inhibitory concentration (MIC) of bile salts for a strain was determined as the lowest concentration totally inhibiting the growth of cells as judged from visual examination of the agar plates.

Total cellular fatty acids analysis

Isolates were grown on tryptone soya broth medium for fatty acid extraction. Fatty acid methyl esters (FAME) were obtained from wet biomass (approx. 40 mg) by saponification, methylation and extraction as described by Osterhout et al. (1991). FAME mixtures were separated using a model 5898A microbial identification system (Microbial ID, Newark, DE, USA) which consisted of a Hewlett-Packard model 3392 gas chromatograph fitted with a 5% phenylmethyl silicone capillary column (0.2 mm x 25 m), a flame ionization detector, a Hewlett-Packard model 3392 integrator, Hewlett-Packard model 7673A automatic sampler and a Hewlett-Packard model 900/300 computer (Hewlett-Packard, Palo Alto, CA, USA). Peaks were automatically integrated and fatty acid names and percentages calculated by the Microbial ID. FAME profiles were compared to a standard library (MIDI Inc.) used with the Sherlock (MIDI Inc.) software system. The system compares samples profiles with a library of known profiles, generating similarity indices (SI) for each unknown profile, with SI values above 0.5 considered as good

Table 1. List of spore forming lactic acid bacteria isolated and purified from sahelian region in Burkina Faso.

Strains	Geographical origin from Burkina Faso	Reference number in bacterial collection of CWBI
B2	Northern Area (Dori)	CWBI-B1082
B6	North (Dori)	CWBI-B1086
BC	North (Dori)	CWBI-B60
C15	North (Dori)	CWBI-B1080
P1	Central area, (Ouagadougou)	CWBI-B1070

matches.

16S rDNA analyses and phylogenetic identifications

The total bacterial DNA was isolated by the method described by Niemann et al. (1997). The 16S-rDNA was amplified by PCR with the couple of universal primers: 16F27 (5'-AGAGTTTGATCCTGGCTCAG-3'), and 16R1522 (5'-AAGGAGGTGATCCAGCCGCA-3'). The PCR-amplified 16S-rDNA's were purified using the QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). The complete sequencing was performed using an Applied Biosystems, Inc. 377 DNA Sequencer according to the protocol of the manufacturer (Perkin-Elmer, Applied Biosystems Div., Foster City, Ca., USA), using the ABI/PRISM™ BigDye™ Terminator Cycle Sequencing ready reaction Kit (With AmpliTaq® DNA Polymerase, Fs). Sequence assembly was performed by the program AutoAssembler™ (Perkin-Elmer, Applied Biosystems Div., Foster City, Ca, USA). The phylogenetic analysis was performed using the software packages Genecompar (Applied, Maths, Belgium) and Vector NTI (Informax, Invitrogen, Maryland, USA) after including the consensus sequence in an alignment of small ribosomal subunit sequences collected from the international nucleotide sequence library EMBL. This alignment was calculated pairwise by using an open gap penalty of 0% and after discarding unknown bases. A resulting tree was constructed using the neighbor-joining method.

RESULTS

Screening and selection of spore-forming lactic acid bacteria

The enrichment procedure was contributed to isolate several acidifying bacteria from each of the six samples used, which showed the presence of significant number of acidifying bacteria in the vicinity of the farms sampled. The majority of the acidifying bacteria isolated, were Gram positives lactic acid producers. However, an important quantity of cocci related to the genus *Pediococcus* and *Enterococcus* was also found. The SFLAB isolated are presented in Table 1. Among the isolates, five strains were chosen of extensive analyses, including CWBI-B1082, CWBI-B1086, CWBI-B1070, CWBC-B1080 and CWBI-B60. The screening criteria were based both on the similarity between the morphological characteristics of the isolate and the SFLAB already known (*Bacillus* and *Sporolactobacillus*), and the amount of lactic acid produce in GYP broth. The strains selected were analyzed to highlight their taxonomic position, growth characteristics and technologic properties.

Physiological and biochemical examination

The microbiological characteristics of the SFLAB isolated are shown in Table 2. All SFLAB isolated were Gram-positive rods, motile, mesophilic, facultative anaerobic and catalase positive. Their optimal growth temperatures and pH were about 35°C and 7.0 respectively. The microscopic observations showed that cells usually form short chains and occasionally long chains. After aerobic growth at 30°C for 2 days, colonies on GYP-agar were generally about 2 to 6 mm in diameter. In the presence of calcium carbonate, the colonies appear more distinct, being surrounded by a transparent halo formed by lactic acid. All isolates were capable to convert glucose to L(+) lactic acid. However the strain CWBI-B60 produced gas from glucose, but the other SFLAB didn't share this characteristic. As one can see through Table 3, the fermentation pattern of carbohydrates varied among the isolates, even with the type species of SFLAB belonging to the genus *Bacillus,* including *B. coagulans, B. laevolacticus* and *B. racemilacticus* used as reference strains. The strain CWBI-B1082 can acidify from various sugars. From a taxonomic point of view, the strain CWBI-B60 has an interesting profile due to its inability to use most carbon sources of the API 50 CHB system. The strain CWBI-B1080 was the only isolate capable to hydrolyse insulin. The technological properties of the isolates, including the production of enzyme and their resistance to bile salts are shown in Table 4. The resistances to acidic conditions are shown in Figure 3. One can note that no isolate was capable to degrade lipids. The strains CWBI-B1082, CWBI-B1070, CWBI-B60 and *B. laevolacticus* LMG 16675^T were capable to degrade starch by means of amylases. The strains CWBI-B1082, CWBI-B1086, CWBI-B1080, CWBI-B1070, *B. coagulans* LMG 6326^T and *B. laevolacticus* LMG 16675^T were capable to degrade casein by means of proteases. The tests of resistance to bile salts showed a great sensitivity of CWBI-B60 with a minimal inhibitory concentration MIC of 0.1% (w/v). The strain CWBI-B60 was also the only strain

Table 2. Microbiological characteristics of spore-forming lactic acid bacteria isolated from poultry farm environments in Burkina Faso.

Microbiological characteristics	CWBI-B1082	CWBI-B1086	CWBI-B60	CWBI-B1080	CWBI-B1070
Colony morphology	Small, slimy, creamy, iregular margin	Small, slimy, creamy-white regular margin	Small, smooth, creamy, iregular margin	Small, slimy, creamy-white regular margin	Small, smooth, creamy-white iregular margin
Cell morphology	Regular rods 1.2 - 1.5 x 4-8 µm	Regular rods 0,9 x 2-4 µm	Regular rods 1.0-1.2 x 4-6 µm	Regular rods 1.0-1.2 x 4-6 µm	Regular rods 1.0 x 2.5-4.0 µm
Spores location	Subterminal not deforming	Terminal Not deforming	subterminal ellipsoidal deforming	subterminal not deforming	subterminal not deforming
Gas from glucose	-	-	+	-	-
Gram stain	+	+	+	+	+
Catalase	+	+	+	+	+
Optimal growth temperature	30 at 40°C	30 at 37°C	30 at 37°C	30 at 37°C	30 at 37°C
Growth at 20°C	+	+	+/-	+/-	+/-
Growth at 45°C	+	+	-	-	+
Growth at 55°C	+/-	-	-	-	-
Optimal pH for growth	6.0-8.0	6.0-8.0	7.0-8.à	7.0-7.5	7.0-8.0
Growth at pH 5.0	+/-	+	+/-	+/-	+/-
Growth with 5% NaCl	+	+	+	+/-	+
Growth with 10% NaCl	-	-	-	-	-

to have a MIC lower than the minimal value recommended for probiotic bacteria selection, that is, 0.3% (Begley et al., 2005). According to the studies of Hyronimus et al. (2000), the SFLAB belonging to the genus *Sporolactobacillus* are more sensitive to bile salts, with a minimal inhibitory concentration MIC lower than 0,1%. However, according to these same studies the SFLAB belonging to the genus, including *B. racemilacticus* and *B. laevolacticus* have better resistances with MIC higher than 1%, but the MIC of *B. coagulans* is about 0.3%. All isolates had good resistances to acidic conditions (Figure 3), with pH 3.0 and pH 2.5 during 3 hours. The resistance of lactic acid bacteria to such conditions is observed only in few species of the genus *Lactobacillus* (Hood and Zoitola, 1988). In addition, the strains CWBI-B1082, CWBI-B1086 and *B. laevolacticus* LMG 16675^T had higher resistance. In conclusion, the levels of resistance observed for bile salts and the acidic conditions in this study are quite sufficient to enable the SFLAB to survive through animal gastro-intestinal tracts.

Cellular fatty acids analysis

The analysis of the bacteria fatty acids profile by gas chromatography is a very selective and sensitive method, which has already allowed the characterisation of numerous spore-forming bacteria related to the genus *Bacillus* (Kämpfer, 2002; Welch, 1991). The analysis of fatty acid composition of the strain CWBI-B1082 showed significant quantities of $_{iso}$-C13:0 and $_{iso}$-C15:0 corresponding respectively to 24 and 28% of total cellular fatty acids. We observed that this strain has only 4% of $_{anteiso}$-C15:0. It ratio $_{anteiso}$-C15:0/$_{iso}$-C15:0 is thus 0.14. The composition of fatty acid in the strain CWBI-B1086 showed a predominance of the iso-C15:0 which corresponds to 54% of the total fatty acids. The second fatty acid present with a proportion of 24% was the $_{anteiso}$-C15:0. The ratio $_{anteiso}$-C15:0/iso-C15:0 of this bacterium is thus 0.44. The composition of cellular fatty acid of the strain CWBI-B1070 is 43.3% of $_{anteiso}$-C15:0 and 7.73% of $_{iso}$-C15:0. It ratio anteiso-C15:0/iso-C15:0 is thus 9.16. The majority of fatty acid in strain CWBI-B1080 is anteiso-C15:0 with a proportion of 40% of the totals fatty acid. In addition we found 24% of C16:0 and 13% of C14:0. The CWBI-B60 strain has an interesting composition in cellular fatty acid from taxonomic point of view, with the presence of three major compounds, including 31% of $_{anteiso}$-C15:0, 31% of C16:0 and 12% of $_{iso}$-C15:0, so its ratio $_{anteiso}$-C15:0/$_{iso}$-C15:0 is about 2.5. The identifications obtained by the analysis of the cellular fatty acids are shown in Table 5.

Phylogenetic analysis and identification

The 16S-rRNA gene sequences of all isolates were deposited in EMBL/GenBank/DDBJ nucleic acid sequence database under the following accession number (CWB-B1082: DQ112249; CWBI-B1080: DQ112248; CWBI-B1070: DQ112247; CWBI-B60: DQ112246; CWBI-B1086: DQ112245).

The sequence obtained from each isolate was aligned with the 16S rDNA sequences from the GenBank database to identify the new microorganisms. The 16S rDNA can allow the identification of bacteria genus,

Table 3. Sugar fermentation patterns of the new spore-forming lactic acid bacteria determined by API 50 CHB system (BioMérieux) in duplicate at 30°C: {+} sugar fermented; {-} sugar not fermented. The reference strains were *Bacillus coagulans* LM 6326*; Bacillus laevolacticus* LMG 16675 *and Bacillus racemilacticus* LMG 6938.

Carbon sources	Isolates					Reference strains		
(API 50CHB)	B1082	B1086	B60	B1080	B1070	LMG 6326	LMG 6938	LMG 16675
(1) Glycerol	-	+	-	-	+	-	+	+
(2) Erythritol	-	-	-	-	-	-	+	-
(3) D-Arabinose	-	-	-	-	-	-	-	-
(4) L-Arabinose	+	+	-	+	-	+	-	-
(5) Ribose	+	+	-	-	+	+	-	-
(6) D-Xylose	+	+	-	+	+	-	+	-
(7) L-Xylose	-	-	-	-	-	-	-	-
(8) Adonitol	+	-	-	-	+	-	-	-
(9) β-Methyl-xyloside	+	-	-	+	-	-	-	-
(10) Galactose	+	-	+	+	+	+	+	+
(11) D-Glucose	+	+	+	+	+	+	+	+
(12) D-Fructose	+	+	+	+	+	+	+	+
(13) D-Mannose	-	+	+	+	+	+	+	+
(14) L-Sorbose	-	-	-	-	-	-	-	-
(15) Rhamnose	+	-	-	+	+	+	-	-
(16) Dulcitol	-	-	-	-	-	-	-	-
(17) Inositol	-	-	-	-	-	-	-	-
(18) Mannitol	+	+	-	-	+	-	-	-
(19) Sorbitol	-	-	-	-	+	+	-	+
(20) α-Methyl-D- mannoside	+	+	-	-	-	+	+	-
(21) α-Methyl-D-Glucoside	+	-	-	-	+	+	+	+
(22) *N*-Acetyl glucosamine	+	+	+	-	+	+	+	+
(23) Amygdaline	+	-	-	+	+	+	-	+
(24) Arbutine	+	+	-	-	+	+	-	+
(25) Esculine	+	+	-	+	+	+	-	+
(26) Salicine	+	+	-	+	+	+	-	+
(27) Cellobiose	+	+	-	+	+	+	-	+
(28) Maltose	+	-	+	+	+	+	-	+
(29) Lactose	+	-	-	+	+	+	+	+
(30) Melibiose	+	-	+	+	-	+	-	+
(31) Saccharose	+	+	+	+	+	+	-	+
(32) Trehalose	+	+	+	+	+	+	+	+
(33) Inuline	-	-	-	+	-	-	-	+
(34) Melezitose	+	-	-	+	-	+	-	+
(35) D-Raffinose	+	-	+	+	-	+	-	+
(36) Amidon	+	-	+	+	-	-	+	+
(37) Glycogène	+	-	-	+	-	-	-	+
(38) Xylitol	-	-	-	-	-	-	-	+
(39) β-Gentiobiose	-	-	-	+	+	+	-	+
(40) D-Turanose	+	-	+	+	+	+	-	-
(41) D-Lyxose	-	-	-	-	-	-	-	-
(42) D-Tagatose	-	+	-	-	+	-	+	-
(43) D-Fucose	-	-	-	-	-	-	-	-
(44) L-Fucose	+	-	-	+	+	-	-	+
(45) D-Arabitol	-	-	-	-	-	-	-	-
(46) L-Arabitol	-	-	-	-	-	-	-	-
(47) Gluconate	-	-	-	-	+	-	-	-
(48) 2 Ceto-gluconate	-	-	-	-	-	-	+	-
(49) 5 Ceto-gluconate	-	-	-	-	-	-	-	-

Table 4. Technological properties of spore forming lactic acid bacteria isolated from poultry farm environments in Burkina Faso compared to known type strains of spore-forming lactic acid bacteria belonging to the genus *Bacillus.* The reference strains were *Bacillus coagulans* LM 6326*; Bacillus laevolacticus* LMG 16675 *and Bacillus racemilacticus* LMG 6938.

Properties	B1082	B1086	B60	B1080	B1070	LMG 6326	LMG 16675	LMG 6938
Tolerance to bile salts (% w/v CMI)	>1	>1	0.1	0.5	>1	0.5	0.1	0.6
Type of lactic acid produce	L(+)	L(+)	L(+)	L(+)	L(+)	L(+)	D(-)	DL
Quantity of lactic acid produce in GYP broth ($g.l^{-1}$)	3.2±0.1	4.2±0.4	4.2±0.2	4.3±0.1	4.5±0.3	5.2±0.5	5.3±0.2	6.6±0.3
Amylase production	+	-	+	-	+	-	+	-
Protease production	+	+	-	+	+	+	+	-
Lipase production	-	-	-	-	-	-	-	-

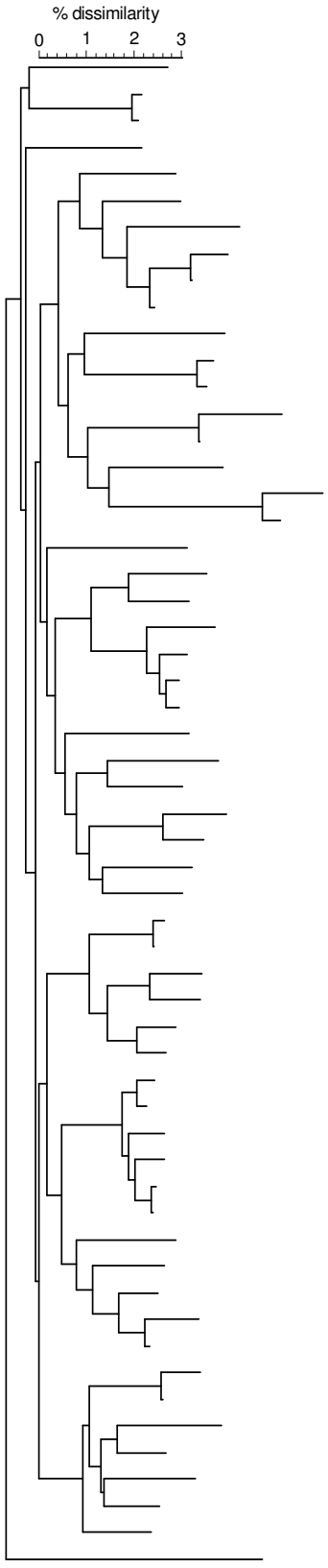
Table 5. Identification by API 50 CHB, Fatty acids and 16S rDNA sequencing of the spore forming lactic acid bacteria isolated from poultry environments in Burkina Faso.

Bacteria	API 50 CHB		Fatty acid analysis		16S rDNA sequencing	
	Identification	Similarity (%)	Identification	Similarity (SI)	Identification	Similarity (%)
CWBI-B1082	*B. circulans*	69.1	*B. cereus*	0.23	*B. cereus*	99.7
CWBI-B1086	*B. pumilus*	99.9	*B. pumilus*	0.92	*B. pumilus*	99.9
CWBI-B60	*B. coagulans*	92.1	*B. lentus*	0.21	*P. stellifer*	99.1
CWBI-B1080	*B. circulans*	87.9	*P. azotofixans*	0.73	*P. borealis*	98.2
CWBI-B1070	*B. licheniformis*	1.0	*P. polymyxa*	0.49	*P. azoreducens*	98.7

specie and even strain level. The results of the phylogenetic analysis of the 16S rDNA analyses are summarized in the phylogenetic trees in the Figures 1 and Figure 2. The analyses showed that the isolates were separated in two distinct genera. Both CWBI-B1082 and CWBI-B1086 belong to the genus Bacillus (Figure 2), and CWBI-B60, CWBI-B1070 and CWBI-B1080 were members of the genus *Paenibacillus* Figure 1. The highest degrees of similarity with the species belonging to both genera are given in Table 5. The most relevant information highlighted from these analyses concerns the net difference between the SFLAB isolated from soil sample in Burkina Faso and the known SFLAB, including species both of *Bacillus* and *Sporolactobacillus*.

DISCUSSION

The functional and technological properties of microorganisms still remain the main characteristics, which should focus the research of new bacteria for industrial applications. On the basis of both characteristics (functional and technological properties), the SFLAB could be excellent candidates in food fermentation industries. The sporulation could undoubtedly give to SFLAB better resistance to environmental stress related to food processing. Most SFLAB produce less lactic acid than the species of the genus *Lactobacillus* especially those related to the genus *Bacillus* (Cavazzoni and Adami, 1933). However, even with this characteristic the SFLAB belonging to the genus *Bacillus* are always interesting in food fermentations. Indeed, these bacteria produce enough lactic acid for such applications and then they have many technological properties nowhere else found in the asporogenous bacteria such as the *Lactobacillus*. In addition the SFLAB are more stable to extreme physicochemical conditions such as heat, mechanic treatments and even to acidic and bile condition. The resistance of the vegetative and spore forms of the SFLAB to osmotic shocks can also be considered, even if this hasn't been tested in this study. The enzymatic activities found in certain SFLAB, concerning the production of amylases and proteases are also interesting, and can be valuable in food fermentation. However, we must recognize that excessive production of casein degrader enzymes (proteases) can be a limiting factor for certain fermentations. Production of bacterial proteases in certain types of fermentation such as dairy products can cause appearance of disadvantageous characteristics, such as unpleasant odour and colour, and so the instability of the products (Larsen et al., 2004). However, to other kinds of fermentation, the proteolytic activities are instead desired. It's particularly the case of certain traditional alkaline fermentations such as those of the African locust beans (*Parkia biglobosa*), Lawal (2004), Ikenebomeh et al. (2006). Several strains of *B. pumilus* already participate to this fermentation (Sarkar et al., 2002; Ouoba et al., 2003). The strain CWBI-B1086 identified as strain of *B. pumilus*, could add value to this type of fermentation. However, this must be verified. The production of a bacteriocin called coaguline



NEM1aT	*Paenibacillus nematophilus*	AF480935
DSM 2T	*Paenibacillus macquariensis*	AB073193
LMG 22078T	*"Paenibacillus antarcticus"*	AJ605292
IAM 12467T	*Paenibacillus macerans*	AB073196
IFO 15660T	*Paenibacillus chitinolyticus*	AB021183
WN9T	*Paenibacillus chinjuensis*	AF164345
ATCC BAA-206T	*Paenibacillus naphthalenovorans*	AF353681
YC300T	*Paenibacillus koreensis*	AF130254
KCTC 3748T	*Paenibacillus ehimensis*	AY116665
SD17T	*"Paenibacillus elgii"*	AY090110
TBT	*Paenibacillus koleovorans*	AB041720
ATCC 9545T	*Paenibacillus larvae subsp. larvae*	X60619
DSM 3615T	*Paenibacillus larvae subsp. pulvifaciens*	AB073204
JCM 9907T	*Paenibacillus illinoisensis*	AB073192
NCIMB 12781T	*Paenibacillus pabuli*	X60630
DSM 3037T	*Paenibacillus validus*	D78320
JCM 9072T	*Paenibacillus chondroitinus*	AB073206
DSM 5050T	*Paenibacillus alginolyticus*	D78465
MOL722T	*Paenibacillus turicensis*	AF378694
IAM 1258T	*Paenibacillus alvei*	AB073200
DSM 5581T	*Paenibacillus apiarius*	AB073201
T168T	*Paenibacillus dendritiformis*	Y16128
ATCC 14707T	*Paenibacillus lentimorbus*	AB073199
IFO 15656T	*Paenibacillus thiaminolyticus*	AB073197
ATCC 14706T	*Paenibacillus popilliae*	AB073198
AP-20T	*Paenibacillus daejeonensis*	AF290916
A30T	*Paenibacillus granivorans*	AF237682
DSM 1355T	*Paenibacillus agaridevorans*	AJ345023
DSM 10247T	*Paenibacillus curdlanolyticus*	AB073202
DSM 10249T	*Paenibacillus kobensis*	AB073363
DS-1T	*Paenibacillus glycanilyticus*	AB042938
DSM 1327T	*Paenibacillus agarexedens*	AJ345020
GMP01T	*Paenibacillus favisporus*	AY208751
LMG 18439	*"Paenibacillus cineris"*	AJ575658
JCM 9905T	*Paenibacillus chibensis*	AB073194
LMG 18419	*"Paenibacillus cookii"*	AJ250317
CM1T	*Paenibacillus azoreducens*	AJ272249
CWBI-P1		
AM49T	*Paenibacillus kribbensis*	AF391123
DSM 8320T	*Paenibacillus peoriae*	AB073186
AM141T	*Paenibacillus terrae*	AF391124
PB172T	*Paenibacillus brasilensis*	AF273740
DSM 36T	*Paenibacillus polymyxa*	AJ320493
CECT 5266T	*Paenibacillus jamilae*	AJ271157
324T	*Paenibacillus campinasensis*	AF021924
MB 1871T	*Paenibacillus lactis*	AY257868
DSM 5162T	*Paenibacillus glucanolyticus*	AB073189
NRRL B-377T	*Paenibacillus amylolyticus*	D85609
JCM 9073T	*Paenibacillus lautus*	AB073188
CWBI-BC		
IS 1T	*Paenibacillus stellifer*	AJ316013
DSM 1735T	*Paenibacillus durus*	X77846
LMG 19079T	*Paenibacillus odorifer*	AJ223990
LMG 19080T	*Paenibacillus graminis*	AJ223987
CWBI-C15		
KK19T	*Paenibacillus borealis*	AJ011322
NCDO 1769T	*Bacillus subtilis subsp. subtilis*	X60646

Figure 1. Taxonomic position of the strains CWBI-B60 (CWBI-BC), CWBI-B1080 (CWBI-C15) and CWBI-B1070 (CWBI-P1) among the type species belonging to the genus *Paenibacillus*. The dendrogram was performed by GeneCompar software according to the 16S rDNA alignments.

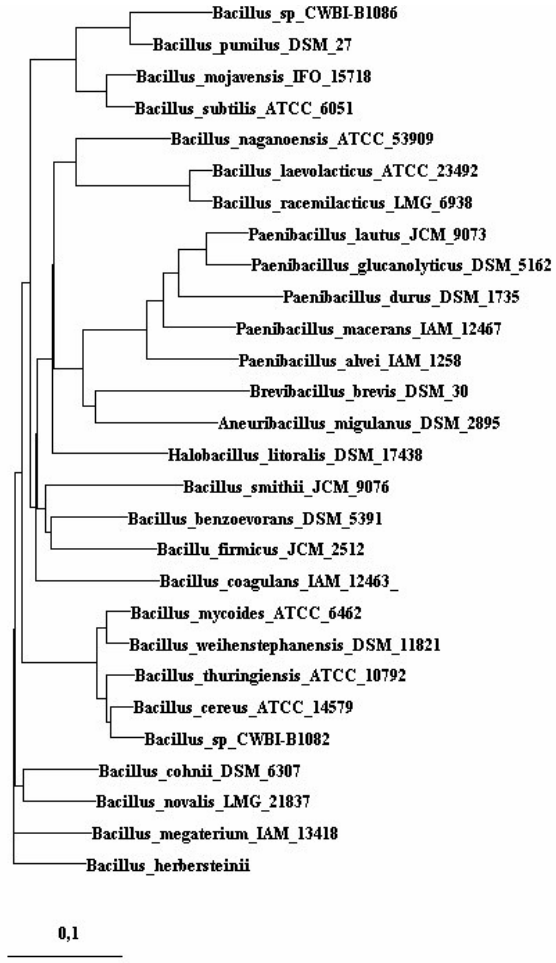


Figure 2. Taxonomic position of strains CWBI-B1082 and CWBI-B1086 within the group of spore-forming bacilli belonging the genus *Bacillus*, according to the 16S rDNA alignments performed with Vector NTI software.

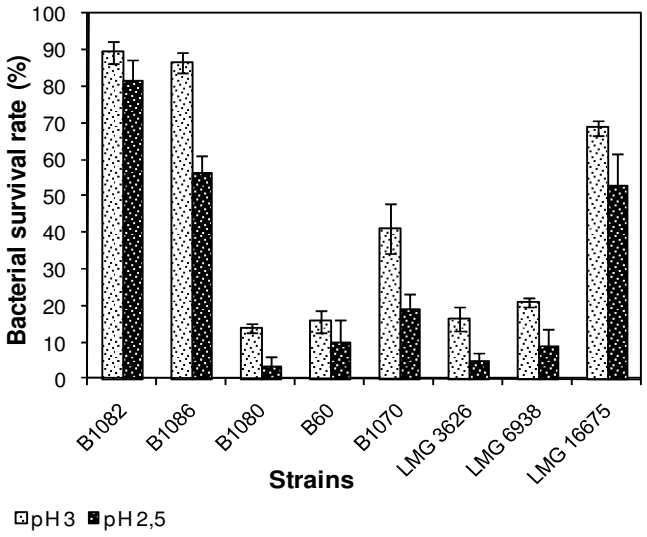


Figure 3. Survival rate (%) of spore forming lactic acid bacteria under acidic conditions after 3 h of incubation.

was identified from a strain of *B. coagulans* by Hyronimus et al. (1998), and its structure was determined by Le Marrec et al. (2000). The coagulin would be identical to the pediocine produced by *Pediococcus acidilactici*, (Le Marrec et al., 2000). This discovery opens the door to the possibility of finding similar characteristics from other SFLAB. In general, the microbiological characteristics of the SFLAB isolated in this study were very close to those of known SFLAB, including *B. coagulans*, *B. laevolacticus* and *B. racemilacticus*. The *Sporolactobacillus* also share certain of these characteristics, but not the production of catalase. The strain CWBI-B1082 identified as a member of the *Bacillus cereus* group is the only isolate for that it would be difficult to find an application in the agro-food sector. Due to the fact, that several members of the genus *B. cereus* are involved in various infectious diseases through the production of toxins and enterotoxins, that is, *B. anthracis*, *B. thuringiensis*, and *B. cereus*. The SFLAB found in current classifications belong to the genera *Bacillus* and *Sporolactobacillus* (Yanagida et al., 1997; Hozapfel and Botha, 1988; Nakayama and Yanoshi, 1967b). The present work shows that some members of this bacterial group can also be found within the genus *Paenibacillus* (CWBI-B1070, CWBI-B60 and CWBI-B1080). Therefore, the isolation of these new SFLAB allows considering a greater diversity of SFAB than what was known to date. The fatty acids extracted from the isolates and more precisely the ratios "anteiso-C_{2n+1}/iso-C_{2n+1}", have highlighted the stress adaptation of some SFLAB. Indeed, the ratios "a-C_{2n+1}/i-C_{2n+1}" and more particularly the ratio "a-$C_{15:0}$/i-$C_{15:0}$" give a good indication on bacterial adaptation to environmental stresses such as heat and osmotic shock (Llarch et al., 1997; Horani and Priest, 1994). The a-$C_{15:0}$ fatty acid with a melting point of 25.8°C is more abundant than the i-$C_{15:0}$ in the cell structure of the mesophilic spore-forming bacteria, while the thermophilic spore-forming bacteria show an otherwise organization where the i-$C_{15:0}$ fatty acid with a melting point of 52.5°C is more abundant (Haque and Rusell, 2004, Kaneda, 1991). Hence the mesophilic spore-forming bacteria possess ratios "a-$C_{15:0}$/i-$C_{15:0}$" higher than one, while the thermophilic species have ratios "a-$C_{15:0}$/i-$C_{15:0}$" lower than one (Zhang and Rock, 2008; Py et al., 1970). The analyses showed ratios "a-$C1_{5:0}$/i-$C_{15:0}$" of 0.15 and 0.44 for the strains CWBI-B1082 and CWBI-B1086 respectively, while both isolates were mesophilic. These values go with such found in the thermophilic member of the genus *Bacillus*. Haque and Rusell (2004), showed that certain microorganisms, such as *Bacillus cereus*, were capable to modify the profile of their cellular fatty acids in response to environmental stresses, including thermal and osmotic stresses. In agreement with this observation we think that the fatty acid composition of the strains CWBI-B1082 and CWBI-B1086 could constitute a mark of their adaptation to the warm and dehydrated environments where the sampling were done (sahelian region). The genetic analyses showed the great diversity of the endospore-forming

Bacilli in nature. The strain CWBI-B1086 was the only isolate whose classification was completed. The classifycation of the strain CWBI-B1082 in the group of *B. cereus* complex is very probable. Nevertheless, this classification highlights the extreme diversity of the members of *B. cereus* group (Claus and Berkeley, 1986). The membership of the strains CWBI-B1080, CWBI-B60 and CWBI-B1070 to the genus *Paenibacillus* is not subject to doubt (Ash et al., 1994). However the exact position of these three strains within the genus *Paenibacillus* needs to be clarified. This will be performed through analysis of their whole DNA by DNA-DNA hybridization technique. The genus *Paenibacillus* was created from the reclassification of the genus *Bacillus* (Ash et al., 1994). The current morphological classification of the members of this genus refers to ellipsoidal and deforming endospores (Heyndrickx et al., 1996). The analyses of the endospores produced by ours isolates with respect to this criterion, shown a positive response only for the strain CWBI-B60. The membership of these isolates to the genus *Paenibacillus* is clear, but we believe that they may both belong to the species revealed by the phylogenetic analyses or constitute new species within this genus. Further investigations will concern the determination of the exact taxonomic position of the isolates related to the genus *Paenibacillus*, but also the characterisation of the isolates not integrated in this study. A screening will be done with larger samples of soil in order to highlight a better understanding of the physiological and technological properties of the lactic acid bacteria housed in farm vicinities in sahelian regions.

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