DNA sequencing, genomes and genetic markers of microbes on fruits and vegetables

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Summary

The development of DNA sequencing technology has provided an effective method for studying foodborne and phytopathogenic microorganisms on fruits and vegetables (F & V). DNA sequencing has successfully proceeded through three generations, including the tens of operating platforms. These advances have significantly promoted microbial whole-genome sequencing (WGS) and DNA polymorphism research. Based on genomic and regional polymorphisms, genetic markers have been widely obtained. These molecular markers are used as targets for PCR or chip analyses to detect microbes at the genetic level. Furthermore, metagenomic analyses conducted by sequencing the hypervariable regions of ribosomal DNA (rDNA) have revealed comprehensive microbial communities in various studies on F & V. This review highlights the basic principles of three generations of DNA sequencing, and summarizes the WGS studies of and available DNA markers for major bacterial foodborne pathogens and phytopathogenic fungi found on F & V. In addition, rDNA sequencing-based bacterial and fungal metagenomics are summarized under three topics. These findings deepen the understanding of DNA sequencing and its application in studies of foodborne and phytopathogenic microbes and shed light on strategies for the monitoring of F & V microbes and quality control.

Introduction

The requirements for the improvement of the quality and safety of horticultural fruits and vegetables (F & V) depend on a better understanding of microorganisms (Dean et al., 2012; Olaimat and Holley, 2012; Siroli et al., 2015). Foodborne pathogens pollute F & V under cultivation in diverse environments, the use of unsterilized agricultural inputs or improper storage. Such contamination can easily cause food poisoning (Olaimat and Holley, 2012). Phytopathogenic fungi cause plant diseases, postharvest deterioration and mycotoxin accumulation, which significantly affect yield, quality and market value (Dean et al., 2012; Kumar et al., 2017). However, several endophytes can also be used as biocontrol agents to provide beneficial conditions for cultivation and postharvest storage (Siroli et al., 2015). Researchers are working to describe and control all of these microorganisms from farmland to consumers.

Microbial genome analysis relies strictly on DNA sequencing technology. The genome is the collection of DNA molecules, in which genes and variable sequences are arranged and provide the basic information for the formation of a microorganism. DNA sequencing, as a general technology applied in life science research, determines the nucleotide sequences of DNA strands. Over the past four decades, DNA sequencing technologies have rapidly developed and proceeded through three generations, resulting in the successful development of tens of platforms (Morey et al., 2013). First-generation sequencing (FGS) was developed in the mid-1970s and was mainly based on Frederick Sanger's DNA chain-termination sequencing method (Sanger...
Next-generation sequencing (NGS) was introduced in the 2000s, involving systems such as the Roche 454 pyrosequencing, Illumina Genome Solexa and Supported Oligo Ligation Detection (SOLiD) platforms (Liu et al., 2012). Third-generation sequencing (TGS) is the most recently introduced advance in this technology, which detects single and longer reads in real-time with a high efficiency (van Dijk et al., 2018). In parallel, DNA sequencing technologies have been applied in various genomic and phylogenetic studies (Rogers et al., 2008; Morey et al., 2013). First, DNA sequencing and whole-genome sequencing (WGS) were applied in microbial studies. Sanger sequencing was first used to determine the genome of phage X174 (5386 bp; Sanger et al., 1977). Subsequently, Sanger et al. verified the sequencing procedure and determined the genome of phage λ (48 502 bp; Sanger et al., 1983). In 1990, Goebel et al. reported the whole genome of vaccinia virus (192 kb), obtained by using the first automatic DNA sequencer, the AB370 system (Goebel et al., 1990). In 1991, Bankier et al. reported the genome of a human cytomegalovirus (229 kb; Bankier et al., 1991). In 1990, genomic research was initiated in Escherichia coli and Saccharomyces cerevisiae as model systems in preparation for the Human Genome Project. In 1995, Fleischmann et al. reported the genome of the first cellular microbe, Haemophilus influenzae Rd (1.83 Mb; Fleischmann et al., 1995). After the AB370 DNA sequencer was upgraded to the AB3730xl system, microbial WGS was greatly promoted. Since then, the genomes of important microbes such as E. coli (Blattner et al., 1997), Salmonella enterica (Parkhill et al., 2001), Listeria monocytogenes (Glaser et al., 2001), Staphylococcus aureus (Kuroda et al., 2001), Campylobacter jejuni (Parkhill et al., 2000) and Shigella flexneri (Jin et al., 2002) have been widely reported. Currently, approximately 100 microbial genomes per day are being registered on the US National Center for Biotechnology Information (NCBI) platform.

Knowledge of DNA polymorphisms improves the understanding of microbial genetic specificity. The microbial genome shows various sequence differences or polymorphisms. Microbial DNA polymorphisms are the basis for explaining the specificity of phenotypes, evolution and taxonomy (Foley et al., 2009). Methods such as the amplified fragment length polymorphism (AFLP), random fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) approaches are important for DNA polymorphism studies. Significantly, polymorphisms in bacterial ribosomal DNA (rDNA) 16S rDNA genes and fungal internal transcribed spacers (ITSs) have been widely used in studies of microbial taxonomy and identification (Sun et al., 2013). The 16S rDNA sequences of bacteria are relatively short, containing several conserved and hypervariable regions, and can provide taxonomic information for bacteria at the genetic level. Fungal rDNA contains tandem repeats of noncoding ITS regions. These ITS regions show a high level of polymorphism, and they are effective for fungal identification. Recently, improved sequencing technologies and powerful databases and software have promoted the development of sequence-based identification methods. In metagenomics, 16S rDNA and ITS sequencing present significant benefits for characterizing overall bacterial and fungal communities.

Notably, DNA sequencing has promoted studies involving F & V microbial WGS, gene identification and specificity analysis. Genetic markers identified in studies of polymorphism have been widely used for polymerase chain reaction- (PCR) and chip-based microbial detection (based, e.g. on conventional PCR, qPCR, multiplex PCR and gene chips; Lüth et al., 2018). These detection methods can monitor foodborne pathogens and phytopathogens on F & V with good accuracy and acceptability (O’Connor and Glynn, 2010). In addition, metagenomic studies have revealed the bacterial and fungal communities on F & V by using 16S rDNA and ITS sequencing (Forbes et al., 2017). The present review highlights the principles of DNA sequencing and outlines the WGS information and genetic markers of major bacterial foodborne pathogens and fungal phytopathogens on F & V. Common foodborne pathogenic species come from the Escherichia, Salmonella, Staphylococcus, Listeria, Shigella and Campylobacter genera (Table 1). Common phytopathogenic fungi come from the Penicillium, Alternaria, Aspergillus, Fusarium, Botrytis, Colletotrichum, Monilinia and Trichothecium genera (Table 2). Furthermore, NGS-based metagenomic references have provided comprehensive data on key aspects of bacterial and fungal communities found on F & V (Table 3). These findings have deepened our understanding of DNA sequencing technology and its application in studies of foodborne and phytopathogenic pathogens, and they have shed light on methods for the microbial monitoring and quality control of F & V.

**DNA sequencing**

**First-generation sequencing**

The initial DNA sequencing methodology was developed in the mid-1970s. Sanger et al. established a method for determining DNA sequences via primed synthesis with DNA polymerase (Sanger et al., 1974; Sanger and Coulson, 1975). Progress in Sanger’s method was made after the introduction of chain-terminating inhibitor dideoxynucleotides (ddNTPs; Sanger et al., 1977). In the same year, Maxam and Gilbert reported a DNA sequencing approach based on chemical modification...
Table 1. Whole-genome sequences of foodborne pathogens registered on NCBI platforms (up to 1 October 2019) and summary of the related genetic markers.

| Species                        | ID number | Number of sequenced strains | Representative strain | Size (Mb) | GC% | Genes | Proteins Specific genetic markers                                                                 |
|--------------------------------|-----------|-----------------------------|-----------------------|-----------|-----|-------|--------------------------------------------------------------------------------------------------|
| Escherichia E. coli            | ID167     | 17 952                      | K-12 substr. MG1655   | 4.64      | 50.8| 4498  | 4140 fiIC, V11, V12 (Gannon et al., 1997); uspA (Osek, 2001); lacZ (Foulds et al., 2002); rBE, eae, stx1, stx2 (Ooka et al., 2009); ipaH (van den Beld and Reubsaat, 2012); lacY, uidA (Mendes Silva and Domingues, 2015); PhoA (Yang et al., 2016); cdTB (Hassan et al., 2018) |
| E. albertii                    | ID 1729   | 89                          | KF1                   | 4.70      | 49.7| 4823  | 4380 cdTB (Maheux et al., 2014); rpOB (Lindsey et al., 2015); EAKF1, ch4033 (Lindsey et al., 2017); 16S rDNA (Grillo et al., 2018) |
| E. fergusonii                  | ID 1877   | 18                          | ATCC 35469T           | 4.64      | 49.9| 4618  | 4349 yidE, EFER, 1569, EFER, 3126 (Simmons et al., 2014); EFER, 0790 (Lindsey et al., 2017) |
| Salmonella S. enterica         | ID 152    | 11 215                      | CT18                  | 5.13      | 51.9| 4829  | 4473 AceK, flIC, iagA, invA, orfC, sfl, setA, ssxA, serA, STM2745, STM4492, 16S rRNA (Lee et al., 2009; Postollec et al., 2011); ssl, spvC (Peterson et al., 2010); hilA, fimA, hns (Jeyasekaran et al., 2011); avrA, stn, stn (Amin et al., 2016); spv, huf, fiB (Aizwghabi et al., 2018) |
| S. bongori                     | ID1089    | 20                          | NCTC 12419            | 4.46      | 51.3| 4382  | 4068 fiB, galD, invA (Lee et al., 2009); flIC, gnd, mltB (Soler-Garcia et al., 2014) |
| Staphylococcus S. aureus       | ID 154    | 10 630                      | NCTC 8325             | 2.82      | 32.9| 2872  | 2767 mecA, nuc, femA-SA, femA-SE, orfX, SCC mec, spa, gyrB, 16S rDNA (Hirvonen, 2014); tuf, rpoB, gap, pyrH, ftsZ (Song et al., 2019); sea, seb, sec, sed, see (Omrenga et al., 2019) |
| S. epidermidis                 | ID 155    | 670                         | ATCC 12228            | 2.56      | 32.1| 2558  | 2482 mecA, emmA, emmB, emmC, and msrA (Martineau et al., 2000); hld, ica, agrA, sarA (Frebourg et al., 2000); 16S rDNA and tuf (Kobayashi et al., 2009); femA-SA, femA-SE (Jukes et al., 2010); allE, gap and mvaA (Kilic and Basustaoglu, 2011); recN (Iorio et al., 2011); adhesin fibrinogen binding protein (Sunagar et al., 2013); hla/yidD and hlb (Pinheiro et al., 2015); Gmk2, pta and SESB (Osmani Bojd et al., 2017); icaA, aap, bhp (Ribic et al., 2017) |
| S. lugdunensis                | ID2548    | 26                          | HKU09-01              | 2.66      | 33.9| 2567  | 2567 Agr (Dufour et al., 2002); rpOB (Drancourt and Raoult, 2002); 16S rRNA (Skow et al., 2005); Fbl (Campos-Pena et al., 2014) |
| S. saprophyticus              | ID1350    | 108                         | ATCC 15305            | 2.58      | 33.1| 2523  | 2351 16S rRNA (Gaszewskas-Mastalzar et al., 1997); femA (Vannuffel et al., 1999); hrcA (Paiva-Santos et al., 2016) |
| S. pseudintermedius           | ID3429    | 223                         | HKU10-03              | 2.62      | 37.5| 2517  | 2384 SpsJ (Versiappan et al., 2017); sps (Phumthanakorn et al., 2017) |
| Listeria L. monocytogenes     | ID 159    | 3063                        | EGD-e                 | 2.94      | 38.0| 3055  | 2867 hly, iap, mpl, prfA, iniA, iniB, actA (Gasanov et al., 2005); pica, 16S rRNA (Xu et al., 2008) |
| L. seeligeri                  | ID 1246   | 6                           | SLCC3954              | 2.80      | 37.4| 2790  | 2663 23S rRNA, iap (Paillard et al., 2003); lse24-315 (Liu et al., 2004); 16S rRNA (Dalmasso et al., 2010); 5’-exonuclease (Hage et al., 2014) |
Table 1. (Continued)

| Species             | ID number | Number of sequenced strains | Representative strain | Size (Mb) | GC%   | Genes | Proteins | Specific genetic markers                                                                 |
|---------------------|-----------|-----------------------------|-----------------------|-----------|-------|-------|----------|------------------------------------------------------------------------------------------|
| Shigella            |           |                             |                       |           |       |       |          |                                                                                           |
| S. flexneri         | ID 182    | 480                         | 301                   | 4.83      | 50.67 | 4788  | 4313     | ipaH, plasmid DNA, ial, 16S rRNA (Warren et al., 2006); she PAI (Farfan et al., 2010); invC, rfc (Ojha et al., 2013); genome marker (Sahl et al., 2015; Kim et al., 2017a) |
| S. boydii           | ID 496    | 113                         | 1221_SBOY             | 4.84      | 50.7  | 5125  | 4745     | ipaH, 16S rRNA (Warren et al., 2006); invC (Ojha et al., 2013); wzy (Radhika et al., 2014); genome marker (Sahl et al., 2015; Kim et al., 2017a) |
| S. sonnei           | ID 417    | 1338                        | 4303                  | 4.55      | 51.1  | 5275  | 4009     | ipaH, 16S rRNA (Warren et al., 2006); IS1 (Hsu et al., 2007); she PAI (Farfan et al., 2010); invC, wbgZ (Ojha et al., 2013); IpaB/D (Farshad et al., 2015); genome marker (Sahl et al., 2015; Kim et al., 2017a) |
| S. dysenteriae      | ID 415    | 67                          | Sd197                 | 4.56      | 50.9  | 4834  | 4294     | il, virA, 16S rRNA (Warren et al., 2006); invC, rfb (Ojha et al., 2013); genome marker (Sahl et al., 2015; Kim et al., 2017a) |
| Campylobacter       |           |                             |                       |           |       |       |          |                                                                                           |
| C. jejuni           | ID149     | 1615                        | NCTC 11168            | 1.64      | 30.5  | 1668  | 1572     | pDT1720 (Ng et al., 1997); CC2, CJ2 (Sails et al., 2001); ciaB, pldA, CDT (Ghorbanalizadgan et al., 2014); GTPase gene, hip, 16S rRNA, rs, cda, porA, 16S-23S ITS, Hpr, ciaA, cepE, hipO, mapA, cefA, askD, gpyA, lpxA, ccoN, ORF-C, poB, oxidoeductase gene, cda, pepT (Frasso et al., 2017); ftaA, cdtF, racR, dnaJ, cdtB, and cdtC (Oh et al., 2017); gyrA (Sierra-Arguello et al., 2018); ask, cdt (Kabir et al., 2019) |
| C. coli             | ID1145    | 928                         | Aerotolerant OR12     | 2.03      | 30.8  | 2185  | 2058     | ansP, ansR, aswC, acO3, acB (Noormohamed and Fakhr, 2013); GTPase gene, hip, cefA, CCCH, cdtB, porA, 16S-23S ITS, cepE, mapA, hipO, gpyA, cefD, oxidoeductase gene, cefA, pepT, inv, C51 (Frasso et al., 2017); cepE (Rodgers et al., 2017); cefD, asp (Pavlova et al., 2016; Banowary et al., 2018) |

and specific cleavage (Maxam and Gilbert, 1977). The first-generation DNA sequencing method of shotgun sequencing was developed based on the principles of both methods (Morey et al., 2013). This technique involves the following steps (Fig. 1). A DNA template is broken down into fragments. Each DNA fragment is then amplified (initially by E. coli cloning and later by PCR). The amplicons of the DNA fragments are then sequenced in a system with four independent PCR arrays. In the four independent PCR assays, four chain-terminating inhibitors (ddATP, ddTTP, ddGTP and ddCTP) are added. In the PCR assays, the majority of DNA polymerase reactions are conducted by adding dNTPs. However, in several reactions, ddNTPs are added for polymerization, thus terminating chain extension. As a result, DNA amplicons with different lengths are obtained. An electrophoresis method is used to separate the terminated DNA amplicons. The continuous DNA sequence is obtained by docking the ends of the electrophoresis-separated products.

Automated DNA sequencing has been available since the introduction of fluorescently labelled ddNTPs and spectrum detection in the mid-1980s. The first automated DNA sequencer (AB370) was announced by Applied Biosystems Co. in 1987 (Bankier et al., 1991). The read length produced by the AB370 platform reached 600 bp, with detection of 96 bp per cycle and 500 kb per day. The AB370 system was upgraded to AB3730xl platform in 1995, with an improved read length (approximately 900 bases), speed (2.88 Mb per day) and accuracy (over 99.99%; Liu et al., 2012). However, compared with NGS platforms, the AB3730xl system was far from

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| Species               | ID number | Number of sequenced strains | Representative strain | Size (Mb) | GC% | Genes | Proteins | Specific genetic markers                                                                 |
|----------------------|-----------|------------------------------|-----------------------|-----------|-----|-------|----------|------------------------------------------------------------------------------------------|
| *Penicillium*        |           |                              |                       |           |     |       |          |                                            |
| *P. expansum*        | ID11336   | 9                            | MD-8                  | 32.36     | 47.5| 11 060| 11 060  | Polygalacturonase (Hesham et al., 2011); PatF (Tannous et al., 2015); idh, 18S, α-tubulin, calmodulin (De et al., 2016; Rharmitt et al., 2016); ITS1-ITS4 (Hammami et al., 2017); PdCYP51 (Hammamoto et al., 2001); α-tubulin (Oshikata et al., 2013); PdCyt b (Zhang et al., 2009); ITS (Liu et al., 2017); RPB1, cmd-3 (Chen et al., 2017b) |
| *P. digitatum*       | ID13384   | 3                            | Pd1                   | 26.05     | 48.9| 8962  | 8961    |                                            |
| *P. griseofulvum*    | ID43141   | 2                            | PG3                   | 29.14     | 47.3| 9630  | 9630    |                                            |
| *P. citrinum*        | ID40785   | 2                            | JCM 22607             | 34.00     | 45.9| –     | –       |                                            |
| *P. italicum*        | ID34360   | 3                            | GL-Gan1               | 31.03     | 45.8| –     | –       |                                            |
| *Alternaria*         |           |                              |                       |           |     |       |          |                                            |
| *A. alternata*       | ID11201   | 6                            | SRC1lrK2f             | 32.99     | 51.4| 13 577| 13 466  | endopolygalacturonase (Garganese et al., 2016); glyceraldehyde-3-phosphate dehydrogenase (Gpd), Alt a1 (Gherbawy et al., 2018); AaSdhB, AaSdhC, AaSdhD (Lichtemberg et al., 2018); β-tubulin (Basim et al., 2018); ITS1, ITS4, histone 3 (Wang et al., 2019) |
| *A. arborescens*     | ID12091   | 5                            | EGS 39-128            | 33.89     | 50.9| –     | –       | ITS (Lorenzini and Zapparoli, 2014); endopolygalacturonase (Garganese et al., 2016); Alt a1, calmodulin, plasma membrane ATPase (Ellar et al., 2018); elongation factor, β-tubulin, glyceraldehyde-3-phosphate dehydrogenase (Somma et al., 2019) |
| *A. brassicicola*    | ID865     | 2                            | Abra43                | 31.04     | 50.8| –     | –       | Cutinase A (Gachon and Saindrenan, 2004); Microsatellite locus ABS28 (Singh et al., 2014); ITS4, ITS5 (Gao et al., 2014); ITS1, ITS2 (Zur et al., 2002); Alt_a1, ITS-ptsAs (Gu et al., 2017); histidine kinase (HK1) (Khan et al., 2018); ITS1, ITS4 (Yousif et al., 2010); RPB1-1, cmd-3 (Chen et al., 2017b); RPB1, RPB2 (Chen et al., 2019) |
| *A. solani*          | ID65961   | 2                            | NL03003               | 32.78     | 51.3| –     | –       |                                            |
| *A. tenuissima*      | ID76065   | 7                            | FERA 1166             | 35.70     | 51.1| 13 653| 13 575  | Aflatoxin (Andersen et al., 2006); histone (Kou et al., 2014) |
| *Aspergillus*        |           |                              |                       |           |     |       |          |                                            |
| *A. flavus*          | ID360     | 60                           | NRRL3357              | 36.89     | 48.3| 13 485| 13 485  | aflS, aflO (Degola et al., 2007); ITS, pksA, omtA (Yin et al., 2008b); β-tubulin (Zarinfar et al., 2015); aflatoxin biosynthesis gene cluster (Cailcott and Cotty, 2015); aflQ (Mahmoud, 2015); ITS1, ITS4 (Mytroie et al., 2016); aflR – aflJ intergenic region (Alouli and El Khoury, 2017); ITS, aflP (1), aflM, aflA, aflID, aflIP(3), aflIP (2), aflR (Al-Shuhaib et al., 2018); norA, omtA (Hua et al., 2018) |
| Species             | ID number | Number of sequenced strains | Representative strain | Size (Mb) | GC%  | Genes | Proteins | Specific genetic markers                                                                                   |
|---------------------|-----------|-----------------------------|-----------------------|-----------|------|-------|----------|----------------------------------------------------------------------------------------------------------|
| A. niger            | ID429     | 14                          | FDAARGOS_311          | 35.74     | 49.7 | 11 602| 11 190  | ITS (Gonzalez-Salgado et al., 2005); acf1F (Tunetevski et al., 2013); β-tubulin gene (Mirhendi et al., 2016); calmodulin gene (Das et al., 2017); benA (von Hertwig et al., 2018)  |
| A. carbonarius      | ID947     | 1                           | ITEM 5010             | 36.15     | 51.6 | 11 735| 11 478  | Calmodulin (Palumbo and O’Keeffe, 2015); β-tubulin (Mirhendi et al., 2016)                                |
| A. tubingensis      | ID18109   | 2                           | CBS 134.48            | 35.15     | 49.2 | 12 592| 12 319  | ITS (Medina et al., 2005); sequence-characterized amplified region (SCAR) (Giaj Merlera et al., 2015); calmodulin (Palumbo and O’Keeffe, 2015); β-tubulin (Mirhendi et al., 2016) |
| A. parasiticus      | ID12976   | 3                           | SU-1                  | 39.47     | 48.1 | 8645 | 8645    | nor-1, ver-1, omf-1, apf-2 (Chen et al., 2002); afiR (Somashekar et al., 2004); ITS (Luo et al., 2009); acf, norA (Yin et al., 2015); afiR-aff intergenic region (Atoui and El Khoury, 2017) |
| A. westerdijkiae    | ID40399   | 1                           | CBS 112803            | 36.07     | 50.2 | –     | –       | ITS (Gil-Sema et al., 2009); pks, p450-B03 (Gil-Sema et al., 2011); β-tubulin (Durand et al., 2019)         |
| Fusarium            |           |                             |                       |           |      |       |          |                                                                                                           |
| F. oxysporum        | ID707     | 115                         | f. sp. lycopersici    | 61.39     | 48.4 | 27 347| 27 347  | FOW1 (Li et al., 2014); IGS, TEF-1α, β-tubulin gene (Kim et al., 2017b); Tfl1 insertion (Ortiz et al., 2017); Bk (Pugliese et al., 2013); ITS (Wu et al., 2019); Efl1 (Chen et al., 2017a); SIX4, SIX5 (Ayukawa et al., 2016); SIX1, SIX3 (Debbui et al., 2018); FEM1, HPEG (van Dam et al., 2018); FocScF/FocScR (Singh and Kapoor, 2018) |
| F. solani           | ID537     | 3                           | 77-13-4 (Coleman et al., 2009) | 51.29     | 50.8 | 15 708| 15 708  | FS1/FS2 (Gasasnovas et al., 2013); EF-1α (Muraosa et al., 2014); ITS (de Souza et al., 2017); RPB (Chitrampalam et al., 2018) |
| (Nectria haematococca) |          |                             |                       |           |      |       |          |                                                                                                           |
| F. fujikuroi        | ID13188   | 15                          | IMI 58289             | 43.83     | 47.5 | 14 943| 14 810  | histone H3 (Steenkamp et al., 1999); MAT allele (Steenkamp et al., 2000); ITS (Llorens et al., 2006); gaoB (Faria et al., 2012); β2-tubulin (Zhang et al., 2015); TEF 1-α (Carneiro et al., 2017) |
| F. verticilloides   | ID488     | 4                           | 7600                  | 41.84     | 48.7 | 20 574| 20 574  | EF-1α (Wu et al., 2016); FUM1; FUM19 (Omoni et al., 2018); ITS (Jedidi et al., 2018); calmodulin (Mulé et al., 2004); EGFP (Gai et al., 2018) |
| F. proliferatum     | ID2434    | 13                          | ET1 (Niehaus et al., 2016) | 45.21     | 48.1 | 16 509| 16 143  | Calmodulin (Mulé et al., 2004); ISR (Borrego-Benjumea et al., 2014); SSR (Moncrief et al., 2016); tef-1α, FUM1 (Gilveze et al., 2017); FUM19 (Proctor and Vaughan, 2017); ITS (Jedidi et al., 2018) |
| Species                | ID number | Number of sequenced strains | Representative strain | Size (Mb) | GC% | Genes | Proteins | Specific genetic markers |
|-----------------------|-----------|-----------------------------|-----------------------|-----------|-----|-------|----------|-------------------------|
| *F. graminearum*      | ID 58     | 11                          | PH-1 (Cuomo et al., 2007) | 36.67     | 48.3 | 13 334 | 13 334   | MGB (Waalwijk et al., 2004); gao4 (de Biazio et al., 2008); cyp51A, beta-tubulin (Yin et al., 2009a); TRI12 (Nielsen et al., 2012); MAT (Demeke et al., 2010); PKS13 (Atoui et al., 2012); Trif7, Trif3 (Tralamazza et al., 2016); TRI (Wang and Cheng, 2017); ITS (Jedidi et al., 2018); tef-1x (Garmandia et al., 2018) |
| *Colletotrichum*      |           |                             |                       |           |     |       |          |                         |
| *C. acutatum*         | ID38530   | 2                           | –                     | 52.13     | 51.7 | –     | –        | ITS (Talhinhas et al., 2002); beta-tubulin 2 (Talhinhas et al., 2005); Calint2 (Jelev et al., 2008); BenA (Polizi et al., 2011); beta-tubulin (TUB), GAPDH (Karimi et al., 2019); MAT1-2 (Furuta et al., 2017) |
| *C. gloeosporioides*  | ID17739   | 6                           | TYU                   | 53.01     | 49.6 | –     | –        | RAPD (Pileggi et al., 2009); CgInt (Lopes et al., 2010); j-tubulin (TUB), actin (ACT), GAPDH (Ramdeen and Rampersad, 2013); act, cal, gapdh, gs, ITS, tub2 (Sharma et al., 2017) |
| *C. coccodes*         | ID56076   | 2                           | NJ-RT1                | 50.12     | 53.8 | –     | –        | RAPD (Dauch et al., 2003); ITS (Baysal-Gurel et al., 2014); CAL, ITS, TUB2 (Silva et al., 2018) |
| *C. fructicola*       | ID57524   | 3                           | 15060 (Aguilar-Pontes et al., 2018) | 55.92     | 53.2 | –     | –        | ITS (Li et al., 2013); ACT, CAL, CHS-1, ITS, TUB, GAPDH (Gan et al., 2017) |
| *Monilinia*           |           |                             |                       |           |     |       |          |                         |
| *M. fructicola*       | ID54919   | 2                           | LMK 125               | 44.68     | 40.1 | –     | –        | MO368-1, Laxa (Cote et al., 2004); 18S rDNA (Fulton and Brown, 1997); j-tubulin (Fan et al., 2014); ITS (Guinet et al., 2016); laccase-2 (Wang et al., 2018b); cytochrome b (Ortega et al., 2019) |
| *M. laxa*             | ID56076   | 2                           | EBR-Ba11b             | 41.84     | 40.2 | –     | –        | 18S rDNA (Fulton and Brown, 1997); MO368-1, Laxa (Cote et al., 2004); ISSR and RAPD (Fazekeas et al., 2014); ITS (Guinet et al., 2016); laccase-2 (Wang et al., 2018b); SCAR (Ortega et al., 2019) |
| *M. fructigena*       | ID66926   | 3                           | ASM290963v1           | 39.33     | 41.7 | –     | –        | 18S rDNA (Fulton and Brown, 1997); MO368-1, Laxa (Cote et al., 2004); j-tubulin (Fan et al., 2014); ITS (Guinet et al., 2016); laccase-2 (Wang et al., 2018b); MO368-1, Laxa (Cote et al., 2004); ITS (Guinet et al., 2016); laccase-2 (Wang et al., 2018b) |
| *M. polystroma*       | ID66925   | 1                           | ASM290964v1           | 44.63     | 39.2 | –     | –        |                                  |
| *Botrytis*            |           |                             |                       |           |     |       |          |                         |
| *B. cinerea*          | ID494     | 4                           | B05.10                | 42.63     | 42.0 | 13 703 | 13 703   | EcoRI (Rigotti et al., 2002); IGS, SCAR (Suarez et al., 2005); IGS (Duguta et al., 2010); G3PDH, HP60, RPB2, NEP1, NEP2 (Fan et al., 2015); ITS, j-tubulin (Reich et al., 2016); necrosis, nep1 (Munoz et al., 2016) |
Table 3. Summary of 16S rDNA and ITS sequencing-based microbiome community studies of fruits and vegetables.

| Samples                      | Bacteria/fungi | Target organs | Study focus                                                                                                   | Technology/sequencing platform | 16S rRNA target/primers | Taxonomic resolution/focused taxonomy                                                                 | References          |
|------------------------------|----------------|---------------|---------------------------------------------------------------------------------------------------------------|-------------------------------|--------------------------|----------------------------------------------------------------------------------------------------------------|---------------------|
| Apple                        | Bacteria       | Flower        | Apple flower Microbiome                                                                                       | Pyrosequencing                | Primers 799 and 1115 reverse | Genera: Acetobacter, Arthrobacter, Burkholderia, Butulaxella, Deinococcus, Escherichia/Shigella, Hymenobacter, Knoellia, Lactobacillus, Methanosarcina, Pantoea, Terrimonas, Truepera | Shade et al. (2013) |
| Apple, Grapes, Lettuce, Peach, Spinach and Tomato | Bacteria       | Fruit; Leaf   | Overall diversity and composition of bacterial communities, and their variations in fruits and vegetables      | Pyrosequencing                | Metabarcoding V4eV7 (universal primer 799F and 1115R) | Family: Bacillaceae, Comamonadaceae, Enterobacteriaceae, Flavobacteriaceae, Leuconostocaceae, Microbacteriaceae, Micrococcaceae, Moraxellaceae, Nocardioidaeae, Oxalobacteraceae, Pseudomonadaceae, Rhizobiales, Xanthomonadaceae | Leff and Fierer (2013) |
| Arugula                      | Bacteria       | Leaf          | Arugula phyllosphere indigenous microbiome                                                                     | Pyrosequencing                | V3-V8                    | Genera: Caulobacteriales, Rhizobiales, Rhodobacteriales, Sphingomonadaceae, Burkholderiales, Rhodocyclales, Enterobacteriales, Pseudomonadales, Xanthomonadales | Cernava et al. (2019) |
| Basil                        | Bacteria       | Leaf          | Naturally bacterial community on basil leaves                                                                  | Pyrosequencing                | V1-V3                    | Genera: Arcicella, Chryseobacterium, Flavobacterium, Sphingobacterium, Altererythrobacter, Novosphingobium, Sphingobium, Sphingomonas, Herbaspirillum, Acinetobacter, Pseudomonas, Rheinheimera, Enterobacter, Erwinia, Klebsiella, Kluyvera, Pantoea, Rahmella, Raoultella | Ceuppens et al. (2015) |
| Blueberry                    | Bacteria       | Root          | Soil properties and plant cultivars cooperatively shaped the variations in bacterial diversity and networks in the rhizosphere | Illumina sequencing           | V4-V5                    | Genera: Azospirillum, Phenylbacterium, Rhodanobacter, Steroidobacter, Pseudomonas, Leteibacter, Acidothermus, Dongia, Rhizomicrobium, Pseudolabrys, Acidothermus, Rhodanobacter, Bradyrhizobium, Rhodoplanes, Pseudolabrys | Jiang et al. (2017)  |
| Cilantro, Cucumber, Mung, Bean and Sprout | Bacteria       | Fruit, Leaf and Stem | Bacterial diversity differences                                                                                   | Illumina sequencing           | V1-V3                    | Genera: Xanthomonas, Sphingobacterium, Stenotrophomonas, Klebsiella, Enterococcus, Corynebacterium, Brachybacterium, Cronobacter, Brevibacterium, Rhizobium, Weissella, Serratia, Acinetobacter, Pantoea, Pseudomonas, Enterobacteriaceae, Bacillus, Enterobacter, Staphylococcus | Jarvis et al. (2018) |
| Samples | Bacteria/ fungi | Target organs | Study focus | Technology/ sequencing platform | 16S rRNA target/primers | Taxonomic resolution/focused taxonomy | References |
|---------|----------------|---------------|-------------|-------------------------------|------------------------|--------------------------------------|------------|
| Grape   | Bacteria       | Leaf          | Characterize the natural microbiome of grapevine | Pyrosequencing         | V6                     | Genera: Neisseriaceae; Sphingomonadaceae; Xanthomonadaceae; Veillonellaceae; Comamonadaceae; Leuconostocaceae; Moraxellaceae; Pseudomonadaceae; Enterobacteriaceae; Streptococcaceae | Pinto et al. (2014) |
| Grape   | Bacteria       | Fruit         | Geographical origin and cultivar of grape by microbiome composition in Northern Italy and Spain Vineyards | Illumina sequencing     | V3-V4                  | Genera: Alphaproteobacteria; Gamaproteobacteria; Actinobacteria; Bacilli; Citrobacter; Shingobactellia; Cytophaga; Blastocellia; Phycisphaerae; Acidimicrobia; Thermoleophilia; Deltaproteobacteria; Solibacteres; Gemmatimonadetes; Actinobacteria; Cyanobacteria | Mezzasalma et al. (2018) |
| Grape   | Bacteria       | Fruit         | Bacterial communities of Grenache and Carignan grape varieties | Not given              | V4                     | Genera: Bacillus; Oenococcus; Acinetobacter; Erwinia; Streptococcus; Pseudomonas; Haemophilus; Enhydrobacter; Methylobacterium; Veillonella; Corynebacterium; Lactobacillus; Niesseria; Gluconobacter; Sphingomonas; Staphylococcus; Micrococcus | Portillo et al. (2016) |
| Grape   | Fungi          | Fruit; Leaf   | Microbial relationship between soil, leaves and fruits | Illumina sequencing     | ITS1                   | Genera: Hydrosporphaera; Mycoarthritis; Chaetomium; Fusarium; Acremonium; Phoma; Cryptococcus; Cladosporium; Guehomyces; Alternaria | Zhang et al. (2017b) |
| Grape   | Fungi          | Leaf          | Natural microbiome of grapevine | Pyrosequencing          | ITS2 and D2            | Genera: Zoophthora; Pandora; Rhizopus; Mucor; Aureobasidium; Sporormiella; Alternaria; Kurztmanomyces; Colacogloea; Lewia; Ustilago; Puccinia; Cronartium | Pinto et al. (2014) |
| Grape   | Fungi          | Fruit         | Wine grape yeast from China | Not given              | ITS1                   | Genera: Zygosaccharomyces; Candida; Issatchenkia; Pichia; Sporidobolus; Hanseniaspora; Cryptococcus; Pichia; Issatchenkia; Metschnikowia | Li et al. (2010) |
| Grape   | Bacteria       | Leaf; Fruit   | Microbial relationship between soil, leaves and grapes | Illumina sequencing     | V5-V7                  | Genera: Kocuria; Microvirga; Flavobacterium; Sphingomonas; Nocardioides; Pseudomonas; Gaiella; Arthrobacter; Bacillus; Blastococcus | Zhang et al. (2017b) |
| Grape   | Bacteria       | Fruit         | Geography and cultivar differences affect wine grape microorganisms | Illumina sequencing     | V4                     | Genera: Acetobacter; Erwinia; Gluconobacter; Hymenobacter; Janthinobacterium; Klebsiella; Lactobacillus; Microbacteriaceae; Sporosarcina; Pseudomonadaceae; Sphingomonas; Leuconostocaceae; Moraxellaceae; Methylobacterium | Bokulich et al. (2014) |
| Samples                          | Technology/sequencing platform | 16S rRNA target/primers | Taxonomic resolution/focused taxonomy | References          |
|---------------------------------|--------------------------------|-------------------------|--------------------------------------|---------------------|
| Fungi                           | Illumina sequencing             | ITS1                    | Genera: *Cladosporium* spp.; *Botryotinia* Fuckelliana, *Penicillium*; *Davidiella*; *Aureobasidium*; *Hanseniaspora*; *Candida* | Bokulich et al. (2014) |
| Fungi                           | Illumina sequencing             | ITS1                    | Genera: *Myrothecium*; *Epicoccum*; *Cryptococcus*; *Mortierella*; *Guehomyces*; *Fusarium*; *Phoma*; *Cladosporium*; *Alternaria* | Zhang et al. (2017a) |
| Fungi                           | Illumina sequencing             | V3-V4                   | Genera: *Acinetobacter*; *Actinobacteria*; *Bacillus*; *Brevundimonae*; *Massillia*; *Methylobacterium*; *Pseudomonas*; *Sphingomonas*; *Streptococcus* | Purahong et al. (2018) |
| Fungi                           | Illumina sequencing             | V3                      | Genera: *Aeromonas*; *Bacillus*; *Citrobacter*; *Curtobacterium*; *Enterobacter*; *Lelliottia*; *Obesumbacterium*; *Pantoea*; *Providencia*; *Pseudomonas*; *Shigella* | Darlison et al. (2019) |
| Fungi                           | Illumina sequencing             | V3                      | Genera: *Dothioraceae*; *Davidiellaceae*; *Tremellales*; *Incertae Sedis*; *Davidiellaceae*; *Cystofilobasidiaceae*; *Tremellales* | Chen et al. (2018) |
| Flower, Fruit, Leaf and Stem    | Pyrosequencing                  | V2                      | Genera: *Pseudomonas*; *Micrococcineae*; *Xanthomonas*; *MethylBacterium*; *Rhizobium*; *Sphingomonas* | Ottesen et al. (2013) |
| Flower, Fruit, Leaf and Stem    | Pyrosequencing                  | EF4 and Fung5           | Genera: *Hypocreac; Aureobasidium; Cryptococcus; Chaetomium; Fusarium; Aspergillus* | Ottesen et al. (2013) |
| Topics | Regional environment and farming practices affect microbiomes |
|--------|-------------------------------------------------------------|
| Cabbage and Lettuce | Bacteria | Leaf | Microbial communities location differences | Pyrosequencing V4-V5 | Genera: Acinetobacter; Alistipes; Barnesiella; Rikenella; Anaerococcus; Aerospiraera; Vagococcus; Parabacteroides; Kluyvera; Peptoclostridium; Lysinibacillus; Lactobacter; Serratia; Macroccus; Weissella; Exiguobacterium; Kosakonia; Candidatus Nardonella; Leclercia; Obesumbacterium; Streptococcus; Escherichia; Clostridium; Aeromonas; Staphylococcus; Lactococcus; Butulaxella; Citrobacter; Pseudomonas; Enterococcus; Klebsiella; Bacillus; Enterobacter; Pantoea | Higgins et al. (2018) |
| Grape | Fungi | Fruit | Fungal diversity in ferment associated with grape microbes | Pyrosequencing | NL1 and NL4 | Genera: Columnosphaeria; Davidella; Cryptococcus | Morrison-Whittle et al. (2017) |
| Grape | Fungi | Fruit | Grape microbiome related to field origin and environmental conditions | Illumina sequencing ITS1 | Family: Botryosphaeriaceae; Dothioraceae; Pleosporaceae; Trichocomaceae; Incertae Sedis; Saccharomycetaceae; Saccharomycodaceae; Hymenochaetales; Peniophoraceae; Chytridiomycota | Mezzasalma et al. (2017) |
| Lettuce | Bacteria | Leaf | Bacterial communities on plant leaf surfaces related to time, space and environment | Pyrosequencing V5-V7 | Genera: Xanthomonas; Pantoea; Pseudomonas; Massilia; Bacillus; Erwinia; Alkanindiges; Acinetobacter; Naxibacter; Duganella; Flavobacterium; Exiguobacterium; Anthrobacter | Rastogi et al. (2012) |
| Lettuce | Bacteria | Leaf | Biotic stress shifted structure and abundance of Enterobacteriaceae in the lettuce microbiome | Pyrosequencing V4-V5 | Genera: Trabulsiella; Serratia; Klusbsela; Yersinia; Rahmelia; Pantoela; Erichichia; Enterobacter; Butulaxella; Cedecea; Citrobacter; Erwinia | Erlacher et al. (2015) |
| Maize | Bacteria | Leaf | Organic fertilization increased antibiotic resistome in phyllosphere | Illumina sequencing V4-V5 | Genera: Sphingomonas; Pseudomonas; Chryseobacterium; Curtobacterium; Methylobacterium | Chen et al. (2018) |
| Mulberry | Fungi | Fruit | Soil fungal community borne related to genotypes and disease occurrence | Illumina sequencing ITS1 and ITS2 | Genera: Humicola; Mortierella; Scleromitra; Schizothecium; Sphaerulina; Lecidella; Corinianius; Russula; Hymenochaete; Mraia | Yu et al. (2016) |
### Table 3. (Continued)

| Topics | Regional environment and farming practices affect microbiomes |
|--------|---------------------------------------------------------------|
| Peach and Nectarines | Fungi Fruit Yeasts traceability of organic and conventional farming types | PCR-DGGE/ GATC Biotech D1/D2 | Species: *Pichia Kluyveri; Pichia Fermentans; Candida; Tremella Flava* | Bigot et al. (2015) |
| Tomato | Bacteria Flower and Fruit Insects introduce variability in bacterial diversity | Illumina sequencing V1-V3 | Family: *Microbacteriaceae; Methylobacteriaceae; Rhizobiales; Alphaproteobacteria; Proteobacteria; Rhizobiaceae; Sphingomonadaceae; Comamonadaceae; Gammaproteobacteria; Shewanellaceae; Enterobacteriaceae; Pseudomonadales; Pseudomonadaceae; Xanthomonadaceae* | Allard et al. (2018) |

### Topics | Artificial treatment and quality control in storage and processing |
|----------|---------------------------------------------------------------|
| Apple | Fungi Fruit Long-term cold storage changed fungal components | Illumina sequencing ITS1 | Genera: *Mucor, Phoma, Cryptococcus; Tilletiopsis; Aspergillus; Penicillium; Acremonium; Aureobasidium* | Shen et al. (2018a) |
| Apple | Fungi Fruit Mycotoxin contamination associated with fungal flora of apple core | AB3730xl sequencing ITS1 and ITS4 | Genera: *Alternaria; Penicillium; Aspergillus; Fusarium; Trichoderma; Epicoccum; Diplodia; Davidiella; Nigrospora; Paraconiothyrium; Bionectria; Botrytis* | Soliman et al. (2015) |
| Arugula, Broccoli, Cabbage, Cauliflower, Horseradish, Radish and Turnip | Bacteria Leaf Microbiomes of vegetables related to plant disease resistance and human health | Illumina sequencing V4 | Genera: *Sphingopyxis; Sphingomonas; Pseudoxanthomonas; Pedobacter; Flavobacterium; Pseudomonas; Acinetobacter; Sphingomonas; Leuconostoc; Acinetobacter; Janthinobacterium; Achromobacter; Methylobacterium* | Wassermann et al. (2017) |
| Asparagus | Bacteria Stem High-hydrostatic pressure treatment for postharvest preservation | Pyrosequencing V1–V3 | Genera: *Streptococcus; Rubrobacter; Pauicibacter; Janibacter; Escherichia; Clostridium; Citrobacter; Bacteroides; Propionibacterium; Brevibacterium; Sphingomonas; Pantoea; Bacillus; Tatumella; Rhodanobacter; Gammaproteobacteria; Halomonadaceae; Brenneria; Raoultella; Acetobacter; Pseudomonas; Proteobacteria; Serratia; Enterobacteriaceae; Rahnella; Acetobacteraceae; Leuconostoc; Gluconobacter* | del Arbol et al. (2016a) |
| Topics                        | Artificial treatment and quality control in storage and processing | Genera                                                                 | Authors/Year       |
|-------------------------------|--------------------------------------------------------------------|------------------------------------------------------------------------|--------------------|
| Baby Spinach                 | Bacteria Leaf, Leaf, Bacterial communities changed by the chlorine dioxide (ClO2) treatment | Illumina sequencing V3-V4, Bacillus, Pseudomonas, Massilia, Pantoea      | Truchado et al. (2016) |
| Bean and Radish              | Bacteria Seed, Bacteria community in the early plant developmental stages | Illumina sequencing Not mentioned, Bacillus, Enterobacter, Pantoce, Pantoea, Streptomyces, Arthrobacter, Bacillus | Torres-Cortes et al. (2018) |
| Cabbage                      | Bacteria Leaf, Microbiota changes during cabbage ferment progress | Illumina sequencing V3-V4, Genes, Bacillus, Pseudomonas, Massilia, Pantoea, Enterobacteriaceae, Pseudomonas, Chryseobacterium, Pseudomonas | Einson et al. (2018) |
| Carrot                       | Bacteria Root, Postharvest gamma irradiation affects surface carrot bacterial community | Illumina sequencing V4-V5, Enterobacteriaceae, Pantoea, Bacillus, Streptomyces, Pseudomonas, Ervinia, Pantoce, Pantoea, Bacillus, Enterobacteriaceae, Pseudomonas | Dharmarha et al. (2019b) |
| Cherry                       | Bacteria Fruit, High-hydrostatic pressure treatment affects bacterial community | Pyrosequencing V1-V3, Streptococcus, Rubrobacter, Escherichia, Clostridium, Citrobacter, Bacteroides, Enterobacteriaceae, Pseudomonas, Enterobacteriaceae, Ureibacillus | del Arbol et al. (2016b) |
| Cherry                       | Bacteria Fruit, High-hydrostatic pressure processing changed the microbiological quality and bacterial biodiversity | Pyrosequencing V1-V3, Enterobacteriaceae, Rahnella, Acetobacteraceae, Leuconostoc, Gluconobacter | Toledo del Arbol et al. (2016) |
| Clementines, Potatoes and Tomatoes | Bacteria Fruit and Root, Food packaging materials affected microbial community | Illumina sequencing V3-V4, Phylum, Proteobacteria, Firmicutes, Actinobacter, Bacteroidetes, Verrucomicrobia, Tenericutes, Planomycetes | Brandwein et al. (2016) |
| Grape                        | Bacteria, Bacterial community and its temporal succession during the fermentation | Pyrosequencing V1–V3, Genes, Gluconobacter, Pedobacter, Spingomonas, Janthinobacterium, Pseudomonas, Enterobacteriaceae, Pseudomonas | Piao et al. (2015) |
| Grape                        | Bacteria, Diversity and evolution of microorganisms during grape wine fermentation | Pyrosequencing Not given, Genes, Orib, Blidobacterium, Wolbachia, Acetobacter, Gluconacetobacter, Lactobacillales, Gluconobacter | Portillo and Mas (2016) |
| Grape                        | Bacteria, Human and animal pathogens related to foodborne diseases in the grapevine endosphere | Pyrosequencing V5-V9, Genes, Propionibacterium, Staphylococcus, Clostridium, Burkholderia | Yousaf et al. (2014) |
| Topics | Artificial treatment and quality control in storage and processing | Methods | Genera | References |
|--------|--------------------------------------------------|---------|--------|------------|
| **Grape** | **Fungi** | **Fr** | **Changes in microbiota and mycobiota during spontaneous fermentation** | **Pyrosequencing** | **ITS1-5.8S–ITS2** | **Nakazawaeae; Hanseniaspora; Aureobasidium; Botrytis; Pichia; Penicillium; Cladosporium; Thelebolus; Geuhomyces** | **Stefanini et al. (2016)** |
| **Grape** | **Fungi** | **Fruit** | **Microbial community in wine fermentation** | **Illumina sequencing** | **BITS and B58S3** | **Alternaria; Aspergillus; Aureobasidium; Botrytis; Brettanomyces; Candida; Cladosporium; Cryptococcus; Erysiphe; Hanseniaspora; Kluyveromyces; Lachancea; Metchnikowia; Meyerozyma; Mucor; Penicillium; Pichia; Rhodospirillum; Rhodotorula; Saccharomyces; Torulaspore; Wicherhamomyces; Zygosaccharomyces** | **Sternes et al. (2017)** |
| **Grape** | **Fungi** | **Fruit** | **Composition and changes of the fungal population during fermentation** | **Pyrosequencing** | **ITS1** | **Sporobolomyces; Periconia; Peniophora; Leptosphaerulina; Issatchenka; Bathyilmia; Diaporthe; Botrytis; Pithomyces; Phoma; Leptosphaerulina; Cryptococcus; Alternaria; Zygosaccharomyces; Metschnikowia; Diploida; Cystospora; Candida** | **Stefanini et al. (2017)** |
| **Grape** | **Fungi** | **Fruit** | **Wine fermentation affected by the metabolome** | **Pyrosequencing** | **ITS1** | **Penicillium; Aspergillus; Botryosphaeria; Starmerella; Saccharomyces; Hanseniaspora; Cladosporium; Aureobasidium** | **Wang et al. (2015)** |
| **Grape** | **Fungi** | **Fruit** | **Soil microbial composition shifts based on under-vine management, but no corresponding changes in fruits** | **Illumina sequencing** | **ITS1** | **Penicillium; Sporobolomyces; Coprinellus; Ischnoderma; Mycosphaerella; Occultifur; Pestalotiopsis; Tilletiopsis** | **Chou et al. (2018)** |
| **Grape** | **Fungi** | **Fruit** | **Microbiome dynamics during spontaneous fermentations of sound grapes in comparison with sour rot and Botrytis infected grapes** | **PCR-DGGE** | **515F/806R** | **Aspergillus; Rhizopus; Saccharomyces cerevisiae; Starmerella; Penicillium; Zygosaccharomyces; Acetobacter; Ameyamaea; Gluconacetobacter; Gluconobacter; Tanticharoenia; Oenococcus; Botrytis; Cladosporium; Hanseniaspora** | **Leixa et al. (2018)** |
| Topics | Artificial treatment and quality control in storage and processing | Species | DNA sequencing, genomes and markers for microbes |
|--------|---------------------------------------------------------------|---------|-------------------------------------------------|
| Grape  | Fungi Fruit Yeast communities on the grape berries affect by fungicides | *Aureobasidium Pullulans, Candida sp.; Candida Zemplinina; Cryptococcus Carnesens; Cryptococcus Dimeanae; Cryptococcus Flavescens; Cryptococcus Magnus; Cryptococcus sp.; Cryptococcus Victoriae; Cryptococcus Weringae; Hanseniospora Uvarum; Issatchenka Terricola; Metschnikowia Pulcherima; Pichia Fermentans; Pichia Membranifaciens; Rhodospiridium Babjevae; Rhodotorula Glutinis; Rhodotorula Nothofagi; Saccharomyces cerevisiae | Milanovic et al. (2013) |
| Grape  | Fungi Fruit Diversity and evolution of microorganisms during grape wine fermentation | *Hanseniaspora; Saccharomyces; Issatchenka; Candida; Saccharomyces* | Portillo and Mas (2016) |
| Lettuce| Bacteria Leaf Chitin amendment changed the microbial composition | *Cellvibrio, Sphingomonas; Pedobacter, Azospirillum; Taibaiella; Nitrospira; Streptomyces; Nocardioctes* | Debode et al. (2016) |
| Lettuce| Bacteria Leaf Gamma irradiation changed the phyllosphere microbiota | *Pseudomonas; Ureibacillus; Escherichia; Bacillus; Symbiobacterium; Thermoactinomycetes* | Dhamarha et al. (2019a) |
| Lettuce| Bacteria Leaf Innovative washing solutions shifted the lettuce microbiota | *Flavobacterium; Pseudomonas; Pedobacter, Oxalabacteria; Comamonadaceae; Chryseobacterium; Agrobacterium; Sphingomonas; Janthinobacterium; Methylphilaeae* | Siroli et al. (2017) |
| Lettuce| Fungi Leaf Chitin amendment changed the microbial composition | *Morteriella; Lecaniciillum; Pseudogymnoascus; Pseudoeurotium* | Debode et al. (2016) |
| Maize  | Fungi Seed Composition and variation in the fungal communities associated with seeds during storage | *Aspergillus; Fusarium; Penicillum; Alternaea; Trichoderma; Chaetomium; Botryothichium; Cladosporium; Bipolaris; Rhizopus* | Xing et al. (2018) |
| Topics         | Artificial treatment and quality control in storage and processing | Genera: | Wang et al. (2018a) |
|---------------|---------------------------------------------------------------|---------|-------------------|
| Orange Peel   | Fungi Fruit Dynamic of fungi communities during the storage | Cyphellophora, Epicoccum; Meyerocyna; Saccharomycopsis; Trichomerum; Colletotrichum; Meira; Cryptostroma; Alteraria; Zymoseptoria; Bryochiton; Talaromyces; Acaeromyces; Cosmopora; Micosphaerella; Pseudopithomyces; Paraphaeosphaeria; Streptiziana; Cyclophye; Picha; Aspergillus; Cladosporium; Hankeriaspora; Ceratothyrium; Schwannomyces; Cystofilobasidium; Penicillium; Candida; Microidium; Wallemia |
| Peanut        | Fungi Seed Dynamic of fungi communities during the peanut storage | Ascomycota, Talaromyces; Nectriaeae; Ceratobasidiaceae; Clonostachys; Erenercella; Eurotium; Phizopus; Penicillium; Aspergillus |
| Pear          | Bacteria Fruit Chlorine drenching and after controlled atmosphere storage affect bacterial populations | Curtobacterium, Pseudomonas, Pantoea; Erwina Bilingiae; Frigoribacterium | Ding et al. (2015) |
| Spinach       | Bacteria Leaf Changes in spinach phylloepiphytic bacteria communities following minimal processing and refrigerated storage | Deinococcus; Exiguobacterium, Sphingomonas; Methylobacterium; Rhizobium; Brevundimonas; Acinetobacter; Pseudomonas; Stenotrophomonas; Pantoea; Escherichia; Ralstonia; Naxibacter; Massilia | Lopez-Velasco et al. (2011) |
meeting the needs for a higher speed, throughput and cost-effectiveness.

**Next-generation sequencing**

Three NGS platforms were introduced during the first decade of the 21st century: the Roche 454 pyrosequencing, Illumina Genome Analyzer and SOLiD sequencing platforms. Compared with FGS, these NGS technologies perform multiparallel sequencing, which improves the throughput and speed and reduces costs.

Roche 454 pyrosequencing was announced by the 454 Life Sciences Co. in 2005. Pyrosequencing is based on sequencing by synthesis and relies on the detection of the released pyrophosphate when a nucleotide polymerizes to the nascent DNA chain (van Dijk et al., 2014). The template DNA is divided into 300- to 500-base single-stranded fragments (Fig. 2). Each DNA fragment was connected with two specific adapters at both ends and then attached to a 20 μm bead and transferred to a PTP hole (29 μm). The bead is emulsified to form a water-in-oil structure. The DNA fragment is amplified by performing emulsion PCR to form thousands of repetitions. In a single pyrosequencing cycle, four dNTPs (dATP, dGTP, dCTP and dTTP) are added to the PTP hole, and only one of them correctly matches the leading chain and is integrated into the nascent DNA. As soon as the correct dNTP is added and polymerized, the pyrophosphate is released to trigger luciferase-mediated light emission. The signal is captured by a spectrum detector. The addition of the other three unpaired dNTPs does not generate a signal, and they will subsequently be removed. The combined data from hundreds of pyrosequencing cycles are used to generate DNA sequence reads. Thousands of PTP holes are arrayed on a PTP board. Therefore, numerous pyrosequencing reactions can be conducted simultaneously.

The Illumina Genome Analyzer was first introduced by Solexa in 2006 and purchased by Illumina in 2007. The Illumina platform is based on sequencing by synthesis (Morey et al., 2013; Ghanbari et al., 2015). The template DNA sequencing, genomes and markers for microbes
DNA is first divided into 100- to 200-base single-stranded fragments. Both ends of the fragment are connected with an oligonucleotide adaptor. The two adaptors are complementarily matched with forward and reverse primers immobilized on a glass surface. The DNA fragment is amplified by performing bridge PCR to generate thousands of repeats. Repeats from a single DNA fragment form a separate strand by linearization. DNA sequencing is then performed by using four specific dNTPs. The dNTPs contain a specific cleavable fluorescent blocking group at the 3'-OH end. In each sequencing cycle, the incorporation of a dNTP into the nascent DNA stimulates the release of a fluorescent signal, which is captured by a detector. Then, the blocking group on 3'-OH is removed to continue the next sequencing step. Combined signals from hundreds of sequencing cycles are used to generate DNA sequence reads.

The SOLiD platform was introduced by Applied Biosystems in 2007. In contrast to sequencing by synthesis, SOLiD relies on the method of sequencing by ligation (Morey et al., 2013). The NDA library preparation method is similar to Roche 454 pyrosequencing. The template DNA is first broken down into 30- to 50-base single-stranded fragments. Each DNA fragment linked with two adaptors at both ends and then attached to a 1 μm bead. The bead is immobilized on a glass slide. The DNA fragment is amplified by performing emulsion PCR. Sequencing is performed by using sixteen classes of 8-base fluorescent nucleotide probes and 5 classes of primers. In the 8-base probe, the 1st and 2nd positions at the 3'-end can be occupied by any combination A, T, G or C, resulting in a total of 16 probes. The 8th probe position is linked with four fluorescent groups, which correspond to the first two nucleotides at the 3'-end (mainly the 1st position). The 3rd and 4th positions of the probe can match any base. The 5th nucleotide is the cleavage site. Five universal primers match the continuous positions (n, n + 1, n + 2, n + 3 and n + 4) on the template DNA. Sequencing is initiated by hybridization. The first primer is hybridized to the template DNA (the initial site, n). Then, an 8-base probe is introduced, which correctly matches the 1st and 2nd positions of the template DNA. Therefore, the fluorescent signal from the probe reflects the nucleotides of the 1st and 2nd positions (mainly the 1st position). After recording the signal, the fluorescent group is removed by cutting at the 5th position of the probe. Another probe matching the 6th and 7th positions of template is connected at the 5th cleavage site. Therefore, the second fluorescent signal reflects the 6th and 7th nucleotides. These steps are repeated until the ligation reaction is complete. The signals of the original consecutive fluorescent codes (n) are obtained, followed by melting. The second universal primer (n + 1) is used to obtain the corresponding fluorescent codes of the 2nd and 3rd positions, the 7th and 8th positions and so on. Then, three other universal primers (n + 2, n + 3 and n + 4) are used to obtain the three consecutive corresponding fluorescent codes. By combining the five signals (n, n + 1, n + 2, n + 3 and n + 4) of the colour codes and the read matrix, the original sequence of the template DNA can be calculated.

The three NGS platforms exhibit different characteristics (Liu et al., 2012). Roche 454 sequencing produces a maximum read length of approximately 700 bases, which is longer than the read lengths generated by Illumina sequencing (150–200 bases) and SOLiD (30-50 bases). In addition, Roche 454 sequencing is faster than Illumina or SOLiD sequencing. However, the Roche 454 platform presents an insufficient sequencing accuracy. Because dNTPs are not added to the last base of the DNA ligation chain, the last base of the sequence cannot be read. The Illumina sequencing platform exhibits the highest sequencing throughput and lowest operating cost per base. However, Illumina sequencing produces a shorter read length and therefore requires a greater sequencing depth. The two-base coding and verification system of the SOLiD platform exhibits the greatest accuracy. However, the computing steps for the colour-coding matrix and the combination of iterative data are complicated. Moreover, the shorter read length of this platform requires a greater sequencing depth. Several shortcomings commonly emerge in NGS platforms (Pushkarev et al., 2009). Generally, the shorter read length of NGS requires that the template DNA be highly fragmented. This demands a greater sequencing depth and complex data computing for obtaining the overall reads. Second, PCR amplification is strictly required. However, PCR results are often inconsistent in regions such as those with a higher GC% or repeated hairpins. PCR amplicons also show variations in abundance under uniform PCR procedures (van Dijk et al., 2018).

Third-generation sequencing

There are three leading TGS technologies: the Heliscope Single Molecule Sequencer (SMS), the single-molecule real-time (SMRT) approach and the Oxford Nanopore MinION sequencer (Reuter et al., 2015; Lu et al., 2016; van Dijk et al., 2018). Compared with NGS, the fundamental improvement achieved by TGS is that a single strain or longer DNA molecules can be sequenced without PCR amplification. In addition, TGS technology shows real-time performance, a higher throughput and reduced costs.

The Heliscope SMS was introduced by Helicos Biosciences in 2009 (Pushkarev et al., 2009; Reuter et al., 2015). The Heliscope SMS approach is based on...
sequencing by single-molecule synthesis. The template DNA is first divided into single-stranded fragments. Then, the 3'-end fragment is linked to a poly-A tail. Sequencing is performed on a HeliScope slide containing millions of flow cells. The flow cells contain a fixed with a poly T tail that both hybridizes with poly-A sequence of the DNA template and provides a primer for DNA synthesis. Sequencing is initiated by the addition of the four fluorescently labelled and 3'-OH-blocked dNTPs, which is similar to Illumina sequencing. In each sequencing cycle, four dNTPs are added in turn. Once the correct dNTP is added and polymerized, the fluorescent signal is released. The signal is captured by a highly sensitive detection system. Then, the blocking group on the 3'-OH is removed to begin the next sequencing step.

The SMRT TGS platform was launched by Pacific Bioscience in 2011 (van Dijk et al., 2018). SMRT sequencing is based on DNA synthesis, and the signal is captured via zero-mode waveguide (ZMW) detection (Fig. 3). The ZMW nanopore is a channel with a diameter of 10 nm, which provides limited space for DNA polymerization. At the bottom of the ZMW nanopore, a DNA polymerase is immobilized. The template DNA is disintegrated into single-stranded fragments of tens of kb. Both ends of a fragment are connected to two closed circular single-stranded DNA adaptors. The DNA fragment is then introduced into the nanopore and ligated to the polymerase via either adaptor. Four fluorescently labelled and 3'-OH-blocked nucleotides are added to the reaction cells to start the synthesis process. Immediately after nucleotide polymerization, a fluorescent signal is generated. At the same time, laser irradiation of the nanopore is performed, and the fluorescent signal is amplified to a detectable level and transmitted to the nanopore-external space. Thus, the undetectable fluorescent signal in the ZMW pore can be captured. Once the blocking group on 3'-OH is removed, the next sequencing cycle continues. There are approximately 150 000 ZNWs in an SMRT unit, which is enough to obtain a sufficient throughput.

MiniION sequencing was introduced by Oxford Nanopore Technologies in 2014 (Mikheyev and Tin, 2014). MiniION sequencing is based on DNA electrophoresis, in which a-haemolysin nanopores distributed across a semipermeable membrane serve as channels for DNA electrophoresis. Cyclodextrins covalently bind to the nanopores to increase nucleotide-channel interactions. First, the template DNA is fragmented by using Covaris g-TUBEs to form single-stranded DNA fragments. The two ends of the DNA fragment are connected with two adapters. The lead adapter (Y adapter) is added to the 5'-end, and the hairpin adapter (HP adapter) is added to the 3'-end. An electric field is applied to both sides of the membrane to provide a driving force for DNA cross-channel electrophoresis. Driven by voltage, DNA fragments enter and pass through the pores and interact with cyclodextrin in the process. Different nucleotide bases (A, T, G and C) interact with cyclodextrin differently and generate corresponding current waves. The ion current is measured and characterized to obtain the sequence of template DNA.

The three TGS platforms have different characteristics. The HeliScope SMS has not been widely used, because of its relatively slower speed, shorter read length and higher price. SMRT is the most commonly used TGS approach. In recent years, SMRT technology has been highly improved to achieve a sufficient throughput and cost-effectiveness. The SMRT PacBio RSII platform produces a read length of 10–15 kb and a throughput of 0.5–1.0 Gb per run (van Dijk et al., 2018). The read length of Nanopore MinION sequencing is similar to that of PacBio RSII. However, the error rate of Nanopore MinION (20–40%) is higher than that of PacBio RSII (10–15%; Lu et al., 2016). However, the Nanopore MinION sequencer has attracted considerable interest.
due to its smaller size, cheaper equipment and lower running costs.

**WGS and genetic marker identification of related microbes on F & V**

**WGS and genetic marker identification of related foodborne pathogen**

The *Escherichia* genus contains three species: *E. coli*, *E. albertii* and *E. fergusonii*. *E. coli* exhibits many strains, which usually colonized the intestines of humans and other mammals and are considered to be part of the intestinal flora. Gut *E. coli* can be released into the environment via feces. Therefore, the count of *E. coli* can reflect the extent of fecal contamination. In addition, several *E. coli* strains are serious opportunistic pathogens that cause food poisoning. To date, 17,952 *E. coli* genomes have been registered (Fig. 4), and approximately 1000 representative references have been summarized (NCBI genome ID 167). The first *E. coli* genome was obtained for strain K-12 MG1655 by shotgun sequencing (Blattner et al., 1997). Subsequently, Hayashi et al. reported the genome of the pathogenic strain *E. coli* O157:H7 RIMD 0509952 (Hayashi et al., 2001). The genome of *E. coli* O157:H7 is 859 kb larger than that of strain K-12 MG1655, and the comparison of their genomes showed extensive polymorphisms (Hayashi et al., 2001). There are fewer reports of *E. albertii* and *E. fergusonii* as pathogens responsible for food poisoning. Genome sequencing has revealed 89 and 18 strains of *E. albertii* and *E. fergusonii* respectively (NCBI data). The representative *E. albertii* strain KF1 was the first to be sequenced and reported (NCBI genome ID 1729; Fedoruk et al., 2014). 16S rDNA sequencing has been used to distinguish *E. coli*, *E. albertii* and *E. fergusonii* (Maifreni et al., 2013). Multiplex PCR based on the *cdgR*, *EAKF1_ch4033* and *EFR_0790* genes can efficiently distinguish *E. coli*, *E. albertii* and *E. fergusonii* (Lindsey et al., 2017). For the identification of *E. coli*, the reported genetic markers mainly include *flIC*, *Vt1*, *Vt2*

![Fig. 4. Genomic information for major foodborne pathogen species of *Escherichia coli* (*E. coli*), *Salmonella enterica* (*S. enterica*), *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* (*L. monocytogenes*), *Shigella sonnei* (*S. sonnei*) and *Campylobacter jejuni* (*C. jejuni*).](image)
The Salmonella genus contains two pathogenic species, and Salmonella enterica and S. bongori. S. enterica can be subdivided into six subspecies: enterica (I), salamae (II), arizonae (Illa), diarizonae (Illb), houtenae (IV) and indica (VI; Agbaje et al., 2011; Lamas et al., 2018). Alternatively, S. enterica can be subdivided into approximately 2,500 different serotypes according to somatic (O) and flagellar (H) antigens (Tindall et al., 2005; Grimont and Weill, 2007). S. enterica subspecies I causes the majority of cases of foodborne diarrhoea (Sanchez-Vargas et al., 2011; Rezende et al., 2016). S. enterica subspecies I exhibits approximately 1531 serotypes (Desai et al., 2013; Lamas et al., 2018). To date, approximately 11,215 strains of S. enterica have been registered (Fig. 4), and approximately 340 representative references concerning S. enterica genomc research have been summarized (NCBI genome ID 152). The genome of the representative S. enterica strain Typhi strain CT18 was the first to be reported (Parkhill et al., 2001). Detailed genomic comparisons between S. enterica typhi, E. coli and S. typhimurium have been reported (McClelland et al., 2001). The genomes of approximately 20 strains of S. bongori have been registered, among which the representative strain is S. bongori NCTC 12419 (NCBI genome ID 1089). The sseL, spvC (Peterson et al., 2010), avrA, stn, stn (Amin et al., 2016), spv, hut, fiJ (Alzwghaibi et al., 2018), hila, fimA and hns (Jeyasekaran et al., 2011) gene regions have been reported as markers for the Salmonella genus. In addition, multiplex PCR analysis based on the fiJB, gatD, invA, mdcA, stn and STM4057 genes is used for Salmonella genus identification (Lee et al., 2009). For S. enterica species, the AceK, fiIC, invA, orfC, sdt, setA, ssaN, STM2745, STM4492 and 16S rRNA genes are widely used for PCR identification and detection (Lee et al., 2009; Postollec et al., 2011). The fiIC, gnd, and mutS genes are used to distinguish S. bongori from other Salmonella pathogens (Soler-Garcia et al., 2014).

The Staphylococcus genus contains several species related to skin infection and food poisoning (mainly Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis, Staphylococcus saprophyticus and Staphylococcus pseudintermedius). Specifically, S. aureus is an important pathogen associated with toxin-related food poisoning. As of recently, the genomes of approximately 10,630 S. aureus strains have been registered (Fig. 4), and approximately 300 representative references have been summarized (NCBI genome ID 154). The genomes of two S. aureus strains, N315 and Mu50, were the first to be determined by performing shotgun sequencing (Kuroda et al., 2001). The characteristics of the genomes of potentially pathogenic species including S. epidermidis, S. lugdunensis, S. saprophyticus and S. pseudintermedius have been summarized (Table 1). Specific regions of mecA, nuc, femA-SA, femA-SE, orfx-SCCmec, spa, gyrB and 16S rRNA are used as markers for S. aureus identification (Hirvonen, 2014). Based on polymorphisms in the femA gene, S. aureus and S. epidermidis can be differentiated (Jukes et al., 2010). Recently, multilocus sequence typing (MLST) was performed on femA, tuf, rpoB, gap, pyrH and ftsZ to identify Staphylococcus strains accurately (Song et al., 2019). The staphylococcal enterotoxin (se) genes of sea, seb, sec and see have been used to monitor toxic S. aureus in food (Omwenga et al., 2019).

The Listeria genus contains the pathogenic species L. monocytogenes and L. seeligeri. L. monocytogenes is an important foodborne pathogen that contaminates various F & V and causes human listeriosis (Buchanan et al., 2017). About the genomes of 3063 strains of L. monocytogenes have been registered to date (Fig. 4), and nearly 80 genomic references have been summarized (NCBI genome ID 159). The genome of the representative L. monocytogenes strain EGD-e was the first to be obtained (Glaser et al., 2001). L. seeligeri is reported less often in food, and its genomic information is summarized in Table 1. Methods for L. monocytogenes identification have been reviewed previously (Gasanov et al., 2005; Välimaa et al., 2015). PCR-based detection has mainly been performed for the hly, iap, mpl, prfA, inlA, inlB, actA (Gasanov et al., 2005), plcA and 16S RNA genes (Xu et al., 2008).

The Shigella genus contains four foodborne pathogens, Shigella flexneri, Shigella boydii, Shigella sonnei and Shigella dysenteriae (Warren et al., 2006). Shigella pathogens contaminate a variety of foods and exhibit diverse occurrences and different epidemiologies (Warren et al., 2006; Levin, 2009; Lin et al., 2010). Shigella sonnei serotype 1 causes deadly epidemics, S. flexneri causes endemic infection, foodborne diseases associated with S. boydii occur mainly in developing countries, and foodborne diseases associated with S. sonnei occur in developed countries (Hale, 1991). To date, approximately 480 strains of Sh. flexneri (NCBI DNA sequencing, genomes and markers for microbes 343

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The genome ID 182), 113 strains of *S. boydii* (genome ID 496), 1338 strains of *S. sonnei* (genome ID 417; Fig. 4) and 67 strains of *S. dysenteriae* (genome ID 415) have been registered (Table 1). The genome of *S. flexneri* strain 2a str. 301 was the first to be obtained (Jin et al., 2002). *S. flexneri* serotype 2a and *E. coli* K12 MG1655 share a high degree of genomic similarity (Stephens and Murray, 2001; Yang et al., 2005). They exhibit a common sequence of approximately 3 Mb (65% in *E. coli*) that encodes 2790 proteins. In PCR-based detection assays, specific gene regions of *ipaH*, virA, *ial* and 16S rRNA have been used as targets for *Shigella* genus identification (Warren et al., 2006). The *ipaH* gene, encoding the invasive plasmid antigen H, is carried by four *Shigella* species (Dutta et al., 2001; Warren et al., 2006). Specific regions of the *rfc* gene of *S. flexneri*, the *wbgZ* gene of *S. sonnei* and the *rfpB* gene of *S. dysenteriae* have been used to differentiate the three *Shigella* species (Ojha et al., 2013). SSR markers that can distinguish *Shigella* species have also been identified (Sahi et al., 2015). A recent study differentiated all four *Shigella* species by performing multiplex PCR analysis of differentiated genes (Kim et al., 2017a), for which a putative restriction endonuclease gene specific to *S. sonnei*, a hypothetical protein gene specific to *S. boydii* and *S. dysenteriae* and a repressor protein gene specific to *S. flexneri* were used.

The Campylobacter genus includes two major species of foodborne pathogens, *C. jejuni* and *C. coli*. To date, about the genomes of 1615 strains of *C. jejuni* have been registered (Fig. 4), and nearly 100 representative references describing genomic research have been summarized (NCBI genome ID 149). The genome of *C. jejuni* is relatively small, with a low GC% (Table 1). The genome of the representative *C. jejuni* strain NCTC 11168 has been reported (Parkhill et al., 2000), and has been indicated to harbour only a few repeat sequences and no transposons, phage remnants or insertion elements (Parkhill et al., 2000; Dorrell et al., 2001). *C. coli* is another pathogen that shows a distinctive epidemiology (Gillespie et al., 2002). To date, 928 genomic sequences have been registered for *C. coli*, and nearly 40 representative references have been summarized (NCBI genome ID 1145). The *hip*, 16S rRNA, *rsr*, *cfaF*, *porA*, *hyp*, *cjaA*, *ceuE*, *hipO*, *mapA*, *ceuA*, *askD*, *glyA*, *lpxA*, *ccoN*, *ORF-C* sequence, *rpoB*, *oxidoreductase gene*, *cfaA* and *pepT* genes are widely used for the PCR identification of *C. jejuni* (Frasao et al., 2017). The other related genetic regions used for *C. coli* identification are summarized in Table 1.

WGS and genetic marker identification of related phytopathogenic fungi

The *Penicillium* genus contains several pathogenic species, particularly *P. expansum*, *P. digitatum*, *P. griseofulvum*, *P. italicum* and *P. citrinum*. The majority of these species are related to the postharvest decay of *F. V. P. chrysogenum* was the first sequenced species in this genus, as it is used as an industrial penicillin producer (van den Berg et al., 2008). *P. expansum* is an important pathogen that accelerates corruption in various produce species (Nie, 2017; Shen et al., 2018a). *P. expansum* also produces the mycotoxins patulin and citrinin. *P. expansum* strain R19 was the first to be sequenced (Yu et al., 2014). To date, the genomes of nine strains of *P. expansum* have been registered (NCBI genome ID 11336; Fig. 5). A representative genome was reported for *P. expansum* from strain MD-8 (Ballester et al., 2015). *P. digitatum* causes postharvest green mould in citrus fruits (Marcet-Houben et al., 2012). The genomes of two *P. digitatum* strains, PHI26 and Pd1, have been reported (Marcet-Houben et al., 2012). *P. italicum* causes postharvest blue mould on citrus fruit (Deng et al., 2018). The representative strain of *P. italicum* PHI1 was reported (Ballester et al., 2015). Genomic comparison showed that *P. expansum* and *P. italicum* present differences in gene clusters related to secondary metabolism (Ballester et al., 2015; Li et al., 2015). Fifteen genes for patulin biosynthesis have been identified in *P. expansum*, which are located in a gene cluster (Ballester et al., 2015; Li et al., 2015). These genes and functions have been reviewed previously (Puel et al., 2010). Several methods, including RAPD (Schena et al., 2000), SNP (Piombo et al., 2018) and microsatellite analysis (Mohmed et al., 2010), have been used for distinguishing *Penicillium* species. The isoeoxypipenyldenogenase (*IDH*) gene is considered to be a useful marker for distinguishing patulin-producing and nonproducing *Penicillium* species (De et al., 2016; Rhamm et al., 2016). Several gene regions, such as the *patF* (Tannous et al., 2015), *ITS* (Hammani et al., 2017), *Pepg1* (Ostry et al., 2018) and polygalacturonase genes (Hesham et al., 2011), have been used for the identification of *P. expansum*. The specific genes employed for the identification of *P. digitatum*, *P. griseofulvum*, *P. italicum* and *P. citrinum* are summarized in Table 2.

The *Alternaria* genus contains several pathogenic species, particularly *A. alternata*, *A. arborescens*, *A. brassicicola* and *A. solani*. These pathogens commonly cause plant diseases and postharvest rot (Harteveld et al., 2014). Additionally, *Alternaria* species can produce host-specific phytotoxins (HSTs), which differ between plant species (Akamatsu et al., 1999). *Alternaria* toxins are a group of mycotoxins produced by *Alternaria* species that mainly include tenuazonic acid (*TeA*), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (Ten) and altenuene (ALT). However, the genes responsible for *Alternaria* toxin biosynthesis have not yet been confirmed. To date, six strains of *A. alternata* have been
registered (NCBI genome ID 11201; Fig. 5). Nguyen et al. (2016) reported the first draft genome of *A. alternata* ATCC 34957, obtained using PacBio SMRT technology, and discussed the gene regions related to mycotoxin metabolism. *A. arborescens* is another pathogen in this genus. Hu, et al. (2012) generated the first sequence for *A. arborescens* and demonstrated the horizontal transfer of the conditionally dispensable chromosome (CDC) carrying HST genes. The genome characteristics of *A. arborescens*, *A. brassicicola*, *A. solani* and *A. tenuissima* are summarized in Table 2.

Several methods have been used for the identification of *Alternaria* species, such as high-resolution melting (HRM) analyses, AFLP and SSR (Lorenzini and Zapparoli, 2014; Wolters et al., 2018). Genes such as histone-3, glyceraldehyde 3-phosphate dehydrogenase (*Gpd*), *Alt a1*, *AaSdhB*, *AaSdhC*, *AaSdhD*, ITS and *j-tubulin* have been used for *Alternaria* species identification (Table 2). The *Alt a1* gene is a widely used marker for *Alternaria* species (Gabriel et al., 2017). The polyketide synthetase (PKS) gene and nonribosomal peptide synthesis (NRPS) gene are essential for *Alternaria* toxin synthesis and regulation and can also be used to identify *Alternaria* toxin-producing species (Chen et al., 2015).

The *Aspergillus* genus is large and contains several saprophytic/pathogenic species, particularly *A. flavus*, *A. parasiticus*, *A. carbonarius*, *A. niger*, *A. tubingensis* and *A. westerdijkiae*. These species cause corruption in various agricultural products and produce the aflatoxin and ochratoxin A mycotoxins. To date, the genomes of a total of 60 *A. flavus* strains have been registered (NCBI data, genome ID 360; Fig. 5). A representative genome of *A. flavus* was reported from strain NRRL3357 (Nierman et al., 2015). Genomic comparison between *A. flavus* strains NRRL3357 and AF70 showed polymorphisms in their aflatoxin toxin gene cluster (Sharma et al., 2018). *A. parasiticus* is another important aflatoxin producer. Two strains of *A. parasiticus* have
been sequenced, and the representative strain is SU-1 (NCBI genome ID 12976). Genomic comparison revealed approximately 98% similarity between the six A. flavus species and 81% similarity between A. flavus and A. parasiticus species (Faustinelli et al., 2016). Fourteen strains of A. niger have been registered, the majority of which have been related to citrate production or sugar metabolism (Aguilar-Pontes et al., 2018; Laotanaachareon et al., 2018). A. carbonarius is another ochratoxin A-producing member of this genus, for which one strain has been sequenced (NCBI genome ID 947). As illustrated in previous reviews, the genes responsible for aflatoxin biosynthesis are integrated as a cluster that contains approximately 25 genes with a total length of 80 kb (Yu et al., 2004; Moore et al., 2010). This gene cluster contains the main regulatory genes aflR and aflS and the biosynthesis genes aflD, aflM, aflP, aflQ aflD, aflO and aflQ. Aflatoxin biosynthesis and regulatory genes have been widely used to identify toxin-producing species (Mahmoud, 2015; Hua et al., 2018). Polymorphisms of the calmodulin gene have been used to identify Aspergillus species (Palumbo and O’Keeffe, 2015). The β-tubulin gene can also be used for the specific identification of several Aspergillus species (Nasri et al., 2015; Falahati et al., 2016). The other genetic markers for Aspergillus species are summarized in Table 2.

The Fusarium genus contains several pathogenic species, particularly F. oxysporum, F. fujikuroi, F. verticillioides, F. proliferatum, F. graminearum and F. sporotrichioides. These pathogens cause serious diseases in crops and vegetables and produce toxic trichotheccene mycotoxins. The genomes of a total of 115 F. oxysporum strains have been registered (NCBI genome ID 707; Fig. 5). The representative strain of F. oxysporum f. sp. lycopersici 4287 has been reported (Ma et al., 2010). Genome comparison between F. graminearum, F. verticillioides and F. oxysporum revealed genomic lineage-specific (LS) regions in the Fusarium genus (Ma et al., 2010). F. fujikuroi is a plant pathogen that causes bakanae disease in rice and produces gibberellins (GAs). A total of 15 strains of this pathogen have been sequenced (NCBI genome ID 13188). The representative F. fujikuroi strain IMI 58289 has been reported (Wiemann et al., 2013). F. proliferatum is also a plant pathogen, and the genomes of 13 strains of this species have been registered (NCBI genome ID 2434). The representative strain F. proliferatum ET1 has been reported (Niehaus et al., 2016). For genetic detection, the Fusarium-specific gene regions that have been used have mainly included the translation elongation factor-1α (tef-1α) gene (Wu et al., 2016), ITS (Jedidi et al., 2018), SIX (Debbi et al., 2018) and FUM gene (Omoni et al., 2018) sequences. The genetic markers used for F. graminearum and F. sporotrichioides are summarized in Table 2.

The Colletotrichum genus contains several pathogenic species, particularly C. gloeosporioides, C. acutatum, C. coccodes and C. fructicola. C. gloeosporioides infects many crops, causing anthracnose diseases. A total of six strains of C. gloeosporioides have been registered, among which the representative strain is C. gloeosporioides SMCG1#C (NCBI genome ID 17739; Fig. 5). C. acutatum widely infects F & V (Cano et al., 2004; Heilmann et al., 2006). Two strains of this species have been registered (NCBI genome ID 38530). In addition, the genomes of two strains of C. coccodes and three strains of C. fructicola have been registered (Table 2). However, they were only registered at the scaffold level, and no related references have been reported. For PCR detection, the ITS, β-tubulin, actin, act, ApMat, cal, chs1, gapdh, gs, his3, tub2, glyceraldehyde-3-phosphate dehydrogenase and oxidase subunit 1 gene have been widely used (Tapia-Tussell et al., 2008; Chung et al., 2010; Ramdeen and Rampersad, 2013; Yang et al., 2015; Sharma et al., 2017).

The Monilinia genus contains several species related to brown rot on F & V, particularly M. laxa, M. fructicola, M. fructigena and M. polystroma. However, the genomes of Monilinia species have rarely been reported. To date, 2 strains of M. laxa, 2 strains of M. fructicola, 3 strains of M. fructigena (Fig. 5) and 1 strain of M. polystroma have been registered. The representative M. laxa strain 8L has been reported (NCBI data, genome ID 66927; Naranjo-Ortiz et al., 2018). The inter-simple sequence repeat (ISSR), RAPD (Fazekas et al., 2014) and HRM (Papavasileiou et al., 2016) methods have been used for interspecies identification. Specific regions of the cytchrome b gene (Ortega et al., 2019), laccase-2 gene (Wang et al., 2018b), β-tubulin gene (Fan et al., 2014), ITS (Guinet et al., 2016), MO368-1, Laxa (Cote et al., 2004) and small subunit (SSU) rDNA (18S) gene (Fulton and Brown, 1997) have been reported as markers for PCR-based detection.

The Botrytis genus contains the pathogenic species B. cinerea. B. cinerea causes serious grey mould diseases on F & V (Reich et al., 2016). Four strains of B. cinerea have been registered, among which the representative strain is B05.10 (NCBI genome ID 494; Fig. 5). Genomic comparative analysis between B. cinerea and Sclerotinia sclerotiorum revealed extensive genetic polymorphisms, but showed few significant polymorphisms in specific pathogenic clusters (Amsellem et al., 2011). The RAPD and HRM methods have been used to identify B. cinerea (Thompson and Latorre, 1999). The genetic markers used for B. cinerea include the ITS (Reich et al., 2016), the necrosis and ethylene-inducing protein gene (Munoz et al., 2016), the Bc-hch locus (Zhang et al., 2018), G3PDH, HSP60 and RPB2 (Zhou et al., 2014), the necrosis and ethylene-inducing protein 1 gene (Fan
et al., 2015), the species-specific sequence-characterized amplified region (SCAR) marker (Suarez et al., 2005) and the intergenic spacer (IGS) region (Diguta et al., 2010).

16S rDNA and ITS sequencing of the microbiome community on F & V

16S rDNA and ITS sequencing-based metagenomics

Metagenomic strategies are technological approaches that are increasingly being used to study the overall microbial community in complex biological samples (Cao et al., 2017). Significantly, metagenomics expands the scope of microbiology research and provides new insights into uncultivable microbes. This method is mainly based on polymorphisms in the bacterial 16S rDNA and fungal ITS regions, combined with powerful sequencing technologies, databases and software platforms (Fig. 6A). Total microbial DNA is directly extracted from research samples under this approach. rDNA PCR-based denaturing gradient gel electrophoresis (PCR-DGGE) was the first method developed to identify differential abundant microbes at a general level (Wang et al., 2015). The distinguished rDNA fragments are obtained by gel cutting, followed by sequencing. Then, the microbes are identified by performing sequence alignment against databases. In the 2000s, NGS could be used to sequence all of the obtained rDNA amplifications, which allows the microbial community to be analysed more deeply and comprehensively. The obtained sequences are assigned to operational taxonomic units (OTUs) based on similarity (Caporaso et al., 2010). Representative OTU sequences are identified (Fig. 6B). Additionally, OTU abundance provides relatively quantitative information for specific microbial taxa. Several analytical software platforms, such as the FLASH and QIIME packages, are used for downstream data analysis (Jüinemann et al., 2017). To address the short read length and PCR dependence of NGS, TGS-based metagenomics is promising and powerful (Uyaguari-Díaz et al., 2016). The long reads obtained via TGS can be used to directly identify microbes at the species or even strain level. Additionally, the sequence abundance accurately represents the number of specific microbes. Once the cost is reduced, TGS will become a powerful tool for metagenomic research.

Many studies have elucidated the bacterial and fungal communities on F & V by using 16S rDNA and ITS sequencing. For this review, we searched references mainly from the Web of Science, NCBI, ScienceDirect and CNKI platforms. A total of 64 original studies describing the microbiomes of various F & V were identified (Table 3). Illumina sequencing and Roche 454 pyrosequencing have been most widely used for these studies. PCR-DGGE combined with AB3730xl sequencing was mainly used in earlier studies. Until recently, no TGS-based metagenomic analyses of F & V had been reported. Based on common themes, we grouped these references into three categories: microbiome diversity between plant species/genotypes; regional/environmental factors and farming practices affecting microbiomes; and microbiomes affected by artificial treatment and quality control procedures in storage and processing.

Microbiome diversity between plant species/genotypes

Plant microbiomes are related to species/genotype specificity (Fig. 7). Recently, differential bacterial and fungal communities have been recorded on diverse fruits, including apples (Soliman et al., 2015), blueberries (Jiang et al., 2017), grapes (Pinto et al., 2014; Zhang et al., 2017a), kiwifruits (Purahong et al., 2018), spinach (Darlison et al., 2019) and tomatoes (Ottesen et al., 2013). Because of the importance of leafy vegetables in food control, the phyllosphere microbiomes have been...
monitored on vegetables such as spinach (Chen et al., 2018), lettuce (Higgins et al., 2018) and rocket (Darlison et al., 2019). In addition, the microbial community may be related to the specificity of plant tissue. The microbiomes differ among plant organs such as the fruits, leaves, flowers (Shade et al., 2013) and roots (Ottesen et al., 2013; Zhang et al., 2017b). However, they also show correlations between several plant tissues (Zhang et al., 2017b). The microbial community on F & V products is related to different characteristics and biological processes. In particular, the bacterial communities on several wine grape varieties have been found to differ, which may affect the fermentation process in winemaking (Bokulich et al., 2014). Additionally, the microbial communities of plants might be related to their chemical compositions. Recently, relationships between phyllosphere minerals and microbial communities have been observed in spinach and rocket (Darlison et al., 2019). Bacterial communities present in the plant rhizosphere could potentially be used as indicators of the soil environment and mineral efficiency (Jiang et al., 2017). Microbiome–host or microbiome–mineral interactions might be widespread. These NGS-based metagenomic studies have shown that the microbiomes of F & V are plant specific.

**Microbiomes affected by regional/environmental factors and farming practices**

The microbiomes of F & V are affected by differences in regional environments, farming practices and disease occurrences (Fig. 8A). Many environmental factors influence microbial activities, including temperature, humidity, light exposure, soil organic matter and mineral compositions (Yu et al., 2016; Higgins et al., 2018). Regional verification based on microbial communities has been conducted on shea fruits (El Sheikha et al., 2011), peaches (Bigot et al., 2015) and grapes (Mezzasalma et al., 2017; Mezzasalma et al., 2018). Recently, a comprehensive review summarized the complex relationships between wine quality, grape microbial communities and regional climates (Droby and Wisniewski, 2018). The natural microbiomes of grapes are important for fruit maturation and wine fermentation, especially regarding the

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Fig. 7. Microbiome specificity in various plant species and tissues.
formation of some secondary metabolites related to wine colour and flavour (Mezzasalma et al., 2017). Agricultural inputs and farming practices such as fertilizers and pesticides influence the formation of microbial communities on agricultural products (Allard et al., 2018; Chen et al., 2018). Fertilization has been reported to be a factor affecting the plant microbiomes of maize plants (Chen et al., 2018). The products and soil associated with organic and conventional farming practices show differences in their microbiome communities. Specifically, F & V such as grapes (Mezzasalma et al., 2017), apples (Abdelfattah et al., 2016), peaches (Bigot et al., 2015), lettuce and spinach (Leff and Fierer, 2013) produced from organic and conventional agriculture systems have been observed to exhibit differences in their microbial communities. However, the significant relationships between microbiomes and the regional environment and farming practices are only just beginning to be revealed.

Effects of artificial treatments in storage and processing on microbiomes

The treatments applied to F & V during postharvest processing and storage affect microbial communities (Fig. 8B). Refrigerated storage is a common approach that is considered to keep food fresh and nutritious. During cold storage, the microbial composition and activity are altered. Variations in microbial communities during cold storage have been reported in apples (Shen et al., 2018a), lettuce and spinach (Lopez-Velasco et al., 2011). Different fungal structures have been identified between harvest-point and stored samples (Shen et al., 2018a). Compared with the harvest-point samples, stored apples show increases in the relative abundance of several genera, particularly Aspergillus, Botrytis, Mucor and Penicillium. The spinach phyllosphere was observed to present a decrease in bacterial diversity after 15 days of storage at 4°C or 10°C, which might be related to the inhibition of bacterial activity by the lower temperature (Lopez-Velasco et al., 2011). In addition, physical and chemical treatments affect the microbial communities of postharvest F & V. Physical gamma irradiation treatment of romaine lettuce alters the leaf bacterial community and reduces the survival rate and regrowth ability of pathogenic bacteria (Dharmarha et al., 2019a). High-hydrostatic pressure treatments change the dynamics of the overall bacterial population and extend the shelf life of sweet cherries and asparagus (del Arbol et al., 2016a, b). Antibiotics and fungicides have been shown to alter the microbial communities and extend the shelf life of several F & V. Hypochlorite treatment alters the structure of bacterial communities and extends the shelf life of carrots (Dharmarha et al., 2019b). The application of fungicides alters the dynamics of yeast communities on grape berries (Milanovic et al., 2013). To improve postharvest storage, the relationships between the postharvest treatment of F & V and their microbial communities require more attention.

Conclusion

The development of DNA sequencing technology has provided an effective method for microbial WGS and genetic analysis. In recent decades, DNA sequencing technologies have been successfully developed including approximately ten operational platforms. By performing DNA sequencing, microbial WGS is largely promoted. Genetic studies provide DNA markers for microbial identification at the genetic level. These markers are extensively used in PCR or chip-based detection. On the basis of 16S rDNA and ITS sequencing, metagenomic approaches are now emerging technologies for analysing the entire microbial community in a complex F & V matrix. The microbiomes of F & V show huge differences between plant species/genotypes. In addition, the microbiomes of F & V are related to factors such as regional/environmental factors, farming practices and postharvest treatments. These studies shed light on
ways to improve F & V cultivation, disease prevention and quality control.

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Conflict of interest

The authors declare no conflict of interest.

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