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Full Length Research Paper

Predominant lactic acid bacteria associated with the traditional malting of sorghum grains

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The traditional processes of sorghum grains malting were investigated in Tamale (Northern Ghana) from the raw materials through to the sun-dried malted sorghum including steeping and germination stages. Samples were taken at each processing stage and cultured for the isolation of LAB. The isolates were characterized by basic phenotyping, ITS-PCR / RFLP analysis and identified by partial sequencing of 16S r RNA genes. Their antimicrobial and amylolytic activities and exopolysaccharides production were also investigated. During steeping, the LAB counts in the steep water increased from 10^5 to 10^9 - 10^{10} cfu/ml and pH of the steep water decreased from 5.08 ± 0.22 to 4.20 ± 0.50. A total of 106 isolates were identified and the predominant isolates belonged to the species *Lactobacillus fermentum* (58.49%), *Pediococcus acidilactici* (22.64%), *Weissella confusa* (11.32%), *Enteroccocus faecium*, *Pediococcus pentosaseus* and *Lactococcus lactis*. Some isolates showed interesting antimicrobial activity (39.62%), slight amylolytic activity (37.73%) and ability to produce exopolysaccharides (90.5%). *L. fermentum* isolates dominated the microbiota from sorghum grains to malted sorghum. These isolates had technological properties comparable to those responsible for the acidification of sorghum beer (dolo, pito) wort produced from sorghum malt (previously studied), suggesting their potential for use as starter cultures. Suitable isolates of *L. fermentum* are promising candidates to be used as starter cultures from the initial step of malting, that is, the steeping and are expected to inhibit the growth and survival of pathogens and spoilage microflora, and to control the lactic fermentation of dolo and pito wort or other sorghum malt-based products like infant formulations.

Key words: Sorghum, sorghum malt, lactic acid bacteria, identification, technological properties.

INTRODUCTION

Malted cereal grains are used as food ingredients for example in the preparation of weaning foods to improve the nutritional quality and increase the energy density of cereals-based gruels; this occurs by liquefying the cereals by the action of the amylases present in the malt (Malleshi et al., 1989; Onyeka and Dibia, 2002; Mbofung and Fombang, 2004; Traoré et al., 2004). Sorghum malt is specifically used to brew traditional opaque beers in many countries in Africa, such as Bantu beer in South Africa, bili-bili in Tchad and North Cameroon, Burukutu or choukoutou in Benin and North Nigeria, Chibuku in Zimbabwe, dolo in Burkina Faso and Mali, pito in Ghana, Togo and Nigeria, tchapalo in Côte d'Ivoire. The production of these traditional beers starts with the malting of the sorghum. Malted sorghum is also used for the pro-duction of non-alcoholic fermented beverages in Nigeria, e.g. otika (Chinyere and Onyekwere, 1996) and fermented sorghum malt-based food in Benin, e.g. gowé (Vieira-Dalodé et al., 2007).

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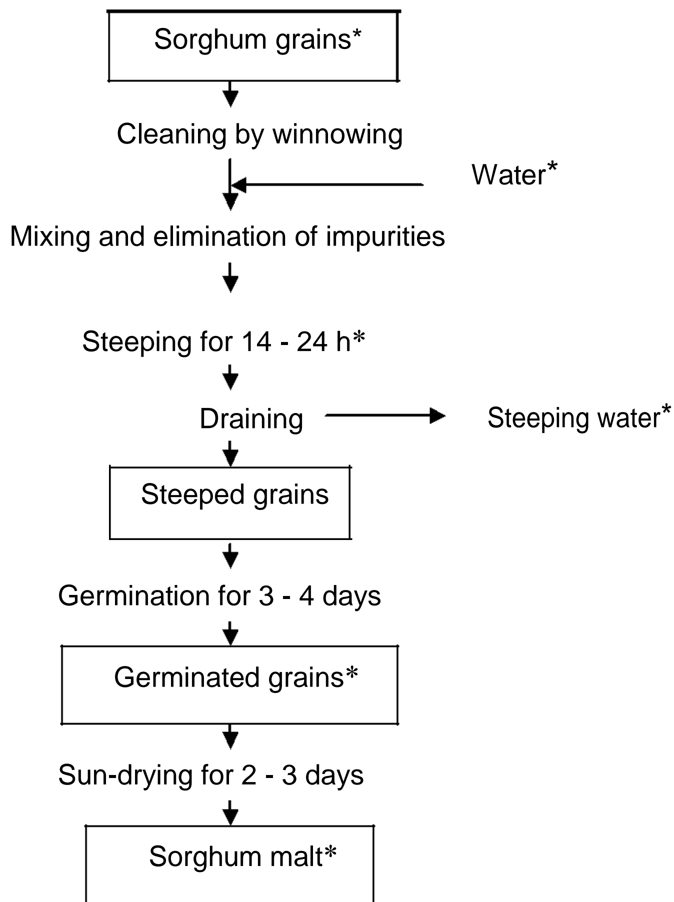

Figure 1. General flow diagram of traditional malting of sorghum grains at the two production sites in Tamale. *: sampling steps.

Malting is the limited germination of cereal grains with the objective to generate the endogenous hydrolytic enzymes (amylases, maltases, proteases …) which break down starch and proteins to provide fermentable sugars, free nitrogen and free amino acids. The malting process also provides aroma and other compounds es-sential for the fermentation and the sensory quality of the beer and modifies the structure of the grain so that it will be readily solubilised during the brewing process to produce the fermentable wort (Demuyakor and Ohta, 1992; Dewar et al., 1997; Agu and Palmer, 1996). The traditional malting process of sorghum grains (Figure 1) in Tamale (Northern Ghana) lasts for 6 to 8 days and involves three main steps: Steeping of grains in water, germination and sun drying of the germinated grains. Steeping is the immersion of grains in water over a period of time and sorghum grains should attain a moisture content of 33 - 36% during the steeping (Agu and Palmer, 1998).

It is a critical stage at which grain microorganisms start to proliferate due to the favourable moisture content (Noots et al., 1999). The bacteria, yeast and moulds which proliferate during the steeping continue to develop during the germination stage (Agu and Palmer, 1997). Some of these survive through the drying step and contribute to the brewing process. Other could cause spoilage and even poisoning if their presence is not controlled (Priest and Campbell, 1996).

A diversity of moulds such as *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp, *Rhizopus, Phoma* spp., *Alternaria* spp., *Mucor* spp. (Ogundiwin et al., 1991; Lefyedi, 2006) and bacteria including *Bacillus cereus*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella aerogenes*, *Staphylococcus aureus, Pseudomonas aeruginosa, Sarcina* spp. and *Lactobacillus* spp. (Ilori et al., 1991; Ogundiwin et al., 1991) have been identified as microorganisms associated with sorghum grains and malt.

As in the case of barley, chemical treatments using formaldehyde, sorbic acid, sodium benzoate, sodium and calcium chlorite… have been investigated to inhibit the growth of microorganisms during malting of sorghum (Ogundiwin et al., 1991; Lefyedi, 2006). Biological me-thods involving inoculation with lactic acid bacteria (LAB) and yeast starter cultures in the steep water have been shown promising for the control of undesirable bacteria and moulds during barley and sorghum malting (Boivin and Malanda, 1997; Lefyedi, 2006). LAB are well known to be able to inhibit the growth and survival of spoilage microflora and pathogens (Holzapfel et al., 1995). Booysen et al. (2002) identified species of *Leuconostoc*, *Weissella*, *Lactobacillus* and *Lactococcus* during the malting of barley.

Antimicrobial-producing LAB species belonging to *Enterococcus* spp and *Lactococcus* spp have been detected from raw barley and sorghum (Hartnett et al., 2002) . *L. fermentum* was found to be present in a signi-ficant number but not dominant during the spontaneous fermentation of ogi and kunun-zaki, fermented sorghum or millet-based products (Olasupo et al., 1997).

Studies on the microbiology of sorghum malting are scarce. In our previous investigation, the predominant LAB involved in the processes of dolo and pito were identified and *L. fermentum* was found to be dominant in the malted sorghum used as raw material, during the mashing and the natural acidification of the wort (Sawadogo-Lingani et al., 2007). Most of *L. fermentum* isolates were also found to be capable of producing exopolysaccharides (EPSs). Interesting antimicrobial activity was detected for some of them and a few isolates showed a slight amylolytic activity (Sawadogo-Lingani et al., 2008).

The aim of the present investigation was to identify the predominant LAB associated with the malting of sorghum grains and to compare their technological properties with those of LAB species involved in the natural fermentation of dolo and pito wort. The purpose is to identify suitable LAB strains that could be used as starter cultures from the initial step of the malting process of sorghum grains

(that is, the steeping) through to the acidification of the wort, to improve the safety of the malt in this process and also in other sorghum malt-based product processes.

MATERIALS AND METHODS

Traditional malting sites investigated and sampling

The malting process was investigated at two different sites in Tamale (Northern Ghana). The processes were carried out by two Dagarti women, one at Tamale town and the other at Nyankpala village. These women produce the malted sorghum to brew *pito*. The malting process by the Dagarti was called the Dagarti process.

The sorghum grains raw material at the sites studied were purchased at local markets. For the Dagarti process, a mixture of red and white sorghum grains (1:1) was used for the malting. Sampling was done twice on two different occasions at each production site at the main steps of the flow diagram shown in Figure 1. For each sampling, two samples of 200 g or 200 ml were taken aseptically into sterilized screw-cap bottles, kept in an icebox and taken to the laboratory where samples were stored at 4°C until analyzed. Analyses were performed within 24 h.

Microbiological analyses, isolation and preliminary phenotypic characterization of LAB isolates

Microbial counts of the samples were carried out at the DANIDA laboratory, University for Development Studies at Tamale (Ghana), as previously described (Sawadogo-Lingani et al., 2007). Basic phenotypic characterization of the isolates was carried out at DTA / IRSAT / CNRST laboratory at Ouagadougou (Burkina Faso). For isolation of aerobic mesophilic bacteria, 10 colonies were randomly chosen from the highest dilution PCA plate of each sample and were purified by continued streaking onto PCA. For lactic acid bacteria, 15 colonies were randomly chosen from the highest dilution MRS plate of each sample and were purified by continued streaking onto MRS agar under anaerobic conditions using anaerobic jar and anaerocult A (Merck).

All pure isolates were characterized by colony morphology, cell morphology using phase contrast microscopy, Gram reaction by the KOH method (Gregersen, 1978), and catalase reaction by the 3% H_2O_2 method (Barrow and Feltham, 1993). In addition, the production of gas by LAB isolates using the semi-solid medium of Gibson and Abd-el-Malek (Guiraud, 1998) and their ability to grow in MRS broth at 15° and 45°C, in MRS broth at pH 9.2 and in MRS broth with 6.5%NaCl, were also tested. A total of 106 LAB isolates obtained from the malting sites in Tamale were subcultured and stored at $-80^{O}C$ in MRS broth with 50% (v/v) glycerol for their identification.

Identification of LAB isolates

Isolates were cultured anaerobically on MRS agar for 48 h as described above and then in MRS broth at $37^{O}C$ for 24 h before use. All of the 106 isolates obtained from Tamale, from samples collected in the main steps of the malting process (Figure 1), were initially grouped by Intergenic Transcribed Spacers-Polymerase Chain Reaction / Restriction Fragment Length Polymorphism (ITS-PCR / RFLP) analysis according to the method previously described (Sawadogo-Lingani et al., 2007). Thirty Two (32) isolates representing all the ITS-PCR / RFLP groups were randomly chosen and tentatively identified by API 50 CHL strips (BioMerieux, Marcy-l´Etoile, France) using APILAB Plus version 3.3.3 (BioMerieux). The identities of the isolates were confirmed by partial sequencing of the16S r RNA gene. The sequencing was carried out with isolates representing all the ITS-PCR / RFPL groups and performed according to the method, reagents and equipments described by Lei and Jakobsen (2004); the template DNA was extracted and amplified as described in a previous investigation (Sawadogo-Lingani et al., 2007). The sequence of the r RNA genes and the identities of the isolates were obtained by BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The partial 16S sequences were submitted to EMBL Nucleotide Sequence Database and given the accession numbers FM163355, FM163356, FM163357 and FM164796 for *L. fermentum* strains N3a6, N3a9, Nsb2 and Nsb8, FM163353 and FM163354 for *Ped. acidilactici* strains Npo8 and N1a5, FM163360 for *P. pentosaceus* strain N2a17, FM163358 and FM163359 for *W. confusa* N1a4 and N2a8, FM163361 for *Lactococcus lactis* strain N2a2, FM163362 for *Enterococcus faecium* strain N2a18a.

Determination of the technological properties of isolates

The 106 Tamale isolates were screened for their antimicrobial activity, amylase production and exopolysaccharides (EPSs) production according to the methods, reagents and media previously described (Sawadogo-Lingani et al., 2008) . For the antimicrobial activity, *Listeria innocua* ATTC 33090, *S. aureus* enterotoxin A producer, *S. aureus* enterotoxin A and B producer, and *E. coli* P1 Etec obtained from Department of Food Science, Food Microbiology at University of Copenhagen, were used as indicator microorganisms. For their ability to produce amylase, isolates were grown on modified MRS media with starch as only source of carbon (MRS-starch agar) under anaerobic conditions, and the culture on MRS-starch agar were sprayed with Lugol´s iodine [0.33% (w/v) iodine (Prolabo, Paris, France), 0.66% (w/v) potassium iodide (Labosi, Paris, France)] to detect starch hydrolysis. The ability of the isolates to grow on MRS-starch agar was also recorded.

For EPSs production, isolates producing EPSs were first selected on the basis of the stickiness of the colonies grown on specific media and confirmed by growing them for 24 h at 30°C in MRS-sucrose (5% w/v) broth without glucose and peptone, pH 5.0 ± 0.2. A volume of 1.5 ml of the 24 h culture was centrifuged at 5 000 g for 10 min (4°C) and 1 ml of the supernatant was put in a glass tube and an equal volume of ethanol 95% was added. In the presence of EPSs, an opaque link was formed at the interface; the positive isolates were classified according to the intensity of the opaque link: ++ for good production; + for fair production; ± for poor production.

RESULTS

Characteristics and identities of the predominant lactic acid bacteria isolates

Counts of dominant microorganisms during malting of sorghum grains

For *Dagarti* malting process in Tamale, sorghum grains used as raw material at the production sites investigated contained LAB, aerobic mesophilic bacteria and yeasts at the levels of 10^5, 10^6 and 10^5 cfu / g respectively (Table 1). During steeping of the grains, the pH of steep water decreased from 5.08 ± 0.22 to 4.20 ± 0.50 and growth of microorganisms was observed: the total LAB counts in the steep water increased from 10^5 to 10^{10} cfu / ml, while total aerobic bacteria counts increased from 2.9 x 10^7 to 4.0 x 10^7 cfu / ml, and yeasts counts increased by about 1 log unit. The proliferation of aerobic bacteria seem to

Table 1. pH and dominant microorganism counts during the malting of sorghum grains at two production sites in Tamale (Northern Ghana).

Samples/Steps of production	pH	Lactic acid bacteria (LAB) (cfu / g or ml)			Aerobic mesophilic bacteria (cfu / g or ml)			Yeast (cfu /g or ml)
		Total LAB	Gram+cat – rods or coccoid rods	Gram+cat –tetrad forming cocci or cocci	Total aerobic mesophilic	Gram–cat+ motile rods	Gram+cat+ no-motile sporing rods	
Sorghum grains	nd	$(7.9 \pm 1.4)10^{5}$	$(5.8 \pm 0.9)10^{5}$	$(2.1 \pm 0.5)10^{5}$	$(4.4 \pm 1.1)10^{6}$	$(4.2 \pm 1.0)10^{6}$	$(1.3 \pm 0.4)10^{5}$	$(2.1 \pm 1.5)10^{5*}$
Water used	5.81 ± 0.11	$(4.1 \pm 1.1)10^{4}$	$(2.4 \pm 0.5)10^{4}$	$(1.7 \pm 0.6)10^{4}$	$(2.1 \pm 1.0)10^{5}$	$(1.7 \pm 1.0)10^{5}$	$(3.2 \pm 1.4)10^{4}$	$(2.8 \pm 1.3)10^{3}$
Water from beginning of steeping	5.08 ± 0.22	$(7.8 \pm 2.5)10^{5}$	$(4.9 \pm 1.0)10^{5}$	$(2.9 \pm 1.5)10^{5}$	$(2.9 \pm 1.1)10^{7}$	$(2.8 \pm 1.0)10^{7}$	$(1.4 \pm 0.3)10^{6}$	$(3.8 \pm 2.2)10^{4*}$
Water from end of steeping	4.20 ± 0.50	$(4.2 \pm 1.7)10^{10}$	$(3.5 \pm 1.5)10^{10}$	$(6.7 \pm 2.2)10^{9}$	$(4.0 \pm 1.3)10^{7}$	$(3.5 \pm 1.1)10^{7}$	$(4.9 \pm 2.3)10^{6}$	$(5.5 \pm 2.3)10^{5}$
Soaked grains	nd	nd	nd	nd	nd	nd	nd	nd
Germinated grains	nd	$(5.0 \pm 1.2)10^{7}$	$(4.3 \pm 1.0)10^{7}$	$(6.5 \pm 1.8)10^{6}$	$(5.3 \pm 2.3)10^{6}$	$(3.4 \pm 0.7)10^{5}$	$(4.9 \pm 2.3)10^{6}$	$(4.8 \pm 1.9)10^{4*}$
Sun-dried sorghum malt	5.42 ± 0.02	$(1.3 \pm 0.7)10^{7}$	$(1.3 \pm 0.7)10^{7}$	$(3.2 \pm 0.3)10^{5}$	$(7.1 \pm 1.0)10^{6}$	$(4.2 \pm 3.1)10^{5}$	$(6.7 \pm 1.6)10^{6}$	$(3.5 \pm 0.4)10^{6*}$

*: presence of moulds. nd: not determined.

be mainly due to the growth of Gram-positive catalase-positive bacteria than the growth of Gram-negative catalase-positive. The counts of the former increased about 3.5 fold, while the counts of the latter increased about 1.2 fold for the Dagarti process samples (Table 1).

Microbial levels of the water used for the steeping was around 10^4, 10^5 and 10^3 cfu / ml respectively for LAB, aerobic mesophilic bac-teria and yeasts. Germinated grains and sun- dried sorghum malt contained high levels of LAB (10^7 cfu / g); aerobic mesophilic bacteria (10^6 cfu / g) and yeasts (10^4 cfu / g and 10^6 cfu / g respect-tively). Moulds were observed in the raw sorghum, the steep water at beginning of steeping, the germinated grains and the sun-dried malted sor-ghum (Table 1). For all the samples of sun-dried malted sorghum analysed, LAB counts were higher than aerobic bacteria counts.

A total number of 106 Gram-positive catalase-negative, non-sporing and non motile rods, coccoid rods, cocci or tetrad forming cocci, were isolated from the production sites investigated. Most of these isolates (70%) were heterofermen-tative bacteria producing gas from glucose.

ITS-PCR / RFLP profiles of the isolates

All the 106 Tamale isolates used for the geno-typing were distributed in four ITS-PCR profiles namely A, B, H and I (Table 2). ITS-PCR profile A (46 isolates) was characterized by five bands of 756, 539, 473, 406 and 289 pb, while ITS- PCR profile B (16 isolates) was characterized by four bands of 756, 473, 406 and 289 pb. Profile H (26 isolates) presented three bands of 510, 439 and 315 pb, while profile I (12 isolates) presented 3 bands of 532, 448 and 335 pb. The remaining isolates showed profiles different from profiles A, B, H and I and were referred to profile O (Table 2).

After digestion with the restriction endo-nuclease *Taq* I, the isolates clustered in the ITS-PCR profile A gave the RFLP profile a with five bands (387, 332, 210, 134 and 98 pb) or c with four bands (332, 210, 134 and 98 pb). The isolates of ITS-PCR profile B presented RFLP profile a or profile d with three bands of 210,134 and 98 pb. These results highlight a similarity between the isolates of profiles A and B. Isolates of ITS-PCR profile H showed RFLP profile h with bands of 404, 320, 249, 194 and 91 bp, and the isolates clustered in the profile ITS-PCR I showed the RFLP profile i with bands of 334, 262, 194, 138 and 77 pb (Table 2).

Identities of the predominant LAB species

For the identification, thirty (30) isolates repre-senting all the ITS-PCR / RFLP groups were tentatively identified by API 50 CHL/APILAB Plus Version 3.3.3 strips and their identities were confirmed by sequencing of the16S r RNA genes. Thus, the isolates of ITS-PCR / RFLP profiles A/a, A/c and B/a were tentatively identified by API 50 CHL strips as *Lactobacillus brevis* , and those of profile B/d were identified as *Lactobacillus cellobiosus*. The isolates of ITS-PCR / RFLP profile H/h were identified as *Pediococcus pentosaceus*, while those of profile I/i were inden-

Table 2. ITS-PCR/RFLP products of the 106 isolates of lactic acid bacteria associated with the malting of sorghum at the production sites of Nyankpala and Tamale town

ITS-PCR products		RFLP products		Number of isolates		
Profiles	**Sizes of bands (fragments) in bp**	**Profiles**	**Sizes of bands (fragments) in bp**	**Total**	**Nyankpala**	**Tamale town**
A (5 bands)	756 ± 25; 539 ± 14; 473 ± 13; 406 ± 11; 289 ± 8	a (28 isolates) c (18 isolates)	387 ± 21; 332 ± 10; 210± 11; 134±10; 98 ± 11 332 ± 10; 210 ± 11;134 ± 10; 98 ± 11	46	24	22
B (4 bands)	756 ± 25; 473 ± 13; 406 ± 11; 289 ± 8	a (12 isolates) d (4 isolates)	387 ± 21; 332 ± 10; 210 ± 11; 134 ± 10; 98 ± 11 210 ± 11; 134 ± 10; 98 ± 11	16	10	6
H (3 bands)	510 ± 11; 439 ± 9; 315 ± 8	h	404 ± 11; 320 ± 9; 249± 9; 194 ± 6; 91 ± 12	26	12	14
I (3 bands)	532 ± 11, 448 ± 9; 335 ± 10	i	334 ± 18; 262 ± 19; 194± 10; 138 ± 11; 77 ± 8	12	7	5
O (other)	nd	nd	nd	6	4	2

nd: not determined.

Table 3. Identification of the 106 isolates of lactic acid bacteria associated with the malting of sorghum at the production sites of Nyankpala and Tamale town.

ITS-PCR/RFLP profiles	API CHL presumptive identification	16S r genes sequencing			% of isolates
		Identities	**% of similarity**	**Accession number**	
A/a	*L. brevis* with 99.9%ID (7 isolates)*	*L. fermentum* (N3a6) **	99.5	FM163355	58.49
A/c	*L. brevis* with 95.5%ID (5 isolates)	*L. fermentum* (N3a9)	99.0	FM163356	
B/a	*L. brevis* with 99.6%ID (5 isolates)	*L. fermentum* (Nsb2)	99.0	FM163357	
B/d	*L. cellobiosus* with 96 %ID (2 isolates)	*L. fermentum* (Nsb8)	99.2	FM164796	
H/i	*P. pentosaceus* with 98%ID (6 isolates)	*P. acidilactici* (N1a5)	99.3	FM163354	22.64
		P. acidilactici (Npo8)	99.0	FM163353	
		P. pentosaceus (N2a17)	99.5	FM163360	1.88
I/i	*L. coprophilus with* 98.8%ID (4 isolates)	*W. confusa* (N1a4)	99.7	FM163358	11.32
		W. confusa (N2a8)	99.7	FM163359	
O	*L. acidophilus with* 89.5%ID (1 isolates)	*Lactococcus. lactis* subsp. *lactis* (N2a2)	95. 7	FM163361	1.88
	Lactococcus lactis with 58.3% ID (2 isolates)	*Enterococcus faecium* (N2a18a)	99.5	FM163362	3.77

*: in brackets, number of isolates used for API CHL identification; **: in brackets, reference of isolates used for sequencing.

identified as *L. coprophilus* (Table 3). The partial sequencing of the 16S r RNA genes (region 968-1401) of the isolates representing ITS-PCR / RFLP profiles showed that all the isolates clustered in ITS-PCR / RFLP profiles A/a, A/c, B/a and B/d were related to *L. fermentum* at 99.5, 99.2 and 99.0% similarity (Table 3); their cells were rod-shaped, producing gas from glucose, growing at 45°C but more and less at 15°C (result not shown).

The isolates of ITS-PCR / RFLP profile H/h were related to *Pediococcus acidilactici* at 99.3

Table 4. Distribution (in % of isolates collected from the sample) of predominant LAB species during the malting of sorghum at the production sites of Nyankpala (N) and Tamale town (Tt); in brackets, counts in cfu / g or ml.

Samples/ steps of production	Nyankpala production site					
	L. fermentum	*P. acidilactici*	*P. pentosaceus*	*W. confusa*	*Lactococcus lactis*	*E. faecium*
Sorghum grains	56.2(4.4 x10^5)	18.7(1.5 x10^5)	-	18.7(1.5 x10^5)	-	6.2(4.9 x10^4)
Water from beginning of steeping	55.5(4.3 x10^5)	11.1(8.6 x10^4)	5.5(4.3 x10^4)	16.2(1.3 x10^5)	5.5(4.3 x10^4)	5.5(4.3 x10^4)
Water from end of steeping	66.7(2.8 x10^{10})	25.0(8.2 10^9)	-	8.3(3.5 x10^9)	-	-
Sun dried sorghum malt	70.0(9.1 x10^6)	30.0(3.9 x10^6)	-	-	-	-
	Tamale town production site					
Sorghum grains	42.8(3.4 x10^5)	28.6(2.2 x10^5)	7.1(5.6 x10^4)	14.3(1.1 x10^5)	-	7.1(5.6 x10^4)
Water from beginning of steeping	54.5(4.2 x10^5)	27.3(2.1 x10^5)	-	9.1(7.1 x10^4)	9.1(7.1 x10^4)	-
Water from end of steeping	61.5(2.6x10^{10})	23.1(9.6 x10^9)	-	15.4(6.4 x10^9)	-	-
Sun-dried sorghum malt	66.7(8.6 x10^6)	25.0(3.2 x10^6)	-	-	-	8.3(1.1 x10^6)

-: not found in the isolates.

and 99.0% similarity; two of them were related to *P. pentosaceus* at 99.5% similarity (Table 3); the cells were tetrad forming cocci, not producing gas from glucose, growing at 15°C and 45°C (result not shown). The isolates clustered in ITS-PCR / RFLP profile I/i were related to *Weissella confusa* at 99.7% similarity (Table 3) and their cells were coccoid rods, single, in pair and short chains (3 - 4 cells); they produced gas from glucose and grew at 15^0 and 45°C (result not shown). The remaining isolates were identified by the sequencing as *E. faecium* at 99.5% similarity (4 isolates) and *Lactococcus lactis* subsp. *lactis* at 95.7% similarity (2 isolates) (Table 3).

The isolates of *E. faecium* were cocci in pairs and short chains, not producing gas from glucose, growing at 15^0 and 45°C, growing in MRS broth with 6.5% NaCl and MRS broth at pH 9.2; the isolates of *Lactococcus lactis* were ovoid cells in pairs and chains, not producing gas from glucose, growing at 15°C but not at 45°C, growing in MRS broth at pH 9.2 but not in MRS broth with 6.5% NaCl. Predominant LAB species associated with the traditional malting of sorghum grains in Tamale (Northern Ghana) for the production of pito, a sorghum beer, belonged to the species *L. fermentum* (58.49% of the isolates), *P. acidilactici* (22.64%), *P. pentosaceus* (1.88%), *W. confusa* (11.32%), *E. faecium* (3.77%) and *Lactococcus lactis* subsp. *lactis* (1.88%).

Distribution of LAB species by steps and production sites

As it can be seen from Table 4, throughout the malting process in Tamale town (Tt) and Nyankpala (N) production sites, *L. fermentum* was the predominant species in the raw sorghum grains (42.8 - 56.2% of the isolates collected from this step), from the beginning (54.5 - 55.5% of the isolates) to the end (61.5 – 66.7% of the isolates) of steeping of grains in water and in the sun-dried sorghum malt (66.7 - 70% of the isolates). *P. acidilactici* represented the second predominant species which has been identified for 11.1 to 30.0% of the isolates according to the step. *W. confusa* was also one of the predominant species found in the raw sorghum grains (14.3 - 18.7% of the isolates from this step), from the beginning (9.1 - 16.2%) to the end (8.3 - 15.4%) of the steeping of grains, but this species was not detected in the sun-dried sorghum malt (Table 4). At both production sites, *L. fermentum*, *P. acidilactici* and *W. confusa* were involved in the spontaneous lactic fermentation which occurred during the steeping of grains in water and their counts in the steep water increased from 10^4 – 10^5 to 10^9 - 10^{10} cfu / ml (Table 4). *Lactococcus lactis* subsp. *lactis* and *E. faecium* were found at a minor level sometimes in the raw grains, the water from the beginning of steeping or the sun dried sorghum malt (Table 4). These results showed that the main origin of *L. fermentum* and *P. acidilactici* found in the malted sorghum was the raw sorghum grains.

Technological properties of the LAB isolates

Carbohydrates fermentation patterns (API 50 CHL) of LAB species identified

The isolates of *L. fermentum, P. acidilactici* and *W. confusa* were capable of fermenting galactose, D-glucose, D-fructose and D-mannose, but did not ferment starch.

In addition, *L. fermentum* isolates were able to ferment L-arabinose, ribose, melibiose, saccharose and gluconate. Maltose, D-raffinose and 5- ketogluconate were fermented by 94% of them, while 88% fermented D-xylose and 70% fermented lactose. Cellobiose, esculin, trehalose, -gentiobiose, salicin and amygdalin were fermented L-arabinose, ribose, rhamnose, cellobiose, meli- by 47% of *L. fermentum* isolates; some of them fermented arbutin (39%), tagatose (35%) and mannitol (12%).

P. acidilactici isolates were also capable of fermenting cellobiose, -gentiobiose and tagatose. 80% of them fermented ribose, trehalose and L- arabinose. Gluconate and mannitol were fermented by 40 and 20% were capable of fermenting glycerol, maltose, lactose and saccharose. *P. pentosaceus* isolates were able to ferment the carbohydrates fermented by *P. acidilactici* isolates except for mannitol and glycerol; in addition, they fermented D-xylose, melibiose, D-raffinose and starch.

W. confusa isolates were able to ferment ribose, D-xylose, cellobiose, maltose, melibiose, saccharose, -gentiobiose and gluconate; 50% of them fermented L-arabinose, lactose, trehalose and raffinose.

Lactococcus lactis subsp. *lactis* isolates fermented galactose, glucose, fructose, D-mannose, esculin, maltose, lactose and saccharose, while the isolates of *E. faecium* in addition to these sugars, fermented biose, trehalose, starch and -gentiobiose (results not shown).

Antimicrobial activity of LAB isolates

The 106 Tamale isolates were screened by the agar spot technique and antimicrobial activity was detected for 39.62, 22.64 and 17.92% of the isolates toward *S. aureus*, *E. coli* and *Listeria innocua* respectively; the sizes of the inhibition zones ranged between 6 and 20 mm for *S. aureus* and between 1 and 5 mm for *E. coli* and *L. innocua*. A total of 42 isolates exerted antagonism against *S. aureus,* and were composed of 35 *Listeria fermentum* isolates, six *W. confusa* isolates and one *P. pentosaceus* isolate; no isolate of *W. confusa* and *Pediococcus* spp. Showed antagonism against *E. coli* and *Listeria innocua* (Table 5). A total of 17.92% of the isolates tested exerted antagonism against all of the four indicator strains with sizes of inhibition zones ranging from 1 to 20 mm (result not shown). No isolate of *P. acidilactici, Lactococcus lactis* and *E. faecium* showed an antimicrobial activity against the four indicator strains used.

Amylase production by LAB isolates

A total of 40 of the 106 isolates screened, that is, 37.73% were capable of growing on modified MRS agar with starch as only source of carbon; these isolates were composed of 34 *L. fermentum* isolates, two *P. acidilactici* isolates, one *P. pentosaceus* isolate, two *W. confusa* isolates and one *E. faecium* isolate (result not shown). Even though a clear zone surrounding the tested colony had not been observed compared with the positive control strain which showed a clear zone of 2 mm, the growth on MRS-starch agar demonstrates a slight amylolytic activity of these isolates.

The ability of the isolates to produce Exopolysaccharides (EPSs)

The screening of the 106 isolates for EPSs production under the experimental conditions described above revealed that most of them (90.5%) have the capacity to produce EPSs. A total of 82 isolates (77.3%) produced fairly EPSs and belonged to the species *L. fermentum* (59 isolates), *P. acidilactici* (13 isolates), *W. confusa* (6 isolates), *P. pentosaceus* (2 isolates) and *Lactococcus. lactis* (2 isolates). Fourteen isolates (13.2%) which produced EPSs poorly belonged to the species *L. fermentum* (3 isolates), *P. acidilactici* (7 isolates) and *W. confusa* (4 isolates) (results not shown).

DISCUSSION

At the two production sites studied, sorghum malt was produced at household level with rudimentary equipments and under uncontrolled environmental conditions. The increase in LAB counts and decrease in pH observed during the steeping of sorghum grains in water, have also been reported in previous studies conducted on steeping of cereals grains for malting or processing of fermented products (Odunfa and Adeyele, 1985; Booysen et al., 2002; Lei and Jakobsen, 2004) . During barley malting for example, it has been reported that the hydration of barley kernels and the secretion of nutrients from kernels into the water, lead to the proliferation of various microorganisms including lactic acid bacteria (Kelly and Briggs, 1992); this is well known as the steeping process and it may also apply for sorghum grains.

The predominance of LAB in the sun-dried sorghum malt is due to the fact that these bacteria, already present in significant numbers in the raw sorghum grains, occurred in a high number during the steeping step which gives an advantage for their dominance in the sun- dried sorghum malt. When observing the succession of the main groups of microorganisms from the raw grains to

Table 5. Antimicrobial activity of the 106 LAB isolates toward pathogen indicator strains.

Pathogen indicator strains	Inhibitory spectrum of LAB isolates (n = 106)					Percentage (%) of isolates exhibiting antimicrobial activity
	L. fermentum	*W. confusa*	*Pediococcus spp.*	*Lactococcus lactis*	*Enterococcus faecium*	
S. aureus Enterotoxin A producer	-(27) ++(12) +++(14) ++++(9)	-(6) +++(3) ++++(3)	-(25) +++(1)	-(2)	-(4)	39.62% (42/106)
S. aureus Enterotoxin A+B producer	-(27) ++(18) +++(12) ++++(5)	-(6) +++(6)	-(25) +++(1)	-(2)	-(4)	39.62% (42/106)
E. coli P1 Etec	-(38) +(24)	-(12)	-(26)	-(2)	-(4)	22.64% (24/106)
Listeria … innocua ATTC 33090	-(43) +(19)	-(12)	-(26)	-(2)	-(4)	17.92% (19/106)

-: no activity; +: antimicrobial activity with inhibition zone of 1 to 5 mm; ++: antimicrobial activity with inhibition zone of 6 to 10 mm; +++: antimicrobial activity with inhibition zone of 11 to 15 mm; ++++: zone antimicrobial activity with inhibition zone of 16 to 20 mm, in brackets: number of isolates.

the sun-dried sorghum malt, it should be mentioned that the fermenting bacteria, that is, LAB, originated mainly from the raw sorghum grains, even though the environment, utensils and material such as the steeping vessel and the water used could also provide microorganisms. Based on that, Chavan and Kadam (1989) repor-ted that the bacteria responsible for the natural fermentation of cereals were essentially the surface flora of seeds and this may also apply to sorghum grains.

Species of *Lactobacillus*, *Weissella*, *Leuconostoc*, *Streptococcus*, *Lactococcus* and *Pediococcus* seem to be predominant LAB associated with the steeping step of cereals grains. During the steeping of sorghum grains for ogibada production, a fermented gruel, the microbial population was mainly constituted by bacterial and yeasts, among which *Lactobacillus* spp. and *Streptococcus* spp. occurred from 10^6 to 10^9 after 72 h of steeping (Odunfa and Adeyele, 1985). Lei and Jakobsen (2004) also showed a decrease in pH from 6.0 to 4.3 and increase in LAB counts up to10^8 cfu / ml during overnight steeping of millet for koko production in the Tamale region, in which *L. fermentum*, *W. confusa* and *L. salivarius* were found as predominating species associated with the steeping step.

Furthermore, in the case of barley malting, LAB counts in barley before steeping and during steeping, increased from10^3 - 10^4 to 10^7 cfu / g, and *Leuconostoc argentinum*, *Leuconostoc lactis*, *W. confusa, L. casei* and *Lactococcus lactis* were found to be the most predominant species in kilned malt (Booysen et al., 2002). For sorghum, *Lactobacillus* spp. and other bacteria have been identified in raw grains and malted sorghum (Ilori et al., 1991; Ogundiwin et al., 1991). In an early study, Vieira-Dalodé et al. (2007) showed that *L. fermentum*, *W. confusa* and *P. acidilactici* constituted the dominant LAB species involved with yeasts in *gowé* fermentation, a fermented sorghum malt- based food from Benin. Previously, it has been shown that *L. fermentum* was the dominant species involved in the processing of dolo and pito, from the sorghum malt to the spontaneously fermented wort. *L. delbrueckii*, *P. acidilactici* and *Lactococcus lactis* were also identified but constituted a minor fraction of LAB population (Sawadogo-Lingani et al., 2007).

When comparing the sugar fermentation pat-terns of *L. fermentum* isolates with those involved in our previous study, a great similarity was observed despite the fact that additional sugars e.g. mannitol, D- tagatose, arbutin and salicin were fermented by 12 to 47% of the isolates involved in sorghum malting. *L. fermentum* has though been

described as positive (> 90%) for ribose, galactose, glucose, fructose, maltose, melibiose, saccharose and raffinose, and negative (< 10%) for esculin, melezitose, amygdalin, mannitol, rhamnose and sorbitol (Hammes et al., 1992; Seseña et al., 2004). Sugar fermentation profiles of the isolates identified as *P. acidilactici* are comparable to those of the isolates involved in *dolo* and *pito* wort processing (Sawadogo-Lingani et al., 2007) and also those reported by Simpson and Taguchi (1995). However some isolates showed the ability to ferment more sugars e.g. N- acetyl-glucosamine, maltose and gluconate. *W. confusa* has been described as positive (> 90%) for cellobiose, galactose, maltose, ribose, saccharose, xylose and esculin, and negative (< 10%) for L-arabinose, melibiose, raffinose and trehalose (Euzéby, 2004); all of our isolates showed the ability to ferment melibiose and 50% of them fermented arabinose, raffinose and treha-lose. Most of the predominant cultures isolated in this study, showed an ability to ferment various carbohydrates which is an important technological trait for the lactic fermentation of food products.

Such as in our previous investigation, these results demonstrate a diversity of LAB isolates within species with regard to their ability of fermenting carbohydrates. Lei and Jakobsen (2004) also demonstrated a considerable biodiversity within the LAB strains isolated from koko through Principal Component Analysis (PCA) of their API profiles. The present investigation highlighted a discrepancy between API 50 CHL"/ APILAB Plus version 3.3.3 and genotypic identification and showed that the use of API 50 CHL analysis only as an identification tool is not reliable for LAB species associated with the traditional malting of sorghum, as also reported by other workers (Nigatu, 2000; Paludan-Müller et al., 2002; Lei and Jakobsen, 2004). This discrepancy should be due to the inter-strain variability in fermentation ability as described above. On this issue, Stiles and Holzapfel (1997) have reported that LAB identification was first based on biochemical and physiological characteristics, but several workers have showed that using this approach only is unreliable, although some physiological characteristics have been used for the differentiation of some species. In other respects *L. fermentum* and *L. brevis* both belong to the obligately heterofermentative lactobacilli group (Novel, 1993; Hammes and Vogel, 1995) and thereby some isolates should present similar physiological characteristics. In addition, it has been revealed by DNA-DNA hybridization that *L. cellobiocus* is identical to *L. fermentum* (Vescovo et al., 1979) and the first name is now considered as synonym to the latter (Hammes et al., 1992). Nowadays, polyphasic approach using both phenotypic and genotypic characterizations is used for bacterial identification (Vandamme et al., 1996; Ehrmann and Vogel, 2005). However, API 50 CHL analysis gives important data about the carbohydrates fermentation patterns of the isolates, which are important for technological aspects.

Concurrently with the decrease in pH and the growth of *L. fermentum*, *P. acidilactici* and *W. confusa* during the steeping of sorghum grains, the growth of Gram-negative bacteria was inhibited, as expected and shown by the use of lactic acid bacteria starter cultures for controlling undesirable microbial growth in malting of barley or sorghum (Haikara and Laitila, 1995; Boivin and Malanda, 1997; Lefyedi, 2006). In such processes, LAB fermentation inhibits the growth of pathogenic and spoilage microorganisms by several mechanisms like the production of organic acids, hydrogen peroxide, antimicrobial substances, as well as lowering pH and oxidation-reduction potential to a point where competing microorganisms are no longer able to grow (Mbugua and Njenga, 1992; Kingamko et al., 1994; Tanasupawat and Komagata, 1995) . *L. fermentum* has been reported as dominant species involved in the spontaneous fermentation of kenkey, a Ghanaian maize-based food product and was selected and used as starter culture for a controlled fermentation (Halm et al., 1993; Halm et al., 1996; Annan et al., 2003).

In developing countries like Burkina Faso, the implementation of such use of starter cultures and Good Manufacturing Practices (GMP) should be considered to improve the bacteriological quality of malted cereals used for several purposes including sorghum malt-based products and infant formulations. The antimicrobial activity, the ability to produce EPSs, as well as the slight amylolitic activity and the sugar fermentation patterns of the dominant isolates (that is, *L. fermentum*) used for the present study are similar to those of *L. fermentum* isolates involved in the spontaneous fermentation of the *dolo* and pito wort produced from malted sorghum and previously investigated (Sawadogo-Lingani et al., 2008). These results showed that the predominant LAB isolates found in the sorghum malt originated mainly from the raw sorghum grains. This suggests that the dominating LAB isolates involved in the natural fermentation of *dolo* and *pito* wort produced from malted sorghum as previously observed (Sawadogo-Lingani et al., 2007) originated from the raw sorghum grains. *L. fermentum* isolates seem well adapted to the substrate and the environment of the production of malted sorghum and dolo and pito wort. Therefore they are potential isolates which can be used as starter cultures during steeping (initial step of malting) and would be expected to contribute to the entire process of dolo and pito fermentation until the wort cooking step. This would improve the hygienic quality of sorghum malt and sorghum malt-based products by inhibiting the growth and survival of pathogenic and spoilage microorganisms and control fermentation during processing. In this regard, suitable strains have to be selected based on their useful technological properties, that is, antimicrobial activity, rapid rate of acidification and ability to produce EPs and safety and processing aspects considered as well (Sawadogo-Lingani et al., 2008). They are also seen

as valuable starter cultures for malted cereal-based products like infant flours formulations.

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