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Metagenomic analysis of bacterial community associated with postharvest *Irvingia* species fruit wastes

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ABSTRACT

The use of culture-dependent methods in studying bacterial community has been proven to be inadequate, and often give misleading results. In this work, metagenomic analysis targeting the 16S ribosomal RNA genes was used to study the diversity and community structure of bacteria associated with postharvest *Irvingia* fruits. Results showed that Simpson's diversity indices and number of Operational Taxonomic Units were dependent on the storage period (days after harvest, DAH) of fruits. Fresh fruits assessed on the day of harvest (DAH = 0) had a Simpson's diversity index of 0.82, while fruits assessed on the 3rd and 6th DAH had diversity indices of 0.69 and 0.72, respectively. Whilst fruits assessed on the day of harvest had 64 OTUs spanning across 7 phyla, 11 Classes, 32 genera with total reads of 23,776, fruits assessed on the 3rd and 6th days after harvest had 58 OTUs spanning across 6 phyla, 9 Classes, 30 genera with 20,949 reads and 66 OTUs spanning across 4 phyla, 6 Classes, 33 genera with 30,722 reads, respectively. Results further showed that majority of the OTUs belonged to two phyla – Proteobacteria and Firmicutes, mostly represented by members of Alphaproteobacteria and Bacilli subdivisions respectively. Predominant among these OTUs were sequences belonging to *Acetobacter ghanensis* (11.94%), *A. okinawensis* (15.36%), *Lactobacillus* species (14.90%), *L. collinoides* (12.35%), *L. plantarum* (7.11%) and *L. vaccinostercus* (2.89%). The relative abundance of these microorganisms indicates that fleshy pericarp of *Irvingia* fruits hitherto treated as wastes could potentially be used as a substrate in food and pharmaceutical industries.

Keywords: Metagenomics, operational taxonomic units, 16S rRNA, *Irvingia*, bacteria.

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INTRODUCTION

Irvingia species (Aubry-Lecomte ex O'Rorke) is a highly, economically important tree that grows in the wild of most tropical forests in West and Central Africa (Harris, 1996; Lowe et al., 2000). It is the most preferred tree of all non-timber forest products for domestication (Ndoye et al., 1997; Agbogidi and Okonta, 2003; Leakey et al., 2003) in West Africa owing to its food and commercial value (Ladipo et al., 1996).

Irvingia species bear mango-like fruits (Matos et al., 2009; Ngondi et al., 2005) of 4 to 7 cm long, green when unripe and yellow when ripe with a fleshy pericarp (Etebu, 2013). Although the fleshy pericarp of the fruits have appreciable beneficial potentials, (Ayuk et al. 1999), locals who harvest the fruits from the wild are more interested in the kernels which are used as thickening condiments in sauce preparation (Matos et al., 2009); the fruits are usually split with a machete to extract the kernels of the seed. Although the locals eat the fleshy juicy pericarp, especially whilst processing the fruit for its kernel, most of the spilt fruits are thrown away and left to rot.

Several recent studies have shown the enormous

nutritional and potential health benefits of this part of the fruit (Etebu, 2012, 2013; Etebu and Tungbulu 2015; Etebu et al., 2016; Tungbulu et al., 2016; Etebu and Oku, 2017). In view of these apparent benefits at the time, Etebu and Tungbulu (2015) studied the bacterial community associated with postharvest *Irvingia* fruit wastes with the goal of assessing the potential role of bacteria in the postharvest quality of the fruits; bacteria being very important determinant of postharvest decay of fruits, especially juicy and succulent fruits. However, their work was centered on culturing bacteria on synthetic media prior to DNA extraction and sequencing. Although conventional sequencing began with a culture of identical cells as a source of DNA, early metagenomics studies have shown that numerous groups of bacteria (≥99%) cannot be cultured and thus cannot be sequenced (Hugenholz et al., 1998; Torsvik et al., 1990). It therefore follows that Etebu and Tungbulu (2015) would have missed out on a vast majority of bacteria associated with decaying postharvest *Irvingia* fruit.

Contemporary metagenomics which could be defined as the application of modern genomics techniques to the study of microbial communities in their natural environments, without recourse to culture of individual species, has greatly enhanced the study of microbial communities. Bacterial metagenomics studies have often targeted the 16S ribosomal RNA gene sequences which are relatively short DNA sequences of prokaryotes, often conserved within a species, and generally different between species (Lane et al., 1985; Coughlan et al., 2015).

Hence in this work, metagenomic analyses targeting the 16S ribosomal RNA gene sequences of bacterial DNA were carried out to study the bacterial community of *Irvingia* fruit wastes at different days after harvest. 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

Sample collection and preparation

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° 7′ 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. From the lot of selected fruits, a total of 900 fresh and green fruits were randomly selected and split with a machete to extract the kernel, and the pericarp which is usually considered waste by locals were thereafter separated into three replicates (300 fruits per replicate). A quadrant measuring about 3 m × 1 m having three equal compartments (representing three replicates) of 1 m × 1 m was constructed and the fruit wastes from each replicate were separately spread into the three compartments of the quadrant. The quadrant was barricaded at the sides with nets to exclude reptiles and the fruits were left to decay for 6 days after harvest (DAH). About 20 *Irvingia* fruits were randomly selected at the 0^{th}, 3^{rd} and 6^{th} days after harvest and surface disinfected in 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 30secs under aseptic conditions and frozen at -20°C until it was needed for metagenomic analyses.

DNA extraction and metagenomic analysis

Genomic DNA from the *Irvingia* samples at the 0^{th} 3^{rd} and 6^{th} days after harvest as described above were separately extracted and purified using ZR Fungal/Bacterial DNA MiniPrep™50 Preps. Model D6005 (Zymo Research, California, USA) according to the Manufacturer's protocol. Briefly, 0.5g of *Irvingia* fruit slurry was suspended in 200µl of deionized sterile water and transferred into a ZR BashingBead™ Lysis Tube. Exactly 750µl Lysis solution was added and microbial cells were lysed by bead beating for 5 min at maximum speed prior to centrifugation at 10,000 x g for 1 min (Zymo Research, California, USA). 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 x g for 1 minute. The filtrate was then mixed with 1.2ml of DNA binding buffer and 800µl of the mixture was thereafter transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10,000 x g for 1 min. The flow through was discarded from the collection tube and the process was repeated. DNA was thereafter washed with 200 µl DNA pre-wash buffer and centrifuge at 10,000 x g for another 1 min. The DNA was finally washed with 500µl of DNA Wash Buffer into the Zymo-Spin™ IIC column and centrifuged at 10,000 x g for 1 min and eluted with 100µl of DNA Elution Buffer into a clean and sterile 1.5 ml Eppendorf tube by centrifugation at 10,000 x g for 30 s.

The DNA samples were thereafter sent to Inqaba Biotechnology Pretoria South Africa for Polymerase Chain Reaction (PCR) targeting the 16S rRNA partial gene sequence through the use of PCR using primers 341F (5'-CCT ACG GGN GGC WGC AG-3') and 785R (5'-GAC TAC HVG GGT ATC TAA TCC-3') respectively (Klindworth et al., 2013). 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.

RESULTS AND DISCUSSION

Postharvest *Irvingia* fruits are colonized by bacteria (Figures 1 and 2). Several workers have shown that fruits harbor a great deal of bacteria (Leff and Fierer, 2013). Universal primers used in this work to amplify partial 16S ribosomal RNA gene sequences showed a comparatively greater affinity for bacterial DNA sequences than those of plant, animal, fungi, protozoa and viruses (Figure 1). In particular, 84.95% of total DNA sequences across all postharvest *Irvingia* fruits amplified using the primers 341-F and 785-R (Klindworth et al., 2013) belonged to bacteria. Whilst 1.73% of sequences cumulatively belonged to plants, fungi, animals, protozoa, and viruses. 13.32% of sequences belonged to organisms that could not be assigned into any known group of organisms

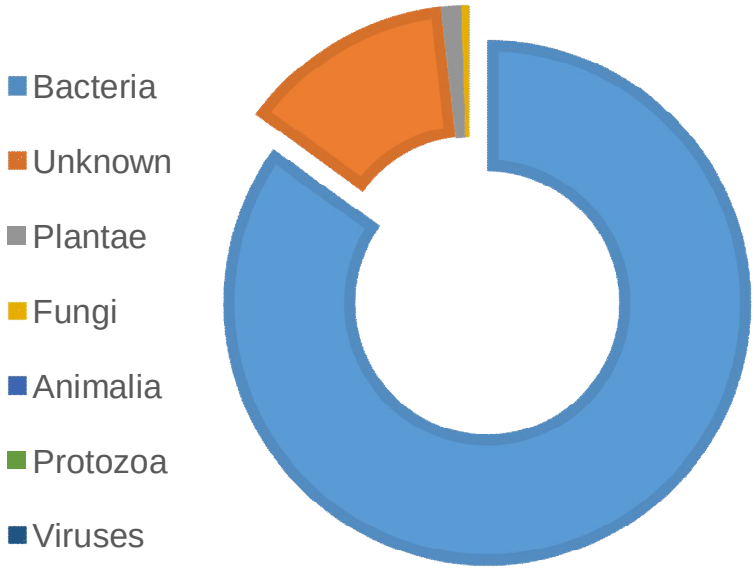

Figure 1. Cumulative proportion of organismal DNA obtained from postharvest *Irvingia* fruit wastes using Primers 27-F (5'-AGA GTT TGA TYM TGG CTC AG-3') and 1492-R (5'-TAC CTT GTT AYG ACT T-3').

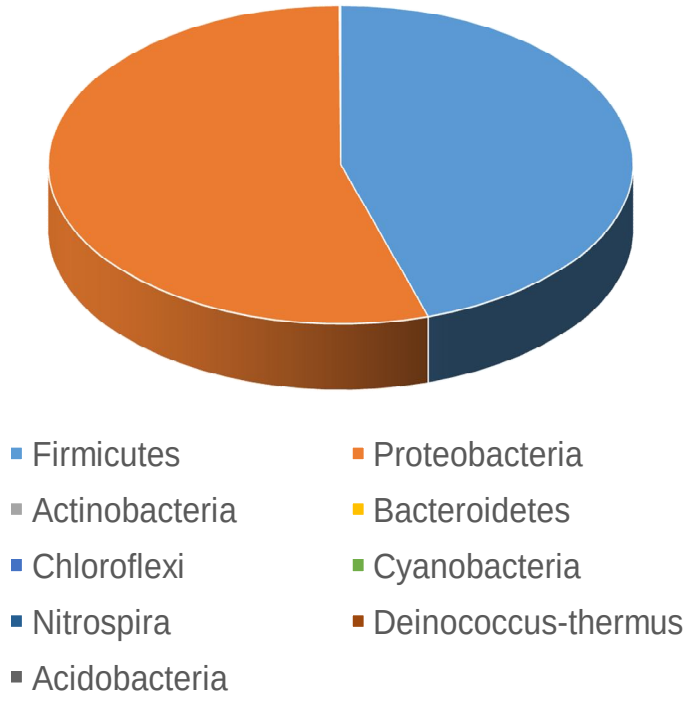

Figure 2. Cumulative proportion of partial 16S Ribosomal RNA gene sequences obtained from different bacterial phyla associated with postharvest *Irvingia* fruit wastes (total number of bacterial sequence reads = 75,447).

(Figure 1). The Primers 341F (5'-CCT ACG GGN GGC WGC AG-3') and 785R (5'-GAC TAC HVG GGT ATC TAA TCC-3') have been used by several workers to amplify bacterial 16S ribosomal RNA genes (Thijs et al. 2017). Findings from this work attest to the suitability of these primers in the metagenomic study of the bacterial community of postharvest *Irvingia* fruits without recourse to culture.

Results from this work showed that the bacterial diversity and taxonomic structure were dependent on the storage period measured herein as 'days after harvest' (Table 1). Fresh fruits assessed on the day of harvest (DAH = 0) had a Simpson's diversity index of 0.82, while fruits assessed on the 3rd and 6th DAH had diversity indices of 0.69 and 0.72, respectively. *Irvingia* fruits assessed on the day they were harvested had a total of 23,776 bacterial sequences belonging to 64 OTUs representing bacterial species in 32 genera, 11 Classes and 7 phyla. Meanwhile fruits assessed on the 3rd day after harvest had a total of 20,949 bacterial sequences belonging to 58 OTUs (Species) in 30 genera belonging to 9 Classes and 6 phyla. The number of bacterial sequence reads increased to 30,722 belonging to 66 OTUs (Species) spanning across 30 genera in 6 Classes and 4 phyla. A relatively recent work that combined both culture dependent and molecular assays showed that bacteria was not isolated from fermenting *Irvingia* fruit wastes after 6 days of harvest (Etebu and Tungbulu, 2015). Although findings from this work showed that bacterial DNA sequences were associated with *Irvingia* fruit wastes on the 6th day after harvest, whether or not they belonged to live bacteria could not be ascertained because Reverse Transcriptase PCR was not performed since RNA gene sequences were not targeted in this work. This notwithstanding, findings clearly showed

Table 1. Effect of postharvest period on the bacterial structure of postharvest *Irvingia* fruit wastes.

Parameter	DAH = 0	DAH = 3	DAH = 6
Number of bacterial phyla	7	6	4
Number of bacterial Classes	11	9	6
Number of bacterial genera	32	30	33
Number of operational taxonomic units (species)	64	58	66
Total number of reads (bacterial counts)	23776	20949	30722
Simpson's diversity index	0.82	0.69	0.72

DAH = Days after harvest.

that *Irvingia* fruit wastes allow a limited group of bacteria to thrive as storage period increased.

A cumulative total of 75,447 bacterial sequence reads representing total bacterial counts were obtained from *Irvingia* fruits across all fruits at the various storage period (days after harvest). On the overall, majority of the bacterial species obtained from the fruits belong to two phyla – Proteobacteria and Firmicutes. Bacterial species belonging to the phylum Proteobacteria alone had a cumulative read counts of 41,304 representing 54.75% and those belonging to the phylum firmicutes had a cumulative read counts of 34,082 representing 45.17%. The remaining 61 species representing 0.08% comprised bacterial species belonging to Actinobacteria, Bacteriodetes, Chloroflexi, Cyanobacteria, Nitrospira, Deinococcus-thermus and Acidobacteria (Figure 2).

In the past bacterial community studies had often focused on fresh foods fruits and vegetables (King et al., 1991; Badosa et al., 2008; Oliveira et al., 2010; Nguyen-the and Carlin, 1994). In this work, the bacterial diversity of fresh *Irvingia* fruits were assessed and studied with time (days after harvest). Expectedly, findings from this work showed that the number of bacterial phyla obtained from *Irvingia* fruits were dependent on the postharvest period (days after harvest) (Table 1). Whilst bacterial sequences obtained from fresh fruits (DAH = 0) spanned across 7 bacterial phyla, the number of bacterial taxonomic groups on the fruits on the 3rd and 6th days after harvest dropped to 6 and 4 phyla respectively (Tables 1 and 2). In particular, bacterial sequences belonging to Firmicutes, Proteobacteria, Actinobacteria and Bacteriodetes were obtained from all *Irvingia* fruits irrespective of the postharvest period. In addition to these bacterial phyla, fresh *Irvingia* fruits (DAH = 0) had chloroflexi, Cyanobacteria and Acidobacteria. Fruits assessed at the 3rd day after harvest had two more phyla (Nitrospirae and Deinococcus-Thermus) in addition to the aforementioned common 4 phyla (Table 2). Previous works have shown that brownish black rot disease, proximate and phytochemical contents of *Irvingia* fruits are influenced by the number of days after harvest (storage periods) (Etebu and Oku, 2017). This relative differential occurrence of different bacteria phyla on the fruits assessed on different days after harvest could be as a result of differences in nutrients availability.

Although proteobacteria and firmicutes dominated the bacterial phyla obtained from the fruits, the relative occurrence of these groups of bacteria was observed to be dependent on the number of days after harvest (storage period) (Table 2). Results showed that 50.89% of bacteria obtained from fresh *Irvingia* fruits on the day of harvest were firmicutes. However, the relative proportion of this group of bacteria decreased as the days after harvest increased. In particular, the relative proportions of firmicutes on the fruits on the 3rd and 6th day after harvest were 42.29 and 42.71% respectively (Table 2). In contrast, relative proportion of proteobacteria obtained from the fruits at DAH =0 was 49.07%, and these increased relatively to 57.65% at DAH = 3 and 57.16% at DAH = 6 (Table 2). Firmicutes are a group of gram positive bacteria which are among the most dominant group of bacteria that forms human microbiome (Flint et al., 2007). The relative dominance of this group of bacteria on fresh *Irvingia* fruits often consumed by locals in the Nigeria and elsewhere is an indication that it potentially contributes to the microbiome of locals that consume them.

Aside firmicutes and proteobacteria, it is pertinent to reiterate that Actinobacteria and Bacteriodetes were also obtained from all *Irvingia* fruits irrespective of the days after harvest. These latter groups of bacteria were, however, observed to be relatively very scanty in number. The huge relative difference in occurrence between bacteriodetes and Firmicutes is worth paying attention to. Studies have shown a link between the number of two bacterial phyla (Bacteriodetes and Firmicutes) and Inflammatory Bowel Disease (IBD). To put it more succinctly, IBD patients have be shown to have lower numbers of Bacteriodetes and Firmicutes in their guts (Lucas et al., 2017). Also, research have shown that a low Bacteriodetes:Firmicutes ratio with a relative greater abundance of Firmicutes contributes to obesity among humans (Rastmanesh, 2011; Singh et al., 2017). Different explanations have been advanced by different workers. Some are of the opinion that Firmicutes are more effective as an energy source than Bacteroidetes, as such is likely to promote more efficient absorption of calories and subsequent weight gain (Turnbaugh et al.,

Table 2. Relative percentage occurrence of bacterial phyla associated with *Irvingia* fruit wastes at different days after harvest.

Bacterial phylum	DAH = 0	DAH = 3	DAH = 6
Firmicutes	50.892	42.293	42.712
Proteobacteria	49.07	57.645	57.161
Actinobacteria	0.017	0.024	0.123
Bacteroidetes	0.004	0.029	0.003
Chloroflexi	0.008	-	-
Cyanobacteria	0.004	-	-
Deinococcus-thermus	-	0.005	-
Acidobacteria	0.004	-	-
Total number of reads	23776	20949	30722

Key: DAH = Days after harvest.

2006; Krajmalnik-Brown et al., 2012). Interestingly, intake of drinks and fruits rich in polyphenols significantly reduce body weight in obese people. This is because phenolic compounds are antimicrobial, and are known to repress Firmicutes than Bacteroidetes. Also, glycans, which are products of microbial metabolism of polyphenol, are more easily degraded by Bacteroidetes than the Firmicutes. These comparative advantages of Bacteroidetes over Firmicutes in the use and tolerance of phenolic compounds would therefore expectedly lead to a greater proliferation of Bacteroidetes than Firmicutes (Rastmanesh, 2011), which invariably would lead to a higher Bacteroidetes:Firmicutes ratio. Although, several works have shown the presence of polyphenols such as Flavonoids in *Irvingia* fruits fleshy pericarp (Etebu, 2013; Etebu et al., 2016; Tungbulu et al., 2016; Etebu and Oku, 2017), findings from this work showed that more Firmicutes were more associated with *Irvingia* fruits than Bacteriodetes irrespective of the storage period (Table 3). This indicates that polyphenol metabolism by microorganisms in the human gut may be significantly different from that obtained within plant tissues. It would be interesting to investigate the potential effect of *Irvingia* fruit consumption on the human gut microbiome.

As stated earlier, Proteobacteria accounted for 54.75% of the total number of bacteria obtained from *Irvingia* fruits across all three postharvest periods (Figure 2). This proportion translated to 41,304 read counts (sequences). Interestingly, a whopping 40,935 read counts of this number are bacterial species belonging to the Alphaproteobacteria subdivision which clearly indicates that 99.11% of the Proteobacteria were of the Class Alphaproteobacteria (Table 3).

Similar to the phylum Proteobacteria, taxonomic and systematic groupings of the 16S rRNA gene sequences belonging to the phylum Firmicutes were predominantly represented by members of the Bacilli subdivision (Table 3). Hence this study revealed that postharvest *Irvingia* fruit wastes are colonized majorly by members of two bacterial Classes; these were Alphaproteobacteria and Bacilli. Whilst 54.11% of bacterial species across all treatments of fruits belonged to Alphaproteobacteria, 45.29% were Bacilli.

It was therefore not surprising to observe a similarity between the pattern of distribution between the phyla Proteobacteria and Firmicutes on one hand and the relative occurrence of Bacilli and alphaproteobacteria Classes of bacteria on the other hand. Similar to the two dominant phyla, Bacilli and Alphaproteobacteria were observed to be dependent on the number of days after harvest (storage period). In particular, whilst bacilli represented 50.88% of total bacteria obtained from fresh *Irvingia* fruits on the day of harvest, the relative proportion of this group of bacteria on the fruits decreased to 42.28% and 42.71% on the 3rd and 6th day after harvest respectively (Table 3). In contrast, the relative proportions of alphaproteobacteria obtained from the fruits at the 0th, 3rd and 6th day after harvest were 48.22, 57.16 and 56.95%, respectively (Table 3). Whilst Bacilli subdivision is a taxonomic Class of bacteria belonging to the phylum Firmicutes (Galperin, 2013), Alphaproteobacteria belong to the phylum Proteobacteria (Madrid et al., 2001).

Remarkably, the Class Alphaproteobacteria is known to constitute the largest bacterial group currently recognized in the domain Bacteria; and being a sub group of the phylum Proteobacteria they occur in diverse shapes, forms and colours. In addition to the varied shapes that characterize the species of this Class of bacteria, they are also very diverse in terms of lifestyle, metabolic capacity and ecological significance (Ettema and Anderrson, 2008).

The relative high abundance of Alphaproteobacteria associated with postharvest *Irvingia* fruits indicates a huge potential for bioremediation of polluted environments. This assertion stems from the fact that Alphaproteobacteria, amongst other subdivisions of proteobacteria, are known to possess nitrite reductase genes that enable them play significant ecological roles as denitrifiers (Vila-Costa et al., 2014; Yu et al., 2014). One of the ways by which polluted environments are remediated through biological means is to enhance the growth of microorganisms capable of degrading

Table 3. Relative percentage occurrence of bacterial classes associated with *Irvingia* fruit wastes at different days after harvest.

Bacterial Class	DAH = 0	DAH = 3	DAH = 6	Mean
Bacilli	50.883	42.279	42.712	45.291
Alphaproteobacteria	48.221	57.158	56.949	54.109
Gammaproteobacteria	0.816	0.153	0.163	0.377
Betaproteobacteria	0.034	0.334	0.049	0.139
Actinobacteria	0.017	0.024	0.124	0.055
Clostridia	0.008	0.014	-	0.008
Chloroflexia	0.004	-	-	0.001
Sphingobacteria	0.004	0.029	-	0.011
Acidobacteria	0.004	-	-	0.001
Anaerolineae	0.004	-	-	0.001
Cytophagia	-	-	0.003	0.001
Nitrospira	-	0.005	-	0.002
Deinococci	-	0.005	-	0.002

DAH = Days after harvest.

pollutants through the addition of nutrients (Das and Chandran, 2011). *Irvingia* fruit wastes could serve as a source of nutrients that would promote the proliferation of Alphaproteobacteria capable of degrading pollutants through denitrification in ecologically disturbed environments.

Members of the Class Alphaproteobacteria obtained from postharvest *Irvingia* fruits in this work spanned across 14 genera – *Acetobacter, Komogataeibacter, Gluconobacter, Nguyenibacter, Gluconacetobacter, Acidiphilium, Asaia, Paracoccus, Swingsia, Methylobacterium, Acidosoma, Neoasaia, Ameyamaea* and *Sphingosinicella* (Table 4). Furthermore, most members of this class of bacteria were observed to belong to the genus Acetobacter; prominent among these, in terms of relative abundance, were *Acetobacter ghanensis* and *A. okinawensis* (Table 5).

The genus Acetobacter, amongst other genera, belongs to the family Acetobacteraceae. Members of this family are Gram-negative, obligate aerobes. They are generally called acetic acid bacteria (abbreviated AAB) because (with the exception of the genus Asaia) they are able to oxidize ethanol to acetic acid under neutral and acidic (pH 4.5) conditions (Cleenwerck et al., 2002; Cleenwerck et al., 2007). A recent work on pH of fresh (DAH = 0) postharvest *Irvingia* fruits has been reported to be 6.42. The pH value of fruits assessed on the 3rd and 6th days after harvest were shown to decrease to 6.31 and 6.22, respectively (Etebu and Tungbulu, 2015). These findings portend that *Irvingia* fruit extract would be a suitable substrate for growth of members of the family Acetobacteraceae.

It is instructive to note that 34.86% amounting to 8288 bacterial sequences obtained from fresh fruits (DAH = 0) were of *Acetobacter ghanensis* but this number on the fruits reduced very sharply as the days after harvest increased. It is interesting to note that *A. ghanensis* has been shown to oxidize ethanol to acetic acid (Cleenwerck et al., 2007; Kersters et al., 2006). Earlier studies show that carbohydrate content accounts for as much as 15% fresh weight of *Irvingia* fruits (Etebu and Tungbulu, 2015). It could be that ethanol was produced from the carbohydrate content, which then became a substrate for *A. ghanensis* to act upon. The apparent depletion of ethanol probably accounts for the relative drastic drop in the population of the bacterium on fruits assessed on the 3rd and 6th days after harvest. Whilst this remains to be studied, the clear relative abundance of *A. ghanensis* on fresh *Irvingia* fruits suggests that the fruits possess a substrate that the bacterium requires for growth but the drop in bacterial population in fruits assessed on the 3rd and 6th days after harvest (Table 5) suggests that the conditions of optimum growth for *A. ghanensis* were compromised in one way or the other.

In contrast to *A. ghanensis*, whilst only 0.04% of bacterial 16S rRNA gene sequences obtained from fresh *Irvingia* fruits (DAH = 0) were identified as belonging to *A. okinawensis*; the proportion of *A. okinawensis* on the fruits increased relatively on the 6th day after harvest. Specifically, 46.04% of the sequences obtained from *Irvingia* fruits on the 6th day after harvest belonged to *A. okinawensis*; indicating a drastic increase in population of this bacterium (Table 5). Apparently, the relative increase in population of *A. okinawensis* associated with the fruits after 6 days of harvest may have been occasioned by changes within the fruits that could have favored the growth of this bacterium.

The class Bacilli in this work included members that spanned across 9 genera which includes *Lactobacillus*, *Leuconostoc, Oenococcus, Enterococcus*, *Lactococcus, Bacillus, Ureibacillus, Pediococcus* and *Sporolactobacillus* (Table 4).

Table 4. Relative mean percentage occurrence of Bacterial genera of Bacillus and Alphaproteobacteria associated with *Irvingia* fruit wastes at different days after harvest.

Genus	Relative % number of reads			Mean
	DAH = 0	DAH = 3	DAH = 6	
Class: Bacillus				
Lactobacillus	31.633	42.055	41.898	38.529
Leuconostoc	14.805	-	-	4.935
Oenococcus	4.433	0.033	0.788	1.751
Enterococcus	0.004	0.191	-	0.065
Lactococcus	-	-	0.016	0.005
Bacilus	-	-	0.007	0.002
Ureibacillus	0.004	-	-	0.001
Pediococcus	0.004	-	-	0.001
Sporolactobacilus	-	-	0.003	0.001
Class: Alphaproteobacteria				
Acetobacter	38.896	2.835	46.878	29.537
Komogotoeibacter	2.885	47.554	1.416	17.285
Gluconobacter	6.212	6.124	0.007	4.114
Nguyenibacter	-	0.010	5.771	1.927
Gluconacetobacter	0.198	0.148	2.835	1.060
Acidiphilium	-	0.473	-	0.158
Asaia	0.021	0.010	0.020	0.017
Paracoccus	-	-	0.010	0.003
Swingsia	-	0.005	0.003	0.003
Methyobacterium	0.004	-	-	0.001
Acidisoma	0.004	-	-	0.001
Neoasaia	-	-	0.003	0.001
Sphingosinicella	-	-	0.003	0.001

DAH = Days after harvest.

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

Operational taxonomic unit (bacterial species)	Relative % number of reads			Mean
	DAH = 0	DAH = 3	DAH = 6	
Class: Bacilli				
Lactobacillus species	11.49	25.99	7.21	14.90
Lactobacillus collinoides	13.88	1.14	22.03	12.35
Lactobacillus plantarum	1.69	10.49	9.16	7.11
Leuconostoc species	11.02	-	-	3.67
Lactobacillus vaccinostercus	1.05	4.34	3.28	2.89
Oenococcus oeni	4.43	0.03	0.79	0.002
Leuconostoc pseudomesenteroides	3.79	-	-	1.26
Class: Alphaproteobacteria				
Komogatoeibacter kakiaceti	-	47.28	-	15.76
Acetobacter okinawensis	0.04	0.01	46.04	15.36
Acetobacter ghanensis	34.86	0.20	0.77	11.94
Gluconobacter species	6.20	6.12	0.01	4.11
Nguyenibacter vanlangensis	-	0.01	5.77	1.93
Komagatoeibacter intermedius	2.87	0.27	1.29	1.48

Table 5. Continues.

Acetobacter senegalensis	3.83	0.45	0.03	1.44
Total number of bacterial reads	23776	20949	30722	25149

DAH = Days after harvest.

Of these 9 genera, the genus *Lactobacillus* was clearly represented by a higher number of bacterial sequence reads than any other genus. Specifically, the genus *Lactobacillus* alone accounted for as much as 38.53% of the total bacterial sequence reads across all treatments (Table 4).

Lactobacillus represents a highly diverse group of Gram-positive, rod or coccobacilli shaped microaerophilic bacteria (Kandler and Weiss, 1986). Members are very prominent amongst Lactic acid bacteria (LAB); known to play significant roles in fermentation processes (Ross et al., 2002). As such, many are used in starter cultures for industrial and agricultural fermentations, and some species are usually part of the human gastrointestinal tract microbiome (Vaughan et al., 2002).

The overall mean percentage abundance of prominent *Lactobacilli* encountered in this work include, not-delineated *Lactobacillus* species (14.90%), *L. collinoides* (12.35%), *L. plantarum* (7.11%) and *L. vaccinostercus* (2.89%) (Table 5). Several members of the genus have been found to be associated with several agricultural produce and products, particularly fruits (Garrido et al., 1997; Ross et al., 2002). Some members have also been described for their probiotic effect; promotes immunomodulation, resistance against pathogens, and reduction of blood cholesterol levels of hosts (Gill and Rutherfurd, 2001; Rosenfeldt et al., 2002; Jones et al., 2004). The relative abundance of several species of this bacterial genus indicates that locals who consume raw *Irvingia* fruits as part of their diet potentially derive these health benefits, without knowing. However, it is important to note that probiotic properties have been shown to be characteristic of each strain, not the genus or even a species (Janković et al., 2012).

Conclusion

Findings from this work have revealed far more bacterial species associated with postharvest *Irvingia* fruit wastes than was previously reported. The fleshy pericarp of *Irvingia* fruits hitherto treated as wastes has the potential of being channeled into more profitable and beneficial use such as agro-food and pharmaceutical industries.

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