



Short communication

PCR-denaturing gradient gel electrophoresis analysis of microbial community in soy-daddawa, a Nigerian fermented soybean (*Glycine max* (L.) Merr.) condiment



Obinna T. Ezeokoli^{a,b}, Arvind K. Gupta^a, Charlotte Mienie^a,
Temitope O.S. Popoola^{b,*}, Cornelius C. Bezuidenhout^a

^a Unit of Environmental Sciences and Management, North-West University, Potchefstroom Campus, 2520, South Africa

^b Department of Microbiology, Federal University of Agriculture, PMB 2240 Abeokuta, Ogun State, Nigeria

ARTICLE INFO

Article history:

Received 9 July 2015

Received in revised form 13 November 2015

Accepted 7 January 2016

Available online 9 January 2016

Keywords:

Soy-daddawa

Fermentation

Bacteria

Fungi

Condiment

PCR-DGGE

Microbial community

ABSTRACT

Soy-daddawa, a fermented soybean (*Glycine max* (L.) Merr.) condiment, plays a significant role in the culinary practice of West Africa. It is essential to understand the microbial community of *soy-daddawa* for a successful starter culture application. This study investigated the microbial community structure of *soy-daddawa* samples collected from Nigerian markets, by PCR-denaturing gradient gel electrophoresis (DGGE) targeting the V3–V5 region of the 16S rRNA gene of bacteria and internal transcribed spacer 2 (ITS2) region of fungi. Six bacterial and 16 fungal (nine yeasts and seven molds) operational taxonomic units (OTUs)/species were obtained at 97% sequence similarity. Taxonomic assignments revealed that bacterial OTUs belonged to the phyla *Firmicutes* and *Actinobacteria*, and included species from the genera *Atopostipes*, *Bacillus*, *Brevibacterium* and *Nosocomiicoccus*. Densitometric analysis of DGGE image/bands revealed that *Bacillus* spp. were the dominant OTU/species in terms of population numbers. Fungal OTUs belonged to the phyla *Ascomycota* and *Zygomycota*, and included species from the genera, *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Dokmaia*, *Issatchenkia*, *Kodamaea*, *Lecytophora*, *Phoma*, *Pichia*, *Rhizopus*, *Saccharomyces* and *Starmerella*. The majority of fungal species have not been previously reported in *soy-daddawa*. Potential opportunistic human pathogens such as *Atopostipes suicloacalis*, *Candida rugosa*, *Candida tropicalis*, and *Kodamaea ohmeri* were detected. Variation in *soy-daddawa* microbial communities amongst samples and presence of potential opportunistic pathogens emphasises the need for starter culture employment and good handling practices in *soy-daddawa* processing.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Soy-daddawa is a traditional food condiment produced through the spontaneous fermentation of soybeans (*Glycine max* (L.) Merr.). The use of fermented protein seeds as condiments is a common culinary practice in West Africa (Omafuvbe et al., 2000). For some households, especially low-income households, such condiments also serve as a significant source of nutritional fortification of meals (Achi, 2005; Popoola and Akueshi, 1986). *Soy-daddawa* is predominantly produced in Benue State, North-Eastern Nigeria (Omafuvbe, 2008). The production process involves the spontaneous fermentation of soybean seeds by a consortium of microorganisms, resulting in the development of a wide range of volatile aroma compounds (Achi, 2005; Popoola and Akueshi, 1985). However, variations in the quality of the final product between batches of production have been identified as a major drawback which can be mitigated by employing starter culture(s) in the production process of *soy-daddawa* (Iwuoha and Eke, 1996).

For an appropriate starter culture application, the knowledge of the microbial community of *soy-daddawa* is paramount. Such knowledge will give insight into the role and interaction of the microbial consortium contributing to the development of the characteristic desirable organoleptic properties of *soy-daddawa*. Furthermore, it will facilitate informed selection and inclusion of starters, optimisation of the fermentation process, and, ultimately, improvement of the quality, consistency amongst batches, safety and shelf-life of the product (Achi, 2005; Omafuvbe et al., 2002).

Previous assessments of the microbial structure of *soy-daddawa* have been based on culture-dependent phenotypic biochemical identification methods (Dakwa et al., 2005; Omafuvbe et al., 2000; Popoola and Akueshi, 1985), which have been the basis for the isolation and identification of starter cultures in a number of controlled studies on *soy-daddawa* fermentation (Omafuvbe et al., 2002). While classical phenotypic methods of microbial identification could be misleading (Spratt, 2004), culture-based methods undermine species richness of microbial communities (Justé et al., 2008). Hence, culture-independent techniques have been developed to mitigate the shortcomings of culture-dependent approaches. Polymerase chain reaction-denaturing gradient

* Corresponding author.

E-mail address: tospopoola@yahoo.com (T.O.S. Popoola).

gel electrophoresis (PCR-DGGE) is a culture-independent method for analyzing microbial communities in a quick and reliable manner (Justé et al., 2008; Muyzer et al., 1993). PCR-DGGE has been applied in studies of microbial communities in fermented foods (Adewumi et al., 2013; Devi et al., 2015; Hamdouche et al., 2015) and environmental samples (Adrados et al., 2014). There is a paucity of culture-independent approaches in *soy-daddawa* microbial ecology.

The aim of the present study was to assess the microbial community structure of *soy-daddawa* products consumed in Nigeria using PCR-DGGE.

2. Materials and methods

2.1. Sampling

Seven *soy-daddawa* products were aseptically collected in triplicates into sterile airtight polythene bags from markets (designated SM, NB, W1, W2, GM, BM) and a household (BH) in Benue State (7° 19' 59.88" N, 8° 45' 0" E), Nigeria (See Supplementary Fig. S1). All samples were collected over a 24 h period, refrigerated (4 °C) and analyzed within a week of collection.

2.2. DNA extraction

Community genomic DNA was extracted from each triplicate sample as follows: 5 g of crushed sample suspension in 15 ml of sterile phosphate buffered saline (pH 7.3) was vortexed and filtered through a layer of muslin cloth. Filtrate was centrifuged at 4 °C for 5 min at 14,000 rpm to pelletize cells. DNA was then extracted from pellets after cell wall lysis by using the ZR Soil Microbe DNA kit (Zymo Research, USA), and by following the manufacturer's protocol. Integrity of extracted DNA was ascertained electrophoretically on 1% (w/v) agarose gel, while concentration and purity were determined using a Nano Drop spectrophotometer (ND-1000, Nano Drop technologies, USA).

2.3. PCR-DGGE

For bacterial community, primer set 341F (5'-CCTACGGAGGCAGC AG-3') and 907R (5'-CCGTCATTCCTTTGAGTTT-3') (Muyzer et al., 1993) were used to amplify the V3–V5 regions of bacterial 16S rRNA gene from community DNA. For fungi, the internal transcribed spacer 2 (ITS2) region was amplified with primer set ITS3 (5'-GCATCGATGA AGAACGAGC-3') (forward primer) and ITS4 (5'-TCCTCCGCTTATTGAT ATGC-3') (reverse primer) (White et al., 1990). A 40 bp GC-rich clamp (CGCCCCGCCGCGCGCGCGGGCGGGCGGGGCACGGGGG) was attached to the 5'-end of all forward primers (Muyzer et al., 1993). All PCRs were performed in a thermal cycler C1000™ (Bio-Rad, USA). Each PCR reaction contained: 0.2 µM of each primer, 12.5 µl of 2 × Dream Taq Master Mix (Thermo Scientific), 50 ng DNA template and nuclease-free water to a final volume of 25 µl. Cycling conditions for 16S rRNA gene amplification included initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 3 min, and a final extension at 72 °C for 5 min. The Fungal ITS2 amplification condition included initial denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 45 s, 52 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min.

For DGGE analysis, amplicons of triplicate samples of each product were pooled in equal proportions. A 30 µl amount of pooled PCR amplicon (containing 5 µl of 6 × loading dye) was loaded onto a 1 mm thick 8% (w/v) polyacrylamide gel at a denaturing gradient of 25% to 55% urea (Merck, Germany) and formamide (Sigma-Aldrich, USA) (100% denaturant is defined as 7 M urea and 40% (v/v) formamide). A reference lane (designated 'R') comprising equal volumes of all PCR amplicons pooled together were loaded alongside sample lanes. DGGE was run on the Dcode™ Universal Mutation Detector System (Bio-Rad, CA, USA) at 60 °C, in 1 × TAE buffer initially at 200 V for 15 min,

then at 100 V for 16 h. Gels were stained in 0.1% (v/v) ethidium bromide solution (in 1 × TAE) for 15 min and photographed using the ChemiDoc™ MP imaging system (Bio-Rad, USA). DGGE was repeated twice to ascertain bands migrated to similar positions and to purify bands.

2.4. Analysis of DGGE community fingerprint

Gene Tools software (v. 4.03.1.0, Syngene, England) was used to perform a densitometric analysis of DGGE gel images. Peaks were first corrected to obtain true peaks by using a number of standard functions in the software. The relative intensities of individual peaks were then calculated and used to compute the Shannon–Weiner diversity index (H') and species evenness (J').

2.5. Sequencing

Major DGGE bands were aseptically excised (at least two bands per migration position). DNA was eluted in 20 µl nuclease-free water overnight at 4 °C. Eluted DNA (10 ng) was used as template for re-amplification by using the above stated PCR primers (without the 40 bp GC clamp) and conditions. PCR re-amplification products were purified and prepared for sequencing PCR using forward primers 341F (for bacteria) and ITS3 (for fungi), and the Big Dye Terminator v3.1 cycle sequence kit (Applied Biosystems, UK). Purified sequencing PCR products were run on a 3130 Genetic analyzer (Applied Biosystems/Hitachi, Japan).

2.6. Sequence analyses and phylogenetic reconstruction

Sequence electropherograms were inspected and edited using FinchTV software (v.1.4.0; <http://www.geospiza.com/Products/finchtv.shtml>). The mothur software pipeline (Schloss et al., 2009) was used to check sequences for chimeras (Bellerophon) and cluster sequences into operational taxonomic units (OTUs) at ≥97% sequence similarity (O'Brien et al., 2005). For taxonomic assignment, bacterial OTU representative sequences were aligned against type strains in the EzTaxon database (<http://www.ezbiocloud.net/eztaxon>), while fungi OTU representative sequences were aligned with sequences on the UNITE ITS database (<https://unite.ut.ee/index.php>). For phylogenetic reconstruction, sequences obtained alongside sequences with closest homology in the GenBank were selected. Multiple sequence alignments of bacterial 16S rRNA sequences and Fungal ITS2 sequences were done using MUSCLE (Edgar, 2004) integrated in MEGA version 6.0 software (Tamura et al., 2013). Multiple sequence alignments were edited and corrected for gaps in DAMBE software (Xia, 2013). Phylogenetic dendrograms were constructed by the neighbor-joining method, using the Tamura–Nei substitution model and a thousand bootstrap replications in MEGA.

Nucleotide sequences obtained in this study have been deposited in GenBank under the accession numbers KR905728–KR905747 and KT021541–KT021542.

3. Results

3.1. PCR-DGGE profile and diversity indices

A snapshot of the DGGE profiles of the bacterial and fungal diversity of *soy-daddawa* is shown in Fig. 1. The band profile varied amongst samples. In Table 1, the Shannon–Weiner index of *soy-daddawa* samples are shown. Overall, the population of individual species was not proportional as suggested by the average species evenness value (0.87). The Shannon–Weiner diversity index (H') ranged from 2.08 (sample NB) to 2.40 (Sample W2 (Table 1)).

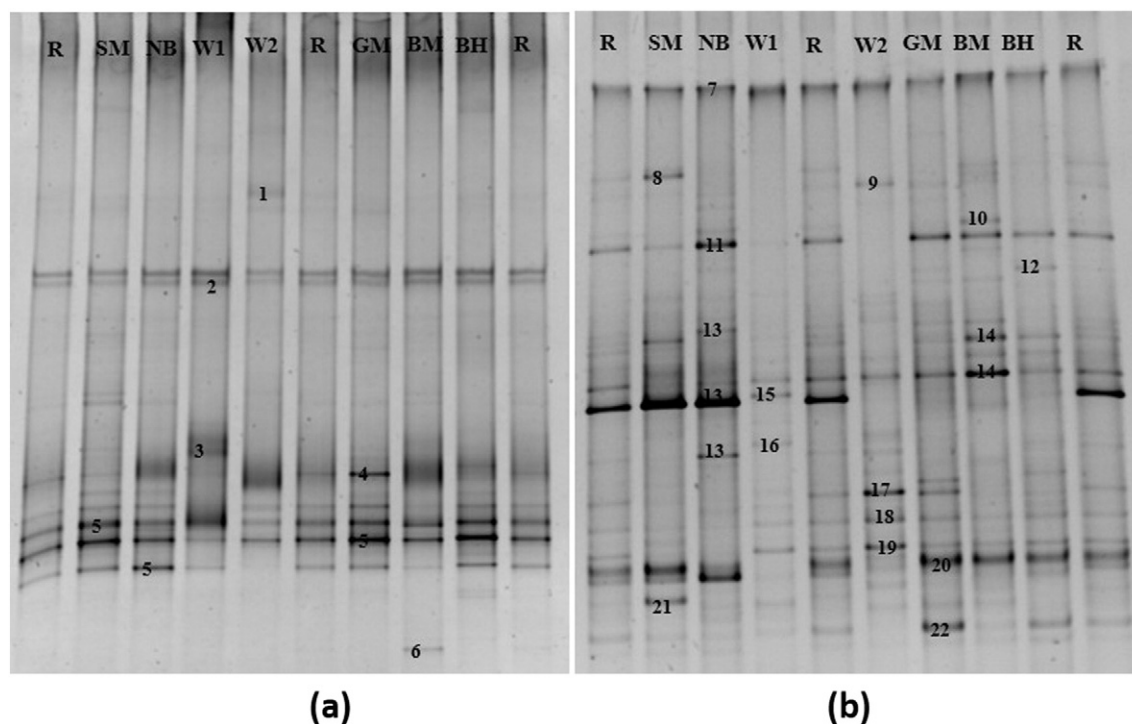


Fig. 1. Corrected DGGE profiles, representing the microbial diversity in seven soy-daddawa products. (a) Bacterial 16S rRNA gene V3–V5 variable regions profile (b) Fungal ITS2 profile. Numbered bands (1–22) indicate major band positions excised. Numbers correspond to OTUs (see Table 1). SM, NB, W1, W2, GM, BM, BH are sampling points. R, reference lane used in densitometric analysis.

3.2. Microbial community structure of soy-daddawa

Six bacterial and 16 fungal (9 yeasts and 7 molds) OTUs were obtained after analyzing 36 bacterial and 48 fungal bands' sequences. Multiple banding positions for designated bacterial OTU 5 (three positions) and fungal OTU 13 (three positions) and OTU 14 (two positions) were observed (Fig. 1). A similar observation has been reported previously (Duarte et al., 2012). According to Duarte et al. (2012), intra-specific or intra-isolate heterogeneity of 16S rRNA genes may give rise to multiple banding patterns in DGGE. OTU 1 and OTU 6 were unique to samples W2 and BM, respectively (Fig. 1a). All other OTUs were present in two or more samples. OTU 15 was only present in sample W1 (Fig. 1b). All other OTUs/band positions were present in at least two of the product samples. Densitometric analyses of the DGGE image indicated that the combined relative intensity (data not shown) of OTU 5s was the highest.

The taxonomic identification of the OTUs is presented in Table 2. Of the six bacterial OTUs obtained, 83.33% belonged to the phylum *Firmicutes*, while the rest (16.6%) belonged to the phylum *Actinobacteria*.

The genus *Bacillus* was dominant (50% of the total number of individual bacterial species) and included *Bacillus tequilensis*, *Bacillus thuringiensis* and *Bacillus pumilus*. Other bacterial species detected included *Brevibacterium massiliensis*, *Nosocomioccocus ampullae*, and *Atopostipes suicloacalis*.

The majority (56.25%) of the fungal OTUs identified were yeasts, while 43.75% was mold (Table 2). The majority (93.75%) belonged to the phylum *Ascomycota*, while, 6.25% belonged to *Basidiomycota*. The *Ascomycota* fungal species included *Alternaria lini*, *Aspergillus brasiliensis*, *Candida rugosa*, *Candida tropicalis*, *Cladosporium cladosporioides*, *Cladosporium oxysporum*, *Dokmaia sp.*, *Issatchenkia orientalis*, *Kodamaea ohmeri*, *Lecythyphora hoffmannii*, *Phoma sp.*, *Pichia kluyveri*, *Pichia rabulensis*, *Saccharomyces cerevisiae*, and *Starmerella sp.* *Rhizopus delemar* was the only *Zygomycota* detected.

Phylogenetic reconstruction (Supplementary Figs. S2 & S3) confirmed taxonomic assignments of bacterial and fungal species: all sequences obtained in the present study grouped together (same clade) with closely related GenBank sequences.

4. Discussion

To our knowledge, this is the first culture-independent study of the microbial community structure of soy-daddawa products in the Nigerian market.

The bacterial community analysis revealed the dominance of *Bacillus* spp. This observation is in agreement with previous culture-dependent studies on soy-daddawa (Omafuvbe et al., 2000; Popoola and Akueshi, 1985) and similar products of soybean fermentation such as, the Japanese *natto* (Nagai and Tamang, 2010) and the Nepalese *kinema* (Sarkar et al., 1997). Importantly, the *Bacillus* species identified in this study belong to taxonomic groups comprising species/subspecies that are not solely distinguishable by 16S rRNA gene sequences (Liu et al., 2013; Wang et al., 2007) (Fig. S2). Hence, the identities of the OTUs belonging to the genus *Bacillus* were not exclusively definitive within the context of a given taxonomic group.

Table 1
Microbial diversity indices of soy-daddawa PCR-DGGE profile.

Sample	H'	J'
SM	2.17	0.85
NB	2.08	0.84
W1	2.28	0.87
W2	2.40	0.94
GM	2.38	0.88
BM	2.39	0.88
BH	2.13	0.86
Mean ± SD	2.26 ± 0.13	0.87 ± 0.03

H' and J' were calculated using combined bacterial and fungal data for each product sample.

$H' = -\sum_{i=1}^S \{P_i \ln(P_i)\}$; $J' = H'/\ln(S)$. Where H', Shannon–Weiner index; J', species evenness; P_i, proportion of all observations in the *i*th species category, S, total number of species.

Table 2

Taxonomic identification of excised major bands from PCR-DGGE of soy-daddawa microbial communities and their percentage similarity from the EzTaxon bacterial 16S rRNA database and UNITE fungal ITS database.

OTU	LAB-ID (accession number)	Sample origin	Closest relative	Sequence similarity (%)
<i>Bacteria (6 OTUs)</i>				
1	SDCB-49 (KT021541)	W2,	[#] <i>Bacillus pumilus</i>	98.6
2	SDCB-30 (KR905731)	SM, NB, W1, W2, GM, BM, BH	<i>Nosocomiicoccus ampullae</i>	99.8
3	SDCB-27 (KR905729)	SM, W1	<i>Atopostipes suicloacalis</i>	98.0
4	SDCB-36 (KP905730)	SM, NB, W1, W2, GM, BM, BH	^{\$} <i>Bacillus thuringiensis</i>	98.6
5	SDCB-10 (KR905728)	SM, NB, W1, W2, GM, BM, BH	[*] <i>Bacillus tequilensis</i>	100.0
6	SDCB-07 (KR905732)	BM	<i>Brevibacterium massiliensis</i>	100.0
<i>Yeasts (9 OTUs)</i>				
7	SDCF-3 (KR905733)	SM, NB, W1, W2, GM, BM, BH	<i>Candida tropicalis</i>	98.0
8	SDCF-4 (KR905734)	SM, BM	<i>Candida rugosa</i>	99.1
9	SDCF-5 (KT021542)	W2, GM, BM	<i>Saccharomyces cerevisiae</i>	97.7
10	SDCF-6 (KR905735)	NB, BM	<i>Kodamaea ohmeri</i>	99.1
12	SDCF-10 (KR905737)	NB, GM, BH	<i>Pichia rabaulensis</i>	98.8
14	SDCF-16 (KR905738)	SM, NB, W1, W2, GM, BM, BH	<i>Starmerella</i> sp.	97.8
17	SDCF-29 (KR905741)	W2, GM	<i>Pichia kluyveri</i>	100.0
20	SDCF-34 (KR905744)	SM, NB, W2, GM, BM, BH	<i>Issatchenkia orientalis</i>	100.0
21	SDCF-36 (KR905745)	SM, NB, W1, BM, GM	<i>Dokmaia</i> sp.	100.0
<i>Molds (7 OTUs)</i>				
11	SDCF-7 (KR905736)	SM, NB, W1, GM, BM, BH	<i>Rhizopus delemar</i>	100.0
13	SDCF-20 (KR905739)	SM, NB, W2, GM, BH,	<i>Lecythophora hoffmannii</i>	97.8
15	SDCF-21 (KR905740)	W1	<i>Alternaria lini</i>	99.3
16	SDCF-25 (KR905747)	W1, W2	Uncultured <i>Phoma</i>	100.0
18	SDCF-31 (KR905742)	SM, W1, W2, GM, BM, BH	<i>Cladosporium cladosporioides</i>	99.6
19	SDCF-33 (KR905743)	W1, W2, GM, BM, BH	<i>Cladosporium oxysporum</i>	100.0
22	SDCF-38 (KR905746)	SM, NB, W1, GM, BM, BH	<i>Aspergillus brasiliensis</i>	99.7

[#], ^{\$} and ^{*}, Taxonomic groups. A taxonomic group includes species/subspecies that are not distinguishable by 16S rRNA sequence.

[#]Member of the *Bacillus pumilus* taxonomic group comprising *Bacillus pumilus* and *Bacillus safensis*.

^{\$}Member of the *Bacillus cereus* taxonomic group comprising *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus toyonensis*.

^{*}Member of the *Bacillus subtilis* taxonomic group comprising *Bacillus subtilis* subsp. *subtilis*, *Bacillus subtilis* subsp. *spizizenii*, *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus mojavensis*, *Brevibacterium halotolerans* and *Bacillus tequilensis*.

Sample origin (see Supplementary Fig. S1): SM, SRS (School of Remedial Studies) junction market, Markudi Local govt. area (LGA); NB, North bank market, in Markudi LGA; W1, Wannune market-producer 1, Tarka LGA; W2, Wannune market-producer 2; GM, Gboko main market; Buruku market, Buruku LGA; BH, a Household in Buruku LGA.

B. tequilensis was identified in all samples of soy-daddawa. However, previous culture-dependent studies have identified *Bacillus subtilis* as the dominant microorganism in soy-daddawa (Omafuvbe et al., 2000; Popoola and Akueshi, 1985) and other fermented-type condiments (Adewumi et al., 2013; Odunfa, 1981). Considering that *Bacillus tequilensis* and *B. subtilis* belong to the same taxonomic group (Wang et al., 2007), it may be concluded that the present finding is congruent with previous reports. *B. pumilus* detected in the present study has been reported in spontaneously fermented soy-daddawa (Omafuvbe et al., 2000), as well as in *soumbala*, a fermented *Parkia biglobosa* condiment in Burkina Faso (Ouoba et al., 2003). Along with *B. subtilis*, they appear to be a safe microbiota of these fermented legume condiments. Their enzymes, proteases, amylases and lipases, are responsible for biochemical changes during fermentation (Achi, 2005; Omafuvbe et al., 2000, 2002).

N. ampullae was the only member of the *Staphylococcaceae* family identified in this study and for the first time in soy-daddawa microbial ecology. Members of the family *Staphylococcaceae* have been previously reported in soy-daddawa (Omafuvbe et al., 2000; Popoola and Akueshi, 1985) and other spontaneously fermented foods (Devi et al., 2015; Adewumi et al., 2013). Their contribution to soy-daddawa fermentation remains unclear. Although the pathogenicity of *N. ampullae* is unknown, closely related members of the same family have been implicated in nosocomial infections (Lory, 2014).

Other bacterial species detected in the present study appear to be food contaminants. *A. suicloacalis* of the order *Lactobacillales*, has been associated with cystic fibrosis patients (Bittar and Rolain, 2010). *B. massiliensis* and *B. thuringiensis* are soil-dwelling microbes, which have also been detected in *iru*, a fermented *P. biglobosa* seed condiment in Nigeria (Adewumi et al., 2013). The presence of these organisms in soy-daddawa is likely associated with human handling and hygiene during soy-daddawa processing, as well as fermentation utensils and raw

materials (Achi, 2005; Iwuoha and Eke, 1996). Their detection questions the microbiological safety of soy-daddawa, and highlights the need for a starter culture application in the fermentation of soy-daddawa and other spontaneously fermented legume condiments in developing countries (Achi, 2005; Holzapfel, 2002; Iwuoha and Eke, 1996).

There is a paucity of information on the fungal diversity of soy-daddawa. Findings of a study by Dakwa et al. (2005) suggested that yeasts do not play a predominant role in the fermentation. However, reemergence in numbers and diversity of fungal species is a possibility, especially during sun-drying and storage of the products, when proteolysis of proteins and associated high alkaline pH of the fermented soy-daddawa matrix is lowered. More importantly, the observed presence of fungal nucleotide sequences in soy-daddawa does not necessarily imply their viability and metabolic activity in the product. We therefore highlight food-safety implications, rather than the role of fungal species detected.

Yeast strains such as *I. orientalis*, *P. kluyveri*, *Pichia rabaulensis*, *S. cerevisiae* and *Starmerella* sp. are considered non-pathogenic and are often involved in food fermentations (Garofalo et al., 2015; Hamdouche et al., 2015). On the other hand, yeast species such as *Candida rugosa*, *C. tropicalis*, *Dokmaia* sp., *L. hoffmannii*, *K. ohmeri* and molds of the genus, *Cladosporium* are documented opportunistic human pathogens (Campbell et al., 2013; Tasic and Tasic, 2007).

Presence of the plant pathogen, *A. lini*, in soy-daddawa may be attributable to materials of plant origin used in soy-daddawa processing (Singh et al., 2014). Other molds, such as *R. delemar* and *A. brasiliensis*, are industrially significant in the production of important biomolecules such as lipases and naphtho- γ -pyrones (Choque et al., 2015). Medically, *A. brasiliensis* and other closely related species, such as *Aspergillus flavus* and *Aspergillus niger*, are known mycotoxin producers, human pathogens and allergens (Hedayati et al., 2007). Although soy-daddawa condiment used in traditional soups undergoes some degree of heating, mycotoxins of *Aspergillus*, especially aflatoxins of *A. flavus*, if present,

are relatively heat-stable and active at temperatures below 2.5 times that of boiling water (Shapara, 2004).

In conclusion, the microbial community of soy-daddawa varied between products. A number of the microbial species are considered pathogenic and raises concerns about the microbiological safety of spontaneously fermented soy-daddawa. Therefore, it is imperative that the processing conditions for soy-daddawa be improved. The use of starter cultures should also be encouraged so as to ensure a product of consistent quality and safety, while also possibly improving the shelf-life of the product.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijfoodmicro.2016.01.003>.

Acknowledgments

This study was funded by the European Union (Grant Agreement 2011- 204072), through the Intra-ACP Academic Mobility Scheme's sponsored Strengthening African Education through Academic Mobility (STREAM) initiative awarded to O.T. Ezeokoli (STRE1101380). Post-doctoral research fellowship awarded by North-West University, South Africa to A.K. Gupta is acknowledged. We sincerely thank Ernest Ekoja for assisting with the collection of samples.

References

- Achi, O.K., 2005. Traditional fermented protein condiments in Nigeria. *Afr. J. Biotechnol.* 4, 1612–1621.
- Adewumi, G.A., Oguntuyinbo, F.A., Keisam, S., Romi, W., Jeyaram, K., 2013. Combination of culture-independent and culture-dependent molecular methods for the determination of bacterial community of *iru*, a fermented *Parkia biglobosa* seeds. *Front. Microbiol.* 3, 1–9.
- Adrados, B., Sánchez, O., Arias, C., Becares, E., Garrido, L., Mas, J., Brix, H., Morató, J., 2014. Microbial communities from different types of natural wastewater treatment systems: vertical and horizontal flow constructed wetlands and biofilters. *Water Res.* 55, 304–312.
- Bittar, F., Rolain, J.-M., 2010. Detection and accurate identification of new or emerging bacteria incystic fibrosis patients. *Clin. Microbiol. Infect.* 16, 809–820.
- Campbell, C.K., Johnson, E.M., Warnock, D.W., 2013. Identification of Pathogenic Fungi. Second ed. Wiley-Blackwell, Oxford, United Kingdom <http://dx.doi.org/10.1002/9781118520055>.
- Choque, E., El Rayess, Y., Raynal, J., Mathieu, F., 2015. Fungal naphtho- γ -pyrones-secondary metabolites of industrial interest. *Appl. Microbiol. Biotechnol.* 99, 1081–1096.
- Dakwa, S., Sakyi-Dawson, E., Diako, C., Annan, N.T., Amoah-Awua, W.K., 2005. Effect of boiling and roasting on the fermentation of soybeans into dawadawa (soy-dawadawa). *Int. J. Food Microbiol.* 104, 69–82.
- Devi, R., Deka, M., Jeyaram, K., 2015. Bacterial dynamics during yearlong spontaneous fermentation for production of *ngari*, a dry fermented fish product of northeast India. *Int. J. Food Microbiol.* 199, 62–71.
- Duarte, S., Pascoal, C., Cassio, F., 2012. Denaturing gradient gel electrophoresis (DGGE) in microbial ecology-insights from freshwaters. In: Magdeldin, S. (Ed.), *Gel Electrophoresis — Principles and Basics*. INTECH, pp. 173–196 <http://dx.doi.org/10.5772/38177>.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797.
- Garofalo, C., Osmani, A., Milanović, V., Aquilanti, L., De Filippis, F., Stellato, G., Di Mauro, S., Turchetti, B., Buzzini, P., Ercolini, D., 2015. Bacteria and yeast microbiota in milk *kefir* grains from different Italian regions. *Food Microbiol.* 49, 123–133.
- Hamdouche, Y., Guehi, T., Durand, N., Kedjebo, K.B.D., Monet, D., Meile, J.C., 2015. Dynamics of microbial ecology during cocoa fermentation and drying: towards the identification of molecular markers. *Food Control* 48, 117–122.
- Hedayati, M., Pasqualotto, A., Warn, P., Bowyer, P., Denning, D., 2007. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology* 153, 1677–1692.
- Holzappel, W.H., 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microbiol.* 75, 197–212.
- Iwuoha, C.I., Eke, O.S., 1996. Nigerian indigenous fermented foods: their traditional process operation, inherent problems, improvements and current status. *Food Res. Int.* 29, 527–540.
- Justé, A., Thomma, B., Lievens, B., 2008. Recent advances in molecular techniques to study microbial communities in food-associated matrices and processes. *Food Microbiol.* 25, 745–761.
- Liu, Y., Lai, Q., Dong, C., Sun, F., Wang, L., Li, G., Shao, Z., 2013. Phylogenetic diversity of the *Bacillus pumilus* group and the marine ecotype revealed by multilocus sequence analysis. *PLoS One* 11, 1–11.
- Lory, S., 2014. The family *Staphylococcaceae*. In: Rosenberg, E., Delong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), *The Prokaryotes*, fourth ed. Springer, pp. 363–366.
- Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59, 695–700.
- Nagai, T., Tamang, J.P., 2010. Fermented legumes: soybean and non-soybean products. In: Tamang, J.P., Kailasapathy, K. (Eds.), *Fermented Foods and Beverage of the World*. CRC Press, Boca Raton, Florida, pp. 191–224.
- O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J.-M., Vilgalys, R., 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Appl. Environ. Microbiol.* 71, 5544–5550.
- Odufa, S.A., 1981. A note on the microorganisms associated with the fermentation of African locust bean (*Parkia filicoidea*) during *iru* production. *J. Plant Foods Hum. Nutr.* 3, 245–250.
- Omafuvbe, B.O., 2008. Effect of temperature on biochemical changes induced by *Bacillus subtilis* (SDA3) during starter culture fermentation of soybean into condiment (soy-daddawa). *Am. J. Food Technol.* 3, 33–41.
- Omafuvbe, B., Shonukan, O., Abiose, S., 2000. Microbiological and biochemical changes in the traditional fermentation of soybean for 'soy-daddawa' — Nigerian food condiment. *Food Microbiol.* 17, 469–474.
- Omafuvbe, B.O., Abiose, S.H., Shonukan, O.O., 2002. Fermentation of soybean (*Glycine max*) for soy-daddawa production by starter cultures of *Bacillus*. *Food Microbiol.* 19, 561–566.
- Ouoba, L.I., Cantor, M.D., Diawara, B., Traore, A.S., Jakobsen, M., 2003. Degradation of African locust bean oil by *Bacillus subtilis* and *Bacillus pumilus* isolated from soumbala, a fermented African locust bean condiment. *J. Appl. Microbiol.* 95, 868–873.
- Popoola, T.O.S., Akueshi, C.O., 1985. Microorganisms associated with the fermentation of soybean for the production of soybean daddawa (a condiment). *Niger. Food J.* 2&3, 194–196.
- Popoola, T.O.S., Akueshi, C.O., 1986. Nutritional evaluation of daddawa, a local spice made from soybean (*Glycine max*). *MIRCEN J. Appl. Microbiol. Biotechnol.* 2, 405–409.
- Sarkar, P.K., Jones, L.J., Craven, G.S., Somerset, S.M., Palme, C., 1997. Amino acid profiles of *kinema*, a soybean fermented food. *Food Chem.* 59, 69–75.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541.
- Shapara, R., 2004. Control of mycotoxins in storage and techniques for their decontamination. In: Magan, N., Olsen, M. (Eds.), *Mycotoxins in Foods: Detection and Control*. Woodhead publishing Ltd., Cambridge, pp. 191–221.
- Singh, R.B., Singh, H.K., Parmar, A., 2014. Yield loss assessment due to *Alternaria* blight and its management in linseed. *Pak. J. Biol. Sci.* 17, 511–516.
- Spratt, D.A., 2004. Significance of bacterial identification by molecular biology methods. *Endod. Top.* 9, 5–14.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729.
- Tasic, S., Tasic, N.M., 2007. *Cladosporium* spp. — cause of opportunistic mycoses. *Acta Fac. Med. Naiss.* 24, 15–19.
- Wang, L.-T., Lee, F.-L., Tai, C.-J., Kasai, H., 2007. Comparison of *gyrB* gene sequences, 16S rRNA gene sequences and DNA–DNA hybridization in the *Bacillus subtilis* group. *Int. J. Syst. Evol. Microbiol.* 57, 1846–1850.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, Inc., New York, pp. 315–322.
- Xia, X., 2013. DABBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Mol. Biol. Evol.* 30, 1720–1728.