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Bacterial species and mycotoxin contamination associated with locust bean, melon and their fermented products in south-western Nigeria

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ABSTRACT

The microbiological safety of spontaneously fermented foods is not always guaranteed due to the undefined fermenting microbial consortium and processing materials. In this study, two commonly consumed traditional condiments (*iru* and *ogiri*) and their respective raw seeds (locust bean and melon) purchased from markets in south-western Nigeria were assessed for bacterial diversity and mycotoxin contamination using 16S rRNA gene sequencing and liquid chromatography tandem mass spectrometry (LC-MS/MS), respectively. Two hundred isolates obtained from the raw seeds and condiments clustered into 10 operational taxonomic units (OTUs) and spanned 3 phyla, 10 genera, 14 species and 2 sub-species. *Bacillus* (25%) and *Staphylococcus* (23.5%) dominated other genera. Potentially pathogenic species such as *Alcaligenes faecalis*, *Bacillus anthracis*, *Proteus mirabilis* and *Staphylococcus sciuri* subsp. *sciuri* occurred in the samples, suggesting poor hygienic practice during production and/or handling of the condiments. A total of 48 microbial metabolites including 7 mycotoxins [3-nitropropionic acid, aflatoxin B_1 (AFB_1), AFB_2, beauvericin, citrinin, ochratoxin A and sterigmatocystin] were quantified in the food samples. Melon and *ogiri* had detectable aflatoxin levels whereas locust bean and *iru* did not; the overall mycotoxin levels in the food samples were low. There is a need to educate processors/vendors of these condiments on good hygienic and processing practices.

1. Introduction

Traditional fermented foods from legumes and oilseeds form a major contribution to the protein requirements of poor households in rural populations across Africa and Asia. Many such fermented foods are used as condiments and widely consumed due to the aroma and flavour they impart on foods (Achi, 2005; Odunfa, 1988). The production of traditional fermented foods in many sub-Saharan African countries is still at small scale/household level (Adejumo et al., 2013; Odunfa, 1985a) and is mostly influenced by chance inoculants which affects the quality characteristics of the finished product.

African locust bean (*Parkia biglobosa*) seeds are very rich in proteins and are spontaneously fermented to produce *iru* (also known as *dawadawa*), a food condiment in Nigeria and parts of West Africa (Odunfa and Oyewole, 1986). *Iru* is important for its flavour and high protein content in soups and stews (Odunfa, 1988). Traditional processing of *iru* is mainly done by women and provides sustainable livelihood for their families (Adejumo et al., 2013). On the other hand, *ogiri* is produced by spontaneous fermentation of oil rich seeds such as those of melon (*Colocynthis citrullus*) (Odunfa, 1981), castor oil bean (*Ricinus communis*)

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(Odunfa, 1985b) and *Telfairia occidentalis* (Barber et al., 1989). *Ogiri* is rich in amino acids such as alanine, lysine and glutamic acid (Odunfa, 1981) and micronutrients (David and Aderibigbe, 2010). *Bacillus* species, especially *Bacillus subtilis*, have been implicated in the fermentation of both condiments (Barber et al., 1988; Odunfa, 1985b; Odunfa and Oyewole, 1986).

Since *iru* and *ogiri* are widely consumed and often used in preparing meals by both households and food vendors, it is important to regularly monitor the safety of these food condiments. It has been previously established that the final microbiological quality and safety of spontaneously fermented food products are influenced by the quality of the raw materials (Steinkraus, 1983), the processing method (Sadiku, 2010) and hygiene of the personnel performing the art of fermentation (Iwuoha and Eke, 1996). Potentially pathogenic bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, coliforms and enterococci (Aderibigbe et al., 2011; Falegan, 2011; Ijabadeniyi, 2007; Oguntoyinbo, 2012; Okanlawon et al., 2010) have been isolated from retailed *ogiri*. Most of the previous studies on microbial ecology of retailed *iru* and *ogiri* have been conducted based on classical biochemical identification methods for bacteria (Ajayi, 2014; Ajayi et al., 2015; Falegan, 2011; Ogunshe et al., 2008) which have several limitations, including the misidentification of species.

Over a decade ago, toxigenic fungal species (e.g. *Aspergillus flavus*, *A. ochraceus* and *Penicillium citrinum*) and/or aflatoxins determined by thin-layer chromatography were reported in either melon or *ogiri* from Nigeria (Bankole et al., 2004, 2006, 2010) and *ogiri* from Sierra Leone (Jonsyn, 1990), but no such information is available on locust bean seeds or *iru*. More recently, diverse mycotoxins including aflatoxins, citrinin, cyclopiazonic acid and ochratoxin A have been reported in high levels in melon seeds analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Ezekiel et al., 2016; Somorin et al., 2016); suggesting the need to analyse fermented condiments for mycotoxins. It is therefore imperative to study the bacterial community and spectrum of microbial metabolites occurring in these fermented condiments because the presence of bacterial pathogens and mycotoxins in *iru* and *ogiri* may constitute a public health concern for consumers. There was no need to study the fungal diversity as mycotoxin profile reflects the mycotoxicological risk posed by the contaminating moulds. Thus, this study aimed to assess the bacteriological and mycotoxicological safety of both food condiments regularly and widely consumed in Nigeria.

2. Material and methods

2.1. Food samples

Locust bean seeds, shelled melon seeds, *iru* and *ogiri* samples were purchased from markets in Lagos (6.6084854 N 3.3915728 E), Ogun (7.1411394 N 3.3478305 E) and Oyo states (7.3905092 N 3.8687164 E), southwest of Nigeria in March 2016. Nine composite samples of each food material were collected to obtain a total of 36 composite samples. Each composite sample (300 g) consisted of three individual sub-samples aggregated from three food vendors. All samples were aseptically collected into sterile polyethylene bags and transported to the laboratory for analysis. Each composite sample was properly homogenized and quartered twice to yield 25 g representative sample for microbiological and mycotoxin analysis. All representative samples were comminuted, stored at 4 °C and processed within 24 h. Each representative sample was batched into two parts: batch A (15 g) for bacteriological analysis and batch B (10 g) for mycotoxin analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). Batch B samples were kept at − 20 °C until mycotoxin analysis.

2.2. Bacteriological analysis of food samples

2.2.1. Isolation of bacteria

Milled portions of the representative food samples were ten-fold serially diluted in sterile distilled water for the isolation of bacteria. Aliquots of serially diluted samples were pour-plated on a range of bacteriological media: plate count agar (PCA) (Oxoid, Basingstoke Hampshire, England) for aerobic bacteria; MacConkey agar (LAB M, Heywood Lancashire England) for coliforms; and mannitol salt agar (MSA) (Oxoid, Basingstoke, Hampshire, England) for staphylococci. All inoculated plates were incubated at 30 °C for 24–48 h. Distinct colonies were streaked on fresh PCA, MSA and MRS agar plates twice to obtain pure cultures, and then maintained on agar slants at 4 °C for further characterization studies.

2.2.2. Preliminary morphological assessment of bacterial isolates

All purified bacterial isolates were microscopically assessed for cell morphology (Gram reaction, cell shape and arrangement). Colony growth pattern, colour, elevation and consistency were also examined. Isolates were then inoculated into nutrient broth E (LAB M, UK) supplemented with 40% glycerol (BDH, Poole, England) and stored at 4 °C for further characterization studies.

2.3. Molecular identification of isolates

2.3.1. DNA extraction

Overnight cultures of pure isolates in Luria-Bertani broth (Acumedia, Michigan, USA) were centrifuged (10,000 × *g* for 1 min) and genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep extraction kit (Zymo Research, California, USA) according to the manufacturer's instructions. Briefly, cells were lysed by bead beating in a lysis buffer and the cell lysate was centrifuged. The supernatant was filtered prior to DNA binding to a column matrix. Bound DNA was further purified and eluted from the column matrix. The integrity of eluted DNA was verified by agarose gel electrophoresis, while quantification was performed using the Qubit 2.0 fluorometer (Thermo Fischer Scientific, Massachusetts, USA).

2.3.2. Partial 16S rRNA gene sequencing

The partial 16S rRNA gene of isolates was amplified using universal primer sets 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). Each reaction tube contained 12.5 μL of 2 × Master mix (Thermo Fisher Scientific, MA, USA), 0.2 μM of each of forward and reverse primers, 20 ng template DNA, and nuclease-free water to a total volume of 25 μL. The PCR protocol involved an initial denaturation at 94 °C for 5 min, 32 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 2 min, and a final extension step of 94 °C for 5 min. All PCRs was performed in a thermal cycler (SimpliAmp, Applied Biosystems, California, USA). Amplicons (~1500 bp) were verified by agarose gel electrophoresis and purified using the NucleoFast 96 PCR clean-up kit (Macherey-Nagel, Duren, Germany) prior to sequencing the PCR products using forward primer 341F (5′-CCTACGGGAGGCAGCAG-3′) and the Big Dye terminator sequencing v3.1 cycle sequencing kit (Applied Biosystems, UK). Sequencing amplicons were purified using Sephadex columns (Princeton Scientific, New Jersey, USA) and analyzed on a genetic analyzer (ABI3730xl, Applied Biosystems, CA, USA).

2.3.3. Taxonomic assignment and phylogenetic reconstruction

For taxonomic assignment, sequence electropherograms were inspected and manually edited using ChromasLite (v.2.1, Technelysium Pty Ltd). Edited sequences were aligned against the EzTaxon server (http://www.ezbiocloud.net; Kim et al., 2012) for identification of isolates on the basis of 16S rRNA gene sequence data. Sequences were further clustered into operational taxonomic units (OTUs) at a sequence similarity of 97% using Mothur software (Schloss et al., 2009) as previously described (Ezeokoli et al., 2016). For phylogenetic