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Acetic acid bacteria in fermented foods and beverages

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Although acetic acid bacteria (AAB) are commonly found in spontaneous or backslopped fermented foods and beverages, rather limited knowledge about their occurrence and functional role in natural food fermentation ecosystems is available. Not only is their cultivation, isolation, and identification difficult, their cells are often present in a viable but not culturable state. Yet, they are promising starter cultures either to better control known food fermentation processes or to produce novel fermented foods and beverages. This review summarizes the most recent findings on the occurrence and functional role of AAB in natural food fermentation processes such as lambic beer, water kefir, kombucha, and cocoa.

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Introduction

The production of fermented foods and beverages was originally performed to enhance the shelf-life of perishable raw materials of agricultural and animal husbandry origin. Today, this bioprocess technology aims at the use of microorganisms and their enzymes, through acidification, alcoholisation, proteolysis, and/or amino acid conversions, to make products with desirable quality characteristics regarding shelf-life, texture, taste, mouthfeel, flavour, and colour [1,2]. Moreover, it plays an important role not only in producing nutritious foods in a natural, rather cheap, and sustainable way worldwide but also in manufacturing foods with health-promoting properties. Lactic acid bacteria (LAB; Firmicutes), yeasts (Fungi), and moulds (Fungi) are predominant in fermented dairy, meat, cereal, vegetable, alcoholic, and other fermented foods and beverages. Also, coryneforms (Actinobacteria) and acetic acid bacteria (AAB; α-Proteobacteria) play a pivotal role in the production of some of these products. However, AAB are not studied to the same extent as many other food-grade and industrially important microorganisms [3]. AAB are predominantly known for their use in the production of vinegar, vitamin C, and cellulose. Moreover, AAB are regarded as undesirable spoilers in alcoholic fermentations (wine, cider, and beer). In this review, several examples are presented on the occurrence and functional role of AAB in natural food fermentation processes, such as lambic beer, water kefir, kombucha, and cocoa (Figure 1).

Cultivation and identification of acetic acid bacteria

One of the reasons why AAB have not been studied widely is that their cultivation, isolation, and identification is cumbersome, in particular during spontaneous food fermentation processes harbouring a wide variety of microorganisms and where they often occur as viable but not culturable (VBNC) cells. Challenging plating of AAB could be accommodated by optimising a selective agar medium, in particular modified deoxycholate–mannitol–sorbitol agar [4,5]. Also, discriminatory high-throughput dereplication and identification techniques and high-throughput sequencing facilitate the determination of the abundance and functionalities of AAB in fermented foods and beverages. Recent examples are matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) [6•,7,8,9•] and sequencing of metagenomic DNA [10•,11•,12•]. A MALDI-TOF MS database containing profiles of approximately 280 reference strains, covering 17 genera and more than 80 species representing the family *Acetobacteraceae*, allows a fast and accurate identification of AAB [9•].

Ecophysiology of acetic acid bacteria

The ecology and physiology of AAB has been described in detail recently [3]. AAB are commonly found on plants, flowers, and fruits. These aerobic environments are rich in carbohydrates, sugar alcohols, and/or ethanol. This enables AAB to rapidly and incompletely oxidize these substrates into organic acids for energy production through a specific respiratory chain. Consequently, an acidification of the environment takes place, thereby preventing the growth of competitors, while the producing cells possess several mechanisms to tolerate the acidity. Also, they can utilize the accumulated organic acids later to further sustain their growth. AAB cells capable of cellulose production form biofilms that allow their retention on the culture surface, which is favourable for the survival of these strictly aerobic bacteria. All these physiological features explain their occurrence and underlines their functional role in the production of

Figure 1

Natural food fermentation processes with acetic acid bacteria. Examples of spontaneous food and beverage fermentation processes in which acetic acid bacteria participate. From left to right: the end of a cocoa bean heap fermentation; fermenting lambic beer in oak casks; water kefir fermentation in a closed jar with the water kefir grains visible as a sediment; and kombucha fermentation in a vessel with the tea fungus visible as a floating cellulose layer.

diverse fermented foods and beverages such as lambic beer, water kefir, kombucha, and cocoa. Alternatively, AAB are associated with different plant and insect species, thereby promoting the growth and development of these species.

Lambic beer

Belgian lambic beers are refreshing, alcoholic, acidic beers with fruity notes and little residual carbohydrates, which become increasingly popular worldwide. They are produced through spontaneous fermentation of water, barley malt, unmalted wheat, and aged dry hops in horizontal wooden casks and mature for up to three years [13]. Most knowledge on the lambic beer production process originates from earlier studies, which relied entirely on culture-dependent microbiological analyses. They have shown a microbial succession of *Enterobacteriaceae* and wild oxidative yeasts, *Saccharomyces cerevisiae* and/or *Saccharomyces pastorianus*, *Pediococcus damnosus* and/or *Lactobacillus brevis*, and *Dekkera (Brettanomyces) bruxellensis*. A revision of the microbial species diversity and community dynamics culture-dependently, making use of MALDI-TOF MS for species identification, has confirmed this succession of microorganisms, albeit that artificial acidification of the wort at the start of the fermentation, which is common practice in today's lambic breweries, does not allow growth of enterobacteria [6•,7]. Also, American coolship ale production mimicking Belgian lambic beer production displays similar microbial profiles, as revealed by amplicon sequencing, targeting both bacterial and yeast species and showing the presence of *Acetobacter* species [14•]. However, AAB are only sporadically recovered throughout the lambic beer fermentation and maturation process, probably due to a VBNC state of their cells [6•,7]. Yet, two new AAB species have been described that seem to be characteristic for acidic beers, namely *Acetobacter lambici* [15] and *Gluconobacter cerevisiae* [16]. It is likely that the obligate aerobic AAB are concentrated at the wort/air interface and, hence, are missed through submerged sampling of the casks. Recent investigations have shown the presence of *Acetobacter orientalis* at the start of the fermentation and *Acetobacter pasteurianus* upon maturation (De Roos J, Vandamme P & De Vuyst L, unpublished results). Similarly, *A. pasteurianus* has been isolated from Belgian red-brown acidic ale productions, whose maturation takes place in vertical wooden casks [17]. Together with the lactic acid produced by the LAB, the AAB are responsible for the acidic flavour of lambic beers through the production of acetic acid.

Water kefir

Water kefir is a sparkling, refreshing, low-alcoholic beverage with acidic and fruity flavours. It is obtained by spontaneous fermentation of water, sucrose, (dried) fruits (e.g., figs), and water kefir grains (dextran grains incorporating microorganisms that serve as the inoculum) in a closed jar at room temperature for 2–4 days [18]. The water kefir grain inoculum determines the grain growth, the microbial species diversity, and the metabolite concentrations [19]. The core microbiota encompasses the LAB species *Lactobacillus hilgardii*, *Lactobacillus nagelii*, and *Lactobacillus paracasei*, and the yeast species *S. cerevisiae*, which mainly produce lactic acid and ethanol plus esters, respectively. Although not considered as part of the core microbiota, AAB (low counts) are often found during water kefir fermentation, as revealed by both culture-dependent [18–21] and culture-independent methods [18–20,22–24]. Growth of AAB particularly occurs under aerobic conditions, leading to increased acetic acid concentrations, which might be undesirable [22–24]. Under anaerobic conditions, they remain in a VBNC state, being metabolically dormant, and when oxygen becomes available they start to grow [24].

Whereas several species of the genera *Acetobacter*, *Gluconacetobacter*, and *Gluconobacter* can be recovered from water kefir fermentation processes, *Acetobacter* species seem to be better adapted to the water kefir ecosystem [18,20–23]. The improved isolation of AAB from water kefir and cider has led to the discovery of the novel species *Acetobacter sicerae* [25]. Water kefir may not be confused with milk kefir, which is an acidic and slightly alcoholic, foaming, fermented milk beverage produced with milk kefir grains in a closed vessel at room temperature for 24–48 h. Moreover, grains of milk kefir are composed of kefiran polysaccharides and harbour a substantially different microbial species diversity, encompassing not only several LAB and yeast species but also AAB, albeit in variable counts [23,26,27].

Kombucha

Kombucha is prepared with water, sugar, tea, and a kombucha culture ('tea fungus') in open vessels at room temperature for 1–3 weeks. This non-alcoholic beverage possesses a sharp acidity and specific flavour. The kombucha cellulose layer that composes the tea fungus and maintains the cells in close contact with oxygen is a consortium of bacteria (AAB and LAB) and yeasts responsible for the fermentation process, as revealed by metagenetic analysis [28,29]. The most prevalent core microbiota in kombucha fermentation are AAB (high counts), which include *Komagataeibacter* and *Gluconobacter* species; *Acetobacter* species are less abundant [30]. *Komagataeibacter xylinus* is responsible for the cellulose formation. Other AAB species have potential in both producing cellulose and fixating nitrogen [31–33]. Although the microbial community composition of kombucha depends on the growth conditions of its members, such as available energy sources, temperature and oxygen tension, AAB are part of the relatively stable bacterial community and are responsible for the production of acetic acid (from ethanol), gluconic acid (from glucose), glucuronic acid (from glucose; detoxifying properties), and D-saccharic acid-1,4-lactone (from glucose; radical-scavenging; some *Gluconacetobacter* species) [29,34,35•,36]. Hence, optimisation of production yields of glucuronic acid and antioxidative activities of kombucha is of importance [36,37].

Cocoa

Fresh cocoa beans need to be fermented through a spontaneous on-farm process, characterised by a fairly strict succession of microbial communities (yeasts, LAB, and AAB), to serve as raw materials for chocolate production [38]. After 4 days of cocoa bean fermentation, the carbohydrate-rich cocoa pulp is fully degraded and the cocoa beans are ready for drying. This degradation process includes cocoa pulp pectin depolymerisation that decreases the viscosity of and allows air ingress into the cocoa pulp-bean mass. Meanwhile, ethanol (from glucose), lactic acid (from glucose and fructose), acetic acid (from citric acid and glucose), and mannitol (from fructose), produced by yeasts and/or LAB, become available as substrates for AAB. All these conditions favour the growth of AAB, which form part of the core microbiota and oxidize those substrates into acetic acid and acetoin [4,5,39,40,41••,42]. Upon fermentation, acetic acid diffuses into the cocoa beans, kills the seed embryo, and initiates a series of enzymatic reactions in the beans that produce the typical cocoa bean flavour and colour [38]. *Acetobacter pasteurianus* is the principal AAB species thriving in and conducting cocoa bean fermentation processes, as shown culture-dependently and culture-independently, thanks to its adapted metabolism, acidic stress responses, and thermotolerance [10•,43,44•]. Poor cocoa bean fermentations suffer from too early acetic acid production during fermentation or from gluconic acid production in a later phase of the fermentation process, causing acidic beans with deviating flavours [45,46]. To optimise the laborious, spontaneous cocoa bean fermentation process, starter cultures are being developed for its standardization, among which AAB [38,47–49]. Although wet coffee processing encompasses a mixed-species fermentation step too, AAB do not prevail; however, they do occur during dry coffee processing, as revealed by metagenetic sequencing [11•].

Conclusions

Although AAB are commonly found in (backslopped) fermented foods and beverages, their role often remains undetermined in terms of strains' functionality and microbial interactions. Therefore, studies focussing on these functionalities would be a welcome addition to today's research, for instance through a combined approach of physiological, high-throughput microbiological, metagenomic, and meta-metabolomic analyses of food fermentation processes [10•,11•,12•,35•,44•]. Furthermore, given the consumption trends towards natural, healthy, and sustainable food products with less sweet flavour and taste profiles, a revival of traditional fermentation processes that make use of AAB may lead to new fermented foods and beverages. For instance, AAB may be applied in the manufacture of healthy all-natural alternatives for soft drinks, among which malt-fermented (*Gluconobacter oxydans*) Bionade is a commercial example (http://www.bionade.de/en/production-process/). Alternatively, AAB may be used to initiate fermentations of, for instance, fruit juices to produce non-alcoholic, acidic, and refreshing beverages. Moreover, many of the fermented beverages mentioned above are thought to contain a number of health-promoting properties, as they may harbour strains with probiotic potential (e.g., kefirs harbour bifidobacteria) [18,19,50], display anti-oxidant activity (e.g., kombucha) [35•], or have other physiological impacts [2]. In this context, kombucha brewing is one of the fastest growing functional beverage production branches.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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