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Full Length Research Paper

Isolation and identification of lactic acid and non-acid lactic bacteria from *“dèguè”* of Western Africa traditional fermented millet-based food

Cheik Amadou Tidiane OUATTARA, Marius Kounbèsiounè SOMDA*, Rachel MOYEN and Alfred Sabedenedjo TRAORE

Research Centre of Biologicals Food and Nutrition Sciences (CRSBAN), Department of Biochemistry and Microbiology, UFR-SVT, University of Ouagadougou. PO Box 7021 Ouagadougou, Burkina-Faso.

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Dégué **is a traditional fermented millet-based food which is consumed in Burkina Faso and other countries of West Africa. A total of 125 strains of bacteria were selected from 16 samples of *dégué*. Isolates were studied by determination of morphological and biochemical characteristics. Among the 125 strains of lactic acid bacteria selected, 68 strains were identified as *Lactobacillus* and 57 strains as *Lactococcus.* The representative species of the *Lactobacillus* were: *Lactobacillus plantarum* (25.6%), *Lactobacillus delbrueckii subsp delbrueckii* (11.2%), *Lactobacillus acidophilus* (7.2%), *Lactobacillus brevis* (4.8%), *Lactobacillus buchneri* (8%). *Lactobacillus cellobiosus* (4%), *Lactobacillus pentosus* (2.4%), *Lactobacillus crispatus* (1.6%), *Lactobacillus fermentum* (1.6%), *Lactobacillus curvatus* (0.8%), *Lactobacillus paracasei subsp paracasei* (0.8%). Among the 57 strains of lactic acid coccus isolated predominated *Pediococcus damnosus* strains (14.4%), followed by *Lactococcus lactis subsp lactis* (6.4%), *Pediococcus pentosaceus* (3.2%), *Pediococcus* sp. (3.2%), *Leuconostoc lactis* (1.6%), *Leuconostoc mesenteroides subsp mesenteroides* (0.8%), *Pediococcus acidilactici* (0.8%), *Lactococcus curvatus* (0.8%) and *Tetragenococcus halophilus* (0.8%). Many Gram negative bacteria were also isolated as coliforms and proteolic strains that can play a negative contribution on the quality of *dègué.***

Key words: Fermented millet, *dèguè*, *Lactobacillus* sp., *Enterobacteria.*

INTRODUCTION

The finger millet (*Eleusine corocana*) is an important food crop in arid and semi-arid regions of the world. In Burkina Faso Finger millet production represents a third of the total consumption of food cereals. Pearl millet *Pennisetum glaucum* is native to the tropical region of western Africa where it is meeting wide all the cultivated and wild varieties (Hama et al., 2009).

These crops are used in production of many traditional fermented products in African and Asian countries (Kumar et al., 2010). Africa is a source of production of

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*Corresponding author. E-mail: somdasmarius@yahoo.fr.

traditional fermented foods and is perhaps the continent with the richest variety of lactic acid fermented foods (Franz et al., 2014).

Some varieties of pearl millet-based foods are produced and consumed in Burkina Faso like: cooked doughs (*tô, déguè*), steamed products (couscous), porridges (*binsalga*), fermented food and alcoholic beverages (*dolo*) (Hama et al., 2009). *Déguè,* made from pearl millet flour is consumed in most of the important cities in Burkina Faso: 32% in Ouagadougou and 25% in Bobo-Dioulasso (Hama et al., 2009).

During fermentation, microorganisms contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture, shelf life and safety. The microbiota of fermented foods is dominated by lactic acid bacteria (LAB), which contribute to their nutritional and sanitary qualities (Nout and Motarjemi, 1997).

Lactic acid bacteria play an important role in traditional fermented foods consumed in different countries (Oguntoyinbo and Narbad, 2012).

The lactic acid fermentation is a process which not only improves the organoleptic and hygienic quality but also the nutritional quality in food; it allows especially the good preservation (Ampe et al., 1999). Other microorganisms as *Enterobacteriaceae*, yeasts and mould were isolated in a lot of based-cereals fermented foods and can influence their quality (Ashenahi, 1994).

LAB may have probiotic characteristics (Lei and Jakobsen, 2004). These bacteria first have to be selected for their ability to survive passage through the gastrointestinal tract.

Probiotic functions may also be associated with other functions that are of interest for nutrition. This is of particular interest for at-risk popula-tions such as pregnant women and young children in developing countries. For instance, the amylase activity of some LAB helps increase the energy content of gruels for the complementary feeding of young children through partial hydrolysis of starch in the food matrix (Songre-Ouattara et al., 2008) but also helps sustain the growth of the microbiota of starchy foods (Tou et al., 2006). Other functions for example, folate and riboflavin synthesis may improve the quality of the food matrix and may be beneficial for the host.

Folate deficiency can lead to neural tube defects, early spontaneous abortion, and megaloblastic anemia, while riboflavin deficiencies can result in growth failure, inflammation of the skin, or vision deterioration (Rohner et al., 2007).

LAB capable of producing B vitamins could be used for fortification of cereal-based foods (Iyer and Tomar, 2009) and as probiotics (Rossi et al., 2011). In this way, bacteria that combine different functional characteristics could be useful for developing improved or new foods made from local raw materials that target specific nutritional needs and health issues.

Many studies have focused on the phenotypic diversity of the LAB in the tropical fermented foods but few works were made on the *dèguè*, which plays nevertheless an important role in the food in the Burkina Faso.

The objective of this work was to isolate, characterize and identify some bacteria occurring in the *déguè* samples. This information could contribute to the better knowledge of the microbiota of this kind of food, and perhaps the development of starter cultures with predictable characteristics for use in small-scale and commercial production of *déguè*

MATERIALS AND METHODS

Sampling

Sixteen samples of « *déguè* » olded from one to three days were obtained from local households of Ouagadougou (Burkina-Faso). Samples were carried out in an ice box for microbial analysis in the Laboratory of Microbiology and Biotechnology (Department of Biochemistry and Microbiology, University of Ouagadougou).

Isolation of lactic acid bacteria (LAB)

The 10^{-1} dilution was made by diluting 10 g of each *dègué* sample in 90 ml of sterile peptone saline water (10 g of peptone, 5 g of NaCl and 1000 ml of water). Further 10-fold serial dilution, ranging from 10^{-2} to 10^{-7} was done.

LAB were isolated in two media: Man, Rogosa and Sharpe (MRS) and Rogosa agar. Spreed-plated MRS and ROGOSA were incubated anaerobically using BBL Gas Pak plus Anaerobic System, Beckon Dickinson Microbiology System (Cockeysville, MD, USA) at 30°C for 48 h. Unit forming colonies were randomly picked from plates at higher dilution 10^{-6} and transferred into 10 ml in test tubes with sterile MRS broth. Pure cultures were made. The isolates were Gram-stained and tested for catalase and oxydase reaction (Harrigan and McCance, 1990). Presumptive LAB were selected based on the morphology, Gram reaction and the catalase test.

Isolation of total coliform (non-LAB)

Coliforms were isolated using the same dilutions, plated on Plate Count agar and Violet Red Bile Lactose Agar, and cultivated for 24-48 h at 37°C.

Characterization and identification of isolated LAB and non-LAB

The carbohydrate fermentation profiles of LAB isolates were investigated using API 50CH strips and API CHL medium according to manufacturer's instructions (API system, Bio-Merieux, France).

Phenotypical identification of non-LAB was done by the API 20 E gallery. Strains were designated to species using APILAB PLUS (Version 3.33, Bio-Merieux) and standard taxonomic descriptions.

RESULTS AND DISCUSSION

Morphological and biochemical characteristics of the LAB strains

All 125 selected isolates were Gram-positive, non -motile,

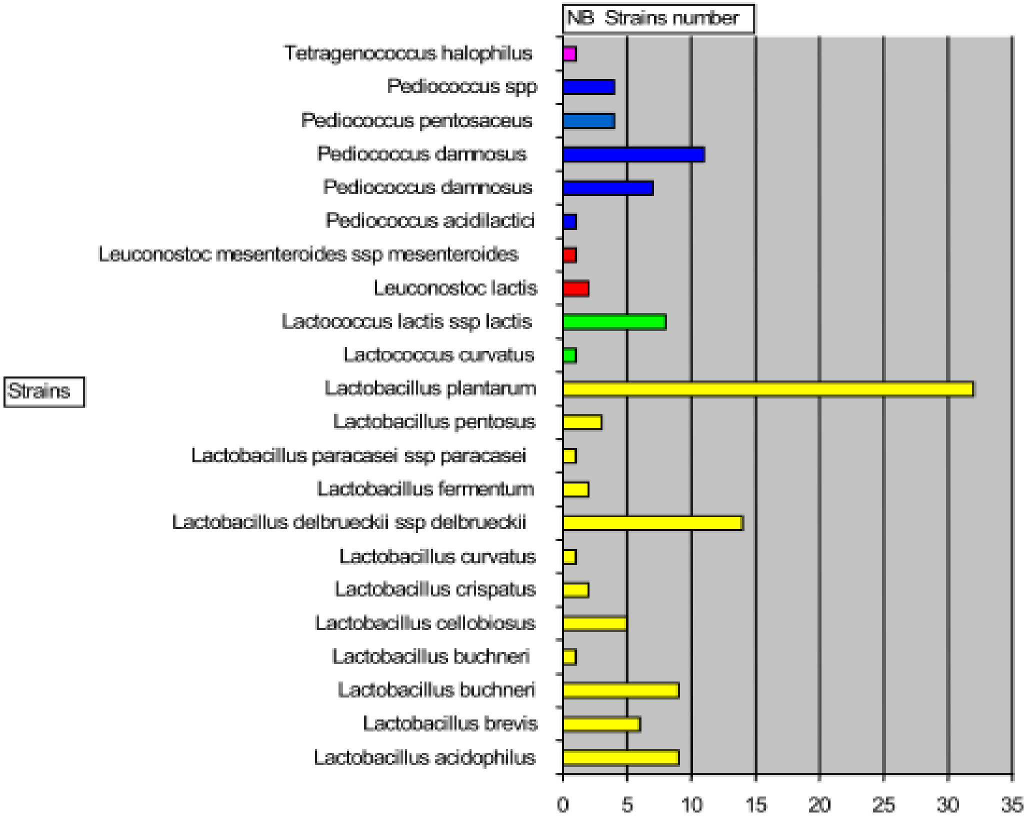

Figure 1. Identified LAB from *dèguè* by API 50.

catalase-oxydase negative and non-spore forming bacteria, they occurred in short rods and in cocci, singly, in pairs or short chains. These characteristics according to Holzapfel (1997) and Bergey's Manual of Systematic Bacteriology indicate lactic acid bacteria. The results are shown in Figure 1.

The LAB isolated were composed of five genera of *Lactobacillus* (68 % from 125 isolates), *Pediococcus* (21.6 %), Lactococcus (7.2 %), *Leuconostoc* (2.4 %), *Tetragenococcus* (0.8 %). *Lactobacillus* sp. was the predominated genera among the isolates.

The following species are involved in the fermentation of one kind of *dèguè*: *Lactobacillus acidophilus* (7.2%), *L. brevis* (4.8%), *L. buchneri (*8%), *L. cellobiosus* (4%), *L. crispatus* (1.6%), *L. curvatus* (0.8%), *L. delbrueckii subsp delbrueckii* (11.2%), *L. paracasei subsp paracasei* (0.8%), *L. fermentum* (1.6%), *L. pentosus* (2.4%), *L. plantarum* (25.6%), *L. curvatus* (0.8%), *L. lactis subsp lactis* (6.4%), *L. lactis* (1.6%), *Leuconostoc mesenteroides subsp. mesenteroides* (0.8%), *Pediococcus acidilactici* (0.8%), *Pediococcus damnosus* (14.4%), *Pediococcus pentosaceus* (3.2%), *Pediococcus spp* (3.2%) and *Tetragenococcus halophilus* (0.8%).

The species *L. plantarum* was the strain isolated in higher number (25.6%), from *dèguè* and is frequently isolated from traditional fermented foods made from cereals (Muyanja et al., 2003; Hama et al., 2009). Other species isolated were: *Pediococcus damnosus* (14.4%), *L. delbrueckii subsp delbrueckii* (11.2%), *L. buchneri (*8%), *L. acidophilus* (7.2%), *L. lactis subsp lactis* (6.4%), *L. brevis* (4.8%), *L. cellobiosus* (4%), *P. pentosaceus* (3.2%), *Pediococcus spp.* (3.2%), *L. pentosus* (2.4%), *L. crispatus* (1.6%), *L. fermentum* (1.6%), *L. lactis* (1.6%), *L. curvatus* (0.8%), *L. paracasei subsp paracasei* (0.8%), *L. curvatus* (0.8%), *L. mesenteroides subsp mesenteroides* (0.8%), *P. acidilactici* (0.8%) and *T. halophilus* (0.8%).

Many of these species were isolated from other fermented foods as *bushera* (an Ugandan traditional fermented drink made from *Sorghum*), *ben-saalga* (a traditional porridge of Burkina) Cassava, *fufu* and *ogi*, fermented doughs made from corn, etc. (Corsetti et al., 2003; Miambi et al., 2003; Tou et al., 2006).

L. plantarum (25.6%) was more frequently isolated than other species of LAB in household *dèguè*.

It has been observed that the presence of *L. plantarum* in the cheese (Cameros) from goat's milk decreased the number of fecal coliforms and other *enterobacteria* in the final product. Indeed LAB starters can contribute to reducing spoilage problems encountered in this domestic fermentation.

Using Api 50 CHL galleries allowed us to find certain strains capable to ferment some complex sugars. For

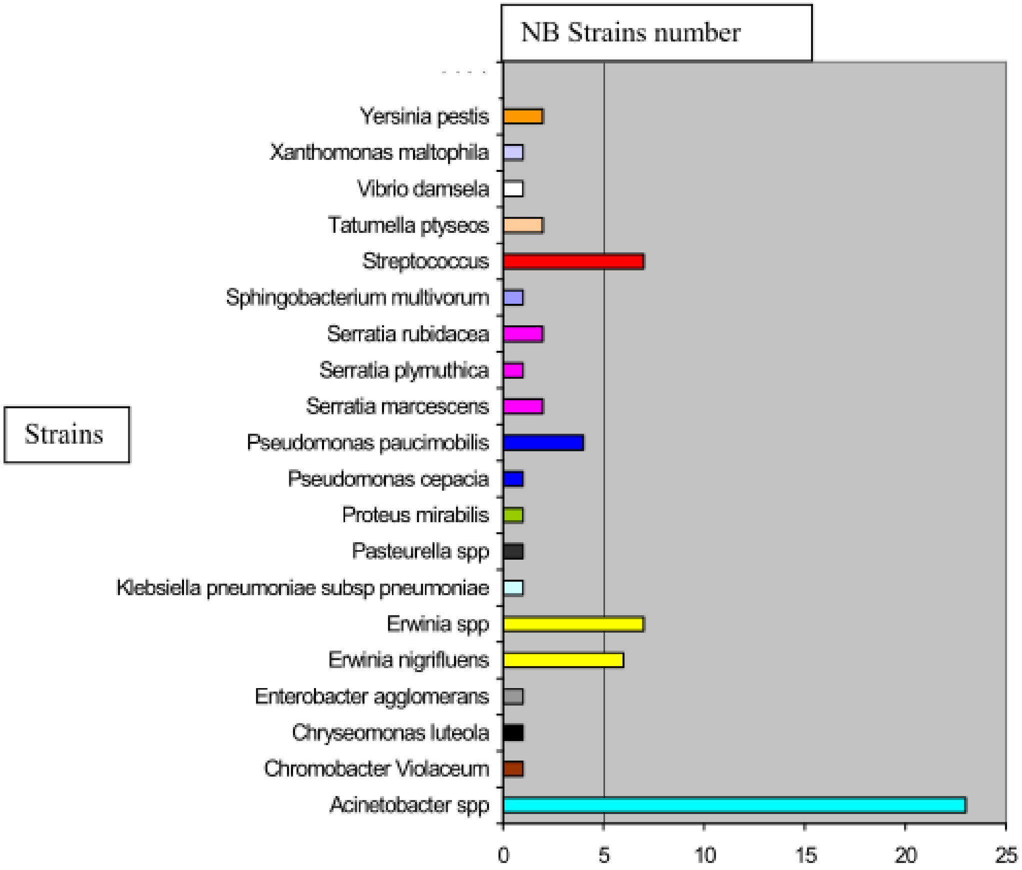

Figure 2. Identified non-LAB by API 20 E.

example, 46 amylolytic lactic acid bacteria *L. plantarum* (21 strains), *L. delbrueckii ssp delbrueckii* (5), *L. lactis ssp lactis* (4), *L. cellobiosus* (3), *L. acidophilus* (3), *L. pentosus* (3), *L. buchneri* (3), *L. fermentum* (2) and *L. crispatus* (2). Amylolytic LAB contributes in pH reduction in the medium inhibiting the growth of some pathogenic microorganisms such some faecal coliforms.

Certain strains isolated presented a potential to ferment raffinose by hydolysing α-galactosidic bonds: *L. pentosus* (2 isolates), *L. buchneri* (8 isolates), and *L. plantarum* (26 isolates). Indeed, raffinose is oligosaccharide that typically occurs in legumes and cereals, and cause flatulence, diarrhoea and indigestion in humans.

Phenotypical identification of non-LAB by the API 20 E gallery

The results of non-LAB are shown in Figure 2. On the total of non-LAB isolates of the majority (39.39%) of the strains belong to the family of *Enterobacteriacaeae.* The following species were identified: *Erwinia spp.* (10.07%), *Erwinia nigrifluens* (10.07%), *Serratia marcescens* (3.03%), *Serratia rubidacea* (3.03%), *Serratia plymuthica* (1.51%), *Tatumella ptyseos,* (3.03%), *Enterobacter agglomerans* (1.51%), *Proteus mirabilis* (1.51%), *Klebsiella pneumonia subsp pneumoniae* (1.51%).

The fecal streptococci isolated from the *dègué* and identified by APILAB PLUS represent 10.60% of non-LAB isolates. The presence of fecal streptococci shows fecal contamination origin of samples. That can be explained by the lack adequate hygiene.

Other strains of non-LAB were identified: 31.81% of isolates are represented by *Acinetobacter* genus*. Pseudomonas* represents 7.57% of isolates; two species were identified: *Pseudomonas paucimobilis* (6.06%) and *Pseudomonas cepacia* (1.51%).

Acinetobacter and *Pseudomonas* are characterized by significant proteolytic and lipolytic activities. The proteolysis leads to the formation of free amino acids then to decarboxylation or desamination reactions. The volatile amines and ammonia formed are responsible of unpleasant odors and savors in food. Lipolysis leads to the release of free fatty acids modifying the gustatory properties and leading to rancid taste.

The following species were also isolated: *Flavimonas oryzihabitans* (1.51%), *Vibrio damsela* (1.51%), *Chromobacter violaceum* (1.51%), *Xanthomonas* *maltophilia* (1.51%), *Pasteurella* spp*.* (1.51%), *Sphingobacterium multivorum* (1.51%) and *Chryseomonas luteola* (1.51 %). The presence of *E. coli* and other coliforms in the samples indicate that manipulations of these foods were not made in good sanitary conditions.

Conclusion

This study brings out that *dègué* contain several microorganisms, lactic acid bacteria and non-lactic acid bacteria. *L. plantarum* was the lactic acid bacteria species isolated in higher numbers*.* Many proteolitic species was observed and could influence the quality of the product.

Author's contributions

The present study was carried out in collaboration with all authors. All authors participated in drafting and revising the manuscript. They also read and approved the final manuscript.

Conflict of interests

The authors did not declare any conflict of interest.

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