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Full Length Research Paper

Assessment of the physiological and biochemical characterization of a *Lactic acid bacterium* isolated from chicken faeces in sahelian region

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The aim of this work was the examination of the microbiological and technological properties of a lactic acid bacterium (CWBI-B623) isolated from chicken faeces in sahelian region (Burkina Faso). The strain CWBI-B623 is a Gram positive rod, asporogenous, catalase-positive, facultatively anaerobic, non motile and mesophilic. The analysis showed that it produce L(+)-lactic acid via homofermentative pathway and it was able to ferment an important number of the carbohydrates of API 50CHL system. The 16S-rDNA-sequence analysis revealed that the isolate was phylogenetically a member of the genus *Lactobacillus* and formed a subline within the *Lactobacillus casei* cluster. The minimal inhibitory concentration of CWBI-B623 for bile salts was higher than 1% and the survival rates to acidity with pH 3.0 and pH 2.5 were 26 and 1.2%, respectively. CWBI-B623 is an *Lactobacillus casei* species based on its physiological and biochemical properties and it could be a good candidate for probiotic formulations.

Key words: Lactic acid bacteria, *Lactobacillus*, probiotic, biodiversity.

INTRODUCTION

From early times until now many Lactic Acid Bacteria (LAB) were used in functional foods manufacture, since they contribute to a healthy nutrition (Holzapfel et al., 1998; Marteau and Rambaud, 1993). The number of organisms involved in these applications is in constant progression. Among the LAB, species within the genera *Lactobacillus* and *Bifidobacterium* are the best known and the most frequently used in food industry. The LAB used as starters play an essential role to inhibit the growth of food spoilage bacteria by producing lactic acid and occasionally antimicrobial compounds like bacte-riocins (Buckenhüskes, 1993; Gomez and Malcata, 1999). The bacteria presenting these later properties are so-called probiotic lactic acid bacteria. Probiotics are more precisely defined as mono or mixed cultures of living microorganisms, which beneficially affect the host (human or animal) by improving the properties of indigenous microflora (Havenaar et al., 1992). The demands of the consumers and requirements of industry direct the research toward new probiotic LAB, which would withstand the technological process. Good probiotic bacteria should be able to guarantee safety, all functional characteristics required but also to undergo food processing and storage without a significant loss of these properties (Berner and O'Donnell, 1998).

Several work have shown that probiotic lactic acid bacteria can be isolated from a variety of habitats, including dairy products, meat products, sewage, humans, plants and animals (Kandler and Weiss, 1986). Some LAB have been isolated from chicken and poultry farms including *L. aviarius* (Fujisawa et al., 1984), *L. fermentum* sub sp. *cellobiosus* and *L. animalis* (Gusils et al, 1999a) from gastro intestinal tracts of chicken, *L. gallinarum* and *L. Johnsonii* from chicken faeces (Fujisawa et al., 1991). These selected bacteria from chickens can be considered as potential ingredients for a chicken probiotic feed formulation intended to control

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salmonellosis and also improve poultry sanitation, but also for other applications (Gusils et al., 1999b). It is within this reason that we have especially focus our research of LAB in the environments of poultry farms with the hope to develop later an animal probiotic feed formulation. This work was undertaken with an aim of constituting a collection of LAB which can interest bioindustries, by a better biological activity and a better resistance to physicochemical properties of digestive tract, desiccation and conservation parameters. The farms environments from Burkina Faso were used for this research search, because the microorganisms which are able to be maintained and to growth in these hot and dehydrated environments have generally a good adaptation to environmental conditions.

MATERIAL AND METHODS

Soil samples and isolation procedure

Samples were collected in poultry farm from sahelian region (Burkina Faso) . 6 samples were collected in 3 farms (2 samples per farm). For each sample, the faeces was separated from the remainder of the components and saved into sterile bags. Microbial isolation was performed on GYP medium containing 1% (w/v) Glucose, 1% (w/v) Yeast extract, 1% (w/v) Peptone and 0.5% (w/v) $CaCO_3$. 5 grams of sample were mixed with 100 ml of GYP broth, and the sample suspension was incubated anaerobically at 30°C for 24 h. 100 µl of the turbid broth was spread onto GYP agar after dilution, and the culture was incubated aerobically at 30°C. Acid producing bacteria were recognized by the appearance of clear zones around colonies. These bacteria were purified by repeated isolation and the purity was checked by plating and by microscope examination. The isolates were maintained as frozen stocks at –80°C in the presence of 30% glycerol as a cryoprotective agent.

Morphological, physiological and biochemical examinations

The cell morphology was examined by phase- contrast microscopy. Gram determination was performed using the KOH method (Gregersen, 1978). All growth tests were performed in MRS medium at 37°C (De Man et al., 1960), including the tolerance to different concentrations of NaCl (Gerhardt et al., 1981). The hydrolysis of starch, caseinate and tributyrine were verified in GYP agar (Larpent and Larpent, 1985). Catalase production and pseudocatalase tests were determined by transferring fresh colonies from agar to a slide glass and adding 5% H_2O_2 or benzidine reagent (Gerhardt et al., 1981). Cell motility was detected by cultivation in a semi-solid medium (Gerhardt et al., 1981). Production of gas from glucose was assayed by growing the bacteria in MRS broth supplemented with Durham tubes (Larpent and Larpent, 1985). Lactic acid enantiomeric configuration was determined by enzymatic method with test kit (Boehringer Mannheim GmbH, Mannheim, Germany) according to the instructions of the manufacturer. Sugar fermentation patterns were determined using API 50 CHL system (BioMérieux sa, Marcy l'étoile, France) in duplicate at 30°C according to the instructions of the manufacturer. The acid tolerance study was performed in sterile phosphate buffer saline (Hyronimus et al., 2000). Cultures were grown in MRS broth at 37°C overnight, and sub cultured in 10 ml of fresh GYP broth adjusted to different pH values (2.5 and 3) with hydrochloric acid (3.0 M). The initial bacterial concentration was 10^6 cfu/ml and was checked by viable count determination on MRS agar. Samples were incubated for 3 h at 30°C. Cells were serially diluted 10-fold in phosphate buffer (0.1 M, pH 6.2) in order to neutralize the medium acidity. The residual viable count was determined by dilution and plate counting on MRS agar after 48 h of incubation. The survival rate was calculated as the percentage of bacteria colonies grown on MRS agar compared to the initial bacterial concentration. The tolerance to bile salts was performed on MRS agar plates (Hyronimus et al., 2000). Samples of overnight cultures (20 µl corresponding to 10^6 cfu/ml) were spotted onto MRS agar containing ox gall bile (0.1% to 1% w/v) (Sigma Chemical Co., St Louis, MO, USA). Plates were incubated at 30°C for 5 days. The minimal inhibitory concentration (MIC) of bile salts for a strain was determined as the lowest concentration totally inhibiting the growth of spots as judged from visual examination of spots.

Identification by 16S-rDNA-sequence analysis

The bacterial genomic DNA was isolated by the method described by Niemann et al. (1997). The 16S-rDNA was amplified by PCR with the universal primers: 16F27 (5'-AGAGTTTGATCCTGGCTCAG-3') and 16R1522 (5'-AAGGAGGTGATCCAGCCGCA-3'). The PCR-amplified 16S-rDNA's were purified using the QIA quick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). The complete sequencing was performed using an Applied Bio systems, Inc. 377 DNA Sequencer and the protocols of the manufacturer (Perkin-Elmer, Applied Bio systems Div., Foster City, Ca., USA) using the "ABI PRISM TM Big Dye TM Terminator Cycle Sequencing ready reaction Kit (With AmpliTaq® DNA Polymerase, Fs)". 5 "forward" and 3 "reverse" primers were used to obtain a partial overlap of sequences, including 16F358 (5'-CTCCTACGGGAGGCAGCAGT-3'), 16F536 (5'-CAGCAGCCGCGGTAATAC-3'), 16F926 (5'-AACTCAAAGGAATTGACGG-3'), 16F1112 (5'-AGTCCCGCAACGAGCGCAAC-3'), 16F1241 (5'-GCTACACACGTGATACAATG-3'), 16R339 (5'-ACTGCTGCCTCCCGTAGGAG-3'), 16R519 (5'-GTATTACCGCGGCTGCTG-3'), 16R1093 (5'-GTTGCGCTCGTTGCGGGACT-3'), ensuring highly reliable assembled data. Sequence assembly was performed by the program AutoAssembler^{TM} (Perkin-Elmer, Applied Biosystems Div., Foster City, Ca, USA). The phylogenetic analysis was performed using the software package Genecompar (Applied, Maths, Belgium) 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 pair wise by using an open gap penalty of 0% and after discarding unknown bases. A resulting tree was constructed using the neighbour-joining method (Saitou and Nei, 1987).

RESULTS

Morphological, biochemical and physiological characteristics

The Morphological, biochemical and physiological examinations of the strain CWBI-B623 are shows in Table 1. CWBI-B623 cells were non-spore-forming rods, Gram-positive, non-motile, with dimensions of 1x1.5-3.0 µm. They are usually single but occasionally in pairs and rarely in short chains. After aerobic growth at 37°C for 2 days, colonies on MRS- agar were 0.5 to 1 mm in diameter. In the presence of calcium carbonate colonies appear more distinct, being surrounded by a transparent

Table 1. Morphological, physiological and biochemical characteristics of strain CWBI-B623 compared to some type strains of *L.* casei group species.

Characteristics	*CWBI-B623*	*L. casei* ATCC 393^T	*L. paracasei* JCM 8130^T	*L. rhamnosus* JCM 1136^T
Cells morphology	Regular rods 1x1.5-3.0 µm	Regular rods 1.1x2.0-4.0 µm	Regular rods 1.1x2.0-4.0 µm	Regular rods 1x2.0-3.0 µm
Gram stain	+	+	+	+
Catalase	+	-	-	-
Pseudocatalase	-	-	-	-
Gas from glucose	-	-	-	-
Lactic acid produce	L (+)	L (+)	L (+)	L (+)
Optimal pH for growth	6.8 ± 0.2	7±0.2	7±0.2	7±0.2
Optimal growth temperature	37°C	37°C	37°C	37°C
Growth at 5°C	-	-	-	-
Growth at 55°C	-	-	-	-
Growth with 5% NaCl	+	+/-	+/-	+/-
Growth with 10% NaCl	-	-	-	-
Amount of lactic acid (g/l)[a]	9.0 ± 0.5	10.4 ± 0.8	10.6 ± 1.1	9.2 ± 1.4
MIC for bile salts (% w/v) [b]	> 1%	0.5%	0.1%	0.4%
Survival rates at pH 2.5 for 3 h	1.2%	0	0	0
Survival rates at pH 3.0 for 3 h	26%	0	0	0

[a]Amount of lactic acid produce in MRS broth after 48 h.
[b]Minimal inhibitory concentration (MIC) of bile salts was determined at 30°C after 5 days of growth.

Table 2. Differences in fermentation of carbohydrates by strain CWBI-B623 and *Lactobacillus casei* group species determined by API 50CHB (BioMérieux) in duplicate at 30°C.

carbohydrates	*CWBI-B623*	*L. casei* ATCC 393^T	*L. rhamnosus* JCM 1136^T	*L. casei subsp. tolerans* JCM 1171^T	*L. paracasei* JCM 8130^T
L-Arabinose	+	-	-	-	-
D-Arabinose	+	-	-	-	-
Ribose	+	-	+	-	+
Adonitol	+	-	-	-	-
Rhamnose	+	-	+	-	-
Mannitol	+	-	+	-	+
D-xylose	-	-	-	-	-
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Maltose	-	-	+	-	+
Lactose	+	+	+	+	+
Melibiose	-	-	-	-	-
Raffinose	-	-	-	-	-
L-fucose	+	-	-	-	-
Gluconate	+	-	-	-	+
2 Ceto-gluconate	+	-	-	-	-

+ sugar fermented; - sugar not fermented.

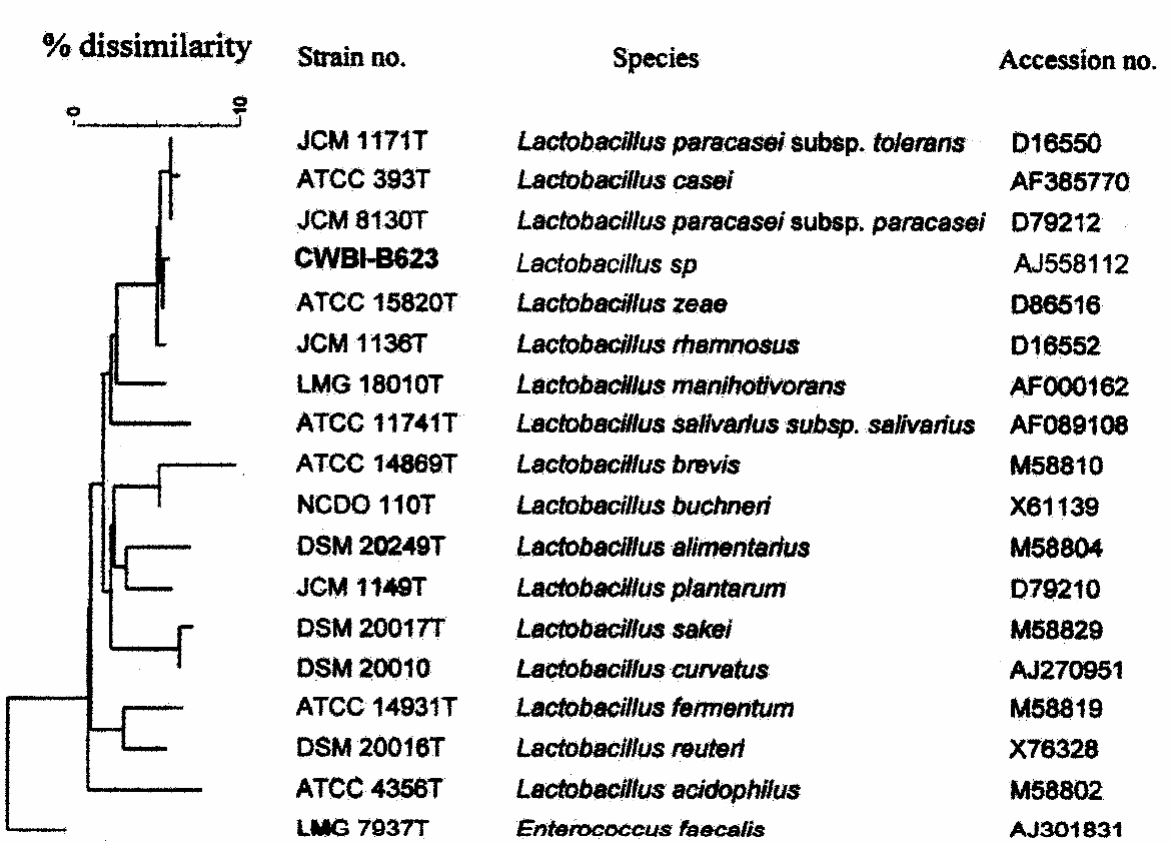

Figure 1. The tree was constructed by 16S-rDNA analysis using the neighbour joining method.

halo formed by the action of lactic acid produced. When they are cultivated in MRS broth, cells precipitate gradually and growth does not occur on the surface. The isolate was mesophilic, facultatively aerobic, and shows a catalase activity, but pseudocatalase was not detected (the result of the benzidine test was negative). The optimal temperature and pH for growth lies around values of 37°C and 6.8, respectively. The determination of fermentation products showed that the isolate converts glucose to L (+)-lactic acid through a typical homo-fermentative pathway. The isolate did not grow at 5 and 50°C, the growth was inhibited by the presence of 10% NaCl (it is neither halophilic nor even halotolerant), it did not hydrolyse caseinate or tributyrine, it showed a good survival rate at pH 2.5 and pH 3 after 2 h of incubation, the averages were 26% at pH 3 and 1.2% at pH 2.5. The minimum inhibitory concentration (MIC) of bile salts was superior to 1%. The patterns of carbon sources fermentation were obtained with API 50CHL kits. The more significance difference between the fermentative pattern of CWBI-B623 and *L. casei* group species were presented in Table 2. Contrary to *L. casei* group strain, the isolate was able to hydrolyse arabinose, adonitol, fucose, 2 aceto-gluconate and particularly rhamnose (hydrolysis only by *L. rhamnosus*).

Phylogenetic analysis and identification

The 16S rDNA sequence of CWBI-B623 was compared to those of the international EMBL catalogue. This analysis indicated that the isolate belongs to the genus *Lactobacillus* (Figure 1). The sequence homology with *L. casei* group species was higher than 99%. This group includes the species *L. zeae*, *L. casei*, *L. paracasei*, *L. rhamnosus* and their respective sub species. The Table 3 give the percentage of similarity between the strain CWBI- B623 and the most significant species of this group.

Nucleotide sequence accession number

The 16S rRNA gene sequence of the strain CWBI-B623 has been deposited in EMBL/GenBank/DDBJ nucleic

Table 3. Comparison of the 16S rRNA sequence of CWBI-B623 to that of other Lactobacilli in the EMBL database. 16S-rDNA similarities (%) as determined by sequence alignment, between the isolate B623 and the sequences collected from EMBL.

Organisms	Similarity to CWBI-B623 (%)
Lactobacillus zeae ATCC 15820^T	99.8
Lactobacillus paracasei subsp. paracasei JCM 8130^T	99.1
Lactobacillus casei ATCC 393^T	99.1
Lactobacillus paracasei subsp. tolerans JCM 1171^T	98.8
Lactobacillus rhamnosus JCM 1136^T	98.8

acid sequence database under accession number AJ558112.

DISCUSSION

The first characteristics observed in the properties of the strain CWBI-B623 were the positive reaction to catalase test and negative reaction to benzidine test. The benzidine test was the most important microbiological test used to determine the presence of the pseudocatalases in microbial culture. The peroxidase produce by CWBI- B623 seems to be different from the catalase found at *Bacillus* species, but we could not establish the bond which could be exist with this enzyme and the pseudocatalase or manganese catalase known at some lactic acid bacteria belonging to the genus *Lactobacillus*. We did not go far enough in this study to determine the nature of the peroxidase produced by the strain CWBI-B623, but we think that the behavior observed in this strain was probably related to the sensitivity of the benzidine test that we have used. From LAB, there are at least three types of pseudocatalase. These peroxidase are generally differentiated by the number of sub-units which compose the enzyme structure and by the oxidation state of their manganese core (Abriouel et al., 2004).

The second characteristic which had held our attention in this analysis relates to the technological properties of the strain CWBI-B623. This strain has several physiological and technological properties which can interest probiotic formulations. The tolerance of this strain to acidity and to bile salts was very exceptional for species of the genus *Lactobacillus*. The highest tolerance to bile salts can be in relation with the hydrolysis of these products, but this hypothesis has to be confirmed. The strain CWBI-B623 has a significant production of lactic acid (9 ± 0, 5 g/l in GYP broth after 48 h) and least nutritional needs; its growth in GYP medium was good. The production of peroxidase in this strain can also be used industrially, in addition to the elimination of hydrogen peroxide, the catalase or pseudocatalase can catalyse the degradation of phenolic compounds. These phenolic compounds are antinutritional factors for their toxicity and their resistance to digestive enzymes. Thus, the catalase can play a role in the detoxification of these phenolic compounds by transforming them into derivatives that are not poisonous (Zamocky et al., 2001). It appears that CWBI-B623 will be a good probiotic candidate for food industries.

On the genetic level, the strain CWBI- B623 was close to the species of *L. casei* group, but on the biochemical level it was rather very close to *L. kunkeii*. *L. kunkeii* produces a pseudocatalase, L(+)-lactic acid from a significant number of carbon sources (Edwards et al., 1998) like CWBI-B623. The alignment of the sequences 16S rDNA of these two bacteria showed that they were genetically different (91% similarity). If the membership of CWBI-B623 to the genus *Lactobacillus* and to the *L. casei* group can be accepted as established, its taxonomic position compared to the members of the *L. casei* group must still be given. The *L. casei* group was regularly reorganized and several new taxa were identified (Collins et al., 1989; Dellaglio et al., 1991; Dellaglio et al., 2002; Disks et al., 1996; Mori et al., 1997; Ward and Timmins, 1999). It includes the species *L. zeae*, *L. casei*, *L. paracasei*, *L. rhamnosus* and their respective subspecies (Kandler and Weiss 1986). The later three species are the best known since they are used as probiotic bacteria in several formulations (Collins et al., 1998). *L. casei* and *L. zeae* type strains were very similar for their 16S-rDNA-sequences and *L. zeae* was proposed as the type strain of this group (Mori et al., 1997). The species of *L. casei* group are present in various environments including plants, dairy products, animals, rhizosphere, etc. (Klein et al., 1998). All strains of this group produce L-Lactic acid and were unable to produce catalase or pseudocatalase. *L. casei* subsp. *tolerans* was remarkable due to its heat resistance at 72°C for 40 s (Disks et al., 1996) and *L. rhamnosus* was remarkable for its ability to hydrolyse rhamnose. The identification of CWBI-B623 could be supplemented by the examination of the total DNA-DNA hybridizing between this bacterium and the standard species of *L. casei* group (Table 2).

In conclusion, an asporogenous catalase-positive LAB has been isolated from chicken faeces in Burkina Faso. The phylogenetic analysis of this bacteria, performed by

the alignment of 16S-rDNA sequences showed that the new strain CWBI-B623 belongs to the genus *Lactobacillus* and formed a subline within the *L. casei* cluster of species, including *L. rhamnosus, L. casei, L. zeae* and *L. paracasei*. The highest homology was obtained with *L. zeae* (type strain of this group). The biochemical and physiological analyses of the strain CWBI-B623 showed that this bacterium had significant qualities for probiotic food formulation, in particular, L(+) lactic acid production in important amount, resistance to bile salts (can be in relation with the hydrolysis of bile salts) and resistance to acidity. The strain B623 should be completely characterized for application in biotechnology and food industry; the future investigations will concern this aspect.

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REFERENCES

Abriouel H, A Herrmann, J Stärke, N Yousif, A Wijaya, B Tauscher, W Holzapfel, C Franz (2004). Cloning and heterologous expression of hematin-dependent catalase produced by *Lactobacillus plantarum* CNRZ 1228. Appl. Environ. Microbiol. 70: 603–606.

Berner AL, AJ O'Donnell (1998). Functional foods and health claims legislation: applications to dairy foods. Int. Dairy J. 8: 355-362.

Buckenhüskes HJ (1993). Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities, FEMS Microbiol. Rev. 12: 253-272.

Collins MD, BA Phillips, P Zanoni (1989). Deoxyribonucleic acid homology studies of *Lactobacillus casei, Lactobacillus paracasei sp. nov., subsp. paracasei* and *tolerans*, and *Lactobacillus rhamnosus sp. nov., comb. nov*; Int. J. Syst. Bacteriol. 39: 105-108.

Collins JK, G Thornton, GO Sullivan (1998). Selection of Probiotic strains for human applications, Int. Dairy J. 8: 487-490.

De Man JC, M Rogosa, ME Sharpe (1960). A medium for the cultivation of lactobacilli, Int. J. Appl. Bacteriol. 23 130-135.

Dellaglio F, LMT Dicks, M du Toit, S Torraniani (1991). Designation of ATCC 334 in place of 393 (NCDO 161) as the neotype strain of *Lactobacillus casei subsp*. casei and rejection of the name *Lactobacillus paracasei* (Collins et al., 1989), Int. J. Syst. Bacteriol. 41: 340-342.

Dellaglio F, G Felis, E Giovanna, S Torriani (2002). The status of *Lactobacillus casei* (Orla-Jensen 1916) Hansen and Lessel 1971 and *Lactobacillus paracasei* Collins et al. 1989. Request for opinion, Int. J. Syst. Evol. Microbiol. 52: 285-287.

Disks LMT., EM. Du Plessis, F. Dellaglio, E. Lauer (1996). Reclassification of *Lactobacillus rhamnosus* ATCC 15820 as *Lactobacillus zeae* nom. rev., designation of ATCC 334 as neotype of *Lactobacillus casei subsp. casei*, and rejection of the name *Lactobacillus paracasei,* Int. J. Syst. Bacteriol. 46: 337-340.

Edwards CG, KM Haag, RA Huston, YC Huang (1998). *Lactobacillus kunkeei sp. nov*.: a spoilage organism associates with grape juice fermentations. J. Appl. Microbiol. 84: 698-702.

Fujisawa T, Y Benno, T Yaeshima, T Mitsuoka (1991). Taxonomic study of the *Lactobacillus acidophilus* group, with recognition of *Lactobacillus gallinarum sp. nov*. and *Lactobacillus johnsonii sp. nov*. and synonymy of *Lactobacillus acidophilus* group A3 (Johnson et al. 1980) with the type strain of *Lactobacillus amylovorus* (Nakamura 1981), Int. J. Syst. Bacteriol. 42: 487- 491.

Fujisawa T, S Shirasaka, J Watabe, T Mitsuoka (1984). *Lactobacillus aviarius sp. nov*: a new species isolated from the intestine of chiken, Syst. Appl. Microbiol. 5: 414-420.

Gerhardt P, RG Murray, RN Costilow, EW Nester, WA Wood, RN Krieg GB Phillips (1981). (Eds), Manual of Methods for General Bacteriology, American Society of Microbiology, Washington, DC. p. 524.

Gomez MP, FX Malcata (1999). *Bifidobacterium spp*. And *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics, Trends Food Sci. Technol. 10: 139-157.

Gregersen T (1978). A rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur. J. Appl. Microbiol. Biotechnol. 5: 123-127.

Gusils C, J Palacios, S Gonzales, G Olivier (1999). Lectin-like protein fractions in lactic acid bacteria isolated from chickens, Biol. Phar. Bull. 22: 11-15.

Gusils C, AC Perez, S Gonzalez, G Oliver (1999). Lactobacilli isolated from chicken intestines: potential use as probiotics. J. Food Prot. 62: 252-256.

Havenaar R, BB Ten, JH Huis in't (1992). Selection of strains for probiotic use. In: Fuller R (Eds). The Scientific Basic, Chapman & Hall, London. pp. 209-224.

Holzapfel WH, P Haberer, J Snel, U Schillinger, JH Huis in't (1998). Overview of gut flora and probiotics, Int. J. Food Microbiol. 41: 85-101.

Hyronimus B, A Le Marrec, SA Hadj, A Deschamps (2000). Acid and bile tolerance of spore-forming lactic acid bacteria, Int. J. Food Micobiol. 61: 193-197.

Kandler O, N Weiss (1986). Regular non-sporing Gram-positive rods, Genus *Lactobacillus* (Beijerinck 1901, 22), In: PHA Sneath, NS Mair, ME Sharpe, JG Holt (Eds.), Bergey's manual of systematic bacteriology, 8th ed, vol. 2, William and Wilkins Co, Baltimore, pp. 1208-1234.

Klein G, A Pack, C Bonaparte, G Reuter (1998). Taxonomy and physiology of probiotic lactic acid bacteria, Int. J. Food Microbiol. 41 : 103-125.

Larpent JP, MG Larpent (Eds.) (1985). Manuel pratique de Microbiologe, Collection Hermann, Paris, France, p. 230.

Marteau P, JC Rambaud (1993). Potential of using lactic acid bacteria for therapy and immunomodulation in man, FEMS Microbiol Rev. 12: 207-220.

Mori K, K Yamazaki, T Ishiyama, M Katsumata, K Kobayashi, Y Kawai, N Inoue, H Shinamo (1997). Comparative sequence analyses of the genes coding for 16S-rDNA of *Lactobacillus casei* related taxa, Int. J. Syst. Bacteriol. 47: 54-57.

Niemann S, A Puehler, HV Tichy, R Simon, W Selbitschka (1997). Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population, J. Appl. Microbiol. 82: 477-484.

Saitou N. and Nei M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

Saul S (1998). Probiotics: Functionality and commercial status, Biotechnol. adv. 16 581-608.

Ward LJH, MJ Timmins (1999). Differentiation of *Lactobacillus casei, Lactobacillus paracasei* and *Lactobacillus rhamnosus* by polymerase chain reaction, Lett. Appl. Microbiol. 29: 90-92.

Zamocky M, J Godocikova, F Koller, B Polek (2001). Potential application of catalase-peroxidase from *Comamonas terrigena* N3H in the biodegradation of phenolic compounds, Antonie Leeuwenhoek. 79: 109-117.