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MINIREVIEW

# What's Inside That Seed We Brew? A New Approach To Mining the Coffee Microbiome

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**Coffee is a critically important agricultural commodity for many tropical states and is a beverage enjoyed by millions of people worldwide. Recent concerns over the sustainability of coffee production have prompted investigations of the coffee microbiome as a tool to improve crop health and bean quality. This review synthesizes literature informing our knowledge of the coffee microbiome, with an emphasis on applications of fruit- and seed-associated microbes in coffee production and processing. A comprehensive inventory of microbial species cited in association with coffee fruits and seeds is presented as reference tool for researchers investigating coffee-microbe associations. It concludes with a discussion of the approaches and techniques that provide a path forward to improve our understanding of the coffee microbiome and its utility, as a whole and as individual components, to help ensure the future sustainability of coffee production.**

The cup of coffee we drink today is prepared from the roasted seeds of *Coffea arabica* and/or *Coffea canephora* var. robusta, which are produced in many tropical nations (1). Coffee exports topped 109 million bags, valued at over $20 billion in the 2011-to-2012 coffee year, and production continues to increase (2, 3). Given its economic importance, an extensive body of scientific literature exists examining agronomic methods and the chemistry of the coffee beverage (4, 5). The microbiology of coffee seeds and changes in microbial communities induced by coffee processing have also been investigated, though less extensively.

Increasing interest in improving coffee quality, protecting coffee production, and creating sustainable coffee markets has prompted recent investigations to understand and exploit the microbial communities associated with coffee at all stages of plant development and seed processing. This review highlights and synthesizes the literature relevant to our understanding of the coffee microbiome with a strong focus on seed- and fruit-associated microbes and their roles in coffee production and processing. This work also reviews how coffee microbes are currently being exploited and how more extensive use of microbes may improve coffee health, production practices, and beverage quality.

## COFFEE PRODUCTION AND THE COFFEE MICROBIOME

In order to understand the nature of the coffee microbiome, one needs to understand the production and processing activities that could affect its structure. Coffee plants are predominantly propagated using seed stock germinated under conditions with various degrees of control in greenhouses or outdoor planters. Seedlings are colonized by seed endophytes, with some microbes adhering to the seed parchment and others present in the germination substrate. After 6 to 12 months, seedlings are transferred to field settings, where they are exposed to prevailing environmental conditions, opening the plant to additional microbial colonization from a variety of sources. Some modes of microbial immigration and emigration, such as splash dispersal and human vectoring, are strongly impacted by crop density and specific cultural practices that vary based on cultivar and growing region. Regardless of origin, coffee plants are actively pruned to increase flowering potential and maintain a manageable plant size (5). Pruning can vector microbes between plants, provide new entry points for colonization, and open new vegetative growth to colonization (6). Another factor generally affecting microbial colonization is the plant variety (7). For example, while they are producers of high-quality seeds, some of the oldest coffee varieties are often susceptible to serious coffee diseases. Breeding programs are actively pursuing new cultivars with improved disease resistance and good crop quality (reviewed in references 7 and 8). Coffee plants flower and fruit biennially, and fruit development is not synchronized. Fruits are generally harvested manually or are harvested mechanically with a mix of mature and immature fruits. Thus, human vectoring of microbial populations onto fruit that enter the processing stream is likely to occur during harvest (9). Following harvest, the coffee fruits, or cherries, are sorted and subjected to various post-harvest processes to ready them for storage and shipment as green coffee. These processes remove fruit debris, allow microbial growth, and promote fermentation, which can change the physical, chemical, and biological properties of the seeds (10).

The different processing methods used in the industry likely impact the seed microbiome in very different ways (1). Dry, or natural, processing involves harvesting ripe coffee fruits and spreading them over a drying surface (e.g., cement patios or packed earth), where they are allowed to dry for 18 to 24 days (1). This extended environmental exposure likely results in a significant lot-to-lot variation in microbial load due to differences in temperature, humidity, and arthropod activity (11–13). Once the cherries are dry, the desiccated fruit tissue is removed to produce parchment coffee, which is stored and subsequently polished to

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remove the endocarp, making green coffee. Parchment and green coffee seeds are stored at 10 to 15% moisture, a level that allows only minimal microbial activity, prior to export and subsequent roasting. Wet processing, or washed coffee, produces more-dramatic but shorter-lived changes in the chemical and microbial environment of the seed. After the fruit exocarp and pulp are removed, the mucilage-covered seeds then ferment for 24 to 72 h in water at ambient temperatures. Microorganisms from the fruit, seed, handlers, water, and processing machinery can all seed the fermentation and have the potential to affect the coffee quality. After fermentation, the degraded mucilaginous pectin layer is removed from the seeds. The seeds are then rinsed and dried to form parchment coffee. A large body of literature exists that explores the impacts of these coffee processing methods on coffee quality, and it is generally accepted that wet-processed beans tend to produce a more acidic cup of coffee with less body (1, 13).

## MICROBIAL POPULATIONS INHABITING COFFEE FRUIT AND SEED: WHO IS THERE?

In an effort to better understand coffee fermentation, a number of studies have examined the microbial diversity of the coffee seeds during the use of the different coffee processing methods (including, e.g., those reviewed in references 1 and 13). Other studies have looked beyond the fermentation process and have monitored microbes throughout the processing chain, from endophytic communities of coffee seeds and fruit to epiphytic communities present during green coffee storage. By synthesizing the results of these studies here, we present a more complete picture of coffee seed-associated microbial communities that can be developed and exploited to improve coffee health and quality.

## FILAMENTOUS FUNGI AND YEASTS

Fungi are known for their metabolic plasticity, variable metabolite production, and highly prevalent plant associations (14), and they comprise a substantial portion of the coffee microbiome. A total of 215 named species of fungi have been found in association with coffee fruit and seed tissues (Table 1; see also Table S1 in the supplemental material) (15–38). These fungi represent 12 classes from three different phyla. The Basidiomycota ($n = 17$ species) and Mucoromycotina ($n = 5$ species) were cited more rarely than the Ascomycota ($n = 193$ species). Over 40% ($n = 80$) of the coffee-associated Ascomycota fungi belong to the Eurotiomycetes and consist almost exclusively of members of the genera *Aspergillus* and *Penicillium*. Of these, *Aspergillus niger* was the most commonly cited species (Table 1).

The extensive knowledge of Eurotiomycetes is likely due, in part, to the emphasis given to unraveling the sources and levels of ochratoxin A (OTA) contamination in coffee (reviewed in references 39 and 40). OTA contamination is linked to *Aspergillus ochraceus* and *Aspergillus carbonarius*, though *Aspergillus niger* and several *Penicillium* species have been implicated as well (41–43). Both *Aspergillus* and *Penicillium* spp., including those that are capable of producing OTA, were encountered very frequently (Table 1) and appear to be ubiquitous in coffee production from fruit to roasting.

The ascomycete yeasts, in the classes Saccharomycetes ($n = 58$) and Schizosaccharomycetes ($n = 1$), accounted for a third of coffee-associated Ascomycota species (Table 1). The majority of these citations have come from studies examining the microbial community dynamics of coffee fermentation. Agate and Bhat (44) and Van Pee and Castellani (45) showed that a number of yeasts from Saccharomycetes derived from wet fermentations. Avallone et al. (46) and Masoud et al. (47) confirmed that *Saccharomyces*, *Pichia*, and *Candida* spp. dominated the yeast communities in these wet fermentations through the use of culture-independent surveys. Silva et al. (48) neatly showed that *Debaryomyces* and *Pichia* spp. dominate natural fermentation communities after the turnover of bacteria from earlier on in the fermentation process.

Sordariomycetes, dominated by *Fusarium* spp., representing 18% of the Ascomycota listed in Table 1, were noted primarily in studies focused on microbial community dynamics during natural coffee processing (9, 48, 49). Other Sordariomycetes, such as *Beauveria bassiana*, were found living endophytically in coffee seeds (50). The remaining 10% of coffee-associated Ascomycota were distributed among the Dothideomycetes ($n = 14$ species) and Leotiomycetes ($n = 2$ species), with a few species of uncertain taxonomic placement (*incertae sedis*, $n = 2$ species). Of these, the dothideomycete *Cladosporium cladosporioides* was cited as appearing in high abundance among species encountered in microbial succession studies and as an endophyte of coffee tissues (9, 48–51).

Members of the other fungal taxa were generally encountered at low frequency. Some members of the Mucoromycotina were cited as spoilage organisms in a number of studies, though poor identification of many of these taxa hindered their inclusion in this review (Table 1). Of note, members of other fungal taxa, especially those from groups with complex systematics such as *Fusarium* spp., are often given genus-only identifications, which may lead to an underestimation of their importance in the coffee microbiome. The coffee pathogen *Hemileia vastatrix* (Basidiomycota) is also commonly encountered in the coffee literature due to its negative impact on coffee production. Most of the Basidiomycota and the rarely cited Ascomycota showed up in surveys of endophytic coffee fungi (50, 52), which suggests a casual association with coffee fruits and seed.

## BACTERIA

Bacteria play critical roles in food processing and fermentation and in plant and soil health and participate in important microbe-microbe interactions (53, 54). Previous studies have noted 106 coffee-associated bacterial species, distributed among six classes belonging to four bacterial phyla (the *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroides*) (Table 2; see also Table S2 in the supplemental material) (15–38). *Proteobacteria* was the most species-rich phylum ($n = 58$ species), followed by *Firmicutes* ($n = 32$) and *Actinobacteria* ($n = 14$). *Bacteroides* was represented by two species in a single class.

Gammaproteobacteria made up 85% of the total *Proteobacteria* from coffee; the *Enterobacteriales* family appears to be the most species rich ($n = 37$) (Table 2). *Enterobacter aerogenes* and *Pantoea agglomerans* were the two most abundantly encountered *Enterobacteriales* species. Members of *Enterobacteriales*, along with members of *Pseudomonadales*, are common soil- and plant-associated Gram-negative bacteria. These groups are expected in humid, nutrient-rich environments like those found during coffee processing.

*Bacillus* spp. constituted 43% of the coffee-associated *Firmicutes* species. Of these, *Bacillus cereus* and *B. subtilis* were cited more commonly than any other bacterial species. Both species were found in both wet and dry fermentations and as endophytes. The

**TABLE 1** Fungal groups reported to be associated with coffee seeds[a]

| Phylum/subphylum | Order | Genus | No. of citations | No. of species | Most commonly cited species |
|---|---|---|---|---|---|
| Ascomycota | Botryosphaeriales | *Botryosphaeria* | 1 | 1 | |
| Ascomycota | Capnodiales | *Cercospora* | 1 | 1 | |
| Ascomycota | Capnodiales | *Cladosporium* | 7 | 4 | *C. cladosporioides* |
| Ascomycota | Capnodiales | *Mycosphaerella* | 1 | 1 | |
| Ascomycota | Dothideales | *Aureobasidium* | 1 | 1 | |
| Ascomycota | Pleosporales | *Alternaria* | 4 | 1 | |
| Ascomycota | Pleosporales | *Bipolaris* | 1 | 1 | |
| Ascomycota | Pleosporales | *Drechslera* | 1 | 1 | |
| Ascomycota | Pleosporales | *Exserohilum* | 1 | 1 | |
| Ascomycota | Pleosporales | *Phoma* | 2 | 1 | |
| Ascomycota | Pleosporales | *Ulocladium* | 1 | 1 | |
| Ascomycota | Eurotiales | *Aspergillus* | 36 | 39 | *A. niger* |
| Ascomycota | Eurotiales | *Eurotium* | 9 | 5 | *E. chevalieri* |
| Ascomycota | Eurotiales | *Paecilomyces* | 3 | 1 | |
| Ascomycota | Eurotiales | *Penicillium* | 18 | 35 | *P. citrinum* |
| Ascomycota | *Incertae sedis* (Ascomycota) | *Fusariella* | 2 | 1 | |
| Ascomycota | *Incertae sedis* (Ascomycota) | *Torulopsis* | 1 | 1 | |
| Ascomycota | Helotiales | *Monilia* | 1 | 1 | |
| Ascomycota | Orbiliales | *Arthrobotrys* | 1 | 1 | |
| Ascomycota | Diaporthales | *Phomopsis* | 2 | 1 | |
| Ascomycota | Glomerellales | *Colletotrichum* | 3 | 4 | *C. kahawae* |
| Ascomycota | Hypocreales | *Acremonium* | 1 | 1 | |
| Ascomycota | Hypocreales | *Beauveria* | 3 | 3 | *B. bassiana* |
| Ascomycota | Hypocreales | *Clavicipitaceae* | 1 | 1 | |
| Ascomycota | Hypocreales | *Cylindrocarpon* | 1 | 1 | |
| Ascomycota | Hypocreales | *Fusarium* | 10 | 14 | *F. solani* |
| Ascomycota | Hypocreales | *Gibberella* | 1 | 1 | |
| Ascomycota | Hypocreales | *Myrothecium* | 1 | 1 | |
| Ascomycota | Hypocreales | *Trichoderma* | 2 | 1 | |
| Ascomycota | Hypocreales | *Verticillium* | 1 | 1 | |
| Ascomycota | Sordariales | *Chaetomium* | 1 | 1 | |
| Ascomycota | Trichosphaeriales | *Nigrospora* | 1 | 2 | *N. oryzae*, *N. sphaerica* |
| Ascomycota | Xylariales | *Nodulisporium* | 1 | 1 | |
| Ascomycota | Xylariales | *Pestalotia* | 1 | 1 | |
| Ascomycota | Xylariales | *Pestalotiopsis* | 1 | 1 | |
| Ascomycota | Xylariales | *Xylaria* | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Arxula* | 3 | 2 | *A. adeninivorans* |
| Ascomycota | Saccharomycetales | *Blastobotrys* | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Candida* | 6 | 17 | *C. fermentati*, *C. membranifaciens* |
| Ascomycota | Saccharomycetales | *Citeromyces* | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Debaryomyces* | 1 | 2 | *D. hansenii*, *D. polymorphus* |
| Ascomycota | Saccharomycetales | *Geotrichum* | 3 | 3 | *G. candidum*, *G. fermentans* |
| Ascomycota | Saccharomycetales | *Hanseniaspora* | 3 | 2 | *H. uvarum* |
| Ascomycota | Saccharomycetales | *Issatchenkia* | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Kloeckera* | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Kluyveromyces* | 3 | 2 | *K. marxianus* |
| Ascomycota | Saccharomycetales | *Kodamaea*§ | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Meyerozyma*§ | 2 | 1 | |
| Ascomycota | Saccharomycetales | *Pichia* | 4 | 13 | *P. anomala* |
| Ascomycota | Saccharomycetales | *Saccharomyces* | 6 | 4 | *S. cerevisiae* |
| Ascomycota | Saccharomycetales | *Saccharomycopsis* | 1 | 2 | *S. fermentans*, *S. fibuligera* |
| Ascomycota | Saccharomycetales | *Sporopachydermia* | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Stephanoascus* | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Torulaspora* | 2 | 1 | |
| Ascomycota | Saccharomycetales | *Wickerhamomyces*§ | 1 | 1 | |
| Ascomycota | Saccharomycetales | *Williopsis* | 1 | 1 | |
| Ascomycota | Schizosaccharomycetales | *Schizosaccharomyces* | 1 | 1 | |
| Basidiomycota | Agaricales | *Schizophyllum* | 1 | 1 | |
| Basidiomycota | *Incertae sedis* (Agaricomycetes) | *Rhizoctonia* | 2 | 1 | |

(Continued on following page)

TABLE 1 (Continued)

| Phylum/subphylum | Order | Genus | No. of citations | No. of species | Most commonly cited species |
|---|---|---|---|---|---|
| Basidiomycota | Polyporales | *Phlebiopsis* | 1 | 1 | |
| Basidiomycota | Polyporales | *Trametes* | 1 | 1 | |
| Basidiomycota | Russulales | *Stereum* | 1 | 1 | |
| Basidiomycota | Exobasidiales | *Meira* | 1 | 1 | |
| Basidiomycota | Tilletiales | *Tilletia* | 1 | 1 | |
| Basidiomycota | *Incertae sedis* (Basidiomycota) | *Trichosporonoides* | 1 | 1 | |
| Basidiomycota | Wallemiales | *Wallemia* | 3 | 2 | *W. sebi* |
| Basidiomycota | *Incertae sedis* (Microbotryomycetes) | *Rhodotorula* | 2 | 1 | |
| Basidiomycota | *Incertae sedis* (Microbotryomycetes) | *Sporobolomyces* | 1 | 1 | |
| Basidiomycota | Pucciniales | *Hemileia* | 1 | 1 | |
| Basidiomycota | Tremellomycetidae | *Cryptococcus* | 2 | 3 | *C. albidus*, *C. laurentii* |
| Basidiomycota | Ustilaginales | *Pseudozyma* | 2 | 1 | |
| Mucoromycotina | Mucorales | *Absidia* | 1 | 1 | |
| Mucoromycotina | Mucorales | *Mucor* | 5 | 2 | *M. hiemalis* |
| Mucoromycotina | Mucorales | *Rhizopus* | 3 | 1 | |
| Mucoromycotina | Mucorales | *Syncephalastrum* | 1 | 1 | |

[a] The number of citations and the total number of species described are listed for each genus. Additionally, the most commonly cited species is listed for each genus containing multiple coffee-associated taxa. §, name updated.

members of *Firmicutes* also include *Lactobacillales* (46% of *Firmicutes*), which were found in high abundance during wet fermentations, especially *Leuconostoc* spp. (46, 51, 55). The *Actinomycetales* ($n = 15$ species) were represented by 12 different genera. The high number of different genera and low citation frequency among the members of this group suggest that they are casually associated with coffee fruits and seeds. Alternatively, the *Actinomycetales* may be involved only casually in coffee fermentations, which have been the focus of the majority of studies on coffee seed microbiology. To date, no reports of Archeae on coffee have been made, a reflection on the lack of application of the appropriate profiling tools.

## MICROBES FROM COFFEE FRUIT AND SEED: WHAT CAN WE DO WITH THEM?

The current understanding of the activities of the coffee seed microbiome is heavily based on investigations of microbial roles in coffee fermentation (13), microbial community composition and dynamics during fermentation (1, 13), and microbial impacts on coffee quality (13, 39, 40). Given the wide variety of conditions that fruit and seeds are exposed to, it is likely that geography- and process-specific variations exist in seed microbiomes beyond what has been recorded to date. However, as the majority of plant microbial populations are chemoheterotrophs, the chemistry of the fruits and seeds, combined with the moisture regimens to which they are subjected, likely contributes to a common set of environmental parameters that select for a core microbiome (56). As the research community begins to move beyond descriptive surveys of microbial diversity, it is from this core of coffee-adapted microbes that the most functionally interesting and useful microbes will emerge. Microbial inoculants have long been used to alter, preserve, and improve food products (57). It is now recognized that diverse microorganisms can be utilized to improve a wide range of agricultural and industrial products and processes beyond fermentation. Here, we highlight several applications where microbes have been, or could be, introduced to help improve coffee production and quality (Fig. 1).

## IMPROVING COFFEE QUALITY WITH MICROBES

OTA is now a major factor in assessing coffee quality, and it is allowable in coffee imports to Europe at levels of <5 ppb (58). Currently, cultural controls are promoted as the best methods for controlling OTA contamination, though the application of fungicides is also cited as a possible preventative measure (41, 59). Recently, interest has turned to trying to prevent OTA contamination through the use of biological agents to outcompete or inhibit the growth of *Aspergillus* spp. during processing (13, 60–63). Djossou et al. (63) examined the use of *Lactobacillus plantarum*, isolated from coffee pulp, as a biological antagonist against OTA-producing fungi. The authors were able to show an inhibitory effect on ochratoxigenic fungal growth; however, no mode of action was ascribed. In their work with yeasts isolated from coffee fermentation, Masoud et al. demonstrated an inhibitory effect on *Aspergillus ochraceus* growth and germination during cocultivation with coffee yeast strains (60, 61). Further, they were able to identify several volatile compounds produced by the yeasts, including 2-phenyl ethyl acetate, which could replicate the growth inhibition when *Aspergillus ochraceus* cultures were exposed to the compound alone. Such mode-of-action studies will become important in developing *in vivo* assays and applications. Velmourougane et al. (64) were able to demonstrate *in vivo* that application of a yeast treatment during processing resulted in a reduction of total ochretoxigenic mold incidence and OTA levels. Combined, these works present a pathway to establish biocontrol alternatives for control of unwanted microbes, namely, *in vivo* control efficacy studies coupled with *in vitro* mode of action studies. This pipeline can be improved on by introducing a more efficient, high-throughput screening strategy for candidate microbe recovery and identification.

**TABLE 2** Bacterial groups reported to be associated with coffee seeds[a]

| Phylum | Order | Genus | No. of citations | No. of species | Species most commonly cited |
|---|---|---|---|---|---|
| *Proteobacteria* | *Sphingomonadales* | *Sphingomonas*§ | 1 | 1 | |
| *Proteobacteria* | *Rhizobiales* | *Agrobacterium* | 1 | 1 | |
| *Proteobacteria* | *Rhizobiales* | *Methylobacterium* | 1 | 1 | |
| *Proteobacteria* | *Rhizobiales* | *Ochrobactrum* | 1 | 1 | |
| *Proteobacteria* | *Burkholderiales* | *Burkholderia* | 3 | 3 | *B. cepacia* |
| *Proteobacteria* | *Neisseriales* | *Chromobacterium* | 1 | 1 | |
| *Proteobacteria* | *Aeromonadales* | *Aeromonas* | 1 | 1 | |
| *Proteobacteria* | *Enterobacteriales* | *Cedecea* | 1 | 1 | |
| *Proteobacteria* | *Enterobacteriales* | *Citrobacter* | 4 | 4 | *C. freundii*, *C. sakazakii* |
| *Proteobacteria* | *Enterobacteriales* | *Enterobacter* | 6 | 5 | *E. aerogenes* |
| *Proteobacteria* | *Enterobacteriales* | *Escherichia* | 4 | 2 | *E. coli* |
| *Proteobacteria* | *Enterobacteriales* | *Hafnia* | 2 | 2 | *H. alvei* |
| *Proteobacteria* | *Enterobacteriales* | *Klebsiella* | 5 | 4 | *K. oxytoca* |
| *Proteobacteria* | *Enterobacteriales* | *Pantoea* | 5 | 3 | *P. agglomerans* |
| *Proteobacteria* | *Enterobacteriales* | *Providencia* | 1 | 2 | *P. alcalifaciens*, *P. mirabilis* |
| *Proteobacteria* | *Enterobacteriales* | *Salmonella* | 2 | 4 | *S. enterica* |
| *Proteobacteria* | *Enterobacteriales* | *Serratia* | 4 | 5 | *S. liquefaciens*, *S. plymutica* |
| *Proteobacteria* | *Enterobacteriales* | *Shigella* | 3 | 2 | *S. dysenteriae* |
| *Proteobacteria* | *Enterobacteriales* | *Tatumella* | 1 | 1 | |
| *Proteobacteria* | *Enterobacteriales* | *Yersinia* | 2 | 2 | *Y. frederiksenii* |
| *Proteobacteria* | *Pasteurellales* | *Mannheimia* | 1 | 1 | |
| *Proteobacteria* | *Pseudomonadales* | *Acinetobacter* | 1 | 1 | |
| *Proteobacteria* | *Pseudomonadales* | *Pseudomonas* | 4 | 8 | |
| *Proteobacteria* | *Xanthomonadales* | *Stenotrophomonas* | 1 | 1 | |
| *Proteobacteria* | *Xanthomonadales* | *Xanthomonas* | 1 | 1 | |
| *Firmicutes* | *Bacillales* | *Bacillus* | 6 | 14 | *B. subtilis* |
| *Firmicutes* | *Bacillales* | *Brochothrix* | 1 | 1 | |
| *Firmicutes* | *Bacillales* | *Kurthia* | 1 | 1 | |
| *Firmicutes* | *Bacillales* | *Paenibacillus* | 1 | 1 | |
| *Firmicutes* | *Lactobacillales* | *Enterococcus* | 1 | 2 | *E. faecalis* |
| *Firmicutes* | *Lactobacillales* | *Lactobacillus* | 5 | 3 | *L. plantarum* |
| *Firmicutes* | *Lactobacillales* | *Lactococcus* | 2 | 2 | *L. lactis* |
| *Firmicutes* | *Lactobacillales* | *Leuconostoc* | 5 | 5 | *L. mesenteroides* |
| *Firmicutes* | *Lactobacillales* | *Streptococcus* | 1 | 1 | |
| *Firmicutes* | *Lactobacillales* | *Weissella* | 1 | 2 | *W. confusa*, *W. thailandensis* |
| *Actinobacteria* | *Actinomycetales* | *Arthrobacter* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Cellulomonas* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Clavibacter* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Corynebacterium* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Curtobacterium* | 1 | 2 | |
| *Actinobacteria* | *Actinomycetales* | *Dermabacter* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Gordonia* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Janibacter* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Kocuria* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Microbacterium* | 2 | 2 | *M. flavescens* |
| *Actinobacteria* | *Actinomycetales* | *Micrococcus* | 1 | 1 | |
| *Actinobacteria* | *Actinomycetales* | *Rhodococcus* | 1 | 1 | |
| *Bacteroides* | *Flavobacteriales* | *Chryseobacterium* | 1 | 1 | |
| *Bacteroides* | *Flavobacteriales* | *Flavobacterium* | 1 | 1 | |

[a] The number of citations and the total number of species described are listed for each genus. Additionally, the most commonly cited species is listed for each genus containing multiple coffee-associated taxa. §, name updated.

## MANAGING COFFEE WASTE

Coffee production and utilization generates byproducts in the form of coffee cherry pulp and hulls, wastewater, and spent coffee grounds. Only 6% of coffee fruit mass contributes to the final coffee product; the rest is considered a byproduct (65). Coffee pulp is one of the most abundant examples of agricultural waste, and, due to its high concentrations of caffeine and phenolic compounds, it is relatively useless for traditional value-added products (e.g., animal feed and supplements) and is often treated as an environmental hazard (66, 67). There has been much research into ways to detoxify or repurpose coffee byproducts (reviewed in references 68 and 69), many using microbial transformation. Several studies have explored the use of microbes isolated from coffee pulp for its remediation, focusing on caffeine detoxification (70–72; reviewed in reference 73). This would allow the creation of either a value-added product in the form of silage for animal feed or, at the very least, the detoxification of the byproducts so that they could be safely stored or composted.

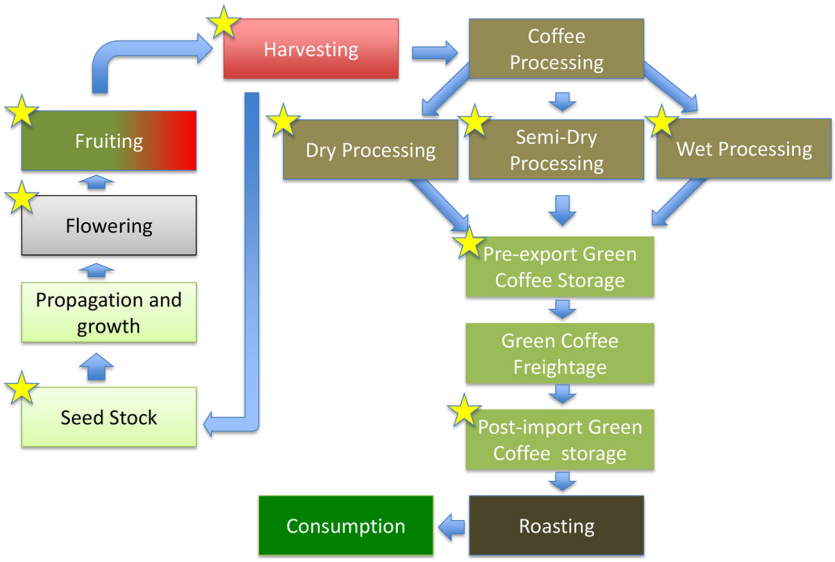


**FIG 1** The coffee processing chain, from fruit to seed. Understanding how microbes are involved in coffee processing will lead to the identification of critical points to control processing, the manipulation of coffee quality, and the management of coffee diseases (stars). Ensuring pathogen-free seed stock would help to prevent many early seedling diseases, while application of beneficial microbes can improve growth and disease resistance once seedlings are planted. Proper sanitation and culling at harvest would lead to a reduction of levels of postharvest spoilage organisms. Similarly, efforts to control the microbial communities active during fermentation could reduce levels of spoilage organisms or ochratoxin-producing fungi before roasting.

The massive amount of waste created each year in the coffee production pipeline can also serve as a substrate for the manufacturing of other valuable products for industry, such as enzymes. Boccas et al. (74) examined the use of coffee pulp as a substrate for the microbial production of pectinase. Others have looked at byproducts of roasting for enzyme production, including α-amylase (75) and β-fructofuranosidase (76) from silver skin and xylanase from spent coffee grounds (77). Others have examined the production of industrially useful small molecules from coffee byproducts. For example, while exploring the phenol- and pectin-degrading abilities of *Gluconacetobacter hansenii* isolated from wine, Rani and Appaiah (78, 79) found that when the bacterium was incubated with coffee cherry extract (another waste product), it was able to alter concentrations of free phenolic acids, such as vanillic acid and tannic acid. These compounds are important flavoring components used in the food industry (80). Given the highly distinctive environment that coffee waste presents, this material is a rich source of microbes able to produce or alter plant metabolites that may transform coffee pulp into a significant resource for many important industrial applications.

## MICROBES TO CONTROL COFFEE DISEASES AND ENVIRONMENTAL STRESSES

Recent changes in the distribution and host range of coffee diseases and pests have made the control of coffee disease of keen interest (81). A number of efforts aimed at breeding resistant cultivars are being pursued, in addition to the targeted use of pesticides. Given the desire to support sustainable cropping systems and reduce the use of toxic pesticides, a number of investigations have been aimed at the use of biological agents to control coffee pests and disease. While some have examined epiphytic microbial communities (82), many have focused on endophytes from coffee tissues (50, 52, 83). Endophytes have been known for their ability to confer increased pest resistance (84, 85), resistance to herbivory (86), and tolerance of environmental stress (87). With regard to disease resistance, both bacterial endophytes (88, 89) and fungal endophytes (85) have been explored as agents to promote resistance to the coffee leaf rust pathogen. Other comprehensive studies have been aimed at isolating endophytic bacteria (83) and fungi (84) to protect crops against the coffee berry borer *Hypothenemus hampei*.

The chemical profile of coffee plants makes them an interesting model for studies in chemical ecology and plant host defense. Looking at how compounds such as caffeine, a compound thought to reduce herbivory (12, 90), respond to microbial pressure would have implications not only for coffee pest control but for microbially mediated host defense in other systems as well. For example, Chaves et al. (91) demonstrated that an endophytic *Aspergillus oryzae* strain isolated from coffee leaves was able to induce the caffeine defense response though kojic acid production, which might protect plants from insect pests. Deciphering the exact roles that different endophytes play in stimulating plant defenses will help inform their utility as biocontrol agents.

## LOOKING FORWARD: NEW APPROACHES TO STUDY THE COFFEE MICROBIOME

The studies reviewed above collectively indicate that diverse microbial populations impact coffee yield and quality. Here, we describe how new approaches, such as marker-assisted selection, could alter the efficiency and speed with which scientists could address some of the larger issues of coffee sustainability in a changing climate. These issues include identifying coffee-adapted microbial agents to help control coffee disease, increase tolerance to environmental stress, and improve coffee quality.

While a majority of plant-associated microbes are considered commensal, some can have positive impacts on plant health through the promotion of plant growth, disease resistance, and tolerance to environmental stresses (53, 92–94). A large body of

literature exists that describes the biocontrol- and plant growth-promoting properties of dozens of microbial agents and the mechanisms through which these benefits are conferred (92, 93, 95). The development of biocontrol agents has depended greatly on traditional isolation and screening procedures for discovery (96), the same procedures employed in most coffee microbiology studies to date. Sifting through hundreds to thousands of isolates to remove redundant genotypes and test their efficacy is very time-consuming and inefficient. Given the rapidity of coffee pest and pathogen spread in recent years (81), a quicker approach to bioprospecting for novel natural products or coffee-adapted biocontrol agents is needed. Benítez et al. (97) employed community profiling to rapidly identify microbes statistically associated with disease suppression. Subsequently, the markers identified were used to direct the isolation and characterization of the potentially disease-suppressing microbes, resulting in the rapid recovery and characterization of two novel species of biocontrol bacteria (98). Together, these studies highlight how adjusting our approach to the use of a popular scientific tool, microbial community profiling, can result in the efficient generation of targets for disease control organisms using limited resources and time. The "next-generation" DNA sequencing technologies have at last become a competitive alternative for community analysis through massively parallel sequencing of community-derived DNA amplicons and direct metagenomic sequencing. These new approaches generate sequence data that can be directly employed to design selective isolation procedures for targeted microbe recovery. Such approaches are currently being used to identify microbial populations associated with increased millet growth in Senegal (99, 100). The application of such marker-assisted selection and recovery to coffee-associated microbes will likely provide new inoculants of value to prevent disease, promote stress tolerance, and improve bean quality.

In order to address the issue of mechanism and timing for specific microbial activities, identifying targets is a critical first step, but it is only a part of the equation. To demonstrate a microbe's impact on a desired trait and, indeed, to exploit it during production, the microbe needs to be recovered in culture. It is agreed that the majority of the microbial diversity has yet to be cultured. This could be due to a number of factors, including improper isolation media or growth conditions (reviewed in reference 101). With this in mind, future coffee microbiome research efforts should focus on sampling and selection factors specific to coffee to increase the isolation of novel microbial targets, such as the yet-to-be studied Archeae. Park et al. (102) present one such model for increasing the isolation efficacy for novel bacterial agents. Using marker-assisted selection and a balanced multifactorial sampling design, they were able to increase the efficiency of recovering novel bacterial genotypes with the targeted function by 5-fold. Such a sampling strategy can be used to select previously unstudied coffee microbes for further phenotypic screening.

Once in culture, microbial strains can be used in manipulative experiments to determine their impacts on many facets of coffee production and quality. The techniques and approaches to studying the coffee microbiome mentioned here will allow us to address more-specific questions concerning coffee microbiology. Are there other microbial endophytes that promote crop health and disease and pest resistance? Can microbes be used to improve coffee tolerance of a changing environment? To what extent are microbes associated with coffee seed defects, and how might they be employed to prevent them? Utilizing the aforementioned techniques, coffee researchers will advance our understanding of the coffee microbiome and help ensure a future for the world's largest-production agricultural commodity.

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