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Probiotic profiling of *Leuconostoc* species isolated from a traditional fermented cassava product

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The various properties of lactic acid bacteria have made them good auxiliary in the manufacturing process in agro food industries and farms. They are widely used as probiotics which can be defined as living microorganisms that have beneficial effects on human health probiotics could replace antibiotic growth promoters in livestock without creating new threats such as that observed with antibiotics. Before their use as probiotics lactic acid bacteria require a perfect knowledge in view to their biochemical and genetic characteristics because it is difficult to differentiate morphologically some *Leuconostoc* and *Lactobacillus* strains using morphological characteristics. This study was undertaken in order to evaluate the probiotics potential of *Leuconostoc* strains isolated from traditional fermented cassava. The results showed that 5 strains of *Leuconostoc* have antibacterial activity against *Staphylococcus aureus* (MetiR), *Klebsiella pneumoniae* (BLSE), *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The molecular identification of species using the conserved region of the 16S rRNA helped distinguish the species *Leuconostoc mesenteroides*. All these results showed that the studied *Leuconostoc* strains could be used as potential probiotics for the biopreservation of various foods.

Key words: *Leuconostoc*, antibacterial activity, polymerase chain reaction (PCR), sequencing, probiotics.

INTRODUCTION

Probiotics are defined as living microorganisms that have beneficial effects on human health (FAO/WHO, 2002). Indeed, several studies have demonstrated that probiotics may enhance growth performance, immunity and disease resistance (Saxelin et al., 2005; Ezendam and van Loveren, 2006; Ström-Bestor and Wiklund, 2011). Despite the numerous definitions, the criteria to select probiotic strains are total safety for the host, resistance to gastric acidity and pancreatic secretions, adhesion to epithelial cells, antimicrobial activity, inhibition of adhesion of pathogenic bacteria, evaluation of resistance to antibiotics, tolerance to food additives and stability in the food matrix (Soccol et al., 2010). In addition, the functional properties of probiotics include

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hypocholesterolemic activity by lowering plasma cholesterol, preventing and treating diarrhoea (Liong and Shah, 2005). The mechanisms by which probiotics exert their beneficial effects on the host include the reduction of luminal pH, competition with pathogens for adhesion sites and nutritional sources, secretion of antimicrobial substances, toxin inactivation, and immune stimulation (Salminen et al., 2004). The most commonly used probiotics are the strains of lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus* (*Streptococcus thermophilus*); the first two are known to resist gastric acid, bile salts and pancreatic enzymes, to adhere to colonic mucosa and readily colonize the intestinal tract (Fioramonti et al., 2003).

The use of selected probiotics from alternative sources known as “unconventional sources” is likely to increase. These unconventional sources include non-intestinal sources and non-dairy fermented food products, such as traditional fermented foods, traditional fermented drinks, vegetables, and fruit juices (Ramirez-Chavarin et al., 2013; Siddiqee et al., 2013). In Côte d’Ivoire, cassava (*Manihot esculenta*) root is traditionally fermented into a traditional microbial starter called “mangnan” that is used to prepare a really appreciated food called “attiéké” defined as fermented and steamed semolina cassava (Assanvo et al., 2006; Dje et al., 2008). Studies on this traditional microbial starter showed that the dominant microflora consists of lactic acid bacteria (LAB) (Assanvo et al., 2006). LAB, a group of Gram-positive, non-spore forming, non-motile microorganisms can produce inhibitory compounds such as lactic acid, bacteriocin and hydrogen peroxide preventing the growth of harmful microorganisms. The role of LAB in improving the shelf life and nutritional quality of fermented foods and beverages, controlling diarrhea, as well as their antimicrobial properties have also been established. However, despite an increasing interest in LAB, there is a paucity of literature regarding novel and emerging uses of LAB as probiotics, especially from traditional African fermented foods. Thus, the objective of the current study was to characterize the potential probiotic properties of *Leuconostoc* species isolated in previous studies (Coulibaly et al., 2016) from traditional fermented cassava.

MATERIALS AND METHODS

LAB and indicator strains

The microbial strains used in this study are shown in Table 1. Five (05) LAB belonging to *Leuconostoc* genus, previously isolated from traditional fermented cassava (Coulibaly et al., 2016) were used as test strains: BL1, BL2, BL7, BL39, BL44, and BL61. The LAB reference strains used were *Lactobacillus plantarum* CWBIBF-76, *Enterococcus faecium* EFTHT and *Weissella confusa* CWBI-B902. The indicators strains (Table 1) were collected from Institute Pasteur of Côte d’Ivoire.

Table 1. LAB and indicators strains.

Test strains
BL1
BL7
BL39
BL44
BL61
Lactobacillus plantarum CWBI BF-76
Enterococcus faecium EFTHT
Weissella confusa CWBI-B902
Indicator strains
Staphylococcus aureus
Escherichia coli
Salmonella typhimurium
Stqphylococcus hemolyticus (Resistant to methicillin: SARM)
*Klebsiella pneumoniae (*Producer of beta-lactamase: ESBL)
Staphylococcus aureus ATCC 25923
Escherichia coli ATCC 25922
Pseudomonas aeruginosa ATCC 27853

Antimicrobial activity determination

The method of agar spots as described by Larpent-Gourgaud et al. (1997) was used to evaluate the antimicrobial activity of the selected LAB strains. For this Petri dish, MRS agar was spotted with a 24-h colony of the LAB strain. The plates were seeded at 37°C for 24 h. At the same time, the indicator strains were subcultured in BCC broth for 3 h and then isolated on selective agar and incubated at their optimum growth temperature for 18 h. Each indicator strain was suspended in 2 ml of 0.85% NaCl and then vortexed. The OD was adjusted to 2.5 Mc Farland. Then, the inoculum was obtained by mixing 1 ml of inoculum of each strain in 9 ml of physiological water. The boxes with the spots were inoculated by flooding and the dishes were observed after 24 h of incubation at 37°C. The size of the zones of inhibition produced was measured.

Morphological, physiological and biochemical identification

The Gram characteristics of the isolates were determined using light microscope (Leica DM 1000, France) following staining. LAB are known to be Gram-positive. Cultures were grown in appropriate MRS media at 37°C for 48 h under anaerobic conditions. Cells from fresh cultures were used for Gram staining.

The determination of fermentation profiles (heterofermentative or homofermentative) was performed by inoculating 10 ml MRS broth containing bell Durhams at 28°C for 24 h (Harir et al., 2009).

For the fermentation of sugars, MRS media broth without glucose and supplemented with bromocresol purple as indicator was used (Mannu et al., 2000). For this, 9 ml of the medium is left in test tubes and sterilized for15 min. One milliliter of the sugar solution (10%, p/v) was aseptically added after filtration on a membrane with 0.45 µm porosity. Incubation was performed at 28°C for 48 h. Tested sugars were arabinose, sucrose, fructose, trehalose and esculin (Garvie, 1983).

The method described by Leveau et al. (1991) was used to determine the dihydrolase arginine (ADH), while the ability to grow at 10, 37 and 44°C was determined after incubation of inoculated Petri dishes at these temperatures.

Table 2. Features of primers used for PCR.

Primers	Tm (°C)	%GC	Sequences (5'--- 3')	Reference
16F27	57.3	50	AGAGTTTGATCCTGGCTCAG	Bayane et al. (2006)
16R1522	44.7	60	AAGGAGGTGATCCAGCCGCA	

Tm: Hybridization temperature; % GC: guanine and cytosine percentage.

Table 3. Diameter of inhibition (mm) of indicators strains.

Test strains	Indicators strains						
	S. aureus	***E. coli***	***S. Typhimurium***	***S. hemolyticus* MétiR**	***K. pneumoniae* (BLSE)**	***S. aureus* ATCC 25923**	***E. coli* ATCC 25922**
BL1	19	12	20	19	17	22	15
BL7	17	11	14	16	16	20	18
BL39	19	16	17	24	21	24	20
BL44	22	13	18	30	19	26	22
BL61	20	11	20	31	19	17	22
Lactobacillus plantarum CWBI BF-76	20	19	22	22	20	23	16
Enterococcus faecium EFTHT	10	8	11	11	11	23	21
Weissella confusa CWBI-B902	15	8	10	16	8	9	12

Molecular identification

DNA extraction and 16s rDNA amplification

A colony from culture was resuspended in 300 µl pure water in 1.5 ml Eppendorf tubes. DNA isolation and purification were realized using *Instagen Matrix kit* (Bio-Rad, USA) according to the manufacturer's instructions. PCR targeting the 16s rDNA of LAB were done as described by Bayane et al. (2006). The amplification reactions of 16S rDNA region were realized in a final volume of 50 µl containing 1X Master Mix (5 PRIME HotMasterMix, 5PRIME), 1 µl of DNA template (approximately 50 ng), 1 µM of each primer (Table 2) and PCR grade water.

The amplification was carried out in a 2720 ABI thermalcycler (Applied Biosystems, Syngapore). PCR conditions started with an initial denaturation at 94°C for 2 min; 36 cycles consisting of 1 min denaturation at 94°C for 30 s, annealing at 58°C and elongation at 65°C for 2 min. Then, a final extension for 7 min at 65°C ended the PCR reaction. PCR products were revealed in 1% agarose gel electrophoresis containing ethidium bromide (1 µg/ml).

Sequencing of the amplified DNA and data analysis

PCR products were sent to a Company (GATC BIOTECH, GERMANY) for sequencing. Sequences obtained were compared to those listed in Genbank (National Center for Biotechnology Information) using the nucleotid BLAST 2.5.0 tool (Zheng et al., 2000; Aleksandr et al., 2008). Similarity percentages were determined between the isolated sequences in this study and the closest sequences listed in GenBank. Sequences were considered similar when they have at least 99% percentage of similarity. Phylogenetic constructions were done after re-alignment of the sequences using MEGA 7.0.14 (Kumar et al., 2016). The maximum likelihood and UPGMA algorithms (USA) were chosen for trees construction.

RESULTS AND DISCUSSION

Antibacterial activity

Among the nine LAB strains tested for antibacterial activity in solid medium, only 5 strains (BL1, BL7, BL39, BL44, and BL61) showed good activity against all the indicator strains. The inhibition diameters measured are superior or equals to those of reference strains (*L. plantarum*, *E. faecium*, and *W. confusa*) (Table 3).

Inhibition diameter is denoted positive when greater than 8 mm (Schillinger et al., 2001). BL39, BL44 and BL61 strains inhibited the growth of all indicators Gram positive strains *Staphylococcus hemolyticus* MetiR and *Staphylococcus aureus* ATCC 25923.

Bacteria of the genus *Leuconostoc,* in association with the mesophilic LAB are capable of inhibiting the growth of pathogenic microorganisms such as *S. aureus*, *Klebsiella pneumoniae* BLSE, *Escherichia coli, Salmonella* Typhimurium and reference test strains, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Indeed, Todorov and Dicks (2005) have shown the growth inhibitory of *E. coli* and *P. aerugionosa* by a product of *Leuconostoc mesenteroides* subsp. *mesenteroides*. The antagonistic action of LAB against pathogens such as *Salmonella*, *S. aureus,* and *E. coli* was also confirmed by studies of Makras et al. (2006).

The diameters of inhibition of the indicator strains *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were higher than those of the three reference strains. The strain BL44 showed a strong

Table 4. Biochemical characteristics of the selected *Leuconostoc* strains.

Parameter	Strains				
	BL1	**BL7**	**BL39**	**BL44**	**BL61**
Arabinose	-	+	+	+	+
Fructose	+	+	+	+	+
Sucrose	+	+	+	+	+
Trehalose	+	+	+	+	+
Hydrolysis of esculin	-	+	+	-	+
ADH	-	-	-	-	-
Type of fermentation	He	He	He	He	He
10°C / 72 h	+	+	+	+	+
37°C 24 h	+	+	+	+	+
40°C / 24 h	+	+	+	+	+
Morphology	Cocci Gram+	Cocci Gram+	Cocci Gram+	Cocci Gram+	Cocci Gram+

He: Heterofermentative.

inhibition (60 mm) of growth of the *P. aeruginosa* ATCC 27853. All strains diameters inhibition of *E. coli* and *Staphylococcus* were similar to those obtained by Labiou et al. (2005).

The antagonist effect as shown is this study is related to the biosynthesis of inhibitor compounds observed in strains of lactic bacteria (Servin, 2004). Indeed, organic acids are able to acidify the cytoplasm after dissociation and inhibit the cellular enzymatic activity of acid-sensitive pathogens (Tou et al., 2006; Djéni et al., 2008). This decrease in pH can therefore affect the viability of pathogenic bacteria (Bruno et al., 2002; Servin, 2004). This inhibition effect may be related to a competition with nutrients. An increase in the number of LAB obtained during a probiotic treatment would make it possible to reduce the substrates available for the implantation of pathogenic microorganisms (Fooks and Gibson, 2002). This justify the fact that all bacteriocins produced by LAB antimicrobial activity against Gram + (Dortu and Philippe, 2009).

Leuconostoc producing bacteriocin can be found in different food products including meat, cereals and milk (Hastings et al., 1994; Wulijideligen et al., 2012).

Morphological, biochemical and physiological characteristics

Morphological, biochemical and physiological examinations of the strains with antibacterial activity are shown in Table 4. Bacterial cells were spherical grouped into short chains and diplococcic. All strains were heterofermentative after 24 h. None of strains possessed hydrolase arginine.

Phylogenetic analysis and identification

The PCR targeting the 16 rDNA showed that all the strains that have demonstrated antibacterial activity belong to the lactic bacteria family. Positive samples detected highlighted a 1500 bp PCR product on agarose gel (Figure 1).

Sequences analysis and phylogeny

The 5 *Leuconostoc* PCR 16 S rDNA products were sequenced. The BLAST*n*'s results confirmed that all the 5 isolates BL1, BL7, BL39, BL44 and BL 61 were genetically related to *L. mesenteroides* with 99% identity. The multiple sequence alignment of the Ivoirian *Leuconostoc* strains with other isolated strains from other region of the world show that all the 5 Ivoirian strains presented identical sequence. The genetic tree analysis showed clearly that those strains form a new genetic group related to *L. mesenteroides* on the basis of Blast results but are genetically distant (Figure 2). The 16S rRNA gene sequences of BL1, BL7, Bl39, BL44, and BL61 where deposited in GenBank nucleic acid sequence database under accession number KM518656 to KM518660.

At genetically sequence alignment of 16S rRNA of BL1, BL7, BL39, BL44 and BL61 strains showed that they were close to *L. mesenteroides* ssp. *mesenteroides* and *L. mesenteroides* ssp. *dextranicum.*

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Zheng et al., 2000). The tree with the highest log likelihood (-2249.2146) is as shown in Figure 2. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions

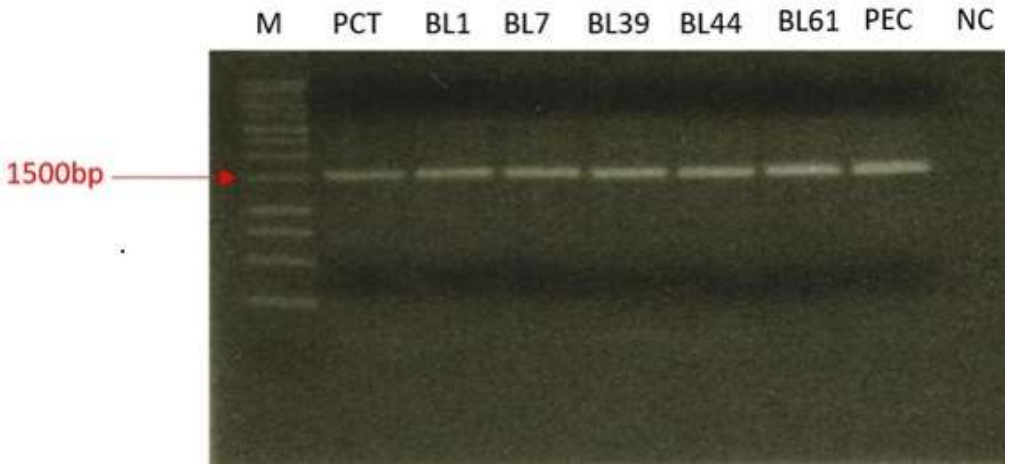

Figure 1. Agarose gel obtained after electrophoresis of PCR's16sDNA product from *Leuconostoc* strains. M: Molecular weight marker (1 kb DNA Ladder); PCT: positive PCR test control; NC: negative control; *Leuconostoc* strains: BL1, BL7, BL39, BL44, BL61.

Figure 2. Molecular phylogenetic analysis by Maximum Likelihood method of *Leuconostoc* strains isolated from traditional fermented Cassava.

per site (next to the branches). The analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 515 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Aleksandr et al., 2008).

Conclusion

In conclusion, it can be stated that strains of LAB that have antibacterial properties belong to *L. mesenteroides*. In view to their strong inhibitory properties on *P. aeruginosa* and on methicillin-resistant *S. aureus*, these strains could be used as biopreservatives of various foods. Furthermore, a better knowledge of other criteria for selecting the probiotics associated with technological properties would make them potential candidates for the formulation of ready to use probiotics

CONFLICT OF INTERESTS

The author(s) have not declared any conflict of interest.

REFERENCES

Aleksandr M, George C, Yan R, Thomas LM, Richa A, Alejandro AS (2008). Database Indexing for Production MegaBLAST Searches Bioinformatics 24:1757-1764.

Assanvo JB, Agbo GN, Behi YEN, Coulin P, Farah Z (2006). Microflora of traditional starter made from cassava for attiéké production in Dabou (Côte d'Ivoire). Food Control 17:37-41.

Bayane A, Dominique R, Robin DD, Jacqueline D, Brehima D, Philippe T (2006). Assessment of the physiological and biochemical characterization of a *Lactic acid bacterium* isolated from chicken faeces in sahelian region. Afr. J. Biotechnol. 5(8):629-634.

Bruno FA, Lankaputhra WEV, Shab NP (2002). Growth, viabilty and activity of bifidobacterium spp in skim milk containing prebiotics. J. Food Sci. 67:2740-2744

Coulibaly KE, Coulibaly KJ, Goualié GB, Akpa EE, Niamké SLA, Mireille D (2016). Benchmarking Loads of Lactic Acid Bacteria from Traditional Ferment of Cassava Used for the Preparation of Cassava Meal in Abidjan. Int. J. Curr. Res. Biosci. Plant Biol. 3(8):69-73.

Djeni NT, N'guessan KF, Dadie AT, Dje KM (2008). Impact of different rates of a traditional starter on biochemical and microbiological changes during the fermentation of cassava for attiéké production. Food 2:145-151.

Dortu C, Philippe T (2009). Les bactériocines des bactéries lactiques: caractéristiques et intérêts pour la bio conservation des produits alimentaires. Biotechnol. Agron. Soc. Environ. 13(1):143-154.

Ezendam J, van Loveren H (2006). Probiotics: immunomodulation and evaluation of safety and efficacy. Nutr. Rev. 64:1-14

FAO/WHO (2002). Guidelines for the evaluation of probiotics in food. Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. World Health Organization, London Ontario, Canada, 2002: 8. Available from: ftp://ftp.fao.org/es/esn/food/wgreport2.pdf

Fioramonti J, Theodorou V, Bueno L (2003). Probiotics: What are they? What are their effects on gut physiology?, Best Pract. Res. Clin. Gastroenterol. 17:711-724.

Fooks IJ, Gibson GR (2002). Probiotics as modulators of the gut flora. Br. J. Nutr. 88(1):S39-S49

Garvie EI (1983). *Leuconostoc mesenteroides subsp cremoris* (Knudsen and Sorensen) comb. Nov. and *Leuconostoc mesenteroides subsp dextranicum* (Beijerinck) comb. nov. Int.J. Syst. Bacterial. 33: 118-119.

Harir A, Ouis N, Sahnouni F, Bouhadi D (2009). Mise en œuvre de la fermentation de certains ferments lactiques dans des milieux à base d'extraits de carouche. Rev. Microbiol. Ind. San. Environ. pp. 37-55.

Hastings JW, Stiles ME, Von HA (1994). Bacteriocins of leuconostocs isolated from meat Int. J. Food Microbiol. 24(1-2):75-81.

Kumar S, Stecher G, Koichiro T (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33(7):1870-1874.

Labiou H, Elmoualdi L, El Yachioui M, Ouhssine M (2005). Sélection de souches de bactéries lactiques antibactériennes. Bull. Soc. Pharm. Bordeaux 144:237-250.

Larpent-Gourgaud M, Odile M, Larpent J-P, Desmasures N, Desmazeaud M, Mangin I, Masson F, Montel M-C, Patrick T (1997). Les ferments lactiques et bactéries apparentées. In. Microbiologie alimentaire techniques de laboratoire. Pp.199-255.

Leveau J-Y, Marielle Bouix, H de Roissart (1991). La flore lactique. In. Techniques d'analyses et de contrôle dans les industries agroalimentaires : le contrôle microbiologique, C. M. Bourgeois et Leveau, J.-Y. Pp. 152-186

Liong MT, Shah P (2005). Optimization of cholesterol removal by probiotics in the presence of prebiotics by using a response surface method. Appl. Environ. Microbiol. 71(4):1745-1753.

Makras L, Triantafyllou V, Fayol-Messaoudi D, Adriany T, Zoumpopoulou G, Tsakalidou E, Servin A, De Vuyst L (2006). Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards Salmonella enterica serovar Typhimurium reveals a role for lactic acid and other inhibitory *compounds.* Res. Microbiol. 157(3):241-247.

Mannu L, Paba A, Pes M, Scintu MF (2000). Genotypic and phenotypic heterogeneity among lactococci from traditional Pecorino Sardo Cheese. J. Appl. Microbiol. 89:191-197.

Ramirez-Chavarin ML, Wacher C, Eslava-Campos CA, Perez-Chabela ML. (2013). Probiotic potential of thermotolerant lactic acid bacteria strains isolated from cooked meat products. Int. Food Res. J. 20:991-1000.

Salminen S, Wright AV, Ouwehand A (2004). Lactic acid bacteria. microbiological and functional aspects. Marcel Dekker. Inc., U.S.A.

Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM (2005). Probiotic and other functional microbes: from markets to mechanisms. Curr. Opin. Biotechnol. 16:204-211.

Schillinger U, Becker B, Vignolo G, Holzapfel WH (2001). Efficacy of nisin in combinaison with protective culture against Listeria monocytogenes Scott A in tofu. Int. J. Food Microbiol. 71:159-168

Servin A (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol. Rev. 28:405-440

Siddiqee MH, Sarker H, Shurovi KM (2013). Assessment of probiotic application of lactic acid bacteria (LAB) isolated from different food items. Stamford J. Microbiol. 2:10-14.

Soccol CR, Vandenberghe LP, Spier MR, Medeiros AB, Yamaguishi CT, Lindner JD, Pandey A, Thomaz-Soccol V (2010). The Potential of Probiotics: A Review. Food Technol. Biotechnol. 48(4):413-434.

Ström-Bestor M, Wiklund T (2011). Inhibitory activity of *Pseudomonas sp.* on Flavobacterium psychrophilum, in vitro. J. Fish Dis. 34:255-264.

Todorov SD, Dicks LMT (2005). Production of Bacteriocin ST33LD, produced by *Leuconostoc mesenteroides* subsp. *mesenteroides*, as Recorded in the Presence of Different Medium Components. World J. Microbiol. Biotechnol. 21(8):1585-90.

Tou EH, Guyot JP, Mouquet-Rivier C, Rochette I, Counil E, Traoré AS, Treche S (2006). Study through surveys and fermentation kinetics of the traditional processing of pearl millet (Pennisetum glaucum) into bensaalga, a fermented gruel from Burkina Faso. Int. J. Food Microbiol. 106:52-60.

Wulijideligen, Asahina T, Hara K, Arakawa K, Nakano H, Miyamoto T (2012). Production of bacteriocin by *Leuconostoc mesenteroides* 406 isolated from Mongolian fermented mare's milk, airag. Anim. Sci. J. 83:704-711.

Zheng Z, Scott S, Lukas W, Webb M (2000). A greedy algorithm for aligning DNA sequences J. Comput. Biol. 7(1-2):203-214.