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PCR screening of an African fermented pearl-millet porridge metagenome to investigate the nutritional potential of its microbiota

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ABSTRACT

Cereals are staple foods in most African countries, and many African cereal-based foods are spontaneously fermented. The nutritional quality of cereal products can be enhanced through fermentation, and traditional cereal-based fermented foods (CBFFs) are possible sources of lactic acid bacteria (LAB) with useful nutritional properties. The nutritional properties of LAB vary depending on the species and even on the strain, and the microbial composition of traditional CBFFs varies from one traditional production unit (TPU) to another. The nutritional quality of traditional CBFFs may thus vary depending on their microbial composition.

As the isolation of potentially useful LAB from traditional CBFFs can be very time consuming, the aim of this study was to use PCR to assess the nutritional potential of LAB directly on the metagenomes of pearl-millet based fermented porridges (*ben-saalga*) from Burkina Faso. Genes encoding enzymes involved in different nutritional activities were screened in 50 metagenomes extracted from samples collected in 10 TPUs in Ouagadougou.

The variability of the genetic potential was recorded. Certain genes were never detected in the metagenomes (genes involved in carotenoid synthesis) while others were frequently detected (genes involved in folate and riboflavin production, starch hydrolysis, polyphenol degradation). Highly variable microbial composition - assessed by real-time PCR - was observed among samples collected in different TPUs, but also among samples from the same TPU. The high frequency of the presence of genes did not necessarily correlate with in situ measurements of the expected products. Indeed, no significant correlation was found between the microbial variability and the variability of the genetic potential. In spite of the high rate of detection (80%) of both genes *folP* and *folK*, encoding enzymes involved in folate synthesis, the folate content in *ben-saalga* was rather low (median: 0.5 μg/100 g fresh weight basis). This work highlighted the limit of evaluating the nutritional potential of the microbiota of traditional fermented foods by the only screening of genes in metagenomes, and suggests that such a screening should be completed by a functional analysis.

1. Introduction

Cereal based fermented foods (CBFFs) are staple food products in many African countries (Guyot, 2012). Some are used as complementary foods for children under five during the period of breast feeding (Mouquet-Rivier et al., 2008; Svanberg, 1995). These foods are mainly fermented by naturally occurring lactic acid bacteria (LAB) (Guyot, 2012; Minervini et al., 2015). For example, the bacteria responsible for spontaneous fermentation in different foods (*ben-saalga*, *koko*, *fura*) made from pearl-millet (*Pennisetum glaucum*) belong to genus *Lactobacillus*, *Weissella*, *Leuconostoc*, *Lactococcus* and *Pediococcus*, with *Lactobacillus fermentum* frequently found to be the dominant species (Humblot and Guyot, 2009; Lei and Jakobsen, 2004; Owusu-Kwarteng et al., 2012). It has been shown that the composition of the microbiota of pearl-millet based fermented foods can vary from one traditional production unit (TPU) to another (Humblot and Guyot, 2009; Owusu-Kwarteng et al., 2012). These differences could be due to different factors, including the raw materials used for the preparation of the CBFF (Owusu-Kwarteng et al., 2012).

As reported in a review by Singh et al. (2015), the nutritional quality of cereals can be enhanced through fermentation by LAB. A set of genes coding for enzymes involved in nutritional functions was screened in 152 LAB strains isolated from *ben-saalga* and showed that the presence or absence of certain genes depended on the species (Turpin et al., 2011). For example, the genes *folP* and *folK* encoding enzymes involved in the synthesis of folate were detected in 100% of the *L. fermentum* strains (n = 70) isolated from *ben-saalga*. On the contrary, the gene *malL* encoding an enzyme involved in the hydrolysis of dextrins and isomaltose was detected only in 7% of the same strains. Therefore, if the genetic potential of these strains is confirmed by phenotypical analysis, the nutritional quality of this CBFF could vary depending on the LAB

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species responsible for the fermentation. Considering the variations in the composition of the microbiota, the nutritional quality may also vary from one TPU to another.

The genetic potential of the bacteria responsible for fermentation may be estimated by PCR screening of genes in metagenomes extracted directly from fermented foods (Turpin et al., 2011). However, in the preliminary work of Turpin et al. (2011) this method was only tested on five samples of pearl-millet based fermented porridges. The objective of the present work was to evaluate the nutritional potential of bacteria directly on the metagenomes of 50 samples collected in 10 TPUs in Burkina Faso using PCR screening. The main bacteria involved in the fermentation of the CBFF products were quantified using real-time PCR. Finally, to validate genetic screening using one simple function, we measured the folate (vitamin B9) content of the samples to check for a relationship between an identified genetic potential and the nutritional reality. Folate is of particular importance since folate deficiency leads to neural tube defects, congenital malformations and megaloblastic anemia, and is still prevalent in many countries (Arsenault et al., 2014; Becquey and Martin-Prevel, 2010; Blount et al., 1997; Rouget et al., 2005; Viñas et al., 2011; Wickramasinghe, 2006).

2. Material and methods

2.1. Samples of fermented porridges

The traditional processing of pearl-millet into *ben-saalga* is well known (Tou et al., 2006). The final steps are spontaneous fermentation, mainly due to LAB (*Lactobacillus*, *Weissella*, *Leuconostoc*, *Lactococcus* and *Pediococcus*) and to a lesser extent yeasts, and a cooking step before consumption (Humblot and Guyot, 2009; Tou et al., 2006). Ten traditional production units (TPUs) were randomly selected in different parts of Ouagadougou (Burkina Faso) based on a previous survey (Tou et al., 2006). For each TPU, five samples of porridges were collected after the cooking step (i.e. food as sold and consumed), on different days. Therefore, the 5 samples collected in a same TPU corresponded to fermentations that occurred on five different days. They were conserved at −20 °C until DNA and folate extraction. The 50 pearl-millet porridge samples were coded with a letter from A to J (corresponding to the TPU) followed by a number from 1 to 5 (corresponding to the day of sampling). Although for example, samples A1 and B1, are both numbered 1, this does not necessarily mean they were collected on the same day.

2.2. Nucleic acid extraction

Total DNA was extracted from 20 g of porridge in two steps after serial centrifugation to eliminate starch and using the Wizard genomic DNA purification kit (Promega, Charbonnières, France) with an additional lysis step using an amalgamator with zirconium beads (VWR, Fontenay-sous-Bois, France), as previously described (Turpin et al., 2011). DNA concentrations and purity were estimated by measuring absorbance at 260 and 280 nm (NanoVue™, GE Healthcare, Uppsala Sweden). The DNA samples were then stored at −20 °C until PCR and real-time PCR analysis.

2.3. PCR amplification for the detection of genes of interest

A set of genes of nutritional interest was selected. These genes are involved in the synthesis of carotenoids, folate and riboflavin (vitamin B2), in starch metabolism, and tannase synthesis. The genes were detected by PCR using primers selected from a previous work (Turpin et

Table 1
List of primers used to screen the food metagenomes.

General function	Gene	Predicted function	Primer name	Primer sequence 5′ to 3′	Primer reference
Carotenoid synthesis	*crtN*	Dehydrosqualene desaturase	crtN/crtM	F_CGCGGAATTCATGAAGCAAGTATCGATTATTGGC	Garrido-Fernández et al. (2010)
	crtM	Dehydrosqualene synthase		R_GATCGAATTCTTAAGCCTCCTTAAGGGCTAGTTC	
	rbscrtN	Dehydrosqualene desaturase	RbscrtN/crtM	F_CTAGGGTACCAAGGGGGAGATTTACTGATGAAGC	
	crtM	Dehydrosqualene synthase		R_GATCGAATTCTTAAGCCTCCTTAAGGGCTAGTTC	
Folate synthesis	*folP*	Dihydropteroate synthase/ dihydropteroate pyrophosphorylase	folP1	F_CCAsGrCsGCTTGCATGAC R_TkACGCCGGACTCCTTTTwy	Turpin et al. (2011)
	folK	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase	folKlfe	F_CCATTTCCAGGTGGGGAATC R_GGGGTGGTCCAAGCAAACTT	Turpin et al. (2011)
Riboflavin synthesis	*ribA*	3,4-dihydroxy-2-butanone 4-phosphate synthase// GTP cyclohydrolase II	ribA2	F_TTTACGGGCGATGTTTTAGG R_CGACCCTCTTGCCGTAAATA	Turpin et al. (2011)
	ribB	Riboflavin synthase subunit alpha	ribBlfe1	F_AGTAAACGGAACGGGCAAGC R_GTTGACCAGGGCACCAACTG	Turpin et al. (2011)
	ribG	Diaminohydroxyphosphoribosylaminopyrimidine deiminase	ribG	F_TGGkAAGACGCCkCCkTGT R_TTCACCAAyCArAATyGCTTGA	Turpin et al. (2011)
	ribH	6,7-dimethyl-8-ribityllumazine synthase	ribHlfe1	F_AGGGCGAAACCGACCACTAC R_CGATTGGGCAGTCATCGAAC	Turpin et al. (2011)
Starch metabolism	*agl*	Alpha-glucosidase	a-glu-lpl1	F_GCsAAAATGCTAGCGACymT R_CCACTGCATyGGyGTACGy	Turpin et al. (2011)
			a-glu-lfe1	F_AACCTGGTGAAATGGCAGAC R_TTGGTCATTCCCAGTTCCTC	Turpin et al. (2011)
	α-amy	Alpha amylase	a-amy-lpl1	F_AGATCAGGCGCAAGTTCAGT R_TTTTATGGGCACACCACTCA	Turpin et al. (2011)
	amyE	Extracellular alpha amylase	amyEV2	F_CATCAACTGCCACTGCAACT R_CGCTTTAGGTCACGACGAAT	Humblot et al. (2014)
	dexC	Neopullulanase	neopul-lpl1	F_CCAGACGAGCAAGAACAACA R_ATTGGCGATACGCCACTTAC	Turpin et al. (2011)
			neopul-lfe1	F_ACTTTTCTGCAGCCTGGTGT R_ACGGCCATTAAACTGTCGTC	Turpin et al. (2011)
	glgP	Glycogen phosphorylase	gP-Lpl1	F_GCGGGTGTTCAAAGTATCGT R_TCTCGAGGGCCTCTTGTAAA	Turpin et al. (2011)
	malL	Oligo-1,6-glucosidase	O1,6glu-lpl1	F_GCGGGTGTTCAAAGTATCGT R_TCTCGAGGGCCTCTTGTAAA	Turpin et al. (2011)
	malP	Maltose phosphorylase	MP-lac1	F_TGCCAyAAyGArTGGGArAT R_ACsCkATCwGCCCArAAAC	Turpin et al. (2011)
Tannase	*tanlpl*	Tannin acylhydrolase	tanlpl	F_TGCTAAGCACTGGCGGATTC R_GGCACAAGCCATCAATCCAG	Iwamoto et al. (2008)

al., 2011) and are listed in Table 1. PCR conditions were the same as those described in Turpin et al. (2011).

2.4. Real-time PCR for the quantification of LAB

Different bacteria, and especially LAB, are involved in the fermentation of *ben-saalga*, including *Lactobacillus* (especially *L. plantarum* and *L. fermentum*), *Weissella*, *Leuconostoc*, *Lactococcus* and *Pediococcus* (Humblot and Guyot, 2009; Turpin et al., 2011). We selected primers corresponding to these groups, genera and species in the literature, and checked them *in silico*. We were unable to find any primers sufficiently specific for *Lactococcus* and *Pediococcus* since *in silico*, their sequences matched other genera or species present in our ecosystem. The primers validated *in silico* are listed in the Table 2.

The strains *L. plantarum* ATCC14917T, *L. fermentum* ATCC14931, *Leuconostoc mesenteroides* ATCC10880, *Pediococcus pentosaceus* ATCC43200 and *Weissella confusa* ATCC10881T were used to generate quantification matrixes, by extracting DNA from samples of pearl-millet flour suspensions in water mixed with each bacterium at different concentrations. The specificity of each previously selected primer (Table 2) was checked by real-time PCR using the DNA extracted from the reference strains and a negative control (sterile water). When necessary, the primers were converted into their reverse complement to perform real-time PCR. For each reaction, 5 μL of the extracted template-DNA was added to 20 μL of PCR mix containing 1 × Mesa green q-PCR Master Mix Plus and 0.3 μM of each primer (Eurogentec, Angers, France). The PCR conditions used were 10 min at 95 °C and 40 cycles of 15 s at 95 °C, then 1 min at 55 °C, followed by a dissociation curve from 55 °C to 95 °C. No template controls (NTC), using water instead of DNA, were included in each plate.

The efficiency of the real-time PCR ranged from 79% to 102% and the correlation coefficients were higher than 0.990. The detection limit was between 10^2 and 10^3 bacteria equivalent (colony forming unit, CFU) per g of porridge depending on the primer.

The primers validated on DNA extracted from reference strains were used for DNA extracted from the 50 samples to estimate bacterial concentrations.

2.5. Total folate assay

The total folate content of the 50 samples was determined in triplicate using the microbiological assay described by Kariluoto and Piironen (2009), modified as follows. Frozen samples were thawed, 1 g was weighed in a 50-ml centrifuge tube, 15 ml of extraction buffer were added (50 mM Ches/50 mM Hepes buffer, 10 mM 2-mercaptoethanol, 2% (w/v) sodium ascorbate, pH 7.85), the tubes were flushed with nitrogen and thoroughly vortexed. The tubes were heated at 100 °C for 10 min, then cooled on ice. The pH was adjusted to 4.7 with acetic acid and 1 ml of α-amylase (Sigma-Aldrich A9857, 20 mg/ml in 1% sodium ascorbate) and 2 ml of conjugase (prepared from desiccated hog kidney, 5 mg/ml in water) were added. The tubes were flushed with nitrogen and incubated for 3 h at 37 °C in a shaking water bath in the dark. Then 2 ml of protease (Sigma-Aldrich P5147, 3 mg/ml in 1% sodium ascorbate) were added. The tubes were flushed with nitrogen and incubated at 37 °C in a shaking water bath in the dark for 1 h. At the end of the incubation, the tubes were heated at 100 °C for 5 min to inactivate the enzymes, then cooled on ice. The pH was adjusted to 6.1 with KOH, the volume was brought to exactly 50 ml with 0.5% sodium ascorbate (pH 6.1), and the tubes were centrifuged at 20,000 *g* at 4 °C for 30 min. The supernatants were then diluted with 0.5% sodium ascorbate (pH 6.1), and the method described by Kariluoto and Piironen (2009) was used to determine the total concentration of folate using 96-well microtiter plates, with *Lactobacillus rhamnosus* ATCC 7469 as the growth indicator organism, folic acid (Sigma-Aldrich, St Louis, MI, USA) as the calibrant, and Folic Acid Casei Medium (Difco, Sparks, MD, USA) as the assay medium. After 18 h of incubation at 37 °C, turbidity was measured with a microplate reader at 590 nm and the values were compared to a standard curve to calculate the concentration of folate. The reliability of the method was confirmed by analyzing a certified reference material (BCR 121 Wholemeal flour, IRMM, Geel, Belgium). A blank sample and the reference sample were analyzed in each set of samples.

The dry matter content of each sample was determined in triplicate by drying the samples in an oven at 105 °C until constant weight.

2.6. Statistical analysis

Statistical analyses were performed using Statgraphics Plus 5.1 software (Rockville, MD, USA). One-way analyses of variance (ANOVA), followed by least significant difference tests (LSD), were carried out to identify the differences between samples. Differences were considered statistically significant for p-values <0.05. Linear correlation tests were also carried out using Pearson's correlation method with a 95% confidence interval. Correlation coefficients (r) were considered significant at p-values <0.05. A principal component analysis (PCA) was performed with R 3.2.2 on the real-time PCR data and a Hierarchical Clustering on Principle Components (HCPC) was also carried out, to separate the samples into statistically different groups, depending on the composition of their microbiota.

3. Results

3.1. Detection of genes coding for enzymes involved in nutritional functions

The nutritional potential of the microbiota of the 50 samples was assessed by screening for the presence or absence of 15 genes. The results of gene detection are presented in Table 3. Even if the primers used were designed to be species specific, there were a few nonspecific detections among the 50 metagenomes. In this study, we considered that a gene was detected even if the detection was nonspecific.

The frequency of detection of the different genes in the metagenomes varied depending on the function. Indeed, the operon

Table 2
Primers used to quantify the genus or species of bacteria from pearl-millet porridge samples using real-time PCR. The primers were designed to match the gene coding for 16S rRNA.

Name	Sequences 5′-3′	Targets	References
338f	ACTCCTACGGGAGGCAGCAG	Bacteria	Muyzer et al. (1993)
338r[a]	CTGCTGCCTCCCGTAGGAGT	Bacteria	Muyzer et al. (1993)
518r	ATTACCGCGGCTGCTGG	Universal	Muyzer et al. (1993)
Lab677r	CACCGCTACACATGGAG	*Lactobacillus*	Heilig et al. (2002)
Lab772r	YCACCGCTACACATGRAGTTCCACT	*Lactobacillus*	Omar and Ampe (2000)
Lab158f	TGGAAACAGRTGCTAATACC	LAB	Omar and Ampe (2000)
Lferm72f[a]	CCTGATTGATTTTGGTCGC	*L. fermentum*	Omar and Ampe (2000)
Lpla72f	ATCATGATTTACATTTGAGTG	*L. plantarum*	Chagnaud et al. (2001)
leu200f[a]	CGGCGTCACCTAGAGATGGATC	*Leuconostoc*	Omar and Ampe (2000)
S-G-Wei-0121-a-S-2	CGTGGGAAACCTACCTCTTA	*Weissella*	Jang et al. (2002)
S-G-Wei-0823-a-A-18	CCCTCAAACATCTAGCAC	*Weissella*	Jang et al. (2002)

[a] Primers from the literature were converted into their reverse complement.

Table 3
Distribution of genes involved in carotenoid, folate and riboflavin synthesis, starch metabolism and tannase synthesis in metagenomes extracted from pearl-millet porridges. Red boxes: the gene was not detected; green boxes: the gene was detected; grey boxes: the gene was detected, but more than one band was observed on the electrophoresis gel (non-specific detection).

General function	Carotenoids	Folate		Riboflavin				Starch metabolism							Tannases
gene	*crtN / crtM*	*folP*	*folK*	*ribA*	*ribB*	*ribG*	*ribH*	*agl*	*α-amy*	*amyE*	*dexC*	*glgP*	*malL*	*malP*	tanlpl
A1															
A2															
A3															
A4															
A5															
B1															
B2															
B3															
B4															
B5															
C1															
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crtNM, encoding dehydrosqualene desaturase and dehydrosqualene synthase, was never detected, suggesting that the LAB present in the *ben-saalga* samples were not able to synthesize carotenoids. The genetic potential of these LAB to synthesize tannases was higher since the gene *tanlpl*, encoding a tannin acyl hydrolase, was detected in 56% of the metagenomes. The LAB displayed a good genetic potential to hydrolyze starch, since the genes *agl*, *glgP* and *dexC* (encoding alpha-glucosidase, glycogen phosphorylase and neopullulanase, respectively) were detected in 74%, 94% and 86% of the metagenomes, respectively. However, the genes *α–amy* and *amyE* (encoding intra and extracellular alpha-amylases, respectively) were detected in only 24% and 0% of the metagenomes, respectively. The genetic potential to hydrolyze maltose and dextrin was lower, since the genes *malP* and *malL* (encoding maltose phosphorylase and oligo-1,6-glucosidase, respectively) were detected in 26% and 54% of the metagenomes respectively. Four genes are required for the biosynthesis of riboflavin (Burgess et al., 2009; Capozzi et al., 2012), and some, such as *ribH* (encoding 6.7-dimethyl-8-ribityllumazine synthase), were very frequently detected (92%) in the metagenomes. However, the low frequency of detection of the gene *ribG* (encoding diaminohydroxyphosphoribosylaminopyrimidine deiminase; 38%), indicates that the LAB in these samples of *ben-saalga* have a low genetic potential to synthesize riboflavin. Conversely, since the two genes *folP* and *folK* (encoding dihydropteroate synthase/dihydropteroate pyrophosphorylase and 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase, respectively) were detected in 80% of the samples, the LAB responsible for the *ben-saalga* fermentation have a high genetic potential to synthesize folate.

3.2. Quantification of targeted LAB populations by real-time PCR

Primers available in the literature and checked *in silico* were tested to quantify Bacteria, the LAB group and the LAB genus and species commonly associated with pearl-millet based fermented foods by real-time PCR (Table 2). Quantification of Bacteria, LAB group and the *Lactobacillus* genus was not possible since there was no linear relationship between the number of bacteria cells and the number of cycles needed to reach a detectable amount of PCR products (Ct). After *in silico* verification, we found that primers designed for Bacteria LAB and *Lactobacillus* also matched chloroplasts from sorghum, which is very close to pearl millet. Thus, we decided not to use them in this study. Only the calibration curves obtained for the *Leuconostoc* and *Weissella* genus and L. *plantarum* and *L. fermentum* species enabled further analysis of the porridge samples (Table 2).

The concentration of microorganisms varied with the sample and was generally lower than 10^5 CFU/g of porridge (Fig. 1). *L. fermentum* was detected in 88% of the samples and was the dominant microorganism. *Leuconostoc* sp. was detected in 60% of the samples, but in none of the samples from TPU C. *L. plantarum* was detected in 32% samples, but in none of the samples from TPU C, F, G and J. *Weissella* sp. was detected in 30% samples, but in none of the samples from TPU C, E, G and J.

The microbiota differed among the samples from the different TPUs. For example, the samples produced in TPU C had the lowest concentrations of microorganisms. In this TPU, only *L. fermentum* was detected. In contrast, the highest concentrations of microorganisms were found in the samples from TPU H. In this TPU, all the genera and species screened were detected. In addition, we observed that the microbiota in the samples produced by the same TPU varied depending on the day the samples were taken. A principal component analysis, followed by a Hierarchical Clustering on Principle Components, was performed (data not shown) on the real-time PCR data, but it showed that the five samples of a same TPU were never grouped together, and thus that the TPUs could not be discriminated on the basis of the samples microbiota.

3.3. Folate content of the pearl-millet porridges

The dry matter content of the 50 samples ranged from 3% to 9% (median: 5.6%). ANOVA showed that the dry matter content of samples differed significantly ($p < 0.05$), depending on the TPU.

The folate content of the samples (Fig. 2) varied from 0 to 3.3 μg/100 g fresh weight basis (median: 0.5 μg/100 g FW). We observed high variability among the samples produced by the same TPU. To determine if the folate content of samples produced by two different TPUs differed, we also compared the TPUs by pairs. The total folate contents of the samples differed significantly ($p < 0.05$) among the different TPUs. A linear correlation test showed that there was a low ($r = 0.3$) but statistically significant correlation ($p < 0.05$) between the folate content and the dry matter content of the porridge samples. On the other hand, there was no significant correlation ($p > 0.05$) between the concentrations of the four main microorganisms investigated and the folate content of the samples.

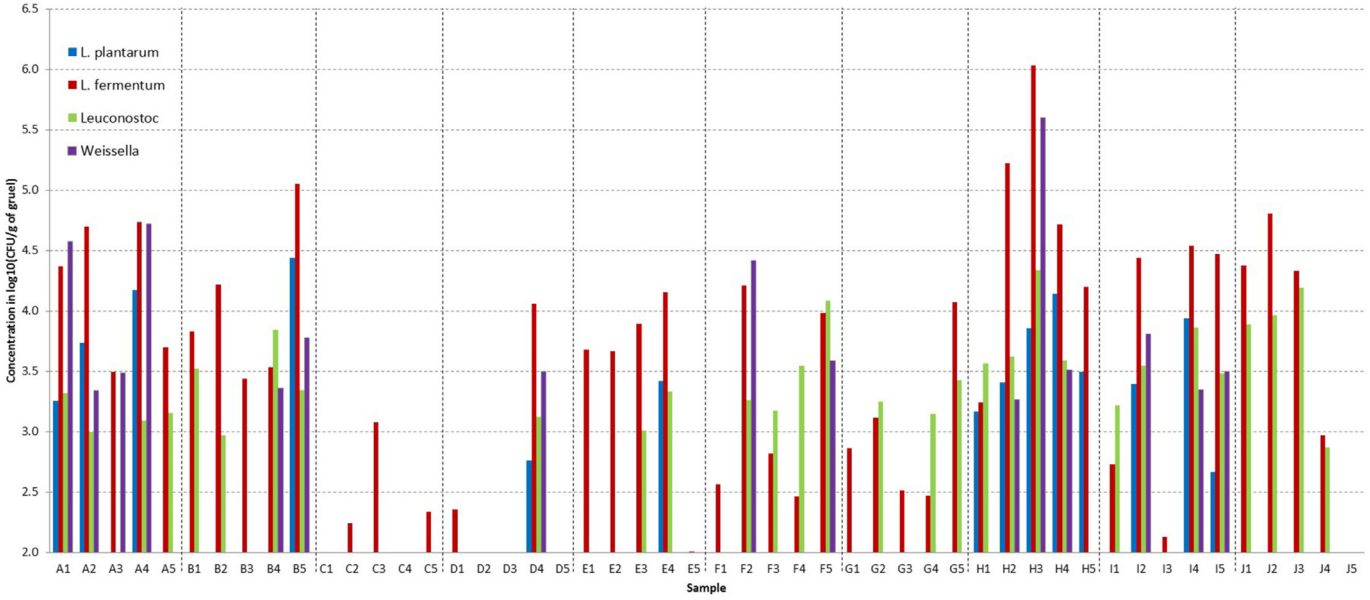

Fig. 1. Real-time PCR quantification of *L. plantarum*, *L. fermentum*, *Leuconostoc* and *Weissella* in fermented pearl-millet based porridges, sampled after the fermentation and the heating steps. Concentrations of microorganisms below the detection limit are not shown in the figure.

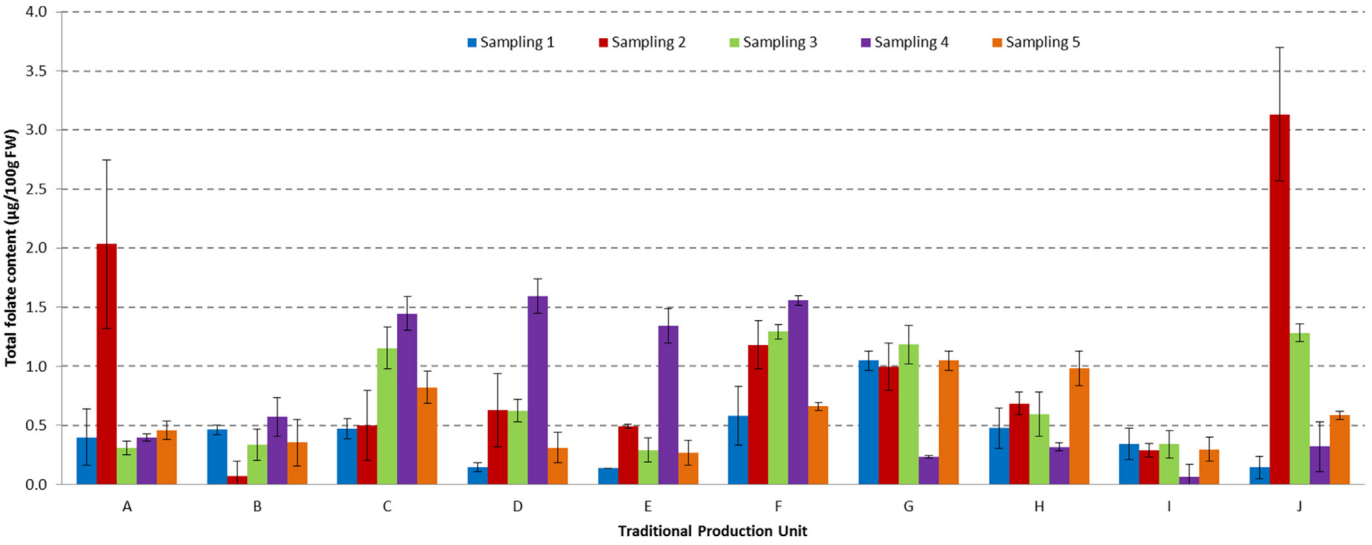

Fig. 2. Folate content of the pearl-millet based porridge samples (FW: fresh weight basis).

4. Discussion

In this study, we estimated the genetic potential of the *ben-saalga* microbiota for different nutritional functions. We also quantified the main LAB described as being responsible for the fermentation of *ben-saalga*: *L. fermentum*, *L. plantarum*, *Leuconostoc* and *Weissella* (Humblot and Guyot, 2009; Turpin et al., 2011). As the genetic potential to synthesize folate was found to be high, we also quantified the folate content of the samples, to investigate if there was any relation between the composition of the main microorganisms investigated, the genes encoding for enzymes involved in folate synthesis and the folate content of the pearl-millet based porridges.

Rather high variability of genetic potential was observed among the samples taken from different TPUs, and also among samples taken from the same TPU. Some genes were detected frequently, such as *agl*, *glgP* and *dexC* (coding for enzymes involved in starch metabolism), while others were not detected at all, such as *crtN* and *crtM* (coding for enzymes involved in carotenoid synthesis). Since the genes *agl*, *glgP* and *dexC* were often detected, the genetic potential of the microbiota of the porridge to metabolize starch appears to be high. However, in a previous study, the three genes were all detected in the non-amylolytic strain *L. plantarum* WCFS1 together with the gene *α–amy* coding for an intracellular alpha-amylase, whereas the gene *amyE* coding for an extracellular alpha-amylase was detected only in the amylolytic strain *L. plantarum* A6 (Humblot et al., 2014). In the present study, the gene *amyE* was not detected in any of the metagenomes. Taken together, these results suggest poor amylolytic potential of the LAB in the porridge.

The genetic potential for the synthesis of carotenoids also appears to be low, since the operon *crtNM* was not detected in our study, whereas it was detected in 37% of LAB wild strains isolated from *ben-saalga* (Turpin et al., 2016). However, this previous work showed that the potential for carotenoid synthesis by these LAB is low, since only 36 LAB strains among the 58 with positive PCR detection were able to produce carotenoids in MRS culture broth. In addition, even the highest carotenoid producing strain produced low amounts of carotenoids in a food matrix made from pearl-millet (<77 μg β-carotene equivalent per 100 g of dry cell weight).

As for microbial diversity, our results are in good agreement with previous studies that showed that *L. fermentum* was the dominant species found in different pearl-millet based fermented foods, including *ben-saalga*, (Lei and Jakobsen, 2004; Owusu-Kwarteng et al., 2012; Turpin et al., 2011). Indeed, in this study, *L. fermentum* was the most frequent species out of the four main species analyzed. Variability in the species investigated was observed among samples produced by different TPUs, which is in good agreement with the literature, but also among samples from the same TPU (Humblot and Guyot, 2009; Owusu-Kwarteng et al., 2012). Different factors could explain this microbial variability, for example the variability of the raw materials used. Vogelmann et al. (2009) demonstrated on sourdoughs prepared from cereals, pseudocereals and cassava that the raw materials have an impact on the composition of the microbiota. According to Tou et al. (2006), porridge producers in Burkina Faso use different aromatic ingredients, such as ginger and pepper, in addition to pearl-millet grains, which could also modify the microbial composition. As reviewed by Minervini et al. (2014), other parameters, such as the duration and temperature of fermentation can also influence the composition of the microbiota. The fermentation of *ben-saalga* is performed at ambient temperature and has a mean duration of 11 h (overnight fermentation) with extreme values (2 to 20 h) only in very few TPU (Tou et al., 2006). In our study, the different samplings in each TPU were carried out on five different days, on the final product ('ready to use' porridge, after cooking at the end of the fermentation). Therefore, the observed variability in the analyzed parameters (microbial variability, folate content, etc.) reflects that of the food as eaten by the consumer.

The variability of the concentrations of the main LAB responsible for the fermentation of *ben-saalga* could have an influence on the variability of the genetic potential of the microbiota. However, the statistical analyses did not allow us to establish clear correlations between the concentration of each individual LAB, and the detection of each gene.

In the present study, the genes *folP* and *folK* (coding for folate synthesis) were detected in 80% of the samples, suggesting that the LAB responsible for the *ben-saalga* fermentation have a high genetic potential to synthesize folate. A complementary mRNA based analysis on the dough before cooking would have indicated if those genes were expressed or not. But to determine if this genetic potential is consistent with the presence of the corresponding micronutrient, we measured total folate content in all 50 samples. However, the folate content of the pearl-millet based porridges was very low (median: 0.5 μg/100 g FW) in comparison to other fermented foods (Souci et al., 2000). This porridge thus cannot be considered as a good source of folate, since 1- to 3-year-old children would have to eat about 3000 g of porridge per day to reach only 10% of their recommended folate intakes, which is 150 μg/day for 1- to 3-year-old children (FAO/WHO, 2005). Considering that the process requires the addition of a large quantity of water to the dough, leading to a dry matter content of <10%, the folate originally present in the food matrix was diluted. Nevertheless, from the folate content of the raw pearl-millet (29 μg/100 g FW; Stadlmayr et al.

2012), we calculated that the final folate content should be higher than 2.5 μg/100 g FW, if there was no loss of folate and only a dilution. This means that folate was probably degraded during the processing of pearl-millet into *ben-saalga*, due to the high sensitivity of this vitamin to oxidation, light, and variations in pH (Strandler et al., 2015). It may also be possible that folate was produced during fermentation, but in small quantities (not sufficient to compensate for the losses), or that it was consumed by the LAB during fermentation. Indeed, folate is involved in essential functions such as metabolism of amino acids, nucleotides synthesis and DNA replication, and therefore each microorganism needs to consume folate to grow (Ball, 2005). Numerous LAB strains belonging to different species such as *L. fermentum* and *L. plantarum*, as well as yeasts, are considered as folate-producing microorganisms since they are able to synthesize higher amount of folate than they consume (Hjortmo et al., 2005; Hugenschmidt et al., 2010; Masuda et al., 2012). But other LAB are considered to be folate-consuming microorganisms, since some of the genes coding for enzymes involved in the synthesis of folate are lacking in these LAB (Rossi et al., 2011). Therefore, the in situ content of folate might have been the result of a balance between auxotrophy and prototrophy.

To conclude, this study highlighted not only the variability of the composition of the microbiota among samples of fermented porridges produced by different TPUs, but also the variability of the composition of the microbiota among the samples produced by the same TPU. This work also shows that it is difficult to draw conclusions concerning the genetic potential of the microbiota of a naturally fermented food only by analyzing genes in metagenomes. The high frequency of the presence of genes does not necessarily correlate with in situ measurements of the expected product, e.g. relation between detection of genes coding for enzymes involved in folate synthesis and the low in situ folate content, observed here. In the case of folate, this could be due to the presence of folate-consuming LAB strains. The choice of another compound, whose consumption by microorganisms is not possible may be more appropriate when using genetic screening. Further studies could aim at optimizing the fermentation step in order to increase the folate content of pearl-millet based porridges. On the contrary, the absence of detection of some genes such as *amyE* appears to be linked with the absence of the corresponding function in the microbiota responsible for the fermentation. Such lack of detection would help, in further studies, to decide whether isolation of LAB with specific nutritional properties from fermented food should be considered or not.

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