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To cite this article: A Djukic-Vukovi et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 854 012025

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Lactic acid bacteria: from food preservation to active packaging

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Abstract. Lactic acid bacteria (LAB) have acted in food fermentations through the ages due to their safety and resilience to specific harsh conditions of high salinity or low pH present in food and gut where they live. Their interaction with human technological development started in food but goes beyond, as some LAB contribute to the health of humans and animals as probiotics. The stress tolerance of LAB also makes them excellent, robust industrial microorganisms for production of lactic acid and other chemicals. The lactic acid market has had a high growth rate in the last decade mainly due to expansion of poly-lactide production. Poly-lactides are biocompatible, thermostable and biodegradable polymers of lactic acid, suitable for use in food packaging or in medicine, as scaffolds, implants or delivery systems. The ability of LAB to grow on complex waste substrates but efficiently produce selected isomers of lactic acid has positioned them at the core of bio-based packaging production, and this field is expected to grow in the future. Therefore, LAB are important for food - for preservation, flavour and packaging, but also beyond food – as probiotics, paraprobiotics and postbiotics. Recent trends in these fields of LAB application are analysed in this work.

1. Introduction

Fermentation was a turning point in food consumption and, consequently, in human history. It prevents food spoilage, human hunger and undernourishment and decreases food wastage [1]. This spontaneous process was the first application of biotechnology for safer, healthier and longer available nutritious food, which contributed to the change from hunter-gathering human communities towards agricultural society [2]. Today, fermented food consumption is rising, as is interest from the scientific community in new ways to apply fermentation as a processing method [3].

Lactic acid bacteria (LAB) are largely responsible for many traditional food fermentations since the dawn of consumption of these foods. All LAB have the common metabolic characteristic of producing lactic acid, and that has been the criterion to categorise a very diverse group of microorganisms as LAB. Taxonomically, Lactobacillus and 22 new related genera [4], Lactococcus,

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Enterococcus, Streptococcus, Pediococcus, Leuconostoc and *Weissella* genera are most often considered LAB [5].

Representatives of LAB are used as starters, surrogates, probiotics, paraprobiotics and postbiotics in food research and technology, but LAB as industrial microorganisms are also used to produce lactic acid in biorefineries. LAB can use variety of substrates rich in fermentable sugars to grow and stereoselectively produce L(+) or D(-) lactic acid isomer for synthesis of FDA-approved polymers – poly-lactides [6]. This puts LAB in a unique place to be used as live or inactivated microorganisms in food and food supplements but also, as industrial microorganisms that can convert food waste and similar complex substrates into important compounds for food, chemical and other industries. As end products, poly-lactides are used for packaging, from nanocapsulated ingredients to disposable caps in the food industry, and from stents to scaffolds in medicine, because of their biocompatibility and thermostability [7]. Thus LAB are highly important microorganisms for the circular economy processes that are expected to dominate production in the next decades.

2. Lactic acid bacteria in food

Lactic acid bacteria in food are present as starters to provide acidity, preserve food and contribute to flavour. When applied as starters, production of acids and other antimicrobial compounds (peroxide, bacteriocins, etc.) plays a key role in food preservation [8,9] while proteolysis and exopolysaccharide production mainly affect food texture [10–12]. Very comprehensive reviews on LAB as starters [5,13], particularly in the dairy [8] and meat [14] industries are available, and commercial formulations contain mostly *Lactobacillus*, *Lactococcus* and *Streptococcus* species. However, artisanal and traditional production of fermented food depend on much wider microbial communities of LAB called non-starter LAB [15,16]. Recent advances in molecular diagnostics equipped scientists to study more deeply the microbiota of fermented food and feed. Processing of feed, through ensiling for example, also depends on naturally occurring microbiota and often results in the variable quality of the final silage [17]. In depth studies are expected to give new strains soon to be applied in industry, particularly for vegetable based food [18], which is increasing in market share. The need to adequately classify fermented food and feed is recognised by relevant bodies with the aim to stimulate and regulate the fermented food market [3].

Metabolic pathways in LAB are the result of an interplay between the abundance of nutrients present in their natural habitat – food or human gut – and stressors naturally existing in these environments, for example high salinity, very low pH or bile salts. The ability of LAB to survive microbial stress involves the "stressome" [19], which makes these bacteria robust enough for industrial application, but also makes them suitable as surrogates in challenge tests. With rising application of non-thermal technologies, LAB are often used to test and optimise treatment conditions, for example food preservation by pulsed electric field [20,21]. With generally recognised as safe (GRAS) status, LAB can be used without hampering or compromising food safety during product development.

3. Lactic acid bacteria beyond food

One subgroup of LAB has positive effects that go beyond safety or flavouring of food – these are probiotic bacteria. Probiotics are: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [3,22]. Not all LAB are probiotics, but probiotic LAB should potentiate food functionality, being a valuable contribution even in the treatment of some diseases. The main requirements for probiotic bacteria are to apply them live and to provide evidence about their health benefit for the host, while fermented food does not need to show any effect over the nutritional value of the food matrix. This is the main difference between fermented food and probiotic fermented food. However, evidence is accumulating that even cell components of probiotics or inactivated whole bacteria could provide health benefits to the host [23,24]. These findings open space for new types of products derived from probiotic bacteria – postbiotics and paraprobiotics. Postbiotics are defined as soluble compounds that can be produced by probiotic bacteria or released after the inactivation and lysis of bacteria [25,26] – enzymes, bacteriocins, organic acids, peptides,

exopolysaccharides etc. Paraprobiotics are inactivated probiotic bacteria which still affect human health in positive manner [27], but also can have some technological advantages over live bacteria – like lower susceptibility to contamination and better process control [28]. These are new concepts in food supplementation introduced in the last five years, but exploited previously in traditional food production, and all rely, firstly, on efficient biotechnological production of LAB biomass.

4. Lactic acid bacteria for food

Selected strains of LAB can produce L(+) or D(-) lactic acid with very high stereo-specificity of above 95% [29] on complex substrates like food wastes, by-products or residues, and which cannot be achieved in chemical processes. This is particularly important for production of poly-lactides. Physicochemical characteristics of poly-lactides are determined by the proportion of L(+) or D(-) lactic acid in the polymer [30], so the ability to produce one selected isomer over a racemic mixture has pushed LAB fermentation to dominate lactic acid's production routes.

Poly-lactide production will consume approximately 50% of all lactic acid produced until 2025, as the main driver of lactic acid demand [31]. This creates pressure to provide sufficient amounts of substrates for LAB fermentation without competing with food production. LAB can effectively use complex waste substrates and by-products rich in fermentable sugars like food waste or agricultural waste. When LAB are used for lactic acid production, the biomass remaining after fermentation could be valorised as high value feed additive [32]. However, to achieve high lactic acid yields on lignocellulose-rich substrates, which are by far the most abundant in nature, it is necessary to use genetically engineered strains or to perform a variety of treatments to release fermentable sugars into the fermentation media. Technological challenges limit faster adoption of novel substrates for lactic acid fermentation, but also, environmental impacts have to be thoroughly examined in order to select the best approach. For assessment of overall sustainability, chemical routes for poly-lactide production and processing also play an important role, but a very limited number of studies have addressed this issue. Integrative studies on lactic acid production from alternative substrates and consecutive polylactide production and processing are limited [30], but it is evident that new value chains for bio-based plastics are in the making. Recent legislation, like the Circular Economy Action Plan 2020 [33], will drive the field forward and clarify the path for best practices, more sustainable production and better end-of-life solutions in future.

5. Conclusions

LAB as safe microorganisms have found many roles in supporting human societies for millennia. They enabled some degree of food safety, contributed to metabolism of food and supported our health mainly through the gut. In the future, LAB will be further exploited, particularly in the production of functional fermented food, with our extended knowledge on microbial communities present in traditional production. Randomised clinical studies are needed for analysis of health benefits and more evidence-based applications of probiotics, paraprobiotics and postbiotics.

In biorefinery, the current challenge is to provide enough substrate for lactic acid fermentation by LAB due to the high demand for lactic acid. New legislation stimulates the circular economy and supports creation of new value chains, which can expand the contribution of bio-based polymers beyond their current market share of just 2%. Poly-lactides will play significant roles in packaging production in the future, especially for high end applications, as poly-lactides are FDA-approved. LAB-based processes will have to be soon expanded to more abundant substrates like lignocellulose, with technological advancements including substrate treatments, strain adaptions and innovations in process design.

Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200135) and Project of Technological

IOP Conf. Series: Earth and Environmental Science 854 (2021) 012025 doi:10.1088/1755-1315/854/1/012025

cooperation Serbia-China #I-1. Part of work was done by AĐV and BL within the framework of EuroMicropH-COST Action CA18113.

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