Future probiotic foods

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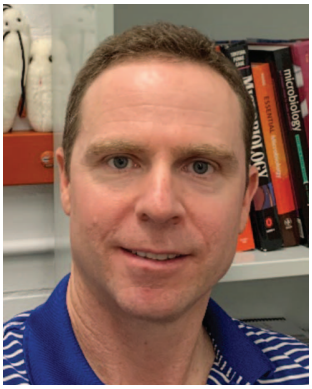

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Abstract. Foods containing edible probiotic bacteria, most commonly *Lactobacillus* and *Bifidobacterium* species, form a multi-billion-dollar industry worldwide. Currently marketed foods containing probiotics are mostly dairy based with yoghurts and fermented milks dominating the industry. Alternative foods as carriers of probiotics are being examined to reduce or eliminate lactose intolerance issues. Food categories including fruit juices, cheese, chocolate and even beer have been shown to be suitable for probiotic delivery. In addition, technologies such as encapsulation in food-grade alginate gels have allowed for improved probiotic survival in certain foodstuffs. We have explored the use of ready-to-eat vegetables such as baby spinach as carriers for commercial probiotics and found that high dose (>8 log CFU/g) can be achieved without having negative effects on appearance, taste or aroma. Leafy greens as well as other foods and beverages may be suitable probiotic containing new food products in the future.

The most commonly used definition for probiotics, initially proposed in 2001 by the Food and Agriculture Organisation of the United Nations (FAO) and supported by the World Health Organization (WHO) is 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'[1]. Most probiotics sold in edible products are *Lactobacillus* and *Bifidobacterium*, while products with *Bacillus, Escherichia coli* and *Saccharomyces* are less commonly available. Probiotic organisms are different to fermentation organisms and the health promoting effects may be only strain specific. More stringency around health claims of probiotics and functional foods in general in various countries has resulted in fewer unsubstantiated marketing claims, which have plagued the probiotic industry for the past three decades. In 2010, the European Food Safety Authority (EFSA) took the strict option of banning all health claims regarding probiotics and until now the only claim that is approved is regarding lactose intolerance prevention through yoghurt ingestion[2]. Nowadays, randomised, double-blind and placebo-controlled studies with high numbers of subjects are the bench mark to demonstrate probiotic efficacy. This is reasonable as probiotics are a major business activity with global sales expected to hit $50 billion by 2022[3]. Despite their controversial history, many scientific studies have demonstrated health promoting activities of specific strains in certain situations. In addition, with the explosion of microbiome insights, 'next generation probiotics', which are defined as 'live microorganisms identified on the basis of comparative microbiota analyses that, when administered in adequate amounts, confer a health benefit on the host', will likely be of significant commercial interest in the coming years[2].

Probiotic foods and beverages

Oral delivery of probiotics can involve a variety of different vehicles. Tablets and capsules containing high doses (e.g. 10 log CFU) of single or mixed strain probiotics are commonplace in pharmacies and supermarkets. The most common foodstuffs containing probiotics are dairy-based, including yoghurts and fermented liquid milks. Other dairy-based foods including Cheddar cheese and chocolate can also support viable probiotic bacteria[4,5] but are yet to make it to market. However, these products can contain high levels of sugar and with around 75% of the world's population being lactose intolerant, alternate non-dairy-based foods which can support probiotic bacteria viability have been investigated[6]. A leading probiotic food producer in the USA, Goodbelly, has developed snack bars containing 9 log viable *Bifidobacterium* BB-12 cells and fruit juice containing *Lactobacillus plantarum* 299v (https://

goodbelly.com/). A new juice product containing alginate micro-encapsulated *Lactobacillus casei* Lc431 cells, called PERKii, was launched in Australia during 2016 (https://perkii.com.au/). Encapsulation of probiotics improves viability during simulated gastrointestinal transit[7] and reduces fermentation of the fruit sugars in the beverage[8].

Other alternatives to dairy-based probiotic foods are cereal, meat and soy-based products. Cereal-based probiotic drinks containing >7.9 log CFU/mL of *L. plantarum* and *Lactobacillus acidophilus* were prepared from single and mixed flours of barley and malt[9]. Interesting recent research has identified that beer can support high survival of probiotic *Lactobacillus paracasei* L26 for 3 weeks before reducing to undetectable levels by 4 weeks[10]. Beer contains several antimicrobial compounds, including alcohol, acid and hops making it a challenging environment for bacteria to survive. A novel approach for the preparation of probiotic breads was developed by coating pan bread slices with sodium alginate film impregnated with *Lactobacillus rhamnosus* GG which could deliver up to 9 log CFU/30–40 g per bread slice[11]. Dry fermented meat products ingested without cooking are potential vehicles to transfer probiotics into the gastrointestinal tract as probiotic cells can be embedded and protected within the meat matrix consisting of protein and fat[12]. When added into fermented sausages, an initial population of 5 log CFU/g of *L. plantarum* 299v increased to 8 log CFU/g after fermentation[13]. Soy protein is also considered as a good protector for probiotics against harsh conditions in the intestine. *Lactobacillus acidophilus* LA-5 showed good growth and survival of >8.7 log CFU/g in a fermented soy beverage stored at 4°C for 21 days[14]. A mix of probiotic bacteria including *Lactobacillus acidophilus*, *L. rhamnosus*, *L. paracasei* and *Bifidobacterium lactis* incorporated into a non-fermented frozen soy dessert exhibited high viable populations exceeding 7 log CFU/g during 6 months storage while maintaining desirable sensory attributes[15].

New probiotic containing vegetable products

To further expand the range of probiotic containing foods, our group has examined fresh ready-to-eat leafy green vegetables as potential carriers. Several probiotic strains that were inoculated onto baby spinach by dipping the leaves for 5 mins in a bacterial suspension resulted in attachment of 7–8 log CFU/g spinach (Figure 1). Viability of probiotic strain A reduced slightly over 7 days, while probiotic strain B increased slightly to >8 log CFU/g. Based on a typical serving size of 60 g of baby spinach a dose of >9.8 log CFU could be achieved, making it equivalent to other high dose probiotic products on the market. We next determined if the probiotics affected the sensory properties of the spinach, such as appearance, aroma and taste. A panel of 40 volunteers, under controlled conditions in a food sensory laboratory, evaluated de-identified spinach samples stored at 4°C for 4 days. Using a triangle sensory test, it was found that there were no statistically significant differences ($P > 0.05$) in the appearance and flavour of spinach leaves inoculated with probiotic strain A or strain B to that of the control samples. Only 12 out of 40 people could differentiate the probiotic strain A containing spinach from the control spinach and 13 out of 40 could differentiate the probiotic strain B spinach from the control spinach. Spinach leaves with and without probiotics had a similar appearance over 7 days of storage at 4°C as shown in Figure 2. It may be concluded that the sensory quality of baby spinach was not adversely affected by the addition of two probiotic strains. In addition, we have found that washing the leaves in various types of salad dressings (e.g. French, Italian, Balsamic) does not detach cells or reduce their viability. Lastly survival of probiotics on spinach in simulated gastrointestinal digestion trials did not reveal any greater reduction in viability compared with

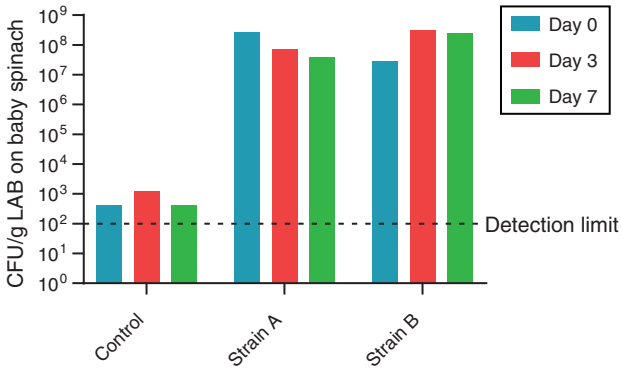

Figure 1. Survival of commercial probiotic strains A and B on baby spinach stored at 4°C. The control is uninoculated spinach, which has low levels of naturally occurring lactic acid bacteria (LAB).

Strain A Strain B Control

Figure 2. Appearance of baby spinach with or without probiotic after 7 days of storage at 4°C.

probiotics suspended in milk. Leafy green vegetables with probiotics may provide an appealing alternative choice for health-conscious consumers in particular.

The future

Our work described here and that of other research groups suggest that there are many unexplored foods which could potentially support good survival of probiotic bacteria. Experimental and industrial trials using these foods are necessary so that factors such as water activity, pH and storage temperature can be optimised for adequate survival of the probiotic. In addition, negative effects on food quality in most cases due to growth and/or fermentation of the food are possible and should be evaluated chemically or using sensory trials. Physiological differences between probiotic species and even strains within species can exist which could mean that only certain probiotics can be incorporated into certain foods. With the explosion of new insights into human health coming from microbiome research, new probiotics and probiotic containing foods and beverages will likely be of significant interest for the food industry and consumers in the future.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

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Biographies

Dr Van Ho received her PhD in food microbiology from the University of New South Wales in 2014. She then worked as a postdoc at the University of Queensland on projects that developed adjunct cultures for Cheddar cheese and biocontrol agents for ready-to-eat vegetables. She is currently working as a R&D Food Technologist at Nu-Mega Ingredients.

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