

Occurrence of Pathogenic Bacteria in Traditional Millet-Based Fermented Gruels for Young Children in West Africa: *Ben-Saalga* and *Ben-Kida* in Ouagadougou (Burkina-Faso)

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Abstract: A study was conducted to evaluate microbiological quality of traditionally millet-based fermented gruels consumed as weaning foods at different stages of the processes as prepared at household level in Ouagadougou, capital of Burkina Faso in 2004, February to May. Our methodology is based on the use of traditional enumeration of four categories of micro-organisms like the enterobacteria that cause fecal contaminations, staphylococcal for the hygiene of producers, *Bacillus* as cereals contaminant and diarrhea and vomiting agents for young children; *Clostridium* like telluric agents. These enumerations were coupled with the identification of the characteristic colonies. Fermentation followed by sufficient cooking remains a good means of reduction of the microbial population, especially non-sporulated micro-organisms. MC agar count at 35°C went from 4.0×10^6 cfu/mL (before fermentation) to 1.9×10^5 cfu/mL (after fermentation) to reach zero values. On BP agar at 35°C, the count was 5.1×10^5 cfu/mL (before fermentation), 2.3×10^5 cfu/mL (after fermentation) and 7.2×10^4 cfu/mL (after cooking). On MYP agar at 35°C, the results are as follows: 9.9×10^6 cfu/mL (before fermentation), 1.0×10^7 cfu/mL (after fermentation) and 1.6×10^3 cfu/mL (after cooking). Finally, we obtained on TSC agar at 46°C about 4.1×10^6 cfu/mL (before fermentation), 2.7×10^7 cfu/mL (after fermentation) and 8.0×10^3 cfu/mL (after cooking). Identifications showed a strong presence of sporulated germs and non-sporulated acid tolerant germs especially after cooking. These results show how difficult these types of germs are to eliminate.

Key words: *Bacillus*, complementary food, cereals, enterobacteria, fermentation, foodborne pathogens, staphylococcal

INTRODUCTION

There is a strong relationship between diarrhea, morbidity and mortality of young children under five ages (Willumsen Juana *et al.*, 1997) in developing countries particularly in the rural and farming population. The incidence is high after weaning (Kunene *et al.*, 1999; Nyatoti *et al.*, 1997). It's proved that large uses of indigenous weaning food, like household cereal-based fermented products, was the mean reason because of hazardous raw materials (Malorny *et al.*, 2003; Manafi, 2000; Kingamkono *et al.*, 1999; Kusumaningrum *et al.*, 2003). As a consequence, the number of microbiological hazards potentially associated with fermented foods can

be correspondingly large. However lactic fermentation which occurs during weaning foods making can improve the microbial quality (Schlundt, 2002). Sure enough, this practice inhibits a number of gram-negative enteropathogens (Kingamkono *et al.*, 1999; Kunene *et al.*, 1999). *Clostridium perfringens* (Brynestad and Granum, 2002), *Bacillus cereus* (Fykse *et al.*, 2003; Manzano *et al.*, 2003a; Manzano *et al.*, 2003b; Shangkuan *et al.*, 2000), and *Staphylococcus aureus*, viral pathogens, and parasites (Kunene *et al.*, 1999; Nyatoti *et al.*, 1997; Tauxe, 2002; te Giffel *et al.*, 1996) are also found in weaning food. Frequently, they have been implicated most in causing illness in neonates and children from 3 days to 4 years of age.

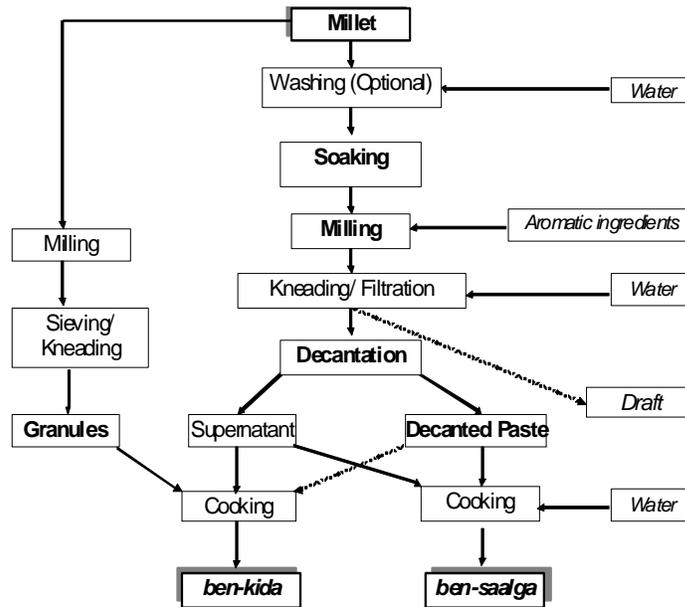


Fig. 1: Flow Diagram for Preparation of *ben-kida* and *ben-saalga* in Ouagadougou (Burkina-Faso)

The purpose of this study was to evaluate the microbial quality during all stages of the making of traditional millet-based fermented gruels consumed as weaning food by young children in Ouagadougou.

MATERIALS AND METHODS

Gruel preparation: In Ouagadougou, Burkina Faso, the preparation of traditional millet-based fermented gruels is conducted manually (Fig. 1). The process is composed of two fermentation steps: soaking and decantation. The most important step is decantation that can last up to 12 h or over. During the fermentation, pH value falls below 4. Fermented paste is kneaded before cooking in the boiling supernatant. Cooking time varies from few minutes to more than an hour.

Sample collection and preparation: A total of 10 households in Ouagadougou (Burkina Faso) were sampled for bacteriological analysis (Motarjemi, 2002). Productions Units (PU) selected during 2004, February to May, cooperate for long time with research unit 106 of Montpellier's Development Research Institute for improvement of fermented foods in deprived populations. They were divided into 7 sectors under 30 in Ouagadougou. Informations were collected on preparation methods, sampling, and storage conditions at the different steps of the process. Sociological aspects were also studied. Approximately 200 mL of food were sampled either before, or after fermentation (just before cooking), cooking, and after granule-making. They were collected using sterile ladle. Immediately, samples were placed into

sterile stomacher bags and transported to the laboratory in an icebox (6-10°C). Bacteriological analysis was initiated one to three hours after sampling. 10 mL (or 10 g for solids) analytical unit of each sample were homogenized with 90 mL of sterile water with NaCl (0.85%) (0.8% buffer peptone-water was used for staphylococcus) for 1 min (Laboratory Blender-Stomacher 400). Then a series of 1/10 dilutions were homogenized (Heidolph TopMix 94323-Bioblock Scientific) with sterile physiological water (0.85% NaCl or 0.8% buffer peptone-water).

Microbial procedures: Each dilution was suspended in the appropriated medium and incubated. All cultured plates were examined after incubation. The average of plates containing 30-300 colony forming units (cfu) was retained. Thereafter, morphological and biochemical tests were conducted for the identification of isolated microorganisms : gram staining (Color Gram 2 - bioMerieux), motility (Mannitol motility nitrate Medium-Biorad), respiration (Meat Liver Iron sulphite Agar - Biorad), production of staphylocoagulase for *Staphylococcus* strains (Lyophilized Rabbit plasma - bioMerieux), oxidase (Oxidase reagent - bioMerieux), catalase (ID color catalase-bioMerieux), use of glucose (Mevag agar - Biorad), lactose (Kligler iron agar - Difco) and mannitol (Mannitol motility nitrate Medium- Biorad). Isolates were then identified using appropriated API kits (bioMerieux) according to the manufacturer's manual. API 20A (bioMerieux) was used for the identification of bacilli Gram-positive anaerobes, API 20E (bioMerieux) for the presumptive *Enterobacteriaceae*, API 20NE (bioMerieux) for non-*Enterobacteriaceae*, API 50 CHB/E (bioMerieux) for *Bacillus* and API STAPH (bioMerieux)

for staphylococcus. Results were interpreted using API Lab Plus software (bioMerieux). For *Bacillus cereus* strains, a BCET-RPLA kit (Oxoid) for detection of enterotoxin (diarrhea type) in food and filtrates by reversed passive latex agglutination was used. In the presence of enterotoxin of *B. cereus*, the agglutination of the latex particles forms a diffuse disorder

Gram-negative organisms: Mac Conkey agar without crystal violet (Biorad) was used for the detection, isolation and enumeration of coliforms and intestinal pathogens. The medium was prepared in distilled water according to the manufacturer's manual. An aliquot of 1 mL of each dilution was surface plated and spread onto duplicate agar plates and incubated aerobically at 35°C for 24 h. All lactose fermenting colonies (appearing pinkish on MacConkey agar plates) and lactose non-fermenting colonies (appearing colorless) were counted.

Bacilli gram-positive aerobic or facultative anaerobe: Mannitol-yolk Polymyxin Agar (MYP Agar) was prepared according to the manufacturer's manual. The medium was then cooled to about 50°C after sterilization and Egg Yolk Emulsion and Polymyxine B sulphate solution (Sigma) were aseptically added into the medium. The well-mixed solution was poured into sterile Petri dishes. 0.1 mL (X 2) of appropriate decimal dilutions was then surface plated using a sterile glass spreader on pre-dried agar plates. Plates were then incubated aerobically at 35°C for 24 h. Typical colonies of *Bacillus cereus*, rough and dry with a violet pink background surrounded by an egg yolk precipitate, as well as atypical colonies were counted.

Staphylococci: Baird Parker agar base (Biorad) was prepared according to the manufacturer's manual and then cooled down to 50°C after sterilization. Egg Yolk Tellurite Emulsion (Biorad) and a sulphamethazine of sodium solution (Biorad) were aseptically added into thoroughly well-mixed agar. The mix was poured after into sterile Petri Dishes. 0.1 mL (X2) aliquots of appropriate dilutions were spread on dried agar surfaces plated. Plates were then incubated aerobically at 35°C and examined after 24 h for typical colonies of plates were re-incubated for an additional 24 h before enumeration.

Bacilli gram-positive anaerobe: Tryptose Sulphite Cycloserine Agar (Biorad) was developed using the basal medium but with D-cycloserine (Fluka) as the selective agent. This medium permitted also the growth of other sulphite-reducing *Clostridium* species (Araujo *et al.*, 2004; de Boer and Beumer, 1999; de Jong *et al.*, 2003). Suspension medium in distilled water was gently heated until agar is completely dissolved. The medium was allowed to cool down to 50°C after sterilization. After adding the D-cycloserine (Fluka) supplement, followed by a thorough mixing, the medium was poured into sterile

Petri dishes. Aliquots of 0.1 mL (X2) of dilutions of the homogenized tested sample was then spread over the surface of the first layer using a sterile swab. The plates were incubated at 46°C for 24 h in an anaerobic jar (Anaerongen AN0025A- Oxoid). Black colonies were considered to be *Cl. perfringens* and counted.

Selective medium for motile nitrate-utilizing microorganisms and use of sugar: Mannitol Motility Nitrate Medium (Biorad) or Mevag agar (Biorad) was dissolved in distilled water, distributed into screw-caps tubes and sterilized at 121°C for 15 min. They were allowed to cool quickly in cold running water and solidify in upright position. The tubes were then inoculated. For each test a negative control without any bacteria was used. Motility was traducted by the presence of diffused growth away from the spot of inoculation. Glucose and Mannitol fermentation, as indicated by a change in the phenol red indicator helped for the differentiation of species.

Specific medium for pre-identification of enterobacteriaceae: Kliger Iron Agar (Difco) was used. Suspension was prepared according to the manufacturer's manuel. After thorough mixing the solution was poured into containers and sterilized at 121°C for 15 min. The containers were then allowed to settle with a slope and bottom. Inoculation was done on the surface in the bottom. The three reactions expected were carbohydrate utilization (acidity or alkalinity), CO₂ production (aerogenic or anaerogenic), H₂S production (blackening or not).

Sorbitol MacConkey Agar (Oxoid) was prepared and poured into plates. The surface was dried when necessary. The plates were inoculated with a suspension and incubated at 35°C for 24 h to produce separated colonies. Bacteria fermenting Sorbitol produce pink to red colonies, some surrounded by precipitated zone of bile, the other, the non-fermenting ones produce colorless colonies.

RESULTS

Level of contamination at different households and processing steps: For each process steps, we observe a difference between the levels of contamination. The bacteria able to grow on Mac Conkey agar are more important after kneading and filtration (4.0×10^6 cfu/mL) (Fig. 2a) and after granules making (2.9×10^7 cfu/g) (Fig. 4a). We note a major reduction of the population after decantation (1.9×10^5 cfu/mL) (Fig. 3a) and cooking to reach the zero value (Fig. 5a) according to the technique used.

In general, counting on Baird Parker agar, at 35°C after 48 hours, was not significant (Fig. 2). We obtained 5.1×10^5 , 2.2×10^6 , 2.3×10^5 , and 7.2×10^4 cfu/mL after kneading and filtration, after granule-making, after decantation and after cooking, respectively (Fig. 2a-b).

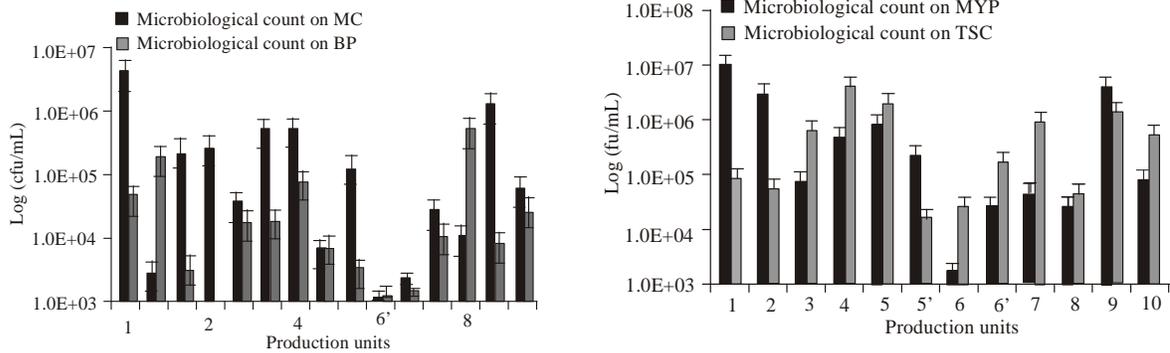


Fig. 2: a, b: Comparison of the microbiological counts after kneading and filtration steps on MacConkey Agar after 24 h and Baird Parker after 48 h at 35°C (b) and on Mannitol Yolk Polymyxin Agar at 35°C and Tryptose Sulphite Cycloserine Agar at 46°C after 24 h (B)

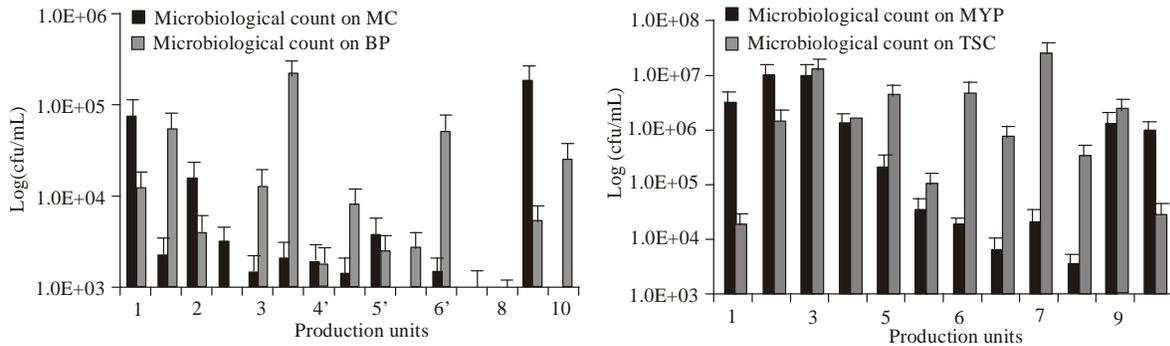


Fig. 3: a, b: Comparison of the microbiological counts after decantation step on MacConkey Agar after 24 h and Baird Parker after 48 h at 35°C (b) and on Mannitol Yolk Polymyxin Agar at 35°C and Tryptose Sulphite Cycloserine Agar at 46°C after 24 h (b)

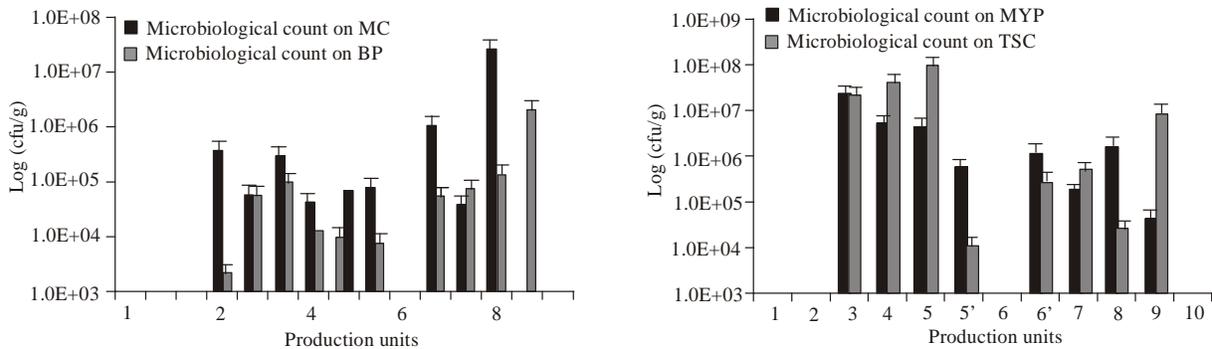


Fig. 4a, b: Comparison of the microbiological counts after granules making on MacConkey Agar after 24 h and Baird Parker after 48 h at 35°C (b) and on Mannitol Yolk Polymyxin Agar at 35°C and Tryptose Sulphite Cycloserine Agar at 46°C after 24 h

However, cooking is sufficient to reduce the population on Baird Parker agar.

For the ten production units visited, the averages on MYP agar are 9.9×10^6 cfu/mL after kneading and filtration (Fig. 2B), 2.2×10^7 cfu/g during granules making (Fig. 4B), 1.0×10^7 cfu/mL after decantation (Fig. 3b) and 1.6×10^3 cfu/mL after cooking (Fig. 5b). Except for few

household, the cooking parameters applied were sufficient to get a good sanitary gruel on MYP agar.

There is a similarity for anaerobic bacteria on TSC agar, at 46°C during 24 h, and bacilli Gram-positive aerobic or facultative anaerobic on MYP agar. The populations obtained were the following: 4.1×10^6 cfu/mL after kneading and filtration (Fig. 2b), 9.2×10^7 cfu/g for

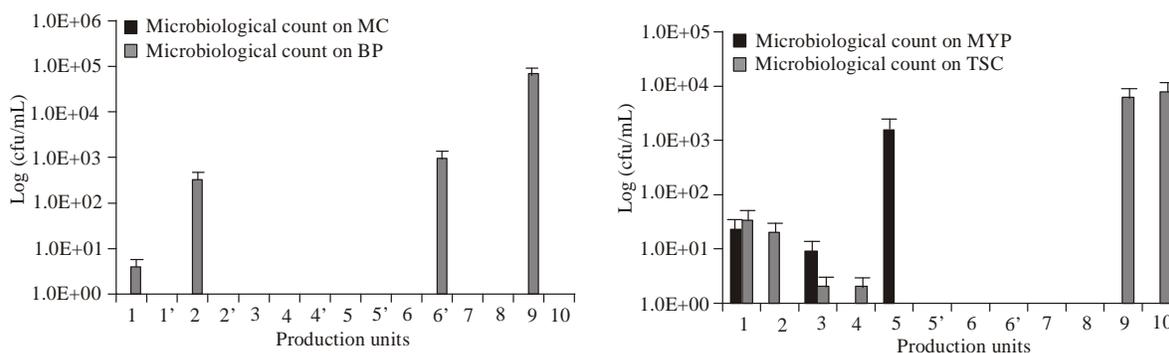


Fig. 5: a, b: Comparison of the microbiological counts after cooking step on MacConkey Agar after 24 h and Baird Parker after 48 h at 35°C (b) and on Mannitol Yolk Polymyxin Agar at 35°C and Tryptose Sulphite Cycloserine Agar at 46°C after 24 h

granules making (Fig. 4b), 2.7×10^7 cfu/mL in the fermented paste (Fig. 3b) and 8.0×10^3 cfu/mL after cooking (Fig. 5B). Except five households (1, 3, 4, 9 and 10), the cooking parameters applied were efficient to make the gruel safe in general (Fig. 5b).

Characterization of the isolated strains: The households are almost all contaminated by *Enterobacteriaceae* (Table 1). Thirty-eight strains have been characterized. The Kneading and the filtration steps are more contaminated by *Enterobacteriaceae* (55.3%). The decantation, the step after fermentation, is characterized by a considerable reduction of *Enterobacteriaceae* (about 10 times). The production unit (PU) 3 with 5 different species and number 8 with 4 different species are the most contaminated by *Enterobacteriaceae*. The species, which appears the most frequently in four households (Table 1), is *Klebsiella pneumo. ssp pneumoniae* (44.7%). *Escherichia coli* 1 (faecal contamination indicator) is found in household 2 and 7.

About 54% of the 24 strains of non-enterobacteria characterized the result from Mac Conckey agar and 29% from Baird Parker agar. Except for PU 2, 4 and 5, the others are contaminated by non-enterobacteria strains (Table 1). In the steps before fermentation and granules making, relatively high levels of contamination occur. The highest levels of contamination are observed at PU 1 and 3, with three different species.

Twenty-nine strains have been characterized as *Bacillus*. They are present in all production units (Table 1). The steps before fermentation and after fermentation are the most contaminated with a maximum the after fermentation step. MYP agar remains a good medium for culture of *Bacillus* strains (more than 62%) followed by BP agar (31%). Five different species were found. *Bacillus cereus* 1 seems to be the most present (48.3%) followed by *B. coagulans* and *B. cereus* 2. PU 7 is the most contaminated followed by PU 2. Three

different species have been identified in each of them (Table 1).

Except for PU 2, 6, 9 and 10, the presence of *Staphylococcus* strains was obvious in the other PU (Table 1). No *Staphylococcus* species were found after the cooking step. PU 5 and 7 with 4 different species of *Staphylococcus* were the most contaminated by these strains. *Staph xylosus* (27.8%) is present in 3 PU (Table 1).

A total of 85 strains isolated on TSC agar have been characterized. The most present is *Clostridium beijerinckii/butyricum*, with a level exceeding 56% of the bacilli Gram-positive anaerobe met. They are found in almost all PU (Table 1).

DISCUSSION

During the process, the pH decreases from 6.2 to 3.8 in the fermented paste. It reaches 4 in the gruel. The temperature of the gruel at the end of the cooking is between 82 and 85°C. The water and the tools used in the process have poor hygiene. Environmental and cooking conditions are bad (Kusumaningrum *et al.*, 2003).

On Mac Conckey agar at 35°C during 24 h, most of contamination in enterobacteria is brought by the raw material, water, ingredients, utensils, environment and handling (Diez-Gonzalez and Russell, 1999; González *et al.*, 2003; Rompré *et al.*, 2002). During fermentation, there is a reduction of pH (3.8) and production of new molecules (lactic acid, bacteriocin etc.). These new parameters would normally lead to the reduction of the level of contamination (Buchanan and Edelson, 1999; Duffy *et al.*, 1999; Mattick *et al.*, 2003; Ogwaro *et al.*, 2002; Ross *et al.*, 2002). Unfortunately, fermented paste show a population which is still too high (1.9×10^5 cfu/mL). We could certainly blame not only the manual practices and the bad environmental conditions for the possible cross-contamination but also an adaptation of the bacteria to the new conditions (Gänzle *et al.*, 1999;

Table 1: Characterization of isolated strains

Type Of Strains	Name	Process Step	Production Unit (PU)
Bacilli Gram-Positive Aerobic Or Facultative Anaerobe	<i>Bacillus amyloliquefaciens</i>	Before fermentation; After fermentation	2 & 10
	<i>Bacillus cereus</i> 1	Before fermentation; After fermentation; Granule making	1; 2; 5; 7 & 9
	<i>Bacillus cereus</i> 2	Before fermentation; After fermentation; After cooking	2; 6 & 8
	<i>Bacillus circulans</i> 2	Before fermentation	7
	<i>Bacillus coagulans</i>	Before fermentation; After fermentation	7 & 8
<i>Enterobacteriaceae</i>	<i>Enterobacter aerogenes</i>	Granule making	3 & 4
	<i>Enterobacter asburiae</i>	Granule making	8
	<i>Enterobacter cloacae</i>	Before fermentation; Granule making	3; 5; 8 & 9
	<i>Enterobacter sakazakii</i>	Before fermentation	8 & 10
	<i>Escherichia coli</i> 1	Before fermentation; Granule making	1 & 7
	<i>Klebsiella pneumo.ssp pneumoniae</i>	Before fermentation; After fermentation; Granule making	3; 8; 9 & 10
	<i>Klebsiella terrigena</i>	Before fermentation	3
	<i>Rahnella aquatilis</i>	After fermentation	9
	<i>Serratia liquefaciens</i>	Before fermentation	3
Others Bacilli Gram-Negative Aerobic Or Facultative Anaerobe	<i>Aeromonas salm. ssp salmonicida</i>	Granule making	3
	<i>Burkholderia cepacia</i>	Before fermentation; Granule making ;	1; 7 & 8
	<i>Chryseomonas luteola</i>	Before fermentation; After fermentation; Granule making	1; 3; 6; 7; 8; 9 & 10
	<i>Pasteurella pneumotropica /haemolytica</i>	Granule making	3
	<i>Stenotrophomonas maltophilia</i>	Before fermentation; After fermentation	1 & 10
<i>Staphylococci</i>	<i>Staph. aureus</i>	Before fermentation; Granule making	5
	<i>Staph. auricularis</i>	After fermentation	7
	<i>Staph. Caprae</i>	Granule making	5 & 7
	<i>Staph. cohnii cohnii</i>	After fermentation	8
	<i>Staph. epidermidis</i>	After fermentation	3
	<i>Staph. lentus</i>	After fermentation	1 & 3
	<i>Staph. saprophyticus</i>	Granule making	7
	<i>Staph. simulans</i>	Before fermentation; Granule making	5 & 7
	<i>staph aureus</i>	Before fermentation	4; 5 & 8
Bacilli Gram-Positive Anaerobe	<i>Clostridium beijerinckii /butyricum</i>	Before fermentation; After fermentation; Granule making;	1; 2; 3; 4; 6; 7; 8 & 10
	<i>Clostridium paraputrificum</i>	After fermentation; Granule making	3
	<i>Clostridium difficile</i>	Before fermentation; After fermentation; Granule making	7; 8 & 9
	<i>Clostridium speticum</i>	Before fermentation	10
Others Bacilli Gram-Positive Facultative Anaerobe Or Anaerobe	<i>Actinomyces israelii</i>	Granule making	7
	<i>Actinomyces naeslundii</i>	Before fermentation	5
	<i>Actinomyces meyeri /odontolyticus</i>	Before fermentation; After fermentation; Granule making	3; 6; 7 & 9
	<i>Bifidobacterim spp 1</i>	Before fermentation; After fermentation; After cooking; Granule making	3; 5; 6 & 8
	<i>Eubacterium lentum</i>	Granule making	8 & 9
	<i>Eubacterium limosum</i>	Before fermentation	8
	<i>Lactobacillus fermentum</i>	Granule making	3
	<i>Propionibacterium propionicus /avidum</i>	Before fermentation; After fermentation	3 & 8

McLay *et al.*, 2002). However, the cooking parameters reduced the population of enterobacteria. The combination of time and temperature during the cooking step remains sufficient to provide gruel free from enterobacteria and good for child consumption. The research of potential pathogenic *E. coli* on MC sorbitol agar (Oxoid) showed that the strain on PU 2 is potentially pathogen (Elliot *et al.*, 2004; Falcão *et al.*, 2004; Gilgen *et al.*, 1998). The non-enterobacteria found were not generally pathogens except for *Aeromonas salm. ssp salmonicida* and *Pasteurella pneumotropica/haemolytica* (Cissé *et al.*, 1997; Grant, 2004; Nejjari *et al.*, 2000).

Enterobacter sakazakii, in PU 8 and 10, (also named Yellow Pigmented *Enterobacter cloacae*), can cause illness like meningitis to new-born babies and the elderly in dehydrated food. Several authors mentioned that *Enterobacter sakazakii*, a gram-negative, rod-shaped bacterium, is a rare cause of invasive infection with high death rates in neonates (Iversen *et al.*, 2004; Iversen and Forsythe, 2004; Kandhai *et al.*, 2004; Nazarowec-White and Farber, 1997; Stoll *et al.*, 2004).

A considerable contamination on Baird Parker agar in paste before fermentation (Fig. 2a) was noted because of the process practices and ingredients used (Aycicek

et al., 2005). BP agar remains a good medium of the *Staphylococcus* selection (approximately 89% of the 18 characterized strains). These bacteria are normally associated with the skin and cutaneous glands. Consequently, their presence would be due to handling of the product (Acco et al., 2003). For the same reasons mentioned for *enterobacteria*, the fermentation step was not sufficient to reduce the contamination (2.3×10^5 cfu/mL) (PU 4) in Baird Parker agar (Fig. 3a). Granules (Fig. 4a) remain an important source of contamination (2.2×10^6 cfu/g) (PU 9) on Baird Parker agar. The staphylocoagulase test with the lyophilised rabbit plasma (bioMerieux) was negative for all characterized strains. After cooking step, strains characterization excluded presence of staphylococcal contamination. The population of 7.2×10^4 cfu/mL (PU 9) found after cooking of fermented paste (Fig. 5a) would be due to *Bacillus* species or non-enterobacteria species (31% of characterized *Bacillus* species or 29% non-enterobacteria isolated on Baird Parker agar).

MYP agar remains a good medium for culture of *Bacillus* strains (more than 62%) but 17% of non-enterobacteria are isolated on MYP agar. Bacteria population of 9.9×10^6 cfu/mL (PU 1) on MYP agar remains important for paste before fermentation (Fig. 5b). It's the same in fermented paste (1.0×10^7 cfu/mL) for the two more contaminated PU (2&3) (Fig. 3b). Gram-positive endospore-forming rod-shaped bacteria, *Bacillus* seem to resist to fermentation (Agata et al., 2002; Beattie and Williams, 2002; Sutherland et al., 1996; Valero et al., 2003). In food, the minimum pH for initiation of growth is 4.35 and the upper limit is greater than 8.8. However, the strains were able to increase the pH of an acid environment (pH 5.0) to a value which was more acceptable for growth and toxin production (Olmez and Aran, 2005; Sutherland et al., 1996). Germination of these species can occur at 5 to 8°C in an acid environment with a pH between 4.5 and 9. These favorable conditions are met during paste fermentation, which could explain this population increase. Fermentation, by reducing the other germs sensitive to the acid stresses present in the samples, allowed *Bacillus* to be able to express itself on the one hand. On the other hand, the treatments undergone by the fermented paste are at the origin of new contaminations. Granules (2.2×10^7 cfu/g) are also great sources of *Bacillus* in the product (PU 3) (Fig. 4b). The cooking treatment (80°C during 15 min) and the possible production of antimicrobial compounds by the lactic bacteria seem to be sufficient to reduce the contamination in vegetative *Bacillus*. The problem remains the presence of toxins (Prüß et al., 1999). Although these enterotoxins are non virulent after the exponential phase of growth (Rajkovic et al., 2005; Ultee and Smid, 2001) in addition with the thermal treatment during cooking that deactivates them, the emetic toxin, a preformed toxin is able to support these conditions, according to Radhika et al. (2002). The

important reduction of *Bacillus* vegetative population (1.6×10^3 cfu/mL) (PU 5) (Fig. 5b) could be explain by cooking parameters. The kit BCET-RPLA (Oxoid) showed that all *Bacillus cereus* strains characterized have diarrhea enterotoxin except the strains found in PU 8 & 9 (Table 1).

The strict anaerobic germs are essentially *Clostridium* (66%). It is one of the most common causes of food borne illnesses. They are present at variable quantities in the paste as well before fermentation, after fermentation, in the granules and after the cooking step. The main problem is *C. perfringens* (vegetative and spore). Firstly, if the cooking temperature is not properly maintained, the spores (100°C for up to 1 h) of *C. perfringens* can germinate, outgrow, and actively multiply to dangerously high dose levels, causing a potential public health risk (Huang, 2003a, b) Secondly, consumption of food products contaminated with large numbers of vegetative cells of this organism can cause symptoms such as acute abdominal pain and diarrhea within 8-15 h after ingestion. In our samples, no *C. perfringens* strains were found. However with the non spores forming germs (enterobacteria, non-enterobacteria and staphylococcal), the spores forming germs (*Bacillus*, *Clostridium*) can survive when the medium is hostile (fermented paste). The destruction of sporulated germs is more difficult, requiring a longer time of cooking.

CONCLUSION

We can say that a good cooking (more than 30 min) combined to a good fermentation (pH~ 3.8) are sufficient conditions to improve hygienic quality of the gruels. The process of fermentation by lactic acid bacteria is able to lower the pH under 4 in food products. The degree of microbiological control achieved by a cooking step is depends on numerous factors including time, temperature of cooking, thermal resistance of the micro-organisms and the composition and physical characteristics of the food. A pragmatic training (initiation to good practices of hygiene) for the producers can help reach these objectives.

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