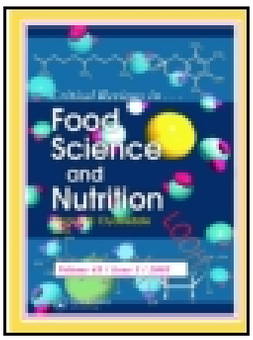

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Vinícius de Melo Pereira Gilberto, Vanete Thomaz Soccol, Satinder Kaur Brar, Ensei Neto & Carlos Ricardo Soccol (2015): Microbial Ecology and Starter Culture Technology in Coffee Processing, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1067759

To link to this article: http://dx.doi.org/10.1080/10408398.2015.1067759

Accepted author version posted online: 13 Oct 2015.

Microbial ecology and starter culture technology in coffee processing

Gilberto Vinícius de Melo Pereira[a], Vanete Thomaz Soccol[a], Satinder Kaur Brar [b], Ensei Neto[c], Carlos Ricardo Soccol[a*]

[a]Bioprocess Engineering and Biotechnology Department,

Federal University of Paraná (UFPR), Curitiba, PR, Brazil

[b]Institut National de la Recherche Scientifique (INRS), Centre Eau, Terre et Environnement, 490, Rue de la Couronne, Quebec(QC) G1K 9A9, Canada

[c]Cafeotech Treinamento & Educação LTDA.,

Patrocínio, MG, Brazil

* Author for correspondence: Carlos Ricardo Soccol

Bioprocess Engineering and Biotechnology Division, Federal University of Paraná

81531-970, BR-Curitiba PR, Brazil.

E-mail address: soccol@ufpr.br (C.R. Soccol).

Tel.: +55 41 33 613 191;

Fax: +55 41 33 613 695.

Abstract

Coffee has been for decades the most commercialized food product and most widely consumed beverage in the world, with over 600 billion cups served per year. Before coffee cherries can be traded and processed into a final industrial product, they have to undergo postharvest processing on farms, which have a direct impact on the cost and quality of a coffee. Three different methods can be used for transforming the coffee cherries into beans, know as wet, dry and semi-dry methods. In all these processing methods, a spontaneous fermentation is carried out in order to

eliminate any mucilage still stuck to the beans and helps improve beverage flavor by microbial metabolites. The microorganisms responsible for the fermentation (e.g., yeasts and lactic acid bacteria) can play a number of roles, such as degradation of mucilage (pectinolytic activity), inhibition of mycotoxin-producing fungi growth and production of flavor-active components. The use of starter cultures (mainly yeast strains) has emerged in recent years as a promising alternative to control the fermentation process and to promote quality development of coffee product. However, scarce information is still available about the effects of controlled starter cultures in coffee fermentation performance and bean quality, making it impossible to use this technology in actual field conditions. A broader knowledge about the ecology, biochemistry and molecular biology could facilitate the understanding and application of starter cultures for coffee fermentation process. This review provides a comprehensive coverage of these issues, while pointing out new directions for exploiting starter cultures in coffee processing.

Keywords: coffee processing, dry processing, wet processing, yeast, bacteria.

Introduction

Since the opening of the first coffee house in Mecca at the end of the fifteenth century, coffee consumption has greatly increased all around the world. The reasons for this continuous increase include, for example, improved cup quality and a change in coffee's image as a functional food (Farah, 2012). A critical step determining the coffee beverage quality is the postharvest practices to make the beans suitable for transport and roasting. Three different methods are employed in producing countries to process coffee fruit, referred to as dry, wet and semi-dry (Pandey et al., 2000). These methods involve removal of the waste from the crop and taking off the outer layers of the beans. Following postharvest processing on farms, coffee beans can be transported to industrial plants, where semi-manufactured or finished products are obtained for commercialization.

Fermentation in coffee refers to the process during which the pulp and mucilage surrounding the seeds are broken down through microbial action. This process produces a vast array of metabolites, such as organic acids, higher alcohols and esters, which will later add complexity and depth to a coffee (Mussatto et al., 2011; Pereira et al., 2014). The microorganisms responsible for the fermentation are indigenous species that originate as natural contaminants of the process, including yeasts (e.g., *Pichia*, *Debaryomyces*, *Sacharomyces* and *Candida* species), bacteria (e.g., members of the family Enterobacteriaceae, lactic acid bacteria and *Bacillus* spp.) and filamentous fungi (e.g., *Aspergillus*, *Penicillium* and *Fusarium* species) (Vaughn et al., 1958; Frank et al., 1965; Van Pee and Castelein, 1972; Gaime-Perra et al., 1993; Masoud et al., 2004; Silva et al., 2008; Vilela et al., 2010; Loew, 2014, Pereira et al., 2014).

The microbial ecology of coffee bean fermentation has been reorganized for over 100 years, and numerous studies have been conducted in different countries to determine the microbial species associated with this process. However, although recent progress has been made in defining the diversity and role of microbial species associated with coffee fermentation, scarce information on the growth and activities of specific microbial groups and their impacts upon final product quality and process efficiency, are available. Consequently, what specific species are essential for the fermentation and development of a good beverage are the questions to be raised and responded. This article reviews the composition and metabolism of coffee fermentation microflora, its impact on the quality of commercial coffee beans, and new directions for exploiting starter cultures in coffee processing

Origin and production of coffee

Coffee comes from the Latin form of the genus *Coffea*, a member of the Rubiaceae family which includes more than 500 genera and 6,000 species of tropical trees and shrubs (Sakiyama and Ferrão, 2014). Botanically, its origin took place in the highlands of Ethiopia, later migrating to Yemen. With a strict control over the transit of the precious coffee seeds, coffee plantations initially scattered in regions where the Islamic religion was predominant. One of the cultivation centers was Indonesia, in Southeast Asia, the world's largest producer until 1890 (Ruiz, 2014). When the habit of drinking coffee spread all over Europe during the seventeenth century, coffee plantations won the New World, where their production expanded through the Central and South America. Today, coffee is grown throughout geographic region between latitudes 30° N and 30° S, known as "the coffee belt" (Fig. 1). Summarized by regions, the main coffee-producing countries are: South America (e.g., Brazil, Colombian and Peru), Central

America (e.g., Honduras and Guatemala), North America (e.g., Mexico), Africa (e.g., Ethiopia), Indonesia and India.

The coffee fruit consists of an orange-red to red skin on ripening (exocarp), a fleshy yellow-white mesocarp (pulp and mucilage), and a plain-yellow parchment (endocarp) and a silver skin (integument) surrounding the endosperm (seeds) (Fig. 2). The mesocarp is subdivided in outer mesocarp (pulp) and inner mesocarp (mucilage). It is a gelatinous, translucent and sweet substance, which is richer in sugar (Elias, 1978). The polysaccharide constitution of mucilage is 30% pectic substances, 8% cellulose and 18% of non-cellulosic polysaccharides (Elias, 1978; Avallone et al., 2001). The presence of the pectic mucilage layer is a serious obstacle to the rapid drying of the beans; also, it is a very favorable medium for the development of different microorganisms.

The annual coffee bean production has reached 8.4 million metric tons, with a turnover close to US $10 billion (ICO, 2011). It is one of the most traded and consumed agricultural products worldwide, at times only surpassed by oil (Selvamurugan et al., 2010; FAO, 2014). Brazil is one of the leading coffee producers, supplying about a third of total world production, followed by Vietnam, Indonesia, Colombia, India, Peru, Honduras, Ethiopia, Guatemala, Mexico and another 60 countries (Fig. 1). The top five consumers are the USA, Brazil, Germany, Japan and France, while the Nordic countries have the world's highest coffee consumption per capita (Petit, 2007).

Postharvest processing

To produce coffee beans suitable for transport and roasting, there is a need to separate the seeds from the outer layers (skin, pulp, mucilage and parchment; Fig. 2), enabling the

reduction of moisture content from 65% to 10-12%. Thus, the postharvest processes aim at removing the components surrounding the beans, so that the beans may be stored for many months without significant change in quality (Trugo and Macrae, 1984; Teixeira et al., 1995). Three different postharvest systems are currently used to process coffee fruits (Fig. 3). The coffee fruits must be processed as soon as possible after harvesting, otherwise, uncontrolled natural fermentation may have negative impacts on the coffee quality.

The dry process, which results in so-called ‘‘unwashed” or ‘‘natural” coffee, is the oldest and simplest method of coffee processing. After harvest, the fruits are separated from impurities (sticks, stones and leaves), subsequently washed and classified according to their maturation. The entire coffee fruits are then spread on the ground (earth, platforms, concrete or tarmac) in layers which are approximately 10 cm thick, heaped at night and re-spread each day. Drying is continued until the beans moisture decreases to 11–12%. To reduce drying time, mechanical dryer can be used after pre-drying in the sun for a few days. During 10 to 25 days of sun-drying, a natural microbial fermentation occurs, when enzymes are secreted which breaks the pulp and mucilage (Silva et al., 2008). The dry processing is mainly used in countries where rainfall is scarce and long periods of sunshine are available to dry the coffee properly, such as Brazil and Ethiopia (Silva et al., 2000). The drying operation is the most important step because it affects the final coffee quality. Over-dried fruits become brittle and produce too many broken (defective) seeds during hulling. On the other hand, fruits that are not sufficiently dried become more vulnerable to deterioration by filamentous fungi and bacteria attacks. Natural coffees that have been properly dry processed can be a quality product with their own market (Duarte et al., 2010).

In wet processing, the pulp (the exocarp and part of the mesocarp) is mechanically removed. The beans are submitted to 24–48 hrs of underwater tank fermentation to allow microbial degradation of remaining mesocarp layer adhering to the parchment. The beans are then sun-dried to 10-12% moisture content (Fig. 3). This process decreases the time and area required for drying. However, the requirement of specific equipment and substantial amounts of water cause additional costs for the process (Bártholo and Guimarães, 1997). This process emerged when *Arabica* coffee began to be cultivated in tropical regions, such as Colombia, Central America and Hawaii. In these countries, the abundant rainfall and warm temperatures caused an immediate undesirable fermentation after harvest. The most practical way to avoid such detrimental fermentation was to remove the outer mesocarp tissue, rich in sugars, and submit the depulped fruits to an underwater tank process to achieve desirable fermentation (Brando, 1999). By this method, the fermentation can be controlled in terms of time, temperature and exchange of water so that spontaneous development of microorganisms can be better managed to minimize any adverse impacts on coffee quality or to encourage desirable quality impacts (Silva, 2014). The final product from the wet processing method is called ‘‘washed” or ‘‘wet” coffee.

The semi-dry processing, also called pulped natural method, presents stages of both dry and wet methods. This process started to be used in Brazil in the early 1990s (Duarte et al., 2010; Vilela et al., 2010). The coffee fruits are selected (only mature red cherries) and de-pulped by mechanical process. The berries are transferred to concrete platforms for the drying stage and the fermentation is also started to remove mucilage adhered to the beans (Vilela et al., 2010). Similar to the dry processing method, the de-pulped fruit is exposed to the local temperature and other

environmental conditions. The aims of the semi-dry process is to go one step beyond the dry process and to mechanically separate unripe cherries from ripe ones in order to treat the ripe ones separately and improve coffee quality (Brando, 2010). This processing method can only occur in countries where the humidity is low and the coffee covered in the sweet mucilage can be rapidly dried without undesirable fermentation. Brazil has made this method famous and produces some of the best semi-dry processed coffees, also called ‘‘pulped natural” coffees (Teixeira et al., 1995).

It is well accepted that green coffees resulting from the wet method yield roasted beans and coffee beverages, respectively, that are characteristically different from those produced with the dry method. Coffees from the wet processing are known to present better quality, less body, higher acidity and more aroma than the ‘unwashed` coffees (Mazzafera and Padilha-Purcino, 2004). These sensorial differences are mainly attributed by the fact that only fully ripe coffee cherries are used for wet processing, whereas fruits of all stages of ripeness are utilized for dry processing (Knopp et al., 2006). In addition, Bytof et al. (2005) showed that during postharvest treatment, various metabolic processes occur inside the coffee seeds which significantly alter the chemical composition of the green beans. This metabolism becomes evident by the conversion of glutamic acid into γ-aminobutyric acid mediated by an enzymatic α-decarboxylation. The reaction is related to a physiological drought stress situation and is specific to the mode of processing applied (Bytof et al., 2005). Such alterations in the pool of free amino acids — components which are considered as essential precursors of flavor and color of the coffee brew (Homma, 2002) — may be suitable to explain some of the sensorial differences between ‘‘washed” and ‘‘unwashed” coffees. Finally, it has been suggested that the intense perception of

‘‘floral” and ‘‘fruity” aromas and high acidity in coffee processed by wet method can be a result of microbial metabolites produced during fermentation stage (Mussatto et al., 2011; Pereira et al., 2014; Pereira et al., 2015).

The semi-dry processed coffees present an intermediate body between ‘‘washed” and ‘‘unwashed” ones. It is often sweeter than wet-processed coffees, has some of the body of a dry-processed coffee, but also retains some of the acidity of a wet-processed coffee. Because of this, pulped natural coffees are strongly appreciated in blends for espresso coffee (Teixeira et al., 1995).

Overview of coffee bean fermentation process

Coffee bean fermentation is conducted as a traditional, on-farm process. Microbial action during fermentation solubilizes the pulp material surrounding the seeds, and produces a range of metabolic end-products (e.g. alcohols and organic acids). Thus, although the major characteristic flavor of coffee originates from the chemical composition of the bean, the microbiota responsible for the fermentation may also contribute to the beverage sensory characteristics and other qualities due to the release of metabolites and their diffusion into the beans during the process (Frank, 1965; Silva, 2014; Pereira et al., 2014)

In wet processing, de-pulped coffee beans are held in fermentation tanks till the mucilage is completely digested and ready for drying. Fermentation times can vary substantially, from 12 to 48 hrs, depending on the environmental temperature. Higher temperatures and thicker mucilage layers accelerate fermentation. Throughout fermentation process, approximately 60% of the sugars are utilized as substrate for microbial growth, which produces significant amounts

of ethanol and acetic and lactic acids, resulting in lowered pH (from 5.5-6.0 to 3.5-4.0) (Avallone et al., 2001). In general, the wet fermentation process can be characterized by action of bacteria and yeast species, while filamentous fungi are rarely found (Avallone et al., 2001; Pereira et al., 2014).

The dry process involves fermentation of whole fruit on the ground. Over the course of 10–25 days of sun drying, natural microbial fermentation occurs and produces ethanol and acetic, lactic, butyric and higher carboxylic acids (Amorim and Amorim, 1977). In general, the microbiota involved in dry fermentation (encompassing bacteria, yeasts and filamentous fungi) are much more varied and complex than those found during wet fermentation (Silva et al., 2000, 2008).

The fermentation in semi-dry processing occurs when the de-pulped fruits are exposed to the local temperature and other environmental conditions (relative humidity, sunlight, rain). The microbial diversity is similar to the dry processing (Silva et al., 2008), except that the presence of filamentous fungi is rarely observed (Vilela et al., 2010).

Source of microorganisms that drive the fermentation

Research on the microbiota of coffee processing sites suggests mechanisms by which microorganisms can access the fermentation stage. Some of these possible sources are summarized in Fig. 4.

Frank et al. (1965) demonstrated that certain bacteria species belonging to the family enterobacteria associated with coffee bean fermentation came from the surfaces of the cherries and the coffee plantation soil, while Avalloni et al. (2001) observed that the water used for pulping was usually highly contaminated by aerobic mesophilic microflora, mainly composed of

enterobacteria. Lactic acid bacteria have also been mainly associated with fresh coffee cherries (Djossou et al., 2012; Leong et al., 2014). With regard to the habitat origins of yeast, Agate and Bhat (1965) reported the presence of certain species on the cherry surfaces and evidence showed that the natural fermentation of coffee was the result of activity of microflora from the cherry surface itself rather than that of flora of air or water.

Thus, most of these studies indicate that the surfaces of coffee cherries are the primary source of microbial contamination in the fermentation process. Many factors can affect the microbial ecology of this surface, e.g., degree of fruit maturity, climatic conditions and applications of agrichemicals. Consequently, the proportion of damaged raw material has dominant influence on the microbial ecology of the overall fermentation process (Fleet et al., 2003; Fleet, 2008; Barata et al., 2012). These conclusions indicate the need for a program of research to understand the microbial ecology of coffee cherries and processing sites.

Evolution of microbiological studies in coffee fermentation

Studies on the microbiology of coffee fermentations have been performed over the lasts 100 years in many coffee-producing countries, such as like Brazil, Mexico, Colombia, Tanzania, India, Ethiopia, Hawaii, Taiwan and Thailand. A timeline for the major events in studies of the coffee fermentation process is shown in Fig. 5. Questions about this microbial action in coffee processing started in 1907 when Loew attributed the fermentation process to yeasts that formed ethyl alcohol, carbon dioxide and acetic acid. Later, Lilienfeld-Toal (1931) isolated several species of yeast and bacteria from coffee fermentation conducted in Brazil. Coliform group was predominant and it was the cause of acid production in fermentation process. The author observed that the mucilage adhering to coffee beans was loosened before a high production of

acid and proposed the hypothesis that the pulp may be decomposed by the natural enzymes of the mature coffee.

Pederson and Breed (1946) reported the first study of lactic acid bacteria associated with coffee fermentation. From coffee cherry samples shipped by air from Mexico and Colombia to Geneva, N.Y., these authors isolated *Leuconostoc mesenteroides, Lactobacillus* ssp. and *Streptococcus faecalis*. Although their isolates were responsible for the acid detected in later stages of fermentation, Pederson and Breed doubted the possible involvement of these bacteria in mucilage-layer decomposition. Years later, Frank et al. (1965) presented a similar hypothesis, which assigned the very low incidence of lactic acid bacteria in coffee fermentation in Hawaii due to their inability to decompose the cherry mucilage layer

Vaughn et al. (1958) was the first to confirm the presence pectinolytic coliform bacteria associated with coffee fermentation. The authors observed that in the first 12 to 24 h of fermentation, the pectin material in Brazilian coffee cherries was degraded by coliform-like bacteria, resembling *Aerobacter* and *Escherichia*. Other pectinolytic bacteria (*Bacillus*) as well as filamentous fungi were also isolated.

All these earlier studies, while valuable in their extension of knowledge, were limited in their contributions because of inadequacies within the ecological and taxonomic methodologies available at the time. More recently, the developed molecular methods for the study of microbial ecology overcome many of these limitations and have lead to major advances in understanding the diversity of yeasts, bacteria and filamentous fungi in coffee fermentations (Silva et al., 2000; Avallone et al., 2001; Masoud et al., 2004; Vilela et al., 2010; Pereira et al., 2014). However, the

overall microbiology and biochemistry of coffee fermentation is poorly studied as yet when compared to other fermentation processes.

A survey on the microbial diversity present in different types of coffee processing and producing countries (Tables 2-4) shown that the distribution of the taxa of yeast and bacteria is highly variable from one ecosystem to another. An important variable is the load of contaminating microorganisms at the start of fermentation and their maximum growth during the process (Table 1). This has been reported to range between 1.0×10^4 to 1.0×10^9 cfu/g for total bacteria and 5.1×10^2 to 5.0×10^7 cfu/g for yeast. Factors affecting this initial load include the quality and integrity of the coffee cherries, plant variety, hygiene of fermentation tank and utensils and water used at the commencement of the fermentation process. Microorganisms grow very fast in coffee pulp at the ambient temperatures of tropical climates (25-30°C). Consequently, short delays among bean removal and transfer to fermentation can have a major impact on the levels of microorganisms at the start of fermentation.

Diversity and function of yeast

Yeasts are among the most frequently isolated microorganisms from fermenting coffee beans. Early studies of coffee yeasts were conducted by Agate and Bhat (1966) and Van Pee and Castelein (1971) in wet fermentations in India and Congo, respectively. A wide diversity of yeast was found, including species of *Saccharomyces marxianus* (*Kluyveromyces marxianus*), *S. bayanus, S. cerevisiae* var. ellipsoideus and *Schizosaccharomyces* spp. (Agate and Bhat, 1966) and *Candida guilliermondii* var. *membranifaciens, C. parapsilosis, C. pelliculosa, Saccharomyces cerevisiae, S. marxianus, Rhodotorula mucilaginosa* and *Torulopsis fumata* (Van Pee and Castelein, 1971).

More detailed ecological studies of coffee yeasts have now been conducted in most coffee producing-countries and the main findings of these studies are summarized in Table 2. Avallone et al. (2001) found that yeast isolated from wet fermentation in Mexico consisted of classical strains found in plants, such as *Cryptococcus laurentii, Kloeckera apis apicuata, Cryptocccus albidus* and *Candida guilliermondii*. Detailed study of yeasts present in coffee fermentation of East Africa was reported by Masoud et al. (2004) who used both traditional cultivation methods and culture-independent molecular tool (viz., denaturing gradient gel electrophoresis (PCR-DGGE)). *Hanseniaspora uvarum* was the predominant yeast with population size of $2.6 \times 10^5 - 1.5 \times 10^7$ cfu/g, while *Kluyveromyces marxianus, Candida pseudointermedia, Issatchenkia orientalis, Pichia ohmeri* and *Torulaspora delbrueckii* occurred in concentrations of 10^3 cfu/g. The authors reported that a good agreement was found between the profiles obtained by the PCR-DGGE technique and the findings obtained by traditional isolation and further identification of yeasts. However, the species *Saccharomyces cerevisiae* and *Candida xestobii* were not identified by cultivation, but by PCR-DGGE. Thus, the authors concluded that PCR-DGGE seemed to be an efficient tool for studying yeast diversity during natural coffee fermentation.

In Brazil, studies on yeast diversity have been performed for all three coffee processing methods, i.e., dry (Silva et al., 2000, 2008), semi-dry (Vilela *et* al., 2010) and wet (Pereira et al., 2014) processing. Silva et al. (2000, 2008) reported the isolation of a rich yeast diversity during fermentation in dry method process, encompassing 15 species, with *Debaryomyces hansenii, Pichia guilliermondii, P. ofunaensis* and *Arxula adeninivorans* being the most prevalent (Table 2). These species were isolated throughout the process, but were most prevalent at 14–18 days

when the total yeast population had increased to maximum values of about 10^6 cfu/g. Later, Silva et al. (2013) showed that seven species (viz., *Debaryomyces hansenii, D. polymorphus, Pichia anomala, P. holstii, P. burtonii, P. guilliermondii* and *Arxula adeninivoran*s) were pectinolytic and may function to break down pulp and mucilage pectin. Vilela *et al*. (2010) applied the first comprehensive polyphasic approach in coffee fermentation, encompassing culture-dependent and culture-independent methods, to study the diversity of yeast in fermentation of semi-dry processing. *Pichia anomala* was the dominant specie throughout the fermentation process, followed by *Rhodotorula mucilaginosa, Saccharomyces bayanus, Saccharomyces* sp. and *Torulaspora delbrueckii* (Table 2). Recently, Pereira et al. (2014) reported the dominance of *Pichia fermentans* in coffee fermentation by the wet method in Brazil, followed by *Candida glabrata, quercitrusa, Saccharomyces* sp., *Pichia guilliermondii*, *Pichia caribbica* and *Hanseniaspora opuntiae*.

The metabolic activity of yeasts is undoubtedly the most studied between the coffee-related microbial groups. Primarily, the yeasts initiate an alcoholic fermentation of pulp sugars to produce ethanol and carbon dioxide, and a vast array of secondary metabolites, such as higher alcohols, organic acids, esters, aldehydes, ketones, sulphur and nitrogen volatiles, as has been well established for other fermented commodities (Romano et al., 2003; Ugliano and Henschke, 2009; Pereira et al., 2012; Pereira et al., 2013; Schwan et al., 2014). These secondary metabolites generally have high flavor impact and will also diffuse into the bean to affect coffee beverage character, although little research has been done on this topic (Evangelista et al., 2014 a,b; Pereira et al., 2014; Pereira et al., 2015). The ability to decrease the number of mycotoxin-producing fungi as well as producing pectinolytic enzymes during coffee fermentation are other

mechanisms by which yeasts are considered to impact coffee bean quality. These aspects will be better described in the starter culture technology for coffee bean fermentation section.

Diversity and function of lactic acid bacteria

Lactic acid bacteria are generally isolated in high populations during wet and semi-dry processing. However, this group of bacteria does not participate in dry coffee processing. Silva et al. (2000) was the only one study that isolated lactic acid bacteria from dry processing, but found a very low population and did not identify them up to species level. It is possible that the anaerobic or low oxygen conditions present in wet fermentation favor the development of lactic acid bacteria (Silva, 2014). Table 4.3 provides a collation of the more significant studies of lactic acid bacteria associated with coffee bean fermentation.

Schillinger et al. (2008) studied the involvement of *Leuconostoc* and *Weissella* species in coffee fermentation in Ethiopia and Tanzania, through a polyphasic taxonomic approach. The 71 strains isolated analyzed belonged to the species *Leuconostoc citreum, L. mesenteroides, L. pseudomesenteroides, Weissella cibaria* and *W. soli*, and one strain consisted of the novel species *Leuconostoc holzapfelii*. Leong et al. (2014) reported that species of *Leuconostoc* (such as *L. pseudomesenteroides* and *L. citreum*) and *Weissella* (such as *W. confusa* and *W. thailandensis*) were found in fresh coffee cherries from three different coffee farms in Taiwan. Other species related in the study were *Lactobacillus plantarum, Lactococcus lactis* subsp. *lactis, Enterococcus* sp. and *Enterococcus faecalis*. Besides, *Leuconostoc* spp., *Weissella* spp. and *Lactobacillus plantarum* were also found in the silage of fresh coffee pulp collected from Ivory Coast (Djossou et al., 2011). These findings indicate that the lactic acid bacteria present in coffee fermentation probably originated from the fresh coffee cherries.

In wet processing, a range of studies have been applied to identify lactic acid bacteria in different coffee producing countries. Avallone et al. (2001) identified the heterofermentative lactic acid bacteria *Leuconostoc mesenteroides dextranicum* and *Lactobacillus brevis* in Mexico. *Leuconostoc mesenteroides* grew primarily during the early phase of fermentation, however, at the final stage, a change of lactic acid population was observed with the *Lactobacillus brevis* appearance.

Vilela et al. (2010) reported the presence of lactic acid bacteria in coffee fermentation by the semi-dry method based on a combination of both traditional molecular methods. *Lactobacillus plantarum* was found as the predominant species followed by *Lactobacillus brevis*, *Leuconostoc mesenteroides* and *Lactococcus lactis*.

So far, only little research has investigated the diversity of lactic acid bacteria in coffee fermentation while some studies have focused on the isolation of this group from the coffee fruit. Thus, the function of lactic acid bacteria is not yet known. However, some species are encountered frequently and probably play some role in the fermentation. To date, the only function assigned to this group was its apparent antifungal activity and bacteriocin-like inhibitory substance-producing capability (Djossou et al., 2011; Leong et al., 2014). Therefore, the potential use of artificial inoculation of lactic acid bacteria to inhibit mould growth could be exploited during coffee processing. In addition, the action of lactic acid bacteria allows the pH to drop, preventing the proliferation of other bacteria and favoring the growth of yeast (Massawe and Lifa, 2010).

Metabolically, homofermentative lactic acid bacteria (e.g., *Lactobacillus plantarum*) convert the available energy source (sugar) almost completely into lactic acid via pyruvate to

produce energy and to equilibrate the redox balance. However, pyruvate can lead to the generation of many other metabolites, such as acetate, ethanol, diacetyl and acetaldehyde. In addition, heterofermentative lactic acid bacteria (e.g., *Leuconostoc mesenteroides*) produce lactic acid and ethanol as well as several short-chain fatty acids, such as acetic acid and formic acid (Helinck et al., 2004). These chemical compounds may contribute to the acidity of fermented coffee beans. For instance, although the diffusion of lactic acid into the coffee beans during fermentation process has not been reported, as it is non-volatile, its excess concentration may not be reduced during drying and can impact the acidity of the beverage. Similar phenomenon has been demonstrated for the cocoa fermentation process to produce chocolate (Pereira et al., 2012; Schwan et al., 2014).

Other bacterial species

A broad bacterial diversity other than those of lactic acid bacteria have been isolated from coffee fermentations in the different processing methods and identified in many genera, such as *Bacillus, Paenibacillus, Acinetobacter, Streptococcus, Pseudomonas, Flavobacterium, Proteus, Aerobacter, Escherichia, Hafnia, Klebsiella, Tatumella, -Paracolobactrum* and *Serratia* (Table 4).

In dry processing, bacterial diversity has been only reported through standard cultural methods (Vaughn et al., 1958; Van Pee and Castelein 1972; Silva et al., 2000, 2008). The presence of bacteria in the exocarp (skin plus pulp) was reported by Van Pee & Castelein (1972), who observed an initial population of 2.5×10^5 cfu/g belonging to the Enterobacteriaceae, nominally *Enterobacter dissolvens, E. aerogenes, E. cloacae, Hafnia* spp., *Klebsiella* spp. The population increased to about 1.5×10^7 cfu/g in the first 24 hrs and then decreased to 3.5×10^6

cfu/g by 72 hrs. Silva et al. (2000, 2008) found a very high bacterial density (10^6 and 10^9 cfu/g) in dry fermentation process in Brazil. Bacterial populations were predominant in coffee cherries sampled on the bush (time 0), where they represented 96.3% of the total isolated microorganisms. The diversity of bacteria isolated from these fermentations encompassed the species *Tatumella ptyseos, Pseudomonas putrefaciens, Proteus mirabilis, E. aerogenes, Acinetobacter* spp., *Bacillus subtilis, B. macerans* and *B. megaterium*. The *Bacillus* species predominated, representing some 50% in the bacterial isolates obtained. A number of these bacteria had the ability to degrade pectin, especially the *Bacillus* species (Silva et al., 2013).

In wet processing, early studies reported species of *Erwinia, Paracolobactrum* and *Escherichia* (Frank and Dela Cruz, 1964; Frank et al., 1965) and *Streptococcus, Pseudomonas, Flavobacterium* and *Proteus* (Agate and Bhat, 1966) in coffee fermentations in Hawaii and India, respectively. In Mexico, during the wet processing of coffee, populations of 10^7–10^8 cfu/mL were observed throughout the fermentation, mainly attributed to the higher microbial contamination from water used in the fermentation tanks (Avallone et al., 2001). Aerobic, Gram-negative bacteria, represented by *Enterobacter herbicola, Klebsiella pneumonia, K. ozaenae* and *K. oxytoca* were the main bacteria species isolated during fermentation.

Vilela et al. (2010) found that bacterial counts in semi-dry fermentation of coffee processing in Brazil varied from 10^2 to 10^7 cfu/g. This population was largest during the first 24 hrs of fermentation (10^7 cfu/g) and decreased with the progress of fermentation (10^2 log cfu/g). *Escherichia coli, Bacillus cereus, B. megaterium, B. macerans* and *Klebsiella pneumoniae* were the predominant bacterial species identified during the fermentation process. Other identified

species were *Acinetobacter* sp., *Bacillus subtilis*, *Bacillus* sp., *Enterobacter herbicola*, *E. coli*, *K. pneumoniae* and *Serratia* sp.

Further research is needed to understand how these "other" bacteria might impact on the fermentation process and coffee bean quality. It is clear that their growth occurs at high levels in the early stages of fermentation, but the only one function attributed to these bacterial groups is the pectinolytic enzymes production and possible contribution to the decomposition of the mucilage layer. Thus, the occurrence and significance of these bacteria species needs more consideration. In the case of the species that can lead to beans with grossly unacceptable odors and flavors, hygienic management throughout the harvesting-fermentation chain is required to avoid this potential problem.

Filamentous fungi

Detailed investigations of the involvement of filamentous fungi in coffee fermentation during dry and semi-dry processing have been reported by Silva et al. (2008) and Vilela et al. (2010), respectively. On the other hand, there are no reports related to the involvement of filamentous fungi during wet processing. This is not unexpected given that the process only lasts for about 48 h and is essentially a high water activity environment that is more conducive to yeast and bacterial growth (Silva, 2014). Thus, although it was suggested the presence of endophytic fungi in cherry fruits and also in the production environment, bacteria and yeast grow faster than filamentous fungi in wet fermentation conditions, competing for nutrient and site of colonization.

In semi-dry processing, Vilela et al. (2014) reported that the population of filamentous fungi was always lower than the bacterial and yeast populations. However, a larger diversity of

filamentous fungal species (14 species) was found in the washed fruits sample, which demonstrated that the washing process was likely insufficient for the physical removal of fungi. In this study, the most frequently identified species were *Aspergillus tubingensis, Aspergillus versicolor, Cladosporium cladosporioides, Aspergillus* sp. and *Penicillium decumbens,* with counts of 10^3-10^5 log cfu/g. Some of these species are associated with good quality coffee, such as *Cladosporium cladosporioides* (Licciardi et al., 2005), while others depreciate the quality, including *Fusarium* and *Penicillium* species (Batista et al., 2003; 2009). Among the mycotoxigenic species already reported (Batista et al., 2003, 2009), only *Aspergillus ochraceus* was detected in semi-dry processing method, indicating that this process minimized the colonization of toxigenic fungi (Vilela et al., 2010).

The dry process allows more fungal contamination and increases the possibility of mycotoxin production. Silva et al. (2001, 2008) found that *Aspergillus, Penicillium, Fusarium* and *Cladosporium* were the most abundant genera during dry processing in Brazil, corresponding to 42.6% of the total fungi isolates (Silva et al., 2008). *Aspergillus flavus, A. niger, A. ochraceus, A. tamarii, A. sydowii, A. foetidius* and *A. dimorphicus* were the most frequent species, being detected starting on the 8th fermentation day for coffee cherries on the ground, but were more abundant during storage, where they represented 59.6% of the total isolates.

Filamentous fungi in coffee processing have been studied due to two main aspects, such as their potential to adversely affect coffee flavor (Liardon et al., 1989; Iamanaka et al., 2011) and produce toxins that adversely affect consumer health and safety (Levi et al., 1974; Mislivec *et al*., 1983; Nakajima et al., 1997). Levi et al. (1974) were the first to report occurrence of

ochratoxin A (OTA) in coffee beans. Since then, several studies have detected both OTA-producing fungi and OTA in green coffee beans (Levi et al., 1974; Mislivec et al., 1983; Nakajima et al., 1997; Taniwaki et al., 2003; Pardo et al., 2004; Chalfoun et al., 2007). The main filamentous fungi that have been found in coffee with the potential to produce OTA were *Aspergillus ochraceus, A. carbonarius and A. niger* (Nakajima et al., 1997; Joosten *et al*., 2001).

The fermentation impact on coffee quality

Over the years, many studies have reported that the coffee bean fermentation process needs to be well controlled to ensure the development of microorganisms that give a high-quality beverage with good coffee aroma (Agate and Bhat, 1965; Frank et al., 1965; Goto and Fukunaga, 1956; Arunga, 1982; Amorim & Amorim, 1977; Evangelista et al., 2014 a,b; Pereira et al., 2014; Pereira et al., 2015). Failure in fermentation can result in the development of microorganisms that adversely affect coffee character and flavor. Coffee beans resulting from such fermentations are often referred to as “stinkers” (Frank et al., 1965; Arunga, 1982).

Unsatisfactory demucilaging can result from insufficient as well as excessive fermentation (Agate and Bhat, 1965). Under-fermentation interferes with the drying process, because the mucilage layer is not completely removed. Beans that are under-fermented contain residual mucilage and sugars that impede the drying process and encourage the growth of spoilage bacteria and fungi. However, it can be determined whether or not the beans are ready for drying by periodically testing small samples from the fermentation tank. The coffee fermentation is adjudged "finished" when the parchment on touch "feels hard" and is free from slippery mucilage. If not, fermentation is continued until demucilaging is completed. Fermented

parchment should be washed immediately after fermentation is completed to avoid these problems (Agate and Bhat, 1965).

Over-fermentation frequently results in adverse changes that affect the flavor and odor of the coffee produced (Goto and Fukunaga, 1956; Silva, 2014). Most frequently, acidogenic fermentations by Enterobacteriaceae and acetic acid bacteria occur. These reasons possibly should be the major cause for cherry spoilage problems arising during processing (Silva et al., 2008). For example, butyric or propionic acids can develop, both of which have an undesirable impact on coffee quality (Amorim & Amorim, 1977). Species of *Bacillus*, especially *B. megaterium*, might be responsible for the propionic acid found in coffees processed via dry method (Silva et al., 2008). The over-fermentation can best be avoided by testing small samples during processing to detect complete demucilaging at its earliest stage, so that washing can be instilled before undesirable changes occur. Because of microbial buildup in machinery and equipment as the season progresses, thorough cleaning of the fermentation vats should be carried out frequently (Agate & Bhat, 1965).

In addition, coffee fermentation and drying must be managed to control the growth of filamentous fungi that can produce off-flavors and mycotoxins (Taniwaki et al., 2003; Suárez-Quiroz et al., 2004; Batista et al., 2009). Studies have demonstrated that the high incidence of *Aspergillus, Penicillium* and *Fusarium* is associated with the loss of sensory quality in the final beverage (Daivasikamani and Kannan, 1986; Pasin et al., 2002; Chalfoun et al., 2005). This is probably due to the production of long-chain carboxylic acids (Daivasikamani and Kannan, 1986; Alves and Castro, 1998; Pasin et al., 2002; Chalfoun et al., 2005).

Pulp pectin degradation during fermentation

In coffee processing, pulping of the coffee cherries removes their skin and pulp, leaving viscous mucilage adhering to the parchment, rich in pectin substances. The highly hydrated tissue is eliminated during the fermentation step through the action of three main enzymes: (i) poligalacturonase, which catalyses the hydrolysis of a-1.4 glycosidic bonds into pectic acid (poligalacturonic acid); (ii) pectin lyase, which catalyses pectin breakage by transelimination, releasing unsaturated galacturonic acids; and (iii) pectin methylesterase, responsible for the de-esterification of the methoxil group of the pectin forming pectic acid and methanol (Silva et al., 2013).

Hypotheses to explain the pectin degradation during coffee fermentation is that mucilage pectic substances are extensively degraded by the natural microflora and/or endogenous coffee enzymes; however, there are still conflicting views in reaching this. Many studies have shown the pectinolytic activity in yeasts (e.g., *Candida, Pichia*, *Kluyveromyces, Schizosaccharomyces* and *Saccharomyces* species) and bacteria (e.g., *Bacillus, Aerobacter*, *Escherichia* and *Erwinia* species) isolated from coffee fermentation process, and suggested their involvement in the degradation of the mucilage (Vaughn et al., 1958; Frank and De La Cruz, 1964; Frank et al., 1965; Agate and Bhat, 1966, Masoud and Jespersen, 2006; Silva et al., 2013; Pereira et al., 2014). However, contrary to this hypothesis, Avallone et al. (2001) showed that the number of pectolytic microorganisms did not increase during fermentation of coffee beans by the wet method. Furthermore, the isolated pectolytic bacterial strains only produced pectate lyase activities that are unable to degrade highly methylesterified coffee pectic substances (Castelein et al., 1976; Avallone *et* al., 2001). Finally, a histological examination of mucilage cells showed

that, after fermentation, their cell walls still contained pectic substances (Avallone et al., 1999). These results lead to the conclusion that depolymerization of pectic substances by pectolytic microorganisms does not occur or is negligible during fermentation.

A third hypothesis suggests that the pectin mucilage is degraded neither by endogenous pectolytic enzymes nor by pectolytic microorganisms, but due to the acidification process (Calle, 1965; Wootton, 1965; Avallone et al., 2002). Microbial growth is necessary but the microflora does not directly participate in mucilage degradation by enzyme production. Its role is to produce metabolites, such as organic acids (lactic and acetic acids) inducing a pH decrease (Calle, 1965; Lopez et al., 1989). As a consequence, the mucilage cell walls swelling capacity in water is modified as well as their bound calcium (Avallone et al., 2002). These alterations loosen the polysaccharide network with a clear textural change. These mechanisms are well known in the cellular response to microbial attack observed in plant/pathogen interactions (D'Auzac, 1996).

Starter culture technology for coffee bean fermentation

Starter cultures are defined as a preparation or material containing large numbers of variable microorganisms, which may be added to accelerate and improve a fermentation process (Holzapfel, 2002). Microorganisms selected to be used as starter cultures are expected to have some characteristics, such as adapting easily to the raw material and process, developing sensory quality, extending shelf life, reducing the processing time and energy during the production, inhibiting food-related pathogenic microorganisms, as well as having non-pathogenic and non-toxigenic properties (Corsetti et al., 2012). The use of functional starter cultures in the food fermentation industry is widely known, such as for cheese, yogurt, bread, beer and wine (Steinkraus, 2004; Schwan et al., 2014). In many cases, the technology has evolved from a

traditional, spontaneous fermentation to a controlled industrialized process based on the use of well defined microbial strains as starter cultures to conduct the fermentation (Steinkraus, 2004).

A controlled coffee fermentation process by use of a starter culture may guarantee a standardized quality and reduce the economic loss for the producer. In the literature, only few studies have been reported for the use of starter culture for coffee fermentation, although the attempt to control this process has existed for over 40 years. Early studies performed by Calle (1957, 1965) and Butty (1973) reported the use of residual waters from a previous coffee fermentation as starter. These authors demonstrated that the time required to degrade the mucilage was shortened from 24 to 12 hrs, without affecting the final quality of the coffee. A study conducted by Agate and Bhat (1965) was the first to effectively introduce a starter culture for coffee fermentation. In particular, they demonstrated that the incorporation of a mixture of three *Saccharomyces* species (viz., *S. marxianus, S. bayanus, S. cerevisiae* var. *ellipsoideus*) aided the process by accelerating the mucilage-layer degradation. These authors also demonstrated that a pooled yeast enzyme preparation from *Saccharomyces* species was indeed very effective in the sense that it brought about complete elimination of pectic substances within 7 to 8 hrs.

However, it was only in the 2000s that subsequent studies on the starter cultures for coffee fermentation were performed again. Avallone et al. (2002) studied the microbial and physicochemical parameters of coffee fermentation inoculated by different pectolytic microorganism strains isolated from a spontaneous process (*viz.*, *Lactobacillus brevis* L166, *Erwinia herbicola* C26, *B. subtilis* C12, *Kluyveromyces fragilis* K211). The authors noted that inoculations with these pectolytic strains do not speed up polysaccharide degradation. It was

observed, however, that organoleptic characteristics of the beverages were not modified by addition of starter cultures, thereby demonstrating that the use of starter culture would be possible to limit off-flavor development and to standardize the final coffee quality. More specifically, the authors suggested that it would be preferable to use lactic acid bacteria in order to stay as close as possible to the natural fermentation.

In study performed by Massawe and Lifa (2010), yeast strains of *Pichia anomala* and *P. kluyveri* and acid lactic bacteria identified as *Leuconostoc*/*Weissella* sp., Homofermentative *Lactobacillus* spp., Heterofermentative *Lactobacillus* spp., and *Enterococcus* strains were used as starter cultures in coffee fermentations against ochratoxin-producing *Aspergillus ochraceus*. The resulted demonstrated that the two yeast species in combination with selected strains of lactic acid bacteria could be used as biocontrol agents against *A. ochraceus*.

Recently, the use of aromatic yeasts in coffee fermentation to promote flavor development in coffee beverages have been investigated during dry (Evangelista et al., 2014b), semi-dry (Evangelista et al., 2014b) and wet (Pereira et al., 2015; Soccol et al., 2015) processing. It has been shown that the metabolic activity of yeast starter cultures (e.g., *Candida parapsilosis*, *S. cerevisiae* and *P. fermentans* strains) in either processing method influences the final volatile fraction of roasted beans and sensory quality of beverages produced thereof. Thus, these recent studies have revealed that yeasts have a complementary role when associated with coffee quality through the synthesis of yeast-specific volatile constituents. However, it is not clear how such volatiles might impact on beverage flavor because, firstly, they must diffuse into the beans and, secondly, it is expected that they would be mostly lost by evaporation or otherwise transformed

during the roasting operation. Further research detailing the kinetics during coffee fermentation process is required.

Criteria for selecting and developing starter cultures for coffee fermentation

Based on literature data of the composition and metabolism of coffee fermentation microflora, criteria for the selection and development of starter cultures can be outlined. Basically, these criteria can be considered in three common categories for the food fermentation industry: (1) properties that affect the performance and efficiency of the fermentation process, (2) properties that determine coffee quality and character and, (3) properties associated with the commercial production of coffee starter cultures.

1) *Properties that affect the performance of the fermentation process*:

Firstly, the ability of the starter culture to dominate the indigenous microbiota is one of the main criteria for its application (Lefeber et al., 2012; Ciani et al., 2010; Perrone et al., 2013). The selection of best-adapted strains offer the possibility of effectively using them over the indigenous microorganisms with lower capacity of adaptation within coffee environment conditions, which might help to develop new, stable, controlled coffee starter cultures for fermentation processes (Pereira et al., 2014)

Next, attention should be paid for selecting strains with capacity to degrade the coffee bean mucilage (pectinolytic activity). The microbial removal of the mucilage can facilitates bean drying and produces metabolites that diffuse into the interior of the coffee beans and react with substances responsible for the flavor of the final beverage (Masoud et al., 2005; Masoud and Jespersen, 2006; Silva et al., 2013; Pereira et al., 2014).

Finally, for the purpose of preventing production of OTA in coffee, it is suggested to use yeasts and/or lactic acid bacteria in biological control of OTA-producing fungi during coffee fermentation. The coffee pulp is a substrate favorable to the production of OTA by *Aspergillus* or *Penicillium* species (Mantle and Chow, 2000). It has been reported that some yeasts and lactic acid bacteria can inhibit growth of filamentous fungi (Masih et al., 2000; Djossou et al., 2011; Leong et al., 2014). Studies conducted by Masoud et al. (2005) and Masoud and Kaltoft (2006) demonstrated that two mechanisms are involved in inhibiting growth of OTA-producing fungi, viz., effect of volatiles produced by yeast metabolism and competition for nutrients.

2) *Properties that determine coffee quality and character*:

The microbial conversion of coffee pulp constituents into flavour-active components has emerged, in recent years, as an important, additional mechanism whereby yeasts substantially impact coffee aroma and flavor (Silva et al., 2013; Evangelista et al., 2014 a,b; Pereira et al., 2014). These profiles vary significantly between yeast species and strains (Pereira et al., 2014), so extensive strain screening is necessary to select for those with positive attributes (e.g. enhanced ester formation) and reject those with distinct negative impacts (e.g. overproduction of acetic, butyric and propionic acids). On this basis, yeast species within *Saccharomyces*, *Candida* and *Pichia* have been shown with the largest potential for enhanced quality of coffee fermentation (Silva et al., 2012; Evangelista et al., 2014 a,b; Pereira et al., 2014; Pereira et al., 2015).

3) *Properties associated with the commercial production of coffee starter cultures*:

Finally, for commercial purpose, the starter culture must be amenable to large-scale cultivation on relatively inexpensive substrates. Subsequently, it needs to be tolerant of the

stresses of drying, packaging, storage and, finally, rehydration and reactivation processes (Soubeyrand et al., 2006). These requirements need to be achieved without loss of the essential and desirable fermentation properties (Fleet, 2008).

Conclusions and future prospects

It can be concluded that the distribution of the taxa of yeast and bacteria, as well as their load at the start of fermentation and maximum growth, are highly variable from one coffee ecosystem to another. Yeast, filamentous fungi and bacteria populations are more diverse and are present in greater numbers in dry processing than that in pulped ones (wet and semi-dry), probable due to the longer exposure period in which the fruits are subjected to contamination during fermentation. On the other hand, lactic acid bacteria are isolated in high numbers in pulped coffees due to the anaerobic or low oxygen conditions present, which favor their development.

In dry processing, the common species are *Bacillus subtilis*, species of Enterobacteriaceae family, *Debaryomyces hansenii*, *Pichia guilliermondii* and *Aspergilus niger*. In pulped coffees (wet and semi-dry processing), bacteria and yeasts represent the most frequently occurring microorganisms, with a predominance of yeasts over bacteria. *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, Enterobacteriaceae, *Bacillus cereus*, *Hanseniaspora uvarum* and *P. fermentans* are commonly isolated. Thus, the common species in all types of processing belong to the Enterobacteriaceae family and *Pichia* genus.

The role of microorganisms in the degradation of the mucilage is still matter of debated and further studies are needed to investigate the ability of bacteria and yeast species to degrade the mucilage in vivo (i.e., during coffee processing).

As coffee beans are generally fermented by various naturally occurring microbiota, the quality of the coffee products varies depending on the raw materials even though it is processed under controlled conditions. The use of starter cultures has recently been suggested as an alternative to control the fermentation process and to promote quality development of coffee product. However, the potential use of starter cultures for coffee fermentation is not yet well studied. The prospect of applying starter cultures in coffee fermentation is to reduce fermentation times (pectinolytic activity), improvement of process control, sensory quality (production of metabolites that confer agreeable flavor to the final product) and safety attributes (inhibition of toxigenic fungi growth). The implementation of coffee starter cultures could be a quite laborious process but, once successfully implemented, can transform an inconsistent process into an economically valuable proposition. In order to explore this avenue, it is first important to understand the ecology, physiology, biochemistry and molecular biology of process. This knowledge can be increased by the applications of more recent, molecular methods, such "omics" technologies, which have never been used in coffee studies. This will open up new horizons in the industrial production of coffee with good taste and high quality.

Acknowledgments

The authors wish to thank the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) and Coordenação de Aperfeiçoamento de Pessoal de Nível de Nível Superior (CAPES) for financial support and scholarships.

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Table 1 - Initial and maximum population of yeast and bacteria in coffee fermentations

Processing method	Country	Yeast cfu/g		Bacteria cfu/g		Reference
		Initial population	Maximum population	Initial population	Maximum population	
Wet	India	4.3×10^4	5.5×10^6	3.2×10^4	6.59×10^5	Agate & Bhat (1966)
Wet	Hawaii	6.2×10^4	6.6×10^4	NA	NA	Frank *et al*. (1965)
Wet	Mexico	3.9×10^4	1.0×10^6	1.0×10^7	1.0×10^8	Avallone *et al*. (2001)
Wet	Tanzania	4.0×10^4	5.0×10^7	NA	NA	Masoud *et al*. (2004)
Wet	Brazil	5.01×10^2	1.4×10^7	NA	NA	Pereira *et al*. (2014)
Semi-dry (Arabica)	India	3.9×10^5	7.9×10^5	1.9×10^5	1.2×10^6	Velmourougane (2013)
Semi-dry (Robusta)	India	2.51×10^5	1.3×10^6	1.3×10^5	6.3×10^5	Velmourougane (2013)
Semi-dry	Brazil	5.0×10^3	7.9×10^6	5.0×10^5	1.0×10^7	Vilela *et al*. (2010)
Dry	Brazil	1.0×10^3	1.0×10^6	1.0×10^4	1.0×10^9	Silva *et al*. (2008)

NA.: Not analyzed

Table 2 - Yeast diversity in coffee fermentation from different countries. Predominant species indicated in bold type.

Country	Processing	Species isolated	Reference
Brazil	Dry	***Arxula adeninivorans***, ***Pichia ofunaensis***, *P. acaciae, P. anomala, P. ciferii, P. jadinii, P. lynferdii, P. sydowiorium, Blastobotrys proliferans, Candida auringiensis,C. glucosophila, C. incommunis, C. paludigena, C. schatarii, C. vartiovaarae, Citeromyces matritensis, Geotrichum fermentans, Saccharomyces cerevisiae, Saccharomycopsis fermentans, S. fibuligera, Schizosaccharomyces pombe, Sporopachydermia cereana, Trichosporonoides oedocephales, Williopsis saturnus* var. *sargentensis.*	Silva *et al*. (2000)
Brazil	Dry	***Debaryomyces hansenii***, *D. polymorphus, D. polymorphus,* ***Pichia guilliermondii***, *P. guilliermondii, P. burtonii, P. anomala, S. smithiae, P. Burtonii, P. sydowiorum, P. subpelliculosa, Candida saitoana, C. fermentati, C. membranifaciens, Stephanoascus smithiae, Saccharomyces cerevisiae, Arxula adeninivorans. A. adeninivorans.*	Silva *et al*.(2008)

Brazil	Semi-dry	***Pichia anomala***, *P. caribbica*, ***Rhodotorula mucilaginosa***, ***Saccharomyces sp., S. bayanus,*** *S. cerevisiae*, ***Torulaspora delbrueckii***, *Arxula sp., Candida ernobii, C. fukuyamaensis, C. membranifaciens, C. carpophila, Hanseniaspora uvarum, Kloeckera sp., Kluyveromyces* sp.	Vilela *et al*. (2010)
Brazil	Wet	***Pichia fermentans***, *P. guilliermondii, P. caribbica, Hanseniaspora opuntiae. Candida glabrata, C. quercitrusa, Saccharomyces* sp.	Pereira *et al*. (2014)
Mexico	Wet	***Kloeckera apis apicuata***, *Cryptococcus laurentii, C. albidus, Candida guilliermondii.*	Avallone *et al*. (2001)
Tanzania	Wet	***Pichia kluyveri***, ***P. Anomala***, ***Hanseniaspora uvarum***, *Candida pseudointermedia, Kluyveromyces marxianus, Issatchenkia orientalis, Torulaspora delbrueckii, Eremothecium coryli.*	Masoud *et al*. (2004)
India	Wet	***Saccharomyces marxianus*** (*Kluyveromyces marxianus*), *S. bayanus, S. cerevisiae* var. *ellipsoideus, Schizosaccharomyces* spp.	Agate & Bhat (1966)
India	Wet	***Saccharomyces marxianus* (*Kluyveromyces marxianus*)**, *S. bayanus, S. cerevisiae var. ellipsoideus, Schizosaccharomyces* sp.	Agate *et al*. (1965)
India	Semi-dry	***Saccharomyces* sp., *Shizosaccharomyces* sp.**	Velmourougane (2013)

Table 3 - Lactic acid bacteria diversity in coffee fermentation from different countries. Predominant species indicated in bold type.

Country	Processing	Species isolated	Reference
Brazil	Dry	*Lactobacillus* sp.	Silva *et al*. (2000)
Brazil	Semi-dry	***Lactobacillus plantarum,*** *L. brevis, , Lactococcus lactis, Leuconostoc mesenteroides.*	Vilela *et al.* (2010)
Mexico	Wet	***Leuconostoc mesenteroides dextranicum***, *Lactobacillus brevis*	Avallone *et a*l. (2001)
Mexico/Colombia	Not mentioned	***Leuconostoc mesenteroides***, *Lactobacillus ssp., Streptococcus faecalis*	Pederson and Breed (1946)
India	Semi-dry	***Lactobacillus* sp., *Leuconostoc* sp.**	Velmourougane (2013)
Ethiopia/Tanzania	Not mentioned	***Leuconostoc citreum***, *L. holzapfelii L. mesenteroides, L. pseudomesenteroides, Weissella cibaria, W. soli.*	Schillinger *et al*. (2008)
Hawaii	Wet	*Leuconostoc mesenteroides*	Frank *et al*. (1965)
Taiwan	Not mentioned	***Leuconostoc pseudomesenteroi, L. citreum***, *Weissella confusa, W. thailandensi, Lactobacillus plantarum, Lactococcus lactis subsp. lactis, Enterococcus sp., Enterococcus faecalis*	Leong *et al.* (2014)

Table 4 Bacterial diversity in coffee fermentation from different countries. Predominant species indicated in bold type.

Country	Processing	Species isolated	Reference
Brazil	Dry	***Enterobacter cloacae,*** *E. aerogenes, E. sakazakii, E. gergoviae,* ***Pseudomonad paucimobilis****, P. pseudoalcaligenes, P. cepacia, P. vesicularis, P. fluorescens, P. aeruginosa, Serratia liquefaciens, S. plymuthica, S. marcescens, Cedecea* sp., *Chromobacter violaceum, Citrobacter freundi, Flavobacterium odoratum, Hafnia alvei, Klebsiella oxytoca, K. ozaenae, Pasteurella haemolytica, Salmonella choleraesuis, S. enterica var. arizonae, S. paratyphi, Shigella dysenteriae, Tatumella ptyseos, Bacillus subtilis, B. cereus, B. anthracis, B. megaterium, B. stearothermophilus, B. laterosporus,* ***Cellulomonas*** **spp.**, *Arthrobacter* spp., *Microbacterium* spp., *Brochothrix* spp., *Dermabacter* spp.	Silva et al. (2000)
Brazil	Dry	*Enterobacter agglomerans, Yersinia* sp., *Arthrobacter,* ***Bacillus cereus****, B. megaterium,* ***B. subtilis****, B. macerans, Acinetobacter* sp., *B. polymyxa.*	Silva et al. 2008
Brazil	Semi-dry	***Enterobacter agglomerans****, Erwinia herbicola,*	

		Escherichia coli, *Klebsiella pneumoniae, Serratia* sp., *Bacillus* sp., ***B. cereus***, ***B.macerans***, ***B. megaterium***, *B.subtilis, Acinetobacter* spp.	
Mexico	Wet	***Klebsiella pneumoniae***, ***K. ozaenae***, ***K. oxytoca,*** ***Enterobacter herbicola***, *Pseudomonas cepaciae, Chrysomonas luteola.*	Avallone et al.(2001)
India	Wet	*Streptococcus* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Proteus* sp.	Agate et al. (1965)
India	Semi-dry	*Pseudomonas* sp., *Flavobacterium* sp., *Bacillus* sp.	Velmourougane (2013)
Hawaii	wet	*Erwinia dissolvens, Paracolobactrum aerogenoides, P. coliforme, P. intermedium, Escherichia intermedium.*	Frank et al., 1965

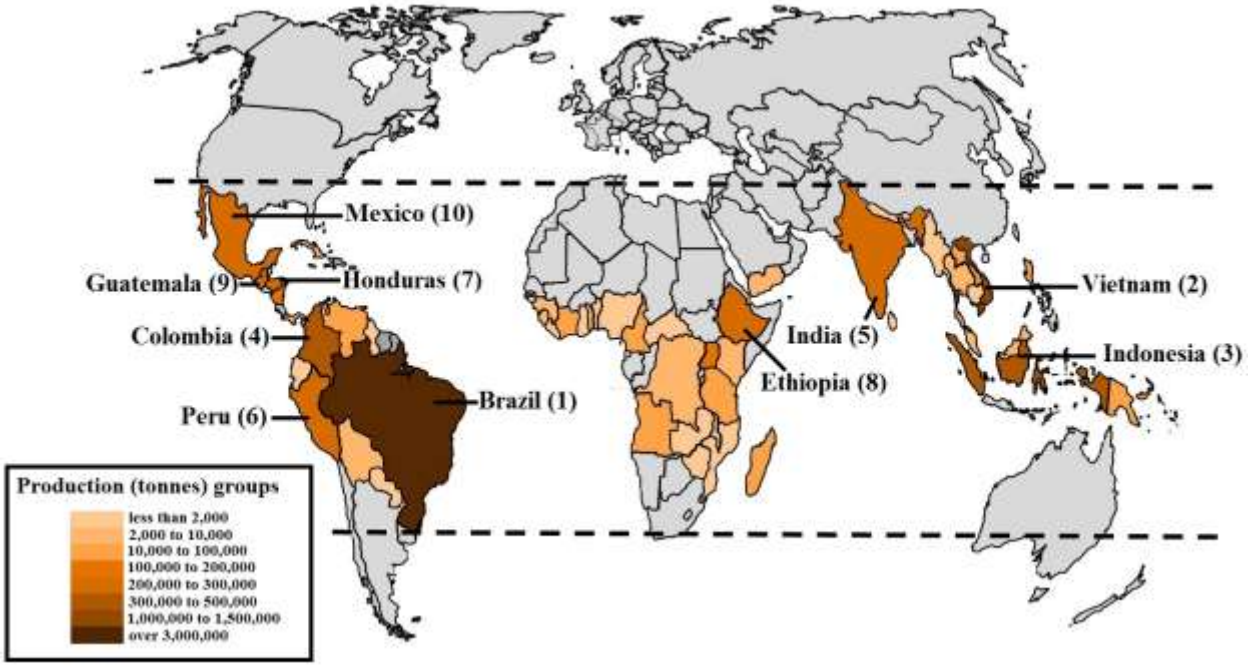

Fig. 1 Geographical distribution of coffee production (Source: FAO, 2014). The dashed lines indicate "the coffee belt" zone. The heatmap was generated by using visual basic for applications (VAB) in conjunction with microsoft® office excel version 7

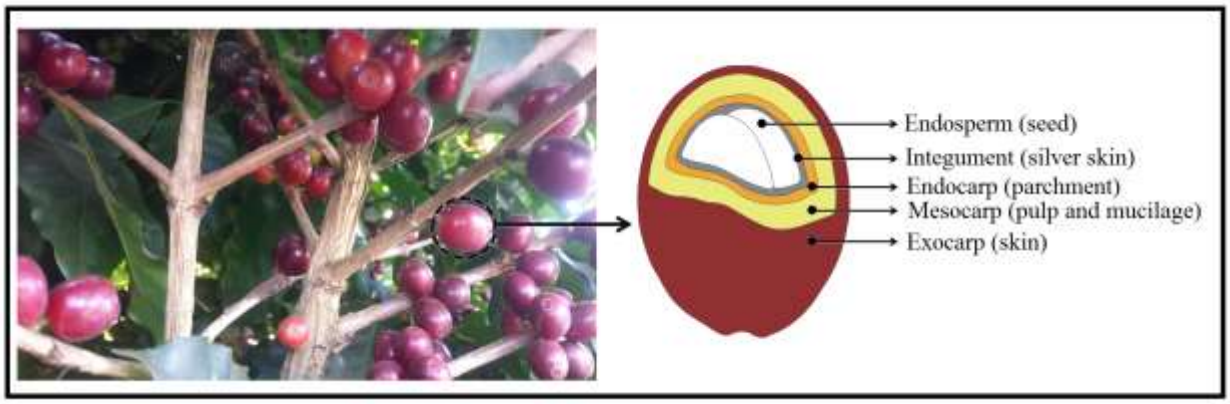

Fig.2 Photographs of coffee fruit and its section

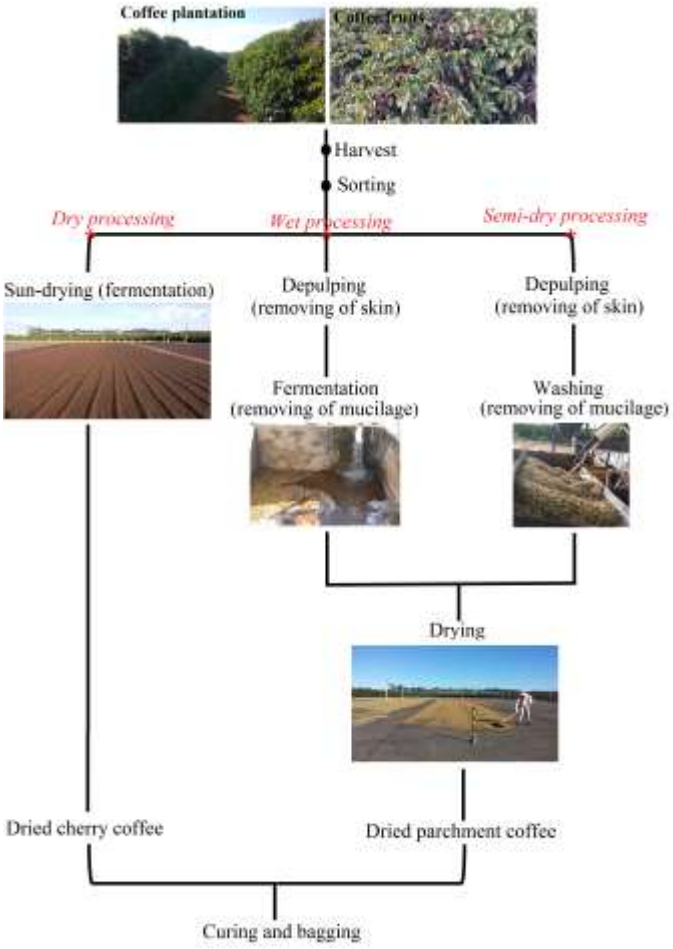

Fig. 3 Different methods employed to process coffee fruits

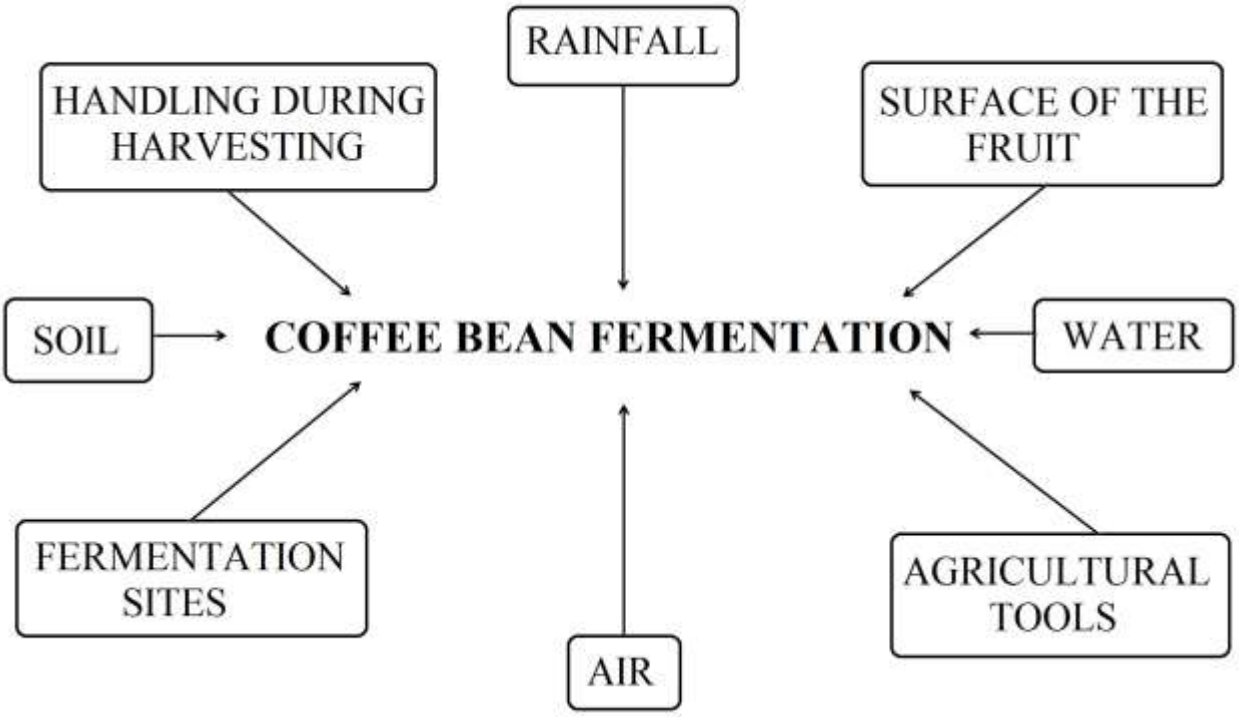

Fig. 4 Different sources that microorganisms can access during coffee fermentation

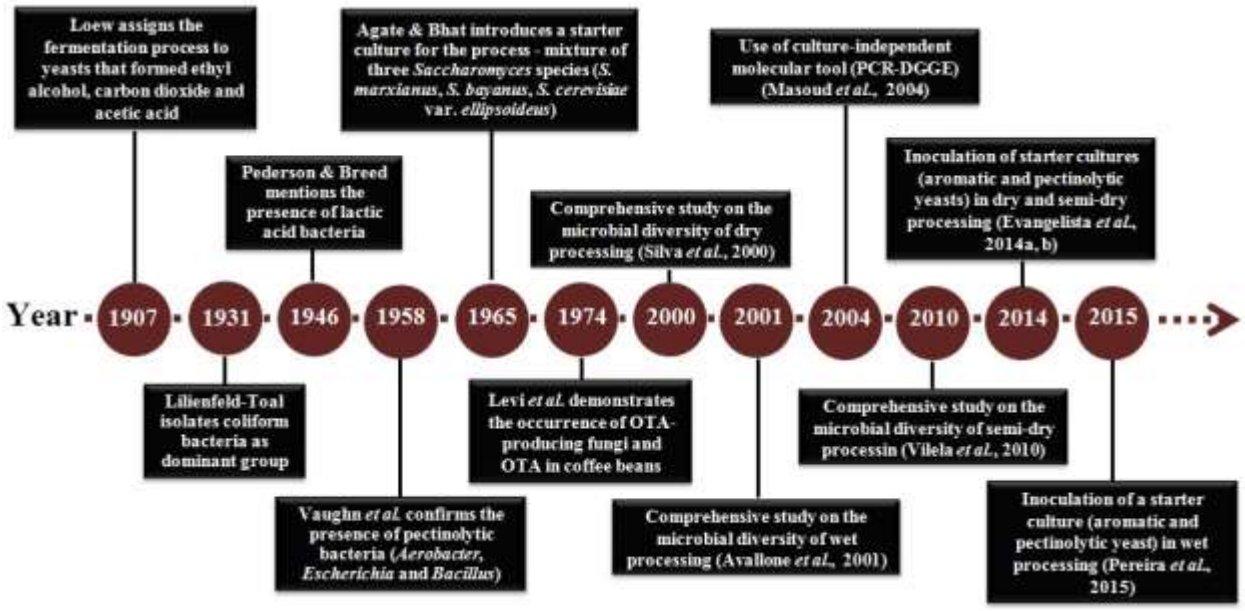

Fig. 5 Timeline for the major events in studies of the coffee fermentation process