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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].