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RESEARCH ARTICLE

# Comparative Bacterial Metagenomics of Soursop (*Annona muricata* L.) and Apple (*Malus domestica* B.)

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## Abstract

Illumina Next Generation Sequencing (NGS) platform targeting the conserved regions of bacteria ribosomal DNA (16s rRNA) was used to identify the bacterial community associated with soursop (*Annona muricata* L.). The aim of this work is to compare the diversities of the bacterial communities of *Annona muricata* and *Malus domestica* [obtained from National Centre for Biotechnology Information (NCBI) database]. The functional genes in these communities were also predicted. A total of 167,693 high quality reads was obtained from *Annona muricata* and *Malus domestica*. Clustering on GREENGENES database revealed 570 Operational Taxonomic Units (OTUs). Alpha-diversity indices indicated high diversity and abundance of microbial community. Taxonomic analysis revealed that bacterial community was grouped into 24 phyla and 455 genera. The microbiome of the samples was dominated by distinct populations of four phyla viz Proteobacteria (58.41%), Bacteroidetes (18.59%), Actinobacteria (11.13%) and Firmicutes (7.29%). The functional genes were predicted for 16S rRNA gene sequences based on Kyoto Encyclopaedia of Genes and Genomes (KEGG) which indicated amino acid metabolism, carbohydrate metabolism, xenobiotics biodegradation and lipid metabolism, metabolism of terpenoids and polypeptides and biosynthesis of other secondary metabolites as predominant metabolic categories. Thus, the study revealed the structure of microbial community and functional genes composition in *A. muricata* and *M. domestica* fruits and this will help to expand the knowledge concerning the structure of plant-associated bacterial communities, revealing valuable information of their impact and indicating their crucial roles in evolutionary and ecological processes.

## Keywords

*Annona muricata*, bacteria, *Malus domestica*, metagenomics, Illumina next-generation sequencing

## Introduction

*Annona muricata* (L.), commonly called soursop belongs to the family Annonaceae (1). It is a multipurpose fruit with various nutritional benefits and has gained global essence because of its medicinal and food values (2). *A. muricata* is a common fruit inherent to the Caribbean and most parts of Central America which is currently being distributed and spread to several tropical areas globally and several other nations in Africa including Southern Nigeria (3). *A. muricata* is susceptible to attack by microorganisms which can lead to reduction in market quality. The International Society for Infec-

tious Disease reported that the impacts of the diseases caused by microbes on plants have increased worldwide (4).

Generally, agricultural food crops of a nation loose appreciable quality in storage due to threatened plant diseases caused by the activities of microorganisms. The interactions between different microorganisms can affect the microbial community structure and can lead to several evolutionary and ecological processes (5). It was explained that early detection of plant diseases is an important key in preventing disease spread with minimal damage to crop production (6). Hypothetically, researchers affirm that microbes living on tropical areas are diverse and defined by associated species of plant (7, 8). As researchers started appreciating and understanding diversity in microbial community, it resulted to understanding the fact that most microbes do not grow under standard culture technique and are not observed by conventional techniques (9). Metagenomics analysis using next-generation sequencing (NGS) has been shown to be an excellent instrument in studying and analyzing microbial diversity in various environments. Interestingly, the relationship between the members of the microbial community and the plant host is crucial to understanding agricultural production systems and management (10). The novel methods in molecular research in addition to bioinformatics have made genetic research for characterizing and identifying microorganisms more practical, revealing diversities which are hidden in environmental samples. Only a limited number of works on microorganisms associated with fruits have been reported and to the best of our knowledge, no reports on *Annona muricata* bacterial microbiota have been presented so far.

This research was carried out to 1) determine the bacterial organisms associated with *Annona muricata* fruits; 2) determine the bacterial community structure of the fruit; and 3) compare the bacterial community structure of *A. muricata* fruit with that of *Malus domestica* obtained from the Sequence Read Archive on National Centre for Biotechnology Information (NCBI) database. This study will contribute to the knowledge of the structure of plant-associated bacterial communities, providing valuable information of their impact.

## Materials and Methods

### *Sample Collection and DNA Extraction*

Ripe *Annona muricata* fruits were obtained from Choba market in Port Harcourt, Rivers State, Nigeria. The fruits were collected in sterile plastic-bags and transported to Regional Centre for Biotechnology and Bio-resources Research Laboratory, University of Port Harcourt under ice condition. DNA was extracted from soursop fruit using Zymo Fungal/Bacterial DNA Miniprep kit (Zymo Research Biotechnology Company, California, U.S.A.) according to the manufacturer's protocol. Half of a gramme (0.5 g) of soursop fruit was used. The fruits used in the study are presented in Fig. 1.


**Fig. 1.** Soursop (**a**) and apple (**b**) fruits

### *Bacterial DNA Amplification*

Bacterial genomic DNA was amplified with the primer pair: 341F (5′-CCT AGC GNG GCG WCG AG-3′) - forward and 785R (5′-GCAC TCA HVG GTG ATC TAA TCC-3′) – reverse (11). This primer pair targets 16S rDNA gene sequence variable regions V3 and V4. The resultant amplicon was gel-purified, end-repaired and Illumina specific adapter sequences were used to ligate each amplicon. After polymerase chain reaction (PCR) quantification, sample was specifically indexed and a second purification was carried out. The amplicon was sequenced on Illumina platform with Miseq v3 (600 cycles) kit, based on manufacturer's protocol. 20Mb data (2 x 300bp long paired end reads) was produced.

### *Data Processing and Analysis*

Raw sequences obtained were first analyzed to remove PCR artifacts and low quality reads with *ngsShort* (Next-generation sequencing Short Reads) trimmer (12). The sequences of *Annona muricata* and *Malus domestica* were normalized to an even sampling depth before diversity analysis. Sequence reads lower than 200 nucleotides, reads with more than 2% of uncertainties or 7% homopolymers were expunged from the data during analysis. The UCLUST algorithm (13) on QIIME (v.1.9.0) pipeline was utilized to cluster sequences at 97% identity threshold, grouping them into OTUs. GREENGENES database was used for open reference picking and highest sequences in each OTU based on alignment of their 16S-rRNA were chosen as representative sequences and utilized for taxonomic assignment. Alpha-diversity analysis (ADA) was done using *vegan* R package (14). ADA indices like Chao1 and Shannon were measured. Sequence reads for *Annona muricata* sample were deposited on National Centre for Biotechnology Information (NCBI) Database under Sequence Read Archive (SRA) Bio-project number PRJNA755909. To compare bacteria metagenomes of *Annona muricata* and *Malus domestica* fruits, four 16S-rRNA meta-sequences of *M. domestica* fruit were obtained from the European Nucleotide Archive (ENA) at the study Bio-project number: PRJEB32455 (run accession numbers - ERR3347698, ERR3347699, ERR3347700 and ERR3347701). The sequences were recovered from a study (15). Analysis of functional group was carried out with PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) software (16). PICRUSt and KEGG were employed to ascertain the relative abundance of the different bacterial genes obtained from the fruits and the functions of those genes.

## Results

### *Composition of Annona muricata and Malus domestica Bacterial Communities*

The clustering of the sequences from *Annona muricata* and *Malus domestica* resulted in 570 OTUs with a total sequence count of 183958. After data processing, a total of 167693 quality reads were generated. The number of OTUs ranged from 346 to 1337 across the samples. The highest number of OTUs was identified in *A. muricata* fruits (1337) whereas the least was observed in *M. domestica* (sample FruH9: 346). The total OTUs observed in *A. muricata* and *M. domestica* were taxonomically assigned into 24 phyla, 76 classes, 133 orders, 248 families and 455 genera.

The diversity of the bacterial community compositions of *A. muricata* and *M. domestica* fruits were explored by comparing the relative abundance at different taxonomic levels, and the alpha diversity indices such as Chao1 and Shannon. The Shannon diversity and Chao1 estimates equally revealed that soursop had higher bacterial diversity compared to apple (Fig. 2, 3).

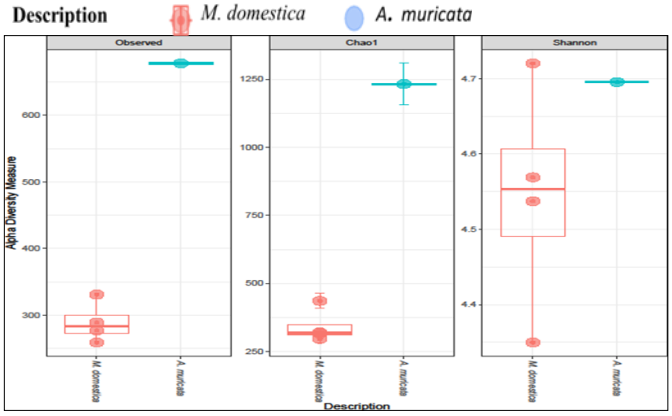


**Fig. 2.** Alpha diversity analysis of the bacterial population when grouped.

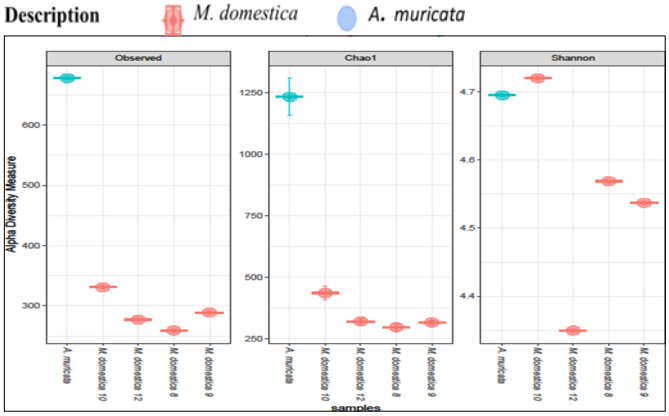


**Fig. 3.** Alpha diversity analysis of the bacterial population for each sample.

### *Distribution of Bacteria Phyla in Annona muricata and Malus domestica fruits*

The dominant phyla across the entire bacterial population were: Proteobacteria (58.41%), Bacteroidetes (18.59%), Actinobacteria (11.13%) and Firmicutes (7.29%) (Fig. 4). Planctomycetes was also detected at a low frequency (0.59%) with other phyla representing less than 1% of the community population.

The most dominant phyla in *Annona muricata* were Actinobacteria (31.64%), Proteobacteria (24.53%), Bacteroidetes (20.73%) and Firmicutes (14.87%). In *Malus domestica*, Proteobacteria (66.88%) was the most prevalent phylum, followed by Bacteroidetes (18.05%), Actinobacteria (6.00%) and Firmicutes (5.39%). The phyla, Acidobacteria

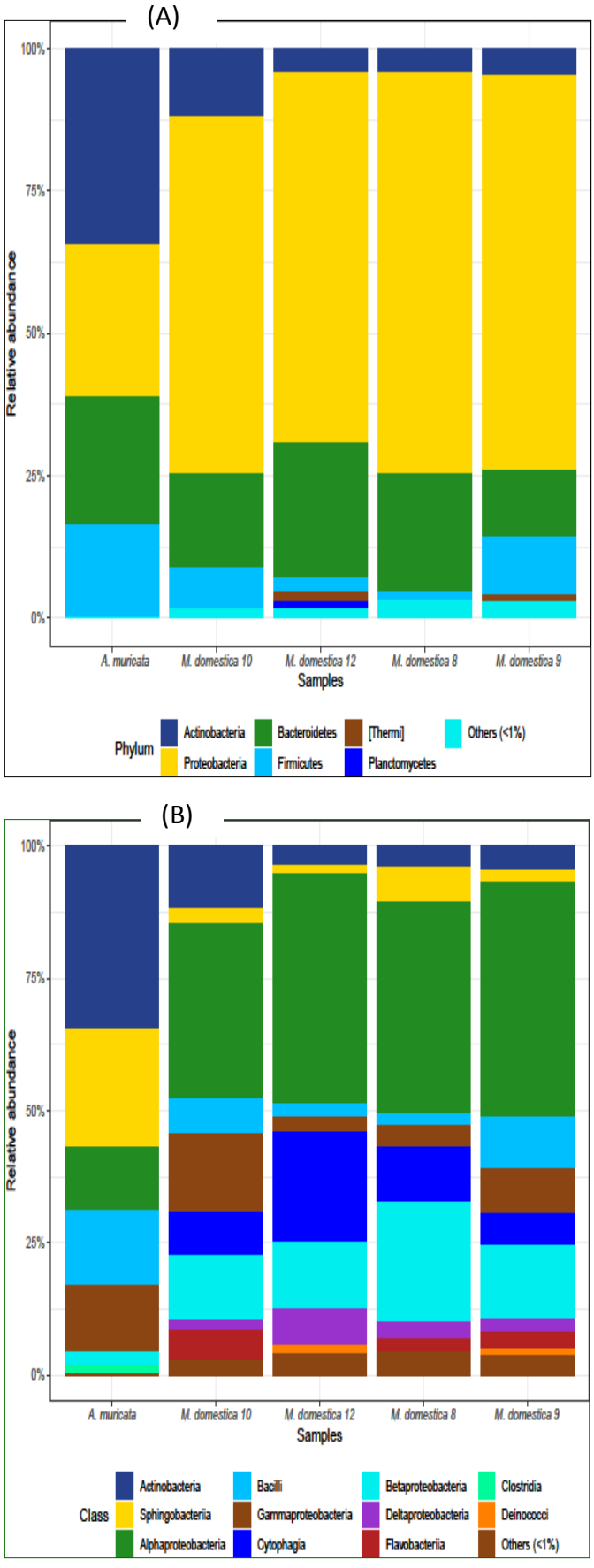


**Fig. 4.** Dominant bacteria phyla (**A**) and classes (**B**) across the bacterial communities for *Annona muricata* and *Malus domestica* fruits.

(0.50%), Armatimonadetes (0.05%), Chlamydiae (0.02%), Fusobacteria (0.06%), Nitrospirae (0.12%), Planctomycetes (0.74%) Spirochaetes (0.12%) and Verrucomicrobia (0.06%) were only present in *M. domestica* fruits.

The most abundant classes across the two fruits were: Alphaproteobacteria (85.89%), Betaproteobacteria (32.24%), Actinobacteria (27.58%) Cytophagia (22.34%), Gammaproteobacteria (21.19%), Bacilli (16.52%), Sphingobacteriia (16.60%), Deltaproteobacteria (6.70%) and Flavobacteria (6.47%) (Fig. 4). In *M. domestica* fruits, Alphaproteobacteria (40.29%), Betaproteobacteria (15.53%), Cytophagia (11.17%), Gammaproteobacteria (7.71%), Actinobacteria (5.89%), Bacilli (4.93%), Deltaproteobacteria (3.35%), Flavobacteria (3.23%) and Sphingobacteriia (3.12%) were the dominant classes while in *A. muricata*, Actinobacteria (31.61%), Sphingobacteriia (20.72%), Bacilli (13.32%), Gammaproteobacteria (11.56%), Alphaproteobacteria (10.62%) and Betaproteobacteria (2.34%) were the predominant classes.

### *Distribution of Bacterial Genera in Annona muricata and Malus domestica Fruits*

The microbial diversity of *Annona muricata* and *Malus domestica* fruits consisted of 455 distinct bacterial genera. The predominant genera across the samples were *Methylobacterium* (12.13%), *Sphingomonas* (8.83%), *Hymenobacter* (7.27%), *Sphinogobacterium* (4.23%), *Pseudomonas* (3.06%), *Ralstonia* (2.98%), *Bacillus* (2.27%), *Flavobacterium* (2.22%), *Brachybacterium* (1.79%), *Erwinia* and (1.52%) (Fig. 5). *Bdellovibrio, Burkholderia, Flavobacterium* and *Pseudomonas* were only observed on *Malus domestica* fruits while *Saccharopolyspora* and *Ochrobactrum* occurred only on *Annona muricata* fruits.

The abundance of *Methylobacterium* which was the predominat genera on *M. domestica* was 15.15%. Other genera present in *M. domestica* fruit include: *Sphingomonas* (11.03%), *Hymenobacter* (9.08%), *Pseudomonas* (3.80%), *Ralstonia* (3.73%), *Flavobacterium* (2.78%), *Bacillus* (2.13%), *Burkholderia* (1.89%) and *Spirosoma* (1.61%). In *A. muricata*, *Sphinogobacterium* (20.72%), *Brachybacterium* (8.94%), *Erwinia* (7.57%), *Brevibacterium* (5.90%), *Paenibacillus* (3.51%), *Staphylococcus* (3.36%), *Ochrobactrum* (3.13%), *Bacillus* (2.80%) and *Saccharopolyspora* (2.12%) were the predominant genera (Fig. 5).

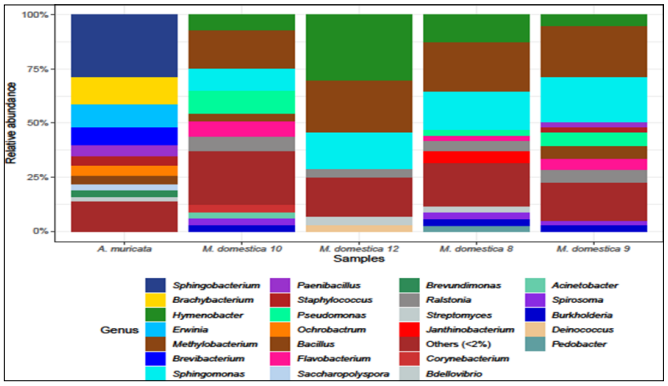


**Fig. 5.** Dominant bacteria genera across the bacterial microbial communities of *Annona muricata* and *Malus domestica* fruits.

### *Functional Prediction of Bacterial Communities for Annona muricata and Malus domestica*

A total of 301 KEGG Orthology (KO) categories were obtained after assigning sequences using KEGG gene database. From these categories, 146 metabolic groups and 12 sub-systems were obtained. The metabolic groups include amino acid metabolism, energy metabolism, metabolism of co-factors and vitamins, lipid metabolism, enzymes families, biosynthesis of other secondary metabolites, metabolism of terpenoids and polyketides, metabolism of other amino acids, xenobiotics biodegradation and metabolism, nucleotide metabolism and carbohydrate metabolic pathways among others (Fig. 6). Lipid metabolism, xenobiotics biodegradation and metabolism, biosynthesis of other

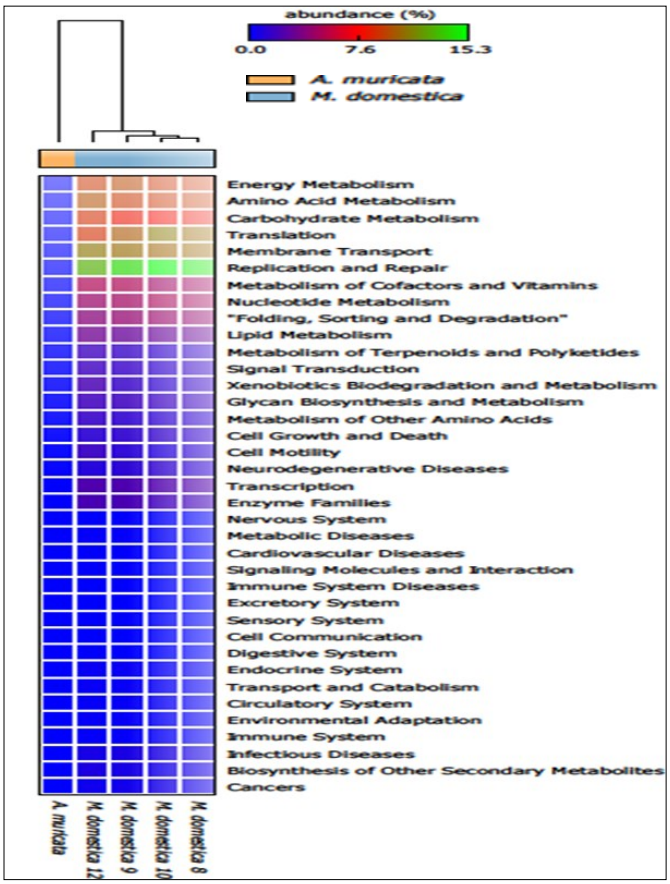


**Fig. 6.** KEGG heat map showing abundances of functional categories.

secondary metabolites, metabolism of terpenoids and polyketides, carbohydrate metabolism and amino acid metabolism were predominant metabolic categories. The genes involved in biosynthesis of streptomycin, novobiocin, isoquinoline alkaloid and phenylpropanoid were the most abundant genes under the biosynthesis of secondary metabolites category (Table 1). These genes were only obtained from *M. domestica* fruits.

## Discussion

### *Bacterial Organisms Associated with Annona muricata and Malus domestica*

Fruits are important components for healthy diet with sufficient amount of nutrients such as minerals, vitamins, protein and water. According to one report, from harvesting to consumption, fruits are highly prone to attack by

**Table 1.** Genes associated with biosynthesis of secondary metabolites in *Annona*

| KEGG Ortholog number | KEGG Pathway | No of genes present | | | | |
|---|---|---|---|---|---|---|
| | | Apple | | | | Soursop |
| | | Fru8 | Fru10 | Fru12 | Fru9 | A11 |
| KO 00940 | Phenylpropanoid biosynthesis | 11693 | 12996 | 12474 | 10575 | 0 |
| KO 00401 | Novobiocin biosynthesis | 38494 | 47875 | 25365 | 24240 | 0 |
| KO 00965 | Betalain biosynthesis | 288 | 325 | 401 | 111 | 0 |
| KO 00944 | Flavone and flavonol biosynthesis | 161 | 159 | 69 | 68 | 0 |
| KO 00941 | Flavonoid biosynthesis | 6084 | 5998 | 7283 | 6666 | 0 |
| KO 00950 | Isoquinoline alkaloid biosynthesis | 31839 | 41627 | 18529 | 18641 | 0 |
| KO 00521 | Streptomycin biosynthesis | 59849 | 70210 | 49469 | 45732 | 0 |
| KO00901 | Indole alkaloid biosynthesis | 227 | 146 | 348 | 54 | 0 |
| KO 00550 | Beta-Lactam resistance | 4936 | 4321 | 4973 | 3245 | 0 |
| KO 00941 | Isoflavonoid biosynthesis | 24 | 5 | 49 | 10 | 0 |
| KO 00311 | Penicillin and cephalosporin biosynthesis | 6414 | 5850 | 6500 | 3707 | 0 |
| KO 00524 | Butirosin, Kanamycin, Gentamicin and neomycin biosynthesis | 5350 | 5626 | 5154 | 4924 | 0 |

several pathogens due to their notable moisture content and this leads to massive economic losses (17). Metagenomic analysis of genomic DNA purified from *A. muricata* and *M. domestica* showed that several bacterial species are associated with the fruits, and this leads to deterioration, rotting or discoloration which may affect their preservation leading to post-harvest fruit losses. Majority of the bacterial organisms obtained from this study revealed that the microbiome of *A. muricata* and *M. domestica* was dominated by four phyla: Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. The number of OTUs represents the abundance of species within each sample (18).

The bacterial community of *A. muricata* was dominated by the genus *Sphingobacterium*. Members of the genus are opportunistic pathogens and contain high amount of sphingophospholipids (lipid components). They are known to possess several antimicrobial susceptibility patterns (19). The bacterial community of *M. domestica* which was dominated by *Methylobacterium* was more diverse when compared to that of *A. muricata*. The genus *Methylobacterium* belongs to the family Methylobacteriaceae and class Alphaproteobacteria. This is a new genus of facultative bacteria that can utilize one-carbon compounds (such as methane or methanol) as the source of carbon for growth (20). *Methylobacterium* species may be related to phytohormone production or interaction with plant pathogens, promoting plant growth and inducing higher photosynthetic activity (21). Some *Methylobacterium* strains have been reported to synthesize cellulose and pectinase suggesting that they are capable of stimulating systemic resistance during plant colonization by pathogens (21). Hence, these species can play an essential role in plant development.

The genus *Hymenobacter* accommodates species that are aerobic and non-motile. The species grow under conditions of extremely low organic substances using limited carbon sources like isoprene, sugars, amino-acids, organic-acids and alcohols (22). *Flavobacterium* spp. are widely distributed in nature. They have been isolated from different habitats such as diseased fish, fresh water, soil and from several cash crops like red-pepper, rice and soybean (23, 24). The isolation of a novel species, *Flavobacterium glycines* from the rhizosphere of *Glycine max* (soyabean) has been reported (24). Consistently, Flavobacteria were also found to be associated with food and products spoilage, and was confirmed that growth of psychrophilic or psychotropic bacteria on products depends on relative humidity of storage environment where such products are kept (25).

*Spirosoma* sp. have been discovered to exist in dust, fresh water, soil and extreme environments such as high Arctic glaciers. The genus contains strains with high activity of glycosyl hydrolases, including L-fucosidase (GH29), xylan-1,4-xylosidase (GH39) and cellulase or hemicellulases, which degrade polysaccharides of plant origin. *Spirosoma* also comprises of strains with high radiation resistance (26).

The genus *Staphylococcus* which belongs to the family Staphylococcaceae and class Bacilli dominated the phyla Firmicutes. The genus consists of gram-positive opportunistic pathogens capable of increasing in aerobic and anaerobic conditions. Firmicutes intake results in obesity among humans (27) and could equally cause infections via toxin production, coagulase enzyme or penetration. These toxins are capable of causing poisoning which can result to life threatening infections, invasive surgery and injuries creating severe clinical issues (28). *Staphylococcus aureus* is of great relevance in the food industry due to its production of enterotoxins, a major cause of food-borne intoxications (29).

It was reported that the genus *Sphingomonas* comprises of over 103 Gram-negative bacteria species that are basically aerobic and contain ubiquinone-10 as their main respiratory quinone (30). They also contain glycosphingolipids (GSLs) and not lipopolysaccharide; and produce

pigmented colonies that are yellow in colour. By 2001, over 20 species with diverse physiological, ecological and phylogenetic properties were added to the genus. Although *Sphingomonas* spp. have been isolated from different environments and the physiological and metabolic properties of selected strains have been examined in detail, relatively little is known regarding the ecology of this diverse group of microorganisms. However, 2 aspects of their metabolism probably contribute to their widespread distribution in the environment: their ability to utilize several organic compounds and their ability to grow and withstand low-nutrient conditions or starvation. Sphingomonads play an essential role in plant tolerance to abiotic stress, biodegradation and bioremediation processes (31).

The genus *Ralstonia* is an aerobic Gram-negative rod-shaped bacterium mainly isolated from plants, soil and contaminated water. These pathogens can cause meningitis, bloodstream infection, endocarditis, pneumonia, spinal osteitis, peritonitis, prostatitis, septic arthritis and osteomyelitis (32). The genus *Burkholderia* was described to include most rRNA group-II pseudomonads which comprises of seven species (33). Two among these species, *Burkholderia mallei* and *Burkholderia pseudomallei* are mainly humans and animals' pathogens. Two species, *Burkholderia gladioli* and *Burkholderia caryophylli* are phytopathogens, 2 other species *Burkholderia pickettii* (a human pathogen) and *Burkholderia solanacearum* (a phytopathogen) were placed under the genus, *Ralstonia* and the last species, *Burkholderia cepacia* was described as the causal agent of bacterial rot of onion bulbs. The study revealed that *Burkholderia* species are diverse and maybe found as free-living species within soil or water, or in association with other hosts, including plants, fungi, animals and humans (34). The bio-geographic distribution of *Burkholderia* spp. is strongly affected by soil pH.

### *Functional Analysis of Annona muricata and Malus domestica Bacterial Communities*

Metabolic profiles and functional ability of microbes from the different samples based on the results of KEGG were predicted. Their results showed that there was significance between phylogenetic calculations and functional profiles of the bacterial genes indicating that the functions of microbiome genes can be predicted from bacterial 16S rRNA sequences.

Research has shifted to the study of compounds in foods to obtain a better understanding of their specialized roles and the mechanisms associated with the prevention and reduction of human diseases. Metagenomic functional analysis of *M. domestica* fruit reveals the abundance of streptomycin, novobiocin, isoquinoline alkaloid and phenylpropanoid biosynthetic genes. A lot of studies have been done on dietary polyphenols, especially the flavonoids (which comprises of 60% of all polyphenols) and phenolic acids (which represents 30% of total polyphenols) (35). Polyphenolic compounds are responsible for the taste, colour, flavours and metabolic activity of foods obtained from plants. The quantity of polyphenols is affected by plant variety and environmental factors such as season, storage and geographical region. *Annona* plant is a source of sweet and fragrant flavor. The fruit is consumed directly or in the form of processed products such as juices, ice cream and alcoholic beverages (36). Apart from this, it is a potential source of bioactive chemicals and nutrients that improve human health. *Annona* fruit contains several natural and beneficial compounds and some phytochemicals that are considered very useful because of their potential health benefits to man. The most important bioactive substances present in the fruit are phenolic compounds, cetogenins (ACGs), essential oils (EOs), alkaloids (ALKs), anthocyanins, cyclopeptides (CPs), minerals, carotenoids, vitamins and amino acids (37).

Agricultural produce that are under various environmental stress that can have deleterious impact on the quality of food, developed several biochemical and physiological mechanisms to adjust and adapt to this stress. Several physiological metabolic reactions are produced in these plants to contain these changes. Thus, functional amino acids metabolism in the fruits shows notable regulatory and metabolic adaptability (38). They are considered as vital precursors for the synthesis of various molecules of great importance, and equally control some major metabolic pathways that are essential to growth, health, development, homeostasis of organisms and reproduction.

Protein and carbohydrate metabolisms are very important determinant factors in response of fruits to stress. It was suggested that ascorbic acid (ASA) metabolism, proline metabolism and other pathways play vital roles during salt stress response (39, 40). Amino acids and carbohydrates are the chief players in various regulatory and metabolic pathways and are responsible for biological cell adaptation. Therefore, amino acid and carbohydrate metabolisms are very important for salt stress response. Also, it has been observed that amino acid metabolism is closely related to abiotic stress tolerance and carbohydrate metabolism holds a vital function in abiotic stress tolerance (41).

Isoflavones have received notable attention due to their health benefits to man. They are believed to be produced only in legumes and are important determinant factors in defense and root nodulation in plants. Isoflavones are involved in symbiotic association with rhizoidal bacteria and defense response in leguminous plants (42). Specialized plant terpenoids are used in medicine because of their biochemical selection for biological activity in animals. Terpenoid families in natural plants are important source of medical discoveries. Isoprenoids and terpenoids are isoprene-based natural products with basic roles in the metabolic process of all organisms. Terpenoid chemical diversity is particularly high in plants where several of them are considered as secondary metabolites (43). Such non-vital, specialized plant terpenoids stimulate several ecological connections between animals and plants (44), acting as allelochemicals to entice pollinators, deter herbivores or attract predators (45).

Xenobiotic compounds are substances which are

foreign to the biosphere. Depending on their occurrence in soil, air, sediment or water, xenobiotic pollutants may become available to microorganisms in different environmental compartments. In fact, the means of degradation and transformation of xenobiotic compounds on earth depends on microorganisms (46). Conversely, microorganisms can utilize xenobiotic compounds as their source of energy, sulfur, nitrogen or carbon. Xenobiotic compounds are naturally persistent however, their persistence is mainly because they are not easily detected by organisms and hence, do not follow common metabolic pathways. They are extremely toxic in nature and can affect both higher and lower eukaryotes. The major challenge in the use of xenobiotics is their toxic nature which is a serious threat to human health. In humans, they interfere with several cellular communication pathways that play vital roles in growth and development (47).

Interestingly, genomics opens molecular channel to the study of metabolic processes in plants which would help in understanding gene coding for some major metabolic enzymes as well as the evolutionary significance of their pathways. This will make way for exploration of several plants for bio-technological advancement, noting that natural products have long been used by humans owing to their beneficial effects. The functional analysis done in this study suggests that the species characterized possess several enzymes that can degrade varied range of carbon for energy. Some of these species possess ability to control aerobic heterotrophic microbes in different land and water environments. Importantly, in the future in order to understand what really happens during post-harvest storage (spoilage), we hope to explore the metabolites of each species at a given time, the part played by chemical reactions and microbes in food quality formation by metabolomics.

## Conclusion

Naturally, microorganisms which are commonly introduced through water, wind, soil, humans and animals are present on every foodstuff, with the ability to cause food borne infections. The findings from this work have revealed bacterial organisms associated with *Annona muricata* and *Malus domestica* some of which are pathogenic and may lead to changes in the durability or nutrient content of the fruits. Pathogenic microorganisms contaminate fruits thereby affecting their market value and promoting health risks on consumers and handlers of these fruits. However, this study has revealed that *A. muricata* and *M. domestica* fruits can host several human, plant and soil pathogens and proper identification of bacterial organisms using metagenomics is a step towards proffering ways to prevent or control microorganisms on fruits.

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## Authors contributions

AEA and NGO conceived and designed the research. Material preparation and data collection were performed by AEA, OM and NGO. NGO performed the sequence filtering and analyzed the data. The first draft of manuscript was written by MO. AEA and NGO commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues**: None.

## References

1. Moreira R, Moreno J, Buitrón J, Orbe K, Hector-Ardisana E, Uguna F, Viera W. Characterization of a soursop population (*Annona muricata*) from the central region of Ecuadorian Littoral using ISSR markers. Int J Plant Res. 2018;31(3):1-5. https://doi.org/10.5958/2229-4473.2018.00067.8
2. Adeola, AA, Aworh OC. Development and sensory evaluation of an improved beverage from Nigeria's tamarind (*Tamarindus indica* L.) fruit. African J Food Agric Nutr Dev. 2010;10(9):4079-92. https://doi.org/10.4314/AJFAND.V10I9.62888
3. Ajayi AA, Peter-Albert CF, Adedeji OM. Modification of cell wall degrading enzymes from soursop (*Annona muricata*) fruit deterioration for improved commercial development of clarified soursop juice (A review). J Medicinal Aromat Plants. 2015;4(1):178. http://dx.doi.org/10.4172/2167-0412.1000178
4. International Society for Infectious Diseases. ProMEDmail; 2018. Available from: https://www.promedmail.org. Retrieved 8 May, 2019.
5. Friesen ML, Porter SS, Stark SC, Von Wettberg EJ, Sachs JL, Martinez-Romero E. Microbially mediated plant functional traits. Annu Rev Ecol Evol Syst. 2011;42:23-46. https://doi.org/10.1146/annurev-ecolsys-102710-145039
6. Yang W, Chen J, Chen G, Wang S, FengFu F. The early diagnosis and fast detection of blast fungus, *Magnaporthe grisea*, in rice plant by using its chitinase as biochemical marker and a rice cDNA encoding mannose-binding lectin as recognition probe. Biosens. Bioelectron. 2013;41(1):820-26. https://doi.org/10.1016/j.bios.2012.10.032
7. Hawksworth DL, Rossman AY. Where are all the undescribed fungi? Phytopathology. 1997;87(9):888-91. https://doi.org/10.1094/PHYTO.1997.87.9.888
8. Pinto C, Pinho D, Sousa S, Pinheiro M, Egas C, Gomes AC. Unravelling the diversity of grapevine microbiome. PLoS One. 2014; 9 (1):1-12. https://doi.org/10.1371/journal.pone.0085622
9. Handelsman J. Metagenomics and microbial communities. University of Wisconsin-Madison, Madison, Wisconsin, USA. Encyclopedia of Life Sciences, John Wiley & Sons, Ltd; 2007. https://doi.org/10.1002/9780470015902.a0020367
10. Liu J, Abdelfattah A, Norelli J, Burchard E, Schena L, Droby S. Apple endophytic microbiota of different rootstock/scion combinations suggests a genotype-specific influence. Microbiome. 2018;6(18):1-11. https://doi.org/10.1186/s40168-018-0403-x

11. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41(1):1-11. https://doi.org/10.1093/nar/gks808
12. Chen C, Khaleel SS, Huang H, Wu CH. Software for pre-processing Illumina next-generation sequencing short read sequences. Source Code Biol Med. 2014;9(8):1-11. https://doi.org/10.1186/1751-0473-9-8
13. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. J Bioinform. 2010;27(16):2194-200. https://doi.org/10.1093/bioinformatics/btr381
14. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Hank S, Wagner H. Vegan: Community Ecology Package. 2019. https://cran.r-project.org
15. Wassermann B, Müller H, Berg G. An Apple a Day: Which Bacteria Do We Eat With Organic and Conventional Apples? Front. Microbiol. 2019;10:1-13. https://doi.org/10.3389/fmicb.2019.01629
16. Langille MG, Zaneveld J, Caporaso JG, Mcdonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RV, Knight R, Beiko RG, Huttenhower C. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol., 2013;31(9):814-21. https://doi.org/10.1038/nbt.2676
17. Eckert JW, Ogawa JM. The chemical control of postharvest diseases: subtropical and tropical fruits. Annu Rev Phytopathol. 1985;23(1) 421-54. https://doi.org/10.1146/annurev.py.23.090185.002225
18. Zhang L, Dai J, Tang Y, Luo X, Wang Y, An H, Fang C, Zhan C. *Hymenobacter deserti* sp. nov., iso- lated from the desert of Xinjiang, China. Int J Syst Evol Microbiol. 2009;59:77-82. https://doi.org/10.1099/ijs.0.000265-0
19. Gupta A, Logan J, Elhag N, Almond M. *Sphingobacterium spiritivorum* infection in a patient with end stage renal disease on haemodialysis. Ann Clin Microbiol Antimicrob. 2018;15(1):25. https://doi.org/10.1186/s12941-016-0141-5
20. Patt TE, Cole GC, Hanson RS. *Methylobacterium*, a new genus of facultatively methylotrophic bacteria. Int J Syst Evol Microbiol. 1976;26:226-29. https://doi.org/10.1099/00207713-26-2-226
21. Tani A, Takai Y, Suzukawa I, Akita M, Murase H, Kimbara K. Practical application of methanol-mediated mutualistic symbiosis between *Methylobacterium* species and a roof greening moss, *Racomitrium japonicum*. PLoS ONE. 2012;7(3):1-9. https://doi.org/10.1371/journal.pone.0033800
22. Marizcurrena JJ, Herrera LM, Cost´abile A, Morales D, Villad´oniga C, Eizmendi A, Davyt D, Castro-Sowinski S. Validating biochemical features at the genome level in the Antarctic bacterium *Hymenobacter* sp. strain UV11. FEMS Microbiol. Lett. 2019;366(14):1-10. https://doi.org/10.1093/femsle/fnz177
23. Yi H, Oh HM, Lee JH, Kim SJ, Chum J. *Flavobacterium antarcticum* sp. nov., a novel psychrotorerant bacterium isolated from the Antarctic. Int J Syst Evol Microbiol. 2005;55:637-47. https://doi.org/10.1099/ijs.0.63423-0
24. Madhaiyan M, Poonguzhal S, Lee J, Lee K, Sundaram S. *Flavobacterium glycines* sp. nov., a facultative methylotroph isolated from the rhizosphere of soybean. Int J Syst Evol Microbiol. 2010;60(Pt 9):2187-192. https://doi.org/10.1099/ijs.0.014019-0
25. De Beer H, Hugo CJ, Joostw PJ, Willems A, Vancanneyt M, Coenye T, Vandamme PA. *Chryseobacterium vrystaatense* sp. nov. isolated from raw chicken in a chicken- processing plant. Int J Syst Evol Microbiol. 2005;55:2149-53. https://doi.org/10.1099/ijs.0.63746-0
26. Pozzo T, Higdon SM, Pattathil S, Hahn MG, Bennett AB. Characterization of novel glycosyl hydrolases discovered by cell wall glycan directed monoclonal antibody screening and metagenome analysis of maize aerial root mucilage. PLoS One. 2018;13(9):1-19. https://doi.org/10.1371/journal.pone.0204525
27. Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med. 2017;15(1):73. https://doi.org/10.1186/s12967-017-1175-y
28. Naber CK. *Staphylococcus aureus* bacteremia: epidemiology, path- ophysiology and management strategies. Clinical Infectious Diseases. 2009;48(Suppl 4):S231–S237. https://doi.org/10.1086/598189
29. Argudin MA, Mendoza MC, Rodico MR. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins. 2010;2(7):1751-73. https://doi.org/.3390/toxins2071751
30. Yabuuchi E, Yano I, Oyaizu H, Hashimoto Y, Ezaki T, Yamamoto H. Proposals of *Sphingomonas paucimobilis* gen. nov., comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., two geno species of the genus *Sphingomonas*. Microbiol. Immunol. 1990;34(2):99-119.https://doi.org/10.1111/j.1348-0421.1990.tb00996.x
31. Yu FB, Shan SD, Luo LP, Guan LB, Qin H. Isolation and characterization of a *Sphingomonas* sp. strain F-7 degrading fenvalerate and its use in bioremediation of contaminated soil. J Environ Sci Health. Part B Pesticides Food Contaminants and Agricultural Wastes.2013;48(3):198-207. https://doi.org/10.1080/03601234.2013.730299
32. Ryan MP, Adley CC. Ralstonia spp.: emerging global opportunistic pathogens. Eur J Clin Microbiol Infect Dis. 2014;33(3):291-304. https://doi.org/10.1007/s10096-013-1975-9
33. Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotta H, Hashimoto Y, Ezaki T, Arakawa M. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. Microbiol Immunol. 1992;36(12):1251-75.https://doi.org/10.1111/j.1348-0421.1992.tb02129.x
34. Stopnisek N, Bodenhausen N, Frey B, Fierer N, Eberl L, Weisskopf L. Genus-wide acid tolerance accounts for the biogeographical distribution of soil *Burkholderia* populations. Environ Microbiol. 2014;16(6):1503-12. https://doi.org/10.1111/1462-2920.12211
35. Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. J Nutr Biochem. 2007;18 (7):427-42. https://doi.org/10.1016/j.jnutbio.2006.11.004
36. González VME. Chirimoya (*Annona cherimola* Miller), Frutal tropical y sub- tropical de valores promisorios. Cult Trop. (3):52-63. ISSN digital: 1819-4087. http://www.redalyc.org/articulo.oa?id=193227533008
37. Bhardwaj R, Pareek S, Sagar NA, Vyas N. Bioactive compounds of *Annona*. In: Murthy HN, Bapat VA editors, Bioactive Compounds in Underutilized Fruits and Nuts; 2019. https://doi.org/10.1007/978-3-030-06120-3_5-1
38. Zhang Z, Mao C, Shi Z, Kou X. The amino acid metabolic and carbohydrate metabolic pathway play important roles during salt-stress response in tomato. Front Plant Sci. 2017;8:1231. https://doi.org/10.3389/fpls.2017.01231
39. Khedr AH, Abbas MA, Wahid AA, Quick WP, Abogadallah GM. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. J Exp Bot. 2003;54(392):2553-62. https://doi.org/10.1093/jxb/erg277
40. Zushi K, Ono M, Matsuzoe N. Light intensity modulates antioxidant systems in salt-stressed tomato (*Solanum lycopersicum* L.

cv. Micro-Tom) fruits. Hortic Sci. 2014;165:384-91. https://doi.org/10.1016/j.scienta.2013.11.033

41. Shi H, Ye T, Chen F, Cheng Z, Wang Y, Yang P, Zhang Y, Chan Z. Manipulation of arginase expression modulates abiotic stress tolerance in Arabidopsis: effect on arginine metabolism and ROS accumulation. J Exp Bot. 2013;64(5):1367-79. https://doi.org/10.1093/jxb/ers400

42. França SC, Roberto PG, Marins MA, Puga RD, Rodrigues A, Pereira JO. Biosynthesis of secondary metabolites in sugarcane. Genet Mol Biol. 2001;24(1-4):243-50.https://doi.org/10.1590/S1415-47572001000100032

43. Bergman ME, Davis B, Phillips MA. Medically useful plant terpenoids: biosynthesis, occurrence and mechanism of action. Molecules. 2019;24(21):3961. https://doi.org/10.3390/molecules24213961

44. McCormick AC, Unsicker SB, Gershenzon J. The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends Plant Sci. 2012;17(5):303-10. https://doi.org/10.1016/j.tplants.2012.03.012

45. Gershenzon J, Dudareva N. The function of terpene natural products in the natural world. Nat Chem Biol. 2007;3(7):408-14.https://doi.org/10.1038/nchembio.2007.5

46. Singh R. Biodegradation of xenobiotics- a way for environmental detoxification. Int J Dev Res. 2017;7(07):14082-87. http://www.journalijdr.com

47. Bhatt P, Gangola S, Chaudhary P, Khati P, Kumar G, Sharma A, Srivastava A. Pesticide induced up-regulation of esterase and aldehyde dehydrogenase in indigenous *Bacillus* spp. BioremediationJ. 2019;23(1):42-52. https://doi.org/.1080/10889868.2019.1569586

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