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Metagenomic analysis of fungal community associated with *Irvingia* species (African mango) fruit wastes

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ABSTRACT

The use of culture-dependent assays in studying microbial communities are limited to the cultivable microorganisms, and do not represent the entire community of a sample. Hence, in this study, metagenomic analysis targeting the internal transcribed spacer (ITS) region of ribosomal RNA genes was used to study the taxonomic diversity and structure of the fungal community associated with postharvest *Irvingia* spp. fruit wastes. Results obtained show that Simpson's diversity and the number of operational taxonomic units (OTUs) were dependent on the fruit storage period (days after harvest, DAH). Fresh fruits assessed on 0th, 3rd and 6th DAH had a Simpson's diversity of 0.61, 0.70 and 0.57, respectively. Whilst a total of 14,403 fungal sequences belonging to 17 OTUs, 11 Genera, 5 Classes and 2 Divisions were amplified from *Irvingia* fruits at 0th DAH, 4,966 fungal sequences belonging to 20 OTUs, 15 Genera, 5 Classes and 2 Divisions were amplified from postharvest *Irvingia* fruits on the 3rd DAH. Meanwhile fungal sequences amplified from *Irvingia* fruits on the 6th DAH were 377 belonging to 11 OTUs, 8 Genera, 3 Classes and 1 Division. Predominant among the OTUs were sequences belonging to *Issatchenkia hanoiensis, Pichia manshurica, Candida asparagi*, *Gliocephalotrichum bulbilium, Pichia* spp., *Saccharomycopsis vini, Schwanniomyces occidentalis* and *Galactomyces candidum*. The majority of these fungi belong to Saccharomycetales; well known as good fermenters of sugar. The comparative relative abundance of these yeasts on postharvest *Irvingia* fruits is a useful indication that these commodities could be a suitable substrate for ethanol production from fruit wastes.

INTRODUCTION

The fruits of *Irvingia* spp. commonly called Bush mango, African mango or wild mango are broadly ellipsoidal, about 4-7 cm long, green when unripe and yellow when ripe with a fleshy mesocarp. The fruit pulp is juicy with a sweet or bitter taste depending on the species (Etukudo, 2000). The fruits are well known and highly valued for their fat and protein rich kernels (nuts) which serve as a sauce thickening condiment (Matos et al., 2009). In addition to their nutritional value, the economic value of *Irvingia* nuts is also equally rewarding. In Cameroon alone, the trade of *Irvingia* kernels to other African countries has been valued at US$ 260,000 per annum (Ndoye et al., 1997). The humid lowlands of Cameroun, Nigeria and Côte d´Ivoire have been identified as the major sources of *Irvingia* kernels in local and international trade (Ayuk et al., 1999). Oyakhilome (1985) showed that one-third of all sauce prepared in Southern Nigeria includes *Irvingia* nuts as part of the ingredients. Furthermore, local farmers in Southwest, Nigeria alone

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harvest as much as 750,000 t of fresh fruits in a year, whilst an estimated total of 1,2,000,000 t of *Irvingia* kernels are traded annually in Nigeria (Ladipo, 2003).

Whilst the nuts are savored for their nutritional and health benefits, the fleshy pericarp which constitutes about 80% of the total weight of the fruit (Etebu, 2013) are often thrown away as wastes by locals who harvest them (Ladipo et al., 1996; Etebu, 2012). Interestingly, the fleshy pericarp of *Irvingia* fruits are beginning to receive attention amongst researchers, owing to recent findings about their rich proximate content, vitamins and phytochemicals (Onimawo et al., 2003; Etebu, 2012; 2013; Etebu and Tungbulu, 2016; Etebu et al., 2016; Tungbulu et al., 2016; Etebu and Oku, 2017). However, fresh *Irvingia* fruits have a short shelf life and this is a major setback militating against their storage and consumption (Joseph and Aworh, 1992; Etebu and Tungbulu, 2016). Previous works have shown that postharvest *Irvingia* fruits can be severely contaminated by microorganisms, a few days after harvest under ambient temperature, resulting to brownish black rot disease (Etebu and Tungbulu, 2016; Etebu et al., 2016). Although fungi are known to be the major culprit responsible for the disease, previous studies on the disease and microorganisms associated to postharvest *Irvingia* fruits have been almost based on culture-dependent assays (Joseph and Aworh, 1992, Etebu, 2012, 2013) without further investigations.

Microbial community studies arising from culture dependent assays have shown to be very limited (Kellenberger, 2001; Price et al., 2009). The recent use of metagenomic approach in the assessment of microbial consortia in their natural environment, without recourse to culture of individual species, have greatly enhanced the study of the taxonomic structure of microbial communities. In recent years, researchers have applied the metagenomic analyses in relation to plant pathology and plant microbial ecological studies (Pinto et al., 2014; Müller and Ruppel, 2014; Taylor et al., 2014). However, metagenomic analyses of fungal consortia associated with postharvest *Irvingia* fruits have recently been studied with respect to bacteria (Etebu et al., 2018); this approach is yet to be extended to study the fungal community structure and diversity.

The metagenomic analyses, targeting the internal transcribed spacer (ITS) region of fungal DNA were carried out in this work to study the fungal community structure associated with *Irvingia* fruit wastes. The results from this work would broaden the knowledge base of researchers to further explore the potential benefits of *Irvingia* fruit fleshy pericarp.

MATERIALS AND METHODS

The experiment was designed according to Etebu (2013) and Etebu et al. (2016) with slight modifications. Briefly, *Irvingia* fruits were harvested from two natural forests situated in Toru-angiama town (Lat. 5° 07′ N Long. 6° 06′ E) in Sagbama Local Government and Amassoma Town (Lat. 4° 58′ 09″ N Long. 6° 06′ 34″ E) in Southern Ijaw Local Government Area, all of Bayelsa State, Nigeria. A total of 900 fresh and green fruits were randomly selected from the selected lots, and split with a machete to extract the kernel. About 200 of the split pericarps were spread within a previously constructed quadrant measuring about 3 m × 1 m. The quadrant was barricaded at the sides with nets to exclude reptiles and the fruits were left to decay for 6 DAH. About 20 *Irvingia* fruits were randomly selected at the 0^{th}, 3^{rd} and 6^{th} DAH and surface disinfected by 0.7% sodium hypochlorite solution as according to Etebu et al. (2003) and rinsed in plenty of sterile distilled water. Thereafter, about 200 g of the fruits' fleshy pericarp was sliced out and blended with a household blender for 30 s under aseptic conditions and frozen in -20°C until needed for metagenomic analyses.

DNA extraction

DNA was separately extracted from the *Irvingia* samples processed at 0^{th} 3^{rd} and 6^{th} DAH as described above. Total DNA was extracted and purified using ZR Fungal/Bacterial DNA MiniPrep™50 Preps. Model D6005 (Zymo Research, California, USA) according to the Manufacturer's protocol as described by Etebu et al. (2018). Briefly, 0.5 g of *Irvingia* fruit slurry was suspended in 200 µl of deionized sterile water in a ZR BashingBead™ Lysis Tube. Exactly 750 µl of Lysis solution was added and microbial cells were lysed by bead beating for 5 min at maximum speed prior to centrifugation at 10,000 × g for 1 min. Thereafter 400 µl of the supernatant containing nucleic acids was transferred into a Zymo-Spin™ IV Spin Filter collection tube and centrifuged at 7,000 × g for 1 min. The filtrate was thereafter mixed with 1.2 ml of DNA binding buffer and 800 µl of the mixture was then transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10,000 × g for 1 min. The flow through was discarded from the collection tube and the process was repeated. Following this, DNA was washed with 200 µl DNA pre-wash buffer and centrifuge at 10,000 × g for another 1 min. The DNA was washed again with 500 µl of DNA Wash Buffer into the Zymo-Spin™ IIC column; centrifuged at 10,000 × g for 1 min and eluted with 100 µl of DNA elution buffer into a clean and sterile 1.5 ml Eppendorf tube by centrifugation at 10,000 × g for 30 s. The DNA samples were thereafter sent to Inqaba Biotechnology Pretoria South Africa for further meta-genomic analysis.

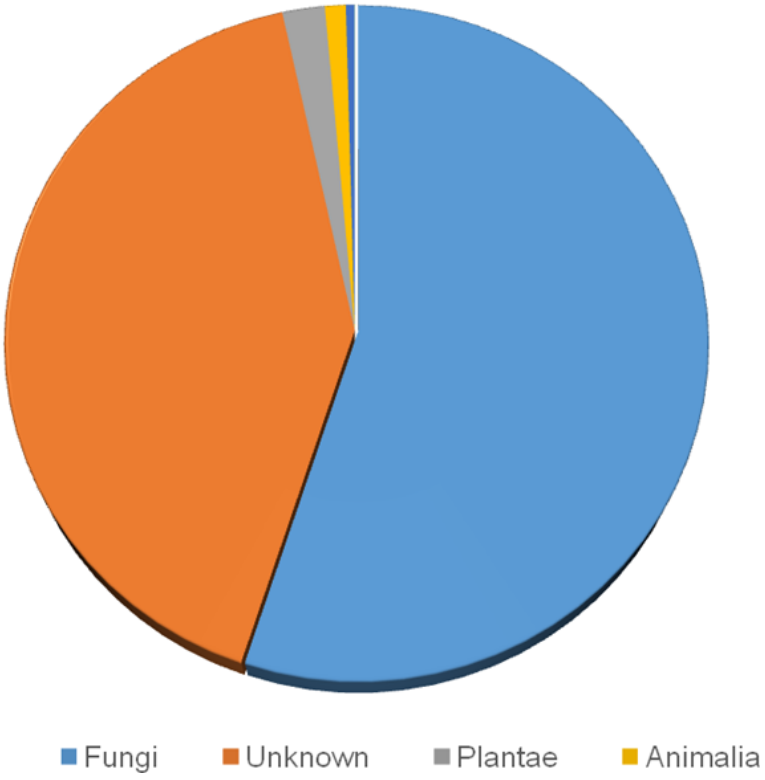

Figure 1. Cummulative proportion of organismal DNA obtained from *Irvingia* fruit wastes after different days of harvest using Primers targeting ITS region.

Polymerase chain reaction and DNA sequencing

Polymerase chain reaction (PCR) as described by Etebu and Osborn (2009) targeting the ITS region using Fungal Primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) described by Gardes and Bruns (1993) and ITS4 (TCC TCC GCT TAT TGA TAT GC) described by White et al. (1990). Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon. Following quantification, the samples were individually indexed, and another bead based purification step was performed. Amplicons were then sequenced on illumina's MiSeq platform, using a MiSeq v3 (600 cycle) kit. 20 Mb of data (2 × 300 bp long paired end reads) were produced for each sample. The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

Sequences of dominant OTUs were also compared to sequences from National Center for Biotechnology Information platform on the web (http://blast.ncbi.nlm.nih.gov), and fungal isolates were identified based on the resultant top hits with maximum percentage identity, minimum gaps and minimum E-score (Altschul et al., 1990).

Simpson's diversity

Simpson's Diversity Index (SDI) used as measure of the diversity of fungi was calculated from the formula:

$$SDI = 1 - \frac{\Sigma n(n-1)}{N(N-1)}$$

Where: SDI, Simpson's diversity index; n, number of the sequence reads of individual OTUs; N, total number of sequences of all OTUs (adapted from Fowler et al., 2005).

RESULTS AND DISCUSSION

Metagenomic analysis of genomic DNA purified from postharvest *Irvingia* fruits showed that several fungal species are associated with the fruits irrespective of postharvest period (Figures 1 and 2). Primers targeting the ITS region of ribosomal RNA gene showed a relatively greater affinity for fungal DNA sequences than those of plants, animals, bacteria and protozoa (Figure 1). In particular, 55.07% of total DNA sequences

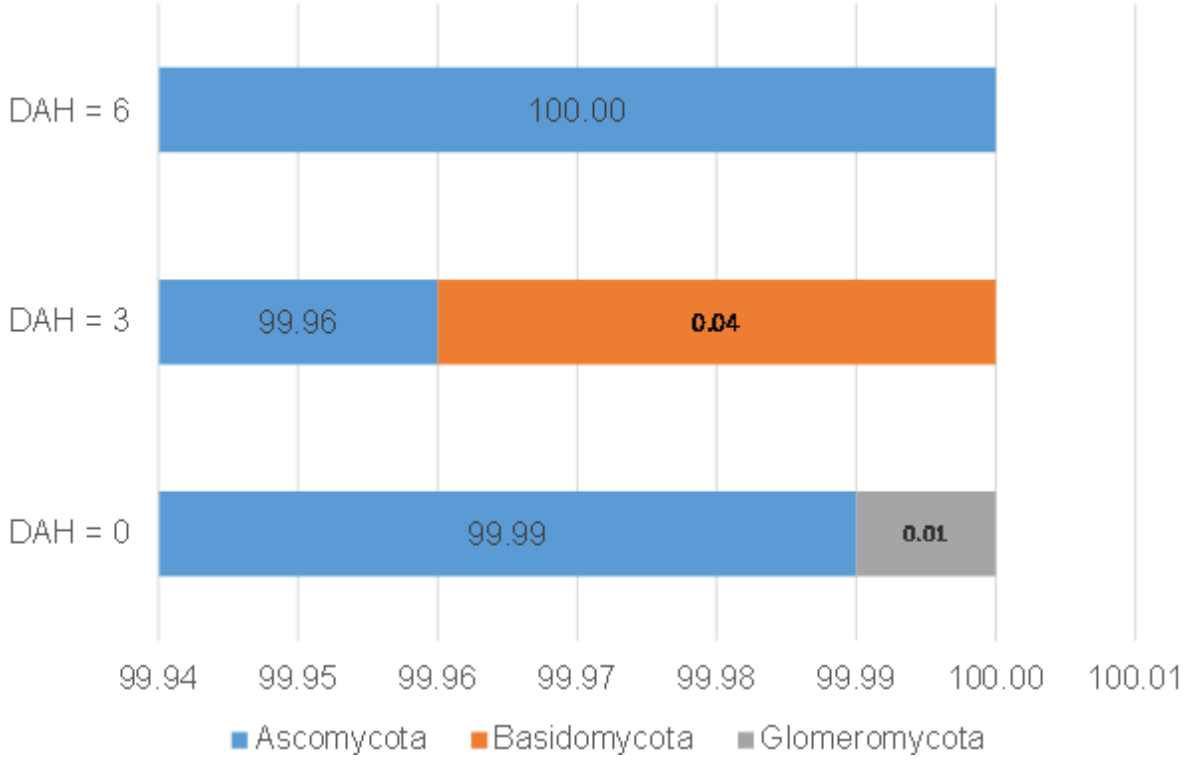

Figure 2. Comparative occurrence of fungal division ITS partial sequences obtained from *Irvingia* fruit wastes at different days after harvest.
Key: DAH, Days after harvest of *Irvingia* fruits.

amplified from postharvest *Irvingia* fruits belonged to fungi while 41.48% of the sequences could not be assigned to any known organism (Figure 1). The use of Primers ITS1F and ITS4 targeting the ITS region of rRNA gene have been shown to be preferred in molecular identification of fungi than some other primers targeting the 18S or 28S of rRNA gene sequences (Etebu, 2008; Etebu and Osborn, 2012). Findings from this study attest to the suitability of these primers (ITS1F and ITS4) in the metagenomic analyses of the fungal community. The ITS regions of ribosomal DNA (rDNA) are the most widely targeted DNA sequences used for fungal identification (Abdelfattah et al., 2016) because these regions of the rRNA sequences are easily amplified and sequenced with universal primers and the corresponding ITS sequence data is highly represented in GenBank and other databases (Kõljalg et al., 2005; Pruesse et al., 2007). Most workers use either the ITS1 or ITS2 as the forward primers because the regions that they amplify share similar properties, and enable similar levels of discrimination (Bazzicalupo et al., 2013).

Results from this work showed that the community structure of fungi associated with postharvest *Irvingia* fruit waste was dependent on the storage period measured herein as DAH (Table 1). *Irvingia* fruits assessed on the day of harvest had a total of 14,403 fungal sequences belonging to 17 OTUs (fungal species) partitioned in 11 Genera, 5 Classes and 2 Divisions. Meanwhile, the number of fungal sequences amplified from fruits assessed on the 3rd day after harvest decreased to 4,966. These sequences were observed to belong to 20 OTUs (fungal species) partitioned in 15 Genera belonging to 5 Classes and 2 Divisions. Likewise, the number of fungal sequences amplified from *Irvingia* fruits assessed on the 6th DAH further decreased to 377 reads, and these belonged to 11 OTUs (fungal species) spanning across 8 Genera in 3 Classes and 1 Division (Table 1). Whilst the total number of fungal sequence reads increased as the DAH increased, the number of OTUs was about the same for fruits assessed on the 0th (17 OTUs) and 3rd (20 OTUs) DAH, but the number of OTUs amplified from the fruits decreased comparatively to 11 on the 6th DAH.

Similar to the number of OTUs, the Simpson's diversity of the fungal community assessed on the 0th, 3rd and 6th DAH were 0.61, 0.70 and 0.57, respectively. This means that the diversity of fungi increased on the 3rd day after fruit harvest compared to fruits assessed on the day of harvest, but dropped drastically on the 6th DAH. This is indicative of a nutrient depletion, and it suggests that fungal nutrients inherent in *Irvingia* fruits were rapidly

Table 1. Summary of fungal taxonomic groups, number and diversity of fungal communities associated with postharvest *Irvingia* fruit wastes.

Parameter	DAH = 0	DAH = 3	DAH = 6
Number of fungal division	2	2	1
Number of fungal classes	5	5	3
Number of fungal genera	11	15	8
Number of OUT (fungal species)	17	20	11
Total number of fungal reads	14403	4966	377
Simpson's diversity index	0.61	0.7	0.57

Key: DAH, Days after harvest; OUT, operational taxonomic units (fungal species).

Table 2. Comparative occurrence of fungal class' sequences from *Irvingia* fruit wastes at different days after harvest.

Fungal class	DAH = 0	DAH = 3	DAH = 6	Mean
Dothideomycetes	0.02	0.50	0.27	0.26
Eurotiomycetes	0.01	0.58	-	0.20
Saccharomycetes	98.56	98.13	79.84	92.17
Sordariomycetes	1.40	0.75	19.89	7.35
Tremellomycetes	0.00	0.04	-	0.01
Glomeromycetes	0.01	-	-	0.00
Total number of fungal reads	14403	4966	377	6582
Simpson's diversity index	0.61	0.7	0.57	0.63

Key: DAH, Days after harvest of *Irvingia* fruits.

depleted after harvest. Previous works have reported the same trend with postharvest *Irvingia* fruits regarding moisture, protein, fibre and lipid (Etebu and Tungbulu, 2016; Etebu and Oku, 2017). On the other hand, carbohydrate was reported in these studies to increase with increase in DAH of the fruits.

Based on the metagenomic data set, members of the Division Ascomycota were dominant in the fruits across all storage period. Ascomycota accounts for as much as 99.99 to 100% of the total number of sequences amplified, irrespective of DAH (Figure 2) Glomeromycota accounted for the remaining 0.01% of fungi for fruits assessed on DAH 0, while Basidiomycota constituted the remaining 0.04% of fungi on fruits assessed on DAH 3.

Ascomycota is known to be the largest fungal Division with approximately 64,000 known species (Kirk et al., 2008). This group is also known to be one of the most diverse and ubiquitous phyla of eukaryotes, occurring in several and diversified ecological niches where they function as saprophytes, mutualists, parasites or pathogens of animals, plants and other fungi. As saprophytes, they are active in natural recycling of the organic matter. It was therefore not unexpected to find members associated with postharvest *Irvingia* fruits. A recent study showed that Ascomycota accounts for as much as 95.60% of fungi associated with strawberry plant (Abdelfattah et al., 2016).

Ascomycetes obtained from this work belonged almost exclusively to Saccharomycetes fungal class subdivision (Table 2). Members of Saccharomycetes constituted an average of 92.17% of the total fungal community of the fruits. This was followed by Sordariomycetes (7.35%), Dothideomycetes (0.26%), Eurotiomycetes (0.20%), Tremellomycetes (0.01%) and Glomeromycetes (0.003%). Findings from this work further showed that eight predominant sequences labelled OTU ID1- OTU ID8 were closely related to ITS sequences belonging to *I. hanoiensis* (Accession number FJ153177.1), *P. manshurica* (KY962538.1), *Candida* spp. (KY101945.1), *G. bulbilium* (KF513258.1), *Pichia* spp. (KP223719.1), *S. vini* (HG939425.1), *S. occidentalis* (KY105385.1) and *G. candidum* (KY103456.1). The percentage identity of the different OTUs to these identified species from the NCBI Gene bank ranged from 78 to 100% (Tables 3 and 4). Previous studies using culture-dependent assays had identified *Botrytis, Fusarium, Mucor, Penicillium* and *Aspergillus* from postharvest *Irvingia* (Etebu, 2012, 2013; Joseph and Aworh, 1992). Although most of these fungi were also found in the present work, metagenomic analyses has nevertheless shown that these fungi occur in relatively low proportion in *Irvingia* fruits, and could not make the list of fungi with a relative abundance of ≥ 1%

Table 3. Fungal ITS sequences of most predominant OTUs amplified from postharvest *Irvingia* fruits.

>OTU ID1
TCCTCCGCTTATTGATATGCTTAAGTTCAGCCGGTAGTCGTACCTGATTTGAGGTCAA CTTTATGAAAGTTATAAGGCCAAGCTTATAATAAAATTCTTGTATTTAATAGCAATA ATTAATGAGTTGGTAAAACCTAATACATTAAAATAGCTATAGAGTACTAAACTTTTT ACTCTTGCCAATTCATTTCAAGGTAGTTTTTTTTAACAAAAACTTATCCCAATACTAA ACCCAAAGGTTTAAGAGAGAAATGACGCTCAAACAGGCATGCACTTTAGAATACTA AAGAGCGCAATATG
>OTU ID2
TCCTCCGCTTATTGATATGCTTAAGTTCAGTGGGTATCCTTACTTGATTTGAGGTCAA AATTTATGATGACAGTTGATTAGGCCGATAACCTAGAGTAACCTAGCTTAGAGGTAT TTATTAAAATGGTAAAACCTAGTAATAAATTCTCCAAAGCTATAAATAGCCACTAAA GGACCTGCCGAGTCATTTCAGAGGAGTGGAAAGAGTATCCCCACTTCCTCCATAACT AAACCTAGAGGTTTAAGAGTGAAATGACGCTCAAACAAGCATACTCTACAGAATAC TATAGAGTGCAATATG
>OTU ID3
TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTACTCCTACCTGATCTGAGGTCGA GCTCAATGATATATTTTCGCTCGGCGGCAAAAGCGTCCCGATGTAGTTCGCTTCGTC CGCAACGTTTCTTTTCGGCCGGGCCAGTGGCACGGCCAATTCTGAATCTTTTTTTAAA AAAAAAACGTTTTATGAAGGAAACGAAGTTGAGACTGGCATGATCGCCGGAATGCG GGCGGGCGACTTGTTGGGTTAATGATTAAGTATATGATGTTGGCTGGTTACTTGTTCT TGCTACTATTTGAC
>OTU ID4
TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTACTCCTACCTGATCTGAGGTCGA GCTCAATGATATTTTCGCTCGGCGGCCAAAGCGTCCCGATGTAGTTCGCTTCGTCCG CAACGTTTCTTTTCGGCCGGGCCAGTGGCACGGCCAGCTCTGGATCTTTCTTTAACA AAAGTTCCAAGAAGGAAACGACGCTCAGACAGGCATGCCCGCCGGAATACCGACG GGCGCAATGTGCGTTCAAGAACTCGATGATTCACGATGGCTGCAATTCACACTAGGT ATCGCATTTCGCTGCGC
>OTU ID5
TCCTCCGCTTATTGATATGCTTAAGTTCATCGGGTACTCCTACTTGATTTGAGGTCAA AATGATGGTCGTGTGAAACGTAGTTAACACAACTCACTCGTTTTCAAGGAAACCGCA GACAAGTCCACAGTAACCCCAGCTCAAAGATTGAACTGACGCTCAAACAAGCATGC CCTGCGGAATACCACAGGGCGCAATGTGCGTTCAAGAACTCGATGATTCACGATGC CTGCAATTCACATTAGTTATCGCATTTCGCTGCGCTCTTCATCGGTGCGAGAGCCAA GAGATCCGTTGTTGAA
>OTU ID6
TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATCCGAGGTCA AACTTCAGAAGTTGGGGGTTTAACGGCTTGGCCGCGCCGCGTTCCAGTGCGAGGTGA GTTACTACGCAGAGGAGGCTACAGCGAGACCGCCACTAGATTTGGGAGACGGCGGC GACCCGGAGGACGCTTGTGCCGATCTCCAACACCAAGCTGGGCTTGAGGGTTGAAA TGACGCTCGAACAGATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGA TTCGATGATTCACTGA
>OTU ID7
CTTGGTCATTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGG AAGGATCATTAAGAATTATAAATATTTGTGAAATTTACACAGCAAACAATAATTTTA TAGTCAAAACAAAAATAATCAAAACTTTTAACAATGGATCTCTTGGTTCTCGTATCG ATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCATCAG TGTTTGAACGCACATTGCACTTTGGGGTATCCCCCAAAGTATACTTGTTTGCACGTTG TTTCTCTCTTGGAATTT

Table 3. Contd.

>OTU ID8
CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGG
AAGGATCATTATTGAAATATCTTACACACTGGATACGATATCTATACAAAATAAATC
TGAATTAACTTCAACTTTACTAAAATTCAAAACTTTCAACAACGGATCTCTTGGTTCT
CGCATCGATGACGCACGCAGCGAATTGCGATACGTAGTATGACTTGCAGACGTGAA
TCATCGAATCTTTGAACGCACATTGCGCTTCGAGGTATTCCTCGCGGCATGCCTGTTT
GAGCTTCGGCCCCCC

Table 4. BLAST analysis result of predominant OUT sequences from NCBI database.

OUT sequence ID number	Topmost hit of fungi with closest related sequence	Accession number	Percentage identity	E-value
OUT ID1	*S. occidentalis*	KY105385.1	78%	2.00E-42
OUT ID2	*S. vini*	HG939425.1	99%	3.00E-150
OUT ID3	*Pichia* sp.	KP223719.1	94%	9.00E-96
OUT ID4	*P. manshurica*	KY962538.1	100%	1.00E-154
OUT ID5	*I. hanoiensis*	FJ153177.1	99%	8.00E-151
OUT ID6	*G. bulbilium*	KF513258.1	100%	4.00E-154
OUT ID7	*G. candidum*	KY103456.1	99%	2.00E-147
OUT ID8	*C. asparagi*	Ky101945.1	91%	2.00E-111

Table 5. Relative abundance of predominant (mean ≥ 1%) fungal species associated with *Irvingia* fruit wastes at different days after harvest.

Fungal species	DAH = 0	DAH = 3	DAH = 6	Mean
I. hanoiensis	42.53	39.17	62.33	48.01
P. manshurica	45.88	28.59	1.06	25.18
Candida sp.	4.68	24.87	-	9.85
G. bulbilium	1.32	0.62	19.63	7.19
Pichia sp.	2.04	-	7.96	3.33
S. vini	0.01	-	3.45	1.15
S. occidentalis	-	-	3.18	1.06
G. candidum	0.03	3.02	-	1.02

Key: DAH, Days after harvest of *Irvingia* fruits.

(Tables 4 and 5).

Mean relative percentage occurrences of the eight predominant fungi obtained from the fruits were *I. hanoiensis* (48.01%), *P. manshurica* (25.18%), *Candida* spp. (9.85%), *G. bulbilium* (7.19%), *Pichia* spp. (3.33%), *S. vini* (1.15%), *S. occidentalis* (1.06%) and *G. candidum* (1.02%) (Table 5). Apart from *G. bulbilium* that belongs to Hypocreales (Sordariomycetes), all of the other 7 fungal species belong to Saccharomycetales (Saccharomycetes). Saccharomycetales constitute the 'true yeasts' whose major and well known characteristic is the ability to ferment sugars resulting to the production of ethanol (Carlson, 1987; Schneiter, 2004). Whether or not these fungi ferment postharvest fruit wastes leading to the production of ethanol in this work was not investigated, but several works have shown that carbohydrate accounts for about 15% of the fresh weight of postharvest *Irvingia* fruit wastes (Etebu and Tungbulu, 2016; Etebu et al., 2016; Etebu and Oku, 2017).

Furthermore, *Irvingia* (Bush mango) fruit juice has been identified as suitable substrate for wine production (Akubor, 1996). Also, the maximal use of disposable

and/or underused plant biomass has been identified as desirable options in the future developments of integrated biorefinery systems (De Corato et al., 2018). The comparative relative abundance of Saccharomycetales in *Irvingia* fruits after harvesting could therefore be a useful suggestion indicating how these wastes could be a suitable source of 2nd generation sugar for ethanol production into a sugary biorefinery.

Conclusion

This work has shown that *Irvingia* fruits are colonized by far more different species of fungi (mainly true yeasts) during the postharvest phase. Fungal species belonging to Saccharomycetales form the bulk of fungi associated with harvested *Irvingia* fruits. This group of fungi are known in literature for a very high adaptation in ethanol production from several sugary sources through alcoholic fermentation. The relative abundance of the true yeasts associated with harvested *Irvingia* fruits is therefore a useful indication that it could be employed as a potential source of 2nd generation sugar for ethanol production into an integrated biorefinery.

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