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Probiotics from an industrial perspective

Sofia D. Forssten [a,*], Connie W. Sindelar [b], Arthur C. Ouwehand [a]

[a] Danisco Bioactives, Health & Nutrition, Sokeritehtaantie 20, 02460 Kantvik, Finland
[b] Danisco USA, 3329 Agriculture drive, Madison, WI 53716, USA

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ABSTRACT

Probiotic products have gained popularity with consumers that expect that the products they consume are healthy and help them maintain health. Hence, the need and preferences of the consumers are translated into a product format concept. Probiotics have been used for a long time as natural components in supplements and functional foods, mainly in fermented dairy products. Most of the strains used as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*. By definition, a strain has to have documented health benefits, in order to be called a probiotic. Although each bacterial strain is unique, there are some points that are essential when selecting a probiotic regarding the genetic stability, survival, and technical properties of a strain. Proper components, food matrices and production processes need to be selected since the matrices may affect the viability of the strain in the product and the intestine. Survival in the product is considered a requirement for the beneficial effects of probiotics.

1. Introduction

It is increasingly acknowledged that a human being is actually a symbiosis of multi-species. In particular the gastrointestinal tract is harbouring a large number of microbes. It is estimated that the diversity of microbes that can be isolated from the human gastrointestinal tract may exceed 1500 [1]. However, a single human being seems to be colonised by a few hundred species. This microbiota has a substantial influence on the health and well being of its host. There have therefore long been sought techniques to modulate the intestinal microbiota composition and or activity. Although it has to be emphasised that modulation of the intestinal microbiota, per se, is not a health benefit.

Antibiotics are most effective at modulating the intestinal microbiota and have, until their ban in the EU, been commonly included in feed as growth promoters. This effect has most likely been obtained by suppressing subclinical infections. However, in human medicine it is well documented that antibiotics can lead to disturbances in the intestinal microbiota; in particular over growth of *Clostridium difficile*, which may lead to antibiotic associated diarrhoea [2]. Other options to modulate the intestinal microbiota include the prebiotic concept: "the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host" [3]. Prebiotics usually are non-digestible oligosaccharides such as fructo-oligosaccharides, galacto-oligosaccharides, inulin, polydextrose, etc. Here we will, however, focus on probiotics; "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [4]. Selected strains of probiotics have specific health effects. These health effects cannot be extrapolated from one strain to another; not even of the same species. The potential health effects of probiotics have been extensively reviewed elsewhere and the reader is referred to selected articles on this topic [5]. Probiotics commonly belong to the genera *Lactobacillus* and *Bifidobacterium*, although also species of other genera are being marketed as probiotics; such as *Saccharomyces cerevisiae (boularii), Bacillus subtilis, Escherichia coli*, etc.

Probiotics, and in particular those belonging to the general *Lactobacillus* and *Bifidobacterium*, are commonly included in fermented dairy products. Due to their physiology, they are very well suited to these kinds of food matrices. However, they can also be included in other matrices as will be discussed below. Another common delivery format is the dietary supplement; especially in these non-lactic acid bacterium probiotics can be found. By definition, probiotics need to be viable at the time of consumption; although non-viable "probiotics" not necessarily are without health effects [6]. This may cause certain food technological challenges as will be discussed. Furthermore, the definition points out that an "adequate amount" should be administered. How much that is, is not defined. As a rule of thumb, a lower limit of 10^9 colony forming units (CFU) per dose is often used; although this may be different depending on the strain, health effect and possibly even the matrix. This has, however, not been investigated to date.

* Corresponding author. Tel.: +358405367810.
E-mail address: sofia.forssten@danisco.com (S.D. Forssten).

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2. Where to find potential probiotics?

There are two major areas of functionality that are required for a probiotic. On one hand, there is the documented health benefit and on the other, the technological properties of the strain. The technological properties include:

- safety
- genetic stability
- ability to be produced at large scale
- stability in the product (maintenance of viability)
- for a fermented product; neutral or positive contribution to flavour or taste

Safety issues will be discussed below. Genetic stability is essential both from a safety perspective, one would not like to have unwanted properties to appear, and from a functionality perspective; nor would one like to have essential properties to disappear. One of those essential properties is the ability of a strain to be grown on large scale; giving a high yield on an economic growth medium. Furthermore, the strain needs to be stable both as a concentrated starter culture and in a product; be it a supplement or a fermented dairy product. Although probiotics and starter cultures have many properties in common, they fulfil different functions in fermented foods. Hence, starter cultures have been defined as "preparations used as food ingredients at one or more stages in the food manufacturing process, which develop the desired metabolic activity during the fermentation or ripening process". They contribute to one or multiple unique properties of a fermented food especially in regard to taste, flavour, colour, texture, safety, preservation, nutritional value, wholesomeness and/or health benefits" [7]. A possibility is therefore to look among existing starter strains from dairy or other fermentations and determine whether these strains may have potential health benefits. A more open minded approach is to collect isolates from sites where one would like the probiotic to exert its health benefits; in practice this often means isolating strains from faecal samples. Although strains can also be isolated from the urogenital tract, oral cavity, etc. this very selection criterion; originating from, e.g. the gastrointestinal tract, often poses challenges from a food technology point of view. Strains of intestinal origin are commonly oxygen sensitive and require specific, often rich, growth media. Thus, although new isolates can be grown on laboratory scale, they may be difficult to grow on industrial scale. For new strains, it is therefore important to make a compromise to have both reasonable chances for industrial up scaling and survival, and success in finding desired health benefits. In addition, a probiotic strain also has to fulfil the needs of the customers, e.g. it has to have good sensory properties and not change the texture or flavour of a product.

3. Safety considerations

Safety criteria for successful probiotics have been defined in several studies [8–10]. It is essential to know the identity of the strain. In the EU; Qualified Presumption of Safety (QPS) is used to evaluate the safety of commonly used food microorganisms. The basis for QPS is the identity and familiarity of with the species.

In order for probiotics to be considered as safe, the strains should be considered to be non-pathogenic, and hence not be associated with diseases. In addition they should not carry any transmissible antibiotic resistance genes. Feeding trials with different probiotic strains have shown that the probiotic strain usually disappears from the gastrointestinal tract within one or two weeks after the ingestion is discontinued. The role of probiotic persistence in the human gastrointestinal tract has therefore been questioned. However, even temporary persistence, which has been noted for most available probiotic strains, may enhance their chances for beneficial functions in the gastrointestinal tract, and is hence considered as a desirable feature. In order to assess the possible effects of probiotic consumption it is helpful to know the viability of probiotics passing through the gastrointestinal tract, as well as their potential translocation and colonization properties. For lactobacilli and bifidobacteria these risks are considered to be small [11].

When assessing the risk of probiotic consumption the target population has to be taken under consideration. If the target group consists of immune compromised individuals the risk-benefit ratio has to be clearly established. A low risk may be acceptable and hence relevant information on the efficacy and safety of the products is required. However, patients from a risk population are under supervision of a physician and the rare cases of lactobacillus infection that have been documented are easily treated, once recognized.

4. Large scale production

In order to provide consumers with products that provide meaningful levels of probiotics, several requirements have to be fulfilled by probiotics selected for industrial production. Important quality-control properties must constantly be monitored and optimized; adhesive properties; bile and acid stability; viability and survival throughout the manufacturing process; effects on carbohydrate, protein, and fat utilization; and, especially, colonization properties and immunogenicity [9]. Most of these properties are related to the physiologic properties of the strain, but long-term industrial processing and storage conditions may influence probiotic properties. Thus, in addition to technologic properties, functional properties should be considered in quality-control measures [12].

The production process of probiotics consists of several different steps, starting with fermentation. For the fermentation process appropriate ingredients need to be selected and possible allergens needs to be properly chosen with regard to the final product. Likewise, possible halal and kosher status need to be taken into consideration. In addition the growth conditions for the probiotic have to be optimized with the right oxygen tension, pH, and temperature [13]. During fermentation, the pH is lowered and different metabolic products like acetic acid, lactic acid, and bacteriocins may appear in the product. After fermentation, a large amount of probiotic biomass has been produced that needs to be concentrated. The traditional method of concentration would involve centrifugation, although nowadays also membrane filtration can be utilized. These methods should be carefully selected to ensure no negative impact on the probiotic.

After the biomass has been concentrated the cells need to be preserved. Industrially, the bacterial preservation technology is to a large extent concentrated on preserving integrity of biological membranes and associated proteins through the drying process, in order to maximize cell recovery following rehydration. Compounds called cryoprotectants are used to stabilize the membrane integrity and to minimize the degrading effects caused during the freezing and drying steps. The stabilized material can then be frozen for the freeze-drying process, and this can take place either inside the freeze-drier (tray freezing) or before the placing the material in the freeze-drier. During this step it is important to minimize ice crystal formation. Thereafter the material can be dried. During freeze-drying, solid water is removed via sublimation, which bypasses the liquid phase, under vacuum and at very low initial temperatures. The freeze-drying step needs to be carefully optimized since

it is a critical process involving careful consideration of finishing temperature and desired residual moisture. The residual moisture content along with the stabilizer components chosen can have a substantial impact on the shelf-life. When the material has been carefully milled to a product with appropriate particle size, it's ready for blending and packaging. Commercial blending options are available for the mixing process that maximizes homogeneity and minimizes mixing time. The final packaging material should be carefully selected in order to ensure a good shelf-life e.g. by minimizing oxygen and moisture transfer into the product.

5. Strain stability

Probiotics have to survive the different production processes with different levels of water activity, pH, oxygen content and temperatures. Additionally, starters culture used in the production of fermented food may influence the stability of the probiotic bacteria and hence the functional properties of the bacteria may be altered. Probiotic strains must therefore retain the characteristics and viability during the production processes and storage in different surroundings [14,15]. Moreover, probiotics have to endure consumption and transit through the gastrointestinal tract [16], even after a long storage time. For products where the probiotic is actively metabolizing, the stability can be a function of the inherent capabilities of the bacteria as well as the physical properties of the food matrix. If stability is defined as the ability to survive under given circumstances, it cannot be firmly linked to the characteristics of a particular genus or species, even though there are typically similarities. However, the exact survival is linked to a specific strain.

It is vital to test the stability in order to ensure that the characteristics are retained in different types of foods. The stability of *Bifidobacterium lactis* HN019 is shown as an example in different matrices in Fig. 1. Viability is not easily defined in microbes. Generally, culturable probiotic strains are considered to be viable. However, microbes can be damaged and enter a so-called viable-but-non-culturable state. It is possible to study the viability of microbes by e.g. staining techniques for determination of the cell membrane integrity or enzyme activity [17]. Some studies have shown that also non-viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host [6]. Hence, for some probiotic strains it might be adequate that they grow well during the initial production steps in order to obtain high enough numbers in the product, but do not necessarily need to retain a good viability during storage and/or passage through the gastrointestinal tract.

The matrix and type of product has a large impact on the viability and shelf-life [18]. The stability and viability of probiotics in dry state are affected by the surrounding temperature and water activity, where water activity is a function of temperature. It is possible to maintain the probiotic viability and retard unfavourable chemical and enzymatic reactions that may lead to cell death by preserving the integrity of the microbial cell membrane by cooler temperatures and low water activities ($<$0.20). Hence, prolonged exposure to elevated temperatures ($>$30 °C) should be avoided. Confectionary has a lower water activity and can be stored at higher temperature (4–30 °C) and have a longer storage time for about 12 months (Fig. 1). Capsules, tablets and powders have a very low water activity and hence the storage time is longer between 12 and 24 months. Dairy products and juices have a high water activity ($>$0.90) and a low storage temperature (4–8 °C) with a short storage time of weeks to months at the most (Fig. 1).

In addition, acid tolerance is an important feature for probiotics. Fermented milks and yoghurts have a relatively low-pH that the probiotic bacteria must survive. The acid tolerance can be tested *in vitro* and hence the ability of the strains to survive in acidic products can be predicted, while *in vivo* testing of the survival of bacteria during transit through the human stomach is more difficult to obtain.

One strategy to improve the survival of an included probiotic is to formulate the food product in such a way that it assists the probiotic. Another strategy is to protect sensitive strains by technological means e.g. by micro-encapsulation of the strain or to use packaging that protects the strain from the food matrix until the moment of consumption.

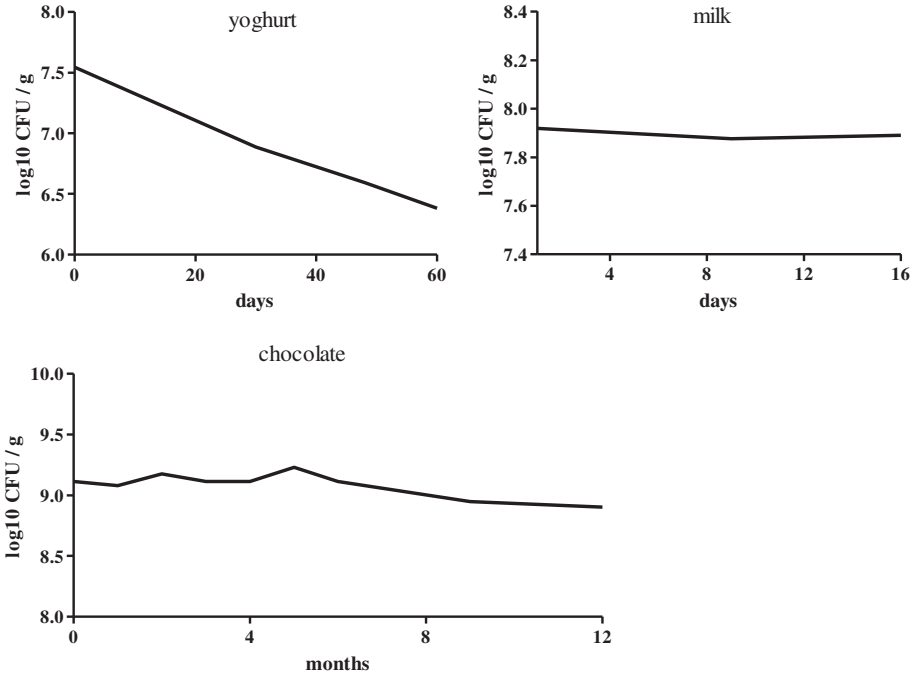

Fig. 1. Stability of *Bifidobacterium lactis* HN019 in yoghurt, milk and chocolate during storage.

6. Conclusions

The knowledge of the bacterial growth and metabolic activity is important in order to be able to optimize the large scale production of bacteria. By using improved bacterial strains in food technology, better yields of fermented products and longer shelf lives can be achieved. The functional requirements of probiotics should be established by using *in vitro* methods and the results of these studies should be reflected in controlled human studies. The use of different food matrices set their own specific requirements on the probiotics and possibly their functionality.

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