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Polyphasic characterization of lactic acid bacteria isolated from Beninese sorghum beer starter

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ABSTRACT

Tchoukoutou is a Beninese traditional sorghum beer obtained by mixed fermentation including yeast and lactic acid bacteria (LAB). The starter's LAB communities as well as their biotechnological importance remain unknown. Furthermore, the sprouted grain of *Sorghum bicolor*, which is used during the beer processing, contains a cyanogenic glucoside (dhurrin). In order to elucidate Beninese sorghum beer starter LAB microbiota, 69 LAB isolated from traditional starters were characterized using a polyphasic approach including phenotypical characterization (physiology and MALDI-TOF MS) and 16S rRNA gene comparison. Based on the enzymes substrate specificity, LAB expressing aryl-β-D-glucosidase and amylase were indexed as potential candidates for dhurrin removal and saccharification improvement. All isolated bacteria belong to the same genus *Lactobacillus* with different strains of the five species *L. fermentum*, *L. plantarum*, *L. helveticus*, *L. paracasei* and *L. brevis* and diverse metabolic pathways. MALDI-TOF MS is a good method for accurate and high-throughput LAB identification. Several facultative heterofermentative LAB such as *L. plantarum* and *L. paracasei* express β-D-glucosidase and amylase. These β-D-glucosidase producers LAB will likely cleave the conjugated glucose of dhurrin, thereby contributing to detoxification if used for controlled sorghum mash bio-acidification.

1. Introduction

African sorghum beer is a traditional drink produced on the basis of ancestral knowledge which is transmitted from one generation to the next. This special beer is called *Tchoukoutou* in Benin and Togo, *Pombe* in Tanzania, *Dolo* in Burkina-Faso, *Amgba* in Cameroon, *Pito* in Ghana, "seven days" beer in Zambia, and *Burukutu* or *Otika* in Nigeria. The production of these special beers follows three main steps such as the malting, mashing and fermentation. However, the manufacturing processes are very variable and depend on the geographical location. The typical traditional Beninese sorghum beer processing is characterized by multi-stage fermentation where sorghum mash is spontaneously acidified by lactic acid bacteria before the main sorghum wort mixed fermentation with the traditional starter (beer deposit) containing different yeast and lactic acid bacteria (Tokpohozin, Fischer, Sacher, & Becker, 2016a). Consumers recognize this special beer by its taste and specific flavor. The aroma compound profile of African traditional beer results from a symbiotic interaction of different yeast species, predominantly *Saccharomyces cerevisiae* (Greppi et al., 2013; Tokpohozin et al., 2016b) cooperating with various species of lactic acid bacteria (LAB). The beer resulting from mixed fermentation with this cocktail of microorganisms is sour with a pH of 3.2–3.5, 2–4% (v/v) alcohol, and unhoped; it is drunk while fermentation continues, without prior maturation. Additional to the resultant short shelf-life, two contaminants pose a severe health risk to consumers: for one, mycotoxins contributed from exogenous sources (Ezekiel et al., 2015; Matumba et al., 2014; Odhav, 2002), for another, the cyanogenic glucoside dhurrin in the raw material of beer processing, namely sprouted grains of *Sorghum bicolor* (Ahmed, Mahgoub, & Babiker, 1996; Traoré, Mouquet, Icard-Vernière, Traoré, & Trèche, 2004). If this secondary metabolite of sprouted sorghum grain remains in the beer, it generates poisonous hydrogen cyanide by the action of enzymes produced by the intestinal microbiota after consumption (Carter, McLafferty, & Goldman, 1980). Several toxico-nutritional diseases are reportedly associated with dietary cyanide, for example "*konzo*" or "tied leg", tropical ataxic neuropathy, goiter, and cretinism (Banea et al., 2015).

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Moreover, as observed by Ryu et al. (2015) and Lachenmeier et al. (2010), this cyanide is the ultimate precursor for the multi-site formation of carcinogenic ethyl carbamate (urethane). However, hydrolyzing dhurrin prior to consumption prevents these adverse effects, so that LAB which express the β-D-glucosidase required for such cleavage are potential candidates for detoxifying sorghum wort. The enzyme, which catalyzes hydrolysis of the β-D-glucosidic bond, may additionally generate good precursors for beer bio-flavoring from sequestered glucosides. To discover which strains of LAB may contribute here, the first challenge is identification: Following a polyphasic approach, we isolated, characterized, and identified LAB in the traditional starter used during *Tchoukoutou* production. Based on their metabolic profile, LAB with interesting biotechnological properties were indexed.

2. Materials and methods

2.1. Sampling

Fifteen samples of *Tchoukoutou* starter (beer deposit) were collected at May 2015 from five localities (Toucountouna, Natitingou, Djougou, Parakou, N'dali) of traditional sorghum beer production in North Benin (Fig. 1) in sterile plastic bottles. To stop the fermentation, the starters were placed in a cooler on ice.

2.2. Lactic acid bacteria isolation and purification

Lactic acid bacteria were isolated from the starter using the standard serial method. A hundred microliters of the dilution (10^{-4} to 10^{-8}) were plated using the spread plate method on MRS agar. The medium's selectiveness was improved by adding 1 mg of cycloheximide to 100 mL of MRS medium to inhibit yeasts. The inoculated plates were incubated at 28 °C for 72 h. Pure cultures were obtained by picking single colonies and streaking them on NBB agar medium following the quadrant streaking technique. Yellow colonies were selected as lactic acid bacteria. These isolated cultures of lactic acid bacteria were stored in glycerol at −80 °C.

2.3. Physiological characterization of lactic acid bacteria

Sixty-nine isolated lactic acid bacteria were phenotypically characterized using different substrates of carbon, including monosaccharides (pentose and hexose), disaccharides, polysaccharides, glucosides, polyalcohols and amino acids as described by Back (2000). Minimal Sharpe medium (10 g/L peptone, 5 g/L yeast extract, 2 g/L potassium hydrogen phosphate, 2 g/L diammonium hydrogen citrate, 5 g/L sodium acetate, 0.1 g/L magnesium sulfate, 0.005 g/L manganese sulfate, 1 g/L Tween 80) was prepared and chlorophenol red (0.04 g/L) was added as indicator of pH change. This minimum medium was supplemented with 1 percent of sugar substrate and the pH was adjusted to 5.8. A color change to yellow during fermentation was appreciated as a positive result; for glucose, maltose, and gluconate, gas production was additionally detected with inverted Durham tubes. For esculin splitting specifically, loss of bluish fluorescence under UV light (366 nm) and a color change from brownish to black indicated a positive result (Edberg, Gam, Bottenbley, & Singer, 1976). After 10 days' incubation, Nessler's reagent (Merck KGaA, Darmstadt,

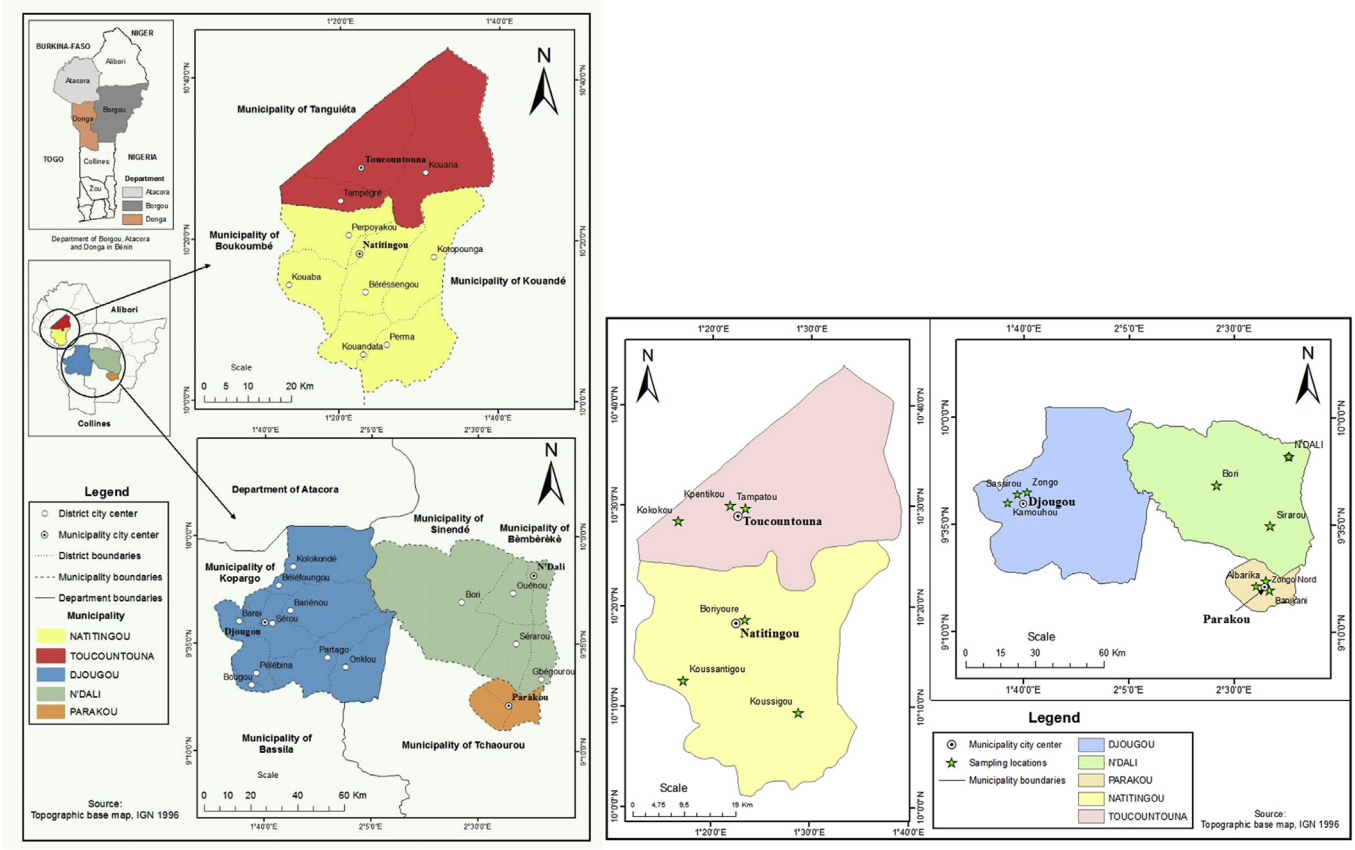

Fig. 1. Carte of situation showing the 5 principal zones (Toucountouna, Natitingou, N'dali, Djougou, Parakou) and the corresponding localities where sorghum starter were collected. The carte of situation and the carte showing the localities where traditional sorghum beer starter were collected were realized on the based of the data of the topographic IGN 1996 of Benin using the ArcGis software version 10.1.