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LAB REPORT
https://doi.org/10.1071/MA22026

Microbiology Australia

Exploring lactic acid bacteria diversity for better fermentation of plant-based dairy alternatives

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Received: 19 March 2022
Accepted: 20 April 2022
Published: 17 May 2022

Cite this:
Huang W *et al.* (2022)
Microbiology Australia
43(2), 79–82. doi:10.1071/MA22026

ABSTRACT

Plant-based foods have risen in popularity in recent years including a number of dairy alternative products. Fermentation has the potential to support the development of innovative plant-based foods with enhanced flavour, texture and nutritional quality. Lactic acid bacteria (LAB) have been used for thousands of years to carry out fermentation of a wide variety of food substrates through production of organic acids and flavour compounds. However, LAB strains used in dairy fermentations are commonly found to be suboptimal in their metabolism of plant substrates, so efforts to identify alternative strains are needed. We provide an overview of the plant-based milk alternative category and explore screening approaches (including citizen-science efforts) to identify new LAB that hold potential in acidification and flavour formation of plant-based substrates.

Keywords: almond, alternative protein, citizen science, dairy-alternatives, fermentation, flavour, lactic acid bacteria, new foods, plant based.

The rise of plant-based foods and the non-dairy category

Consumer interest, preference, and market share for plant-based foods has grown significantly in recent years. This has been due to greater health conscientiousness in consumers, concerns around animal welfare and environmental impacts of greenhouse gas emissions, health-related issues such as dairy allergies and lactose intolerance, as well as the increased promotion of plant-rich diets and interest in veganism.

Plant-based food products include natural (fruits and vegetables) or processed (plant-based meat and milk alternatives). As milk is one of the most widespread and nutritious food sources, its plant-based alternatives have also seen high demand over the past decade.[1] This article will focus on plant-based milk alternatives (PBMA) and explore the potential of fermentation to enhance their flavour, texture, and nutrition.

In 2021, the worldwide PBMA revenue was US$19 billion.[2] The most common sources of PBMA include soy, almond, coconut, rice, and oat, with others including macadamia, walnut, pea, banana, and flax being less common. In Australia, the most popular PBMA currently is soy-based; however, demand for almond PBMA is growing rapidly.[3,4] The PBMA market share captured by almond-based PMBA increased from 18% to 44% between 2015 and 2020, while soy-based PBMA reduced from 69% to 48% in the same period. Australia is the world's second largest producer of almonds with ~$1 billion grown each year, and almonds are our most valuable horticultural export,[5] making this an attractive target for fermented PBMA product development.

In addition to being naturally lactose-free, PBMA contain bioactive compounds that are absent or have low bioavailability in cow milk, such as dietary fibres, antioxidants and phytoestrogens.[6,7] Furthermore, legume- and seed-based PBMA are promising plant-based alternative protein sources.[8] However, PBMA can have several disadvantages including undesirable flavour profiles (e.g. beany flavour in soymilk), a less comprehensive nutritional profile compared with cow milk, anti-nutritional compounds and potential allergenic activity. Fermentation is one potential option that can be investigated to improve PMBA nutritional properties and sensory attributes, and reduce allergenicity.[9]

Lactic acid bacteria (LAB) and their potential in PBMA fermentations

The most common group of bacteria used in food fermentations are LAB. For industrialised (controlled) fermentations, defined LAB strains are used as inoculants. LAB can be grouped into 'starter cultures', whose primary role is to produce lactic acid or 'adjunct cultures' that are used for flavour formation. Many fermented dairy foods including cheese, yoghurt, sour cream, cultured buttermilk, kefir and kumis all utilise LAB (such as *Lactococcus*, *Lactobacillus* and *Streptococcus*). Large culture supply companies provide a wide range of well characterised strains in live freeze-dried powders for dairy applications. However, these strains are adapted to dairy substrates and can be less suited for dairy-free plant bases due to differences in sugar, protein and sensitivity to growth inhibitors.[10,11] In preliminary work, we compared the ability of a cheese starter culture strain (strain A) to acidify cow milk and almond milk (a model PBMA). It acidified cow milk but was incapable of lowering the pH of almond PBMA (Fig. 1). Therefore, there is significant interest in identifying new strains better suited for acidification and flavour formation in plant-based substrates. LAB are commonly found on plants and wild strains exhibit greater metabolic capability than industrialised (domesticated) strains.[12] Therefore, searching for strains from plant niches suitable for plant-based fermentations appears logical.

In some preliminary work using the almond PBMA model, we have screened a collection of ~600 LAB, sourced from a wide variety of vegetables, fruits, and herbs for their ability to acidify almond PBMA. Previous work has shown that this LAB collection can be a novel source of diverse strains that have beneficial anti-bacterial activity, anti-fungal activity or food flavour production.[13–16] While >90% of the plant-derived strains of LAB acidified almond PBMA poorly, several strains possessed medium-to-strong almond PBMA acidification. As an example, strain B was identified as a strong acidifier, strain C a medium acidifier and strain D a non-acidifier (Fig. 2). Further work identifying the strains and also understanding why different LAB acidify almond PBMA differently using whole genome sequencing, metabolic and genetic methods is underway.

Citizen science as a tool for microbial discovery

Obtaining and screening LAB strain collections, as we have done previously as described above,[16] prior to determining their potential in plant-based food fermentations is however both costly and laborious. There is a need to first collect samples, grow, isolate, and identify microbes before even beginning to filter through the strains for applicable candidates. One way to raise efficiency and lower the costs is to make use of Citizen Science (CS), where the public is involved in some or all of the processes. CS is a strong tool for massive sample and data collecting on a low budget and in a short time span, exemplified by the Danish 2018 project Mass Experiment[17] (http://www.bacteriadanica.dk/) where school children collected over 30 000 environmental samples. The project ended up identifying ten new species of LAB. In another study[18] researchers were able to collect more than 500 unique sourdough starter culture samples from four different continents within 3 months, showing the potential of wide geographical coverage when using the public for sampling. CS has even been used to strengthen the possible origin of ancient yoghurt production, which includes the initiation of fermentation in milk, by adding twigs and leaves of specific plants.[19] Here, researchers collected hundreds of plant samples, from which the traditional yoghurt species *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* were isolated. CS in natural sciences is already well established, even though it might be called different names. A well-known phenomenon is the BioBlitz, where both experts and hobbyists contribute to the identification of species in a defined area within a short period. This type of CS is often used for identification of plants, insects, or larger animals, but could in principle just as well be used for microbial identification. Today's social media platforms, global connectivity, and technological advances in terms of e.g. genomic screening, microbial identification and characterisation can enable much faster discoveries, than if CS was used just 20 years ago. This further strengthens the arguments for implementing CS in more research-based work.

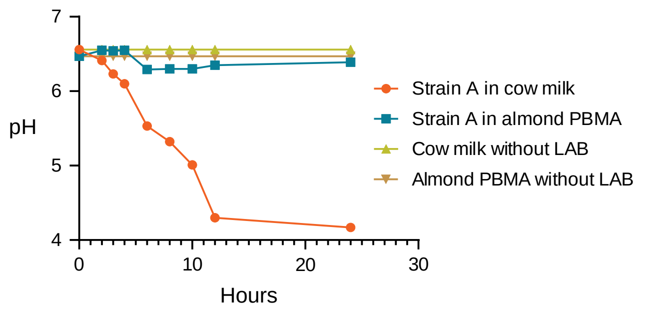

Fig. 1. Acidification bystrain A incubated in cow milk and almond PBMA held in non-shaking tubes at 30°C. Data are representative of at least two independent trials.

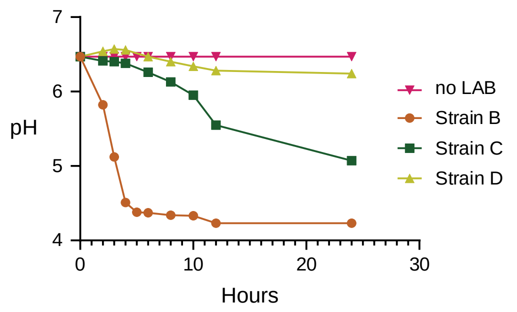

Fig. 2. Acidification bythree plant-derived LAB strains incubated in almond PBMA held in non-shaking tubes at 30°C. Data are representative of at least two independent trials.

Importance of microbial fermentation for flavour formation in plant-based alternatives

To optimise a plant-based fermentation for animal-based alternatives, flavour is key to success. As an example, various compounds produced during lactic acid fermentation are integral factors in obtaining a flavour profile like that of dairy yoghurt. This includes lactate and acetate (acidic), acetoin, diacetyl (buttery), and acetaldehyde (green apple), and even ethanol to name a few.[9,20] The production of these compounds relies on specific precursors in the plant base, as well as LAB capable of fermenting these precursors. Lactic acid fermentation can also decrease the amount of 'beany' off-flavours found in soy products, while also providing dairy related flavour compounds.[21] Madsen *et al.*[11] proved the importance of specific bacterial strain selection, and showed that dairy-adapted yoghurt starter cultures were unable to compete with plant-adapted ones in a mixed soy and malt base. The number of studies characterising flavour development of fermented PBMAs are much fewer than that for meat alternatives.[22] Kaczmarska *et al.*[23] investigated the chemical composition and sensory profile of various meat analogues, as well as fermented plant products such as tempeh and tofu, which are often regarded as meat-like substitutes. The results showed that only a few alternatives had a sensory profile vaguely resembling a meaty taste, and vastly different flavor profiles. Still, the study argued the possibility of using natto and tempeh as an ingredient in a meat substitute.

Future opportunities/challenges

With the recent public surge in interest of plant-based alternatives, the outlook of better quality products is promising. Still, there are several hurdles that need to be overcome, in order to create 'true alternatives'. Nutritional values are hard to reach without additives such as B12 and calcium,[9] texture of cheese alternatives is difficult due to the lack of casein proteins in plants. Likewise, it is complicated to correctly balance the flavor profiles of meat analogues due to the complicated landscape of various contributing compounds.[24] Several start-up companies and non-profit organisations, such as Perfect Day and Real Vegan Cheese, are already trying to tackle many of these obstacles through genetically modified yeast or bacteria producing the main milk protein casein, enabling a way of producing vegan alternatives. But this is still very much on a theoretical stage, and a complicated procedure. The use of plant-derived, non-modified microbes could still prove useful in this regard. Projects such as the Mass Experiment and large sourdough starter culture study[18] has proven that CS is a strong tool, and that nature still holds more potential in terms of new tools for food fermentation. Hopefully future plant-based fermentation research will pave the way for new applications.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare no conflicts of interest.

Declaration of funding. Claus Heiner Bang-Berthelsen has been funded by the Danish national grant GUDP (MycoProtein) [grant number 34009-20-1682]. Anders Peter Wätjen has been funded by the EIT Climate-KIC grant: From brewery waste to new taste (BSG 2.0) [project ID 200820]. Both Wenkang Huang and Anders Peter Wätjen were awarded scholarships from a joint PhD alliance initiative between UQ and DTU.

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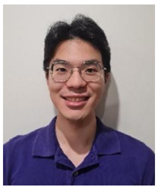
Biographies



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