

## THE ROLE OF PROBIOTICS IN DAIRY PRODUCTS

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### ABSTRACT

Dairy products have been a primary source of probiotics since the turn of the 20th century, serving as the foundation for creating functional food products. Dairy products containing live microorganisms have been used to improve intestinal health and serve as the foundation for creating new functional food products. Probiotic cultures are commonly used in dairy products due to their optimized manufacturing processes for enhancing microorganism survival. Probiotics are categorized into next-generation probiotics (NGPs), non-LAB probiotics, and LAB-based classical probiotics. Commercial applications of probiotics include taste, aroma, and shelf life enhancement. Probiotic-based fermentation and incorporating probiotic strains into fermented or nonfermented food items are two recent trends in the development of probiotic dairy products or functional meals. Probiotics can be used alone or in conjunction with conventional starter cultures. In the dairy sector, fermented milk is the main source of probiotics, and adding probiotic strains to it can improve organoleptic and textural properties and promote health. Yogurt can also benefit from probiotic-based fermentation, as some probiotics can significantly reduce the amount of bisphenol A in yogurt.

Probiotics are found in various foods, such as butter, ice cream, milk powder, and infant mixes. Technological advancements can address probiotic viability issues by choosing oxygen-resistant strains, using oxygen-scavenging compounds, using passive barriers, and using oxygen-impermeable packaging materials. Research on the microbial ecology of fermented dairy products is crucial for exploitation.

Food is vital to health because it supplies energy, sustains the appropriate operation of physiological processes, and serves as a source of nutrients. In the realm of functional foods, milk and dairy products were the forerunners, and they remain the primary means of delivering probiotics through diet today. Another source of probiotics, which are mostly known to improve gut health, is food (Sanchez *et al.*, 2009). These probiotics may be found in dairy products such as cheese, yogurt, milk, and even ice cream. Probiotic bacteria thrive in dairy products because they can be in the right physical conditions (temperature, pH, and time) with the right kind of substrate (Helle, 2001).

Dairy products that have undergone fermentation and contain live microorganisms have long been utilized to improve intestinal health (Sanchez *et al.*, 2009). In this sense, dairy products serve as the foundation for the creation of new functional food products that include either unique probiotic strains or functional substances obtained

from dairy. The idea of probiotics originated around the turn of the 20th century with a theory put up by Russian scientist Elie Metchnikoff, who won the Nobel Prize, who claimed that Bulgarians' long, healthy lives were caused by their use of fermented milk products containing probiotics. The term "probiotics" is derived from the Greek term's "pro" (in favour) and "biotic" (life). Probiotics are characterized as "live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal micro flora" (Sanchez *et al.*, 2009). Probiotics and direct-fed microbials are interchangeable terminology (Helle, 2001). Mono or mixed cultures of living microorganisms that, when administered to humans or animals, benefit the host by enhancing the characteristics of the native microbiota is a more modern definition. Probiotics are beneficial microorganisms that improve host health and are suitable for commercial use in food and medicine. They must maintain their original properties, have high vitality, stability, and

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colonization ability. Fermented foods (such as beer, bread, kefir, wine, and cheese) have a lengthy history, dating back thousands of years, even before the Middle Eastern and Egyptian civilizations (3500 BC). A graphical overview of the discovery of probiotics is presented in Figure 1, which also offers a basic understanding of the terms probiotics, prebiotics, symbiotic, Para probiotics, and postbiotics that have been developed since 1905 (Cuevas-González *et al.*, 2020; Fuller, 1991; Hill *et al.*, 2014; Lee & O'Sullivan, 2010; Lilly & Stillwell, 1965; Mackowiak, 2013; Martin & Langella, 2019).

Probiotic cultures can be added to almost any type of food, but dairy products are clearly preferred because their manufacturing processes have historically been optimized to ensure and improve the survival of microorganisms involved in fermentation processes, requiring only minor modifications to already-existing manufacturing technologies and/or processes.

The first food product likely to include probiotics was fermented milk, however probiotics are currently present in hundreds of different food matrices. Traditionally, choosing bacterial strains for fermentation involved either naturally occurring strains of the bacteria or the bacteria that were technologically significant for the end product's flavor and aroma, making it solely a matter of taste, aroma, and shelf life.

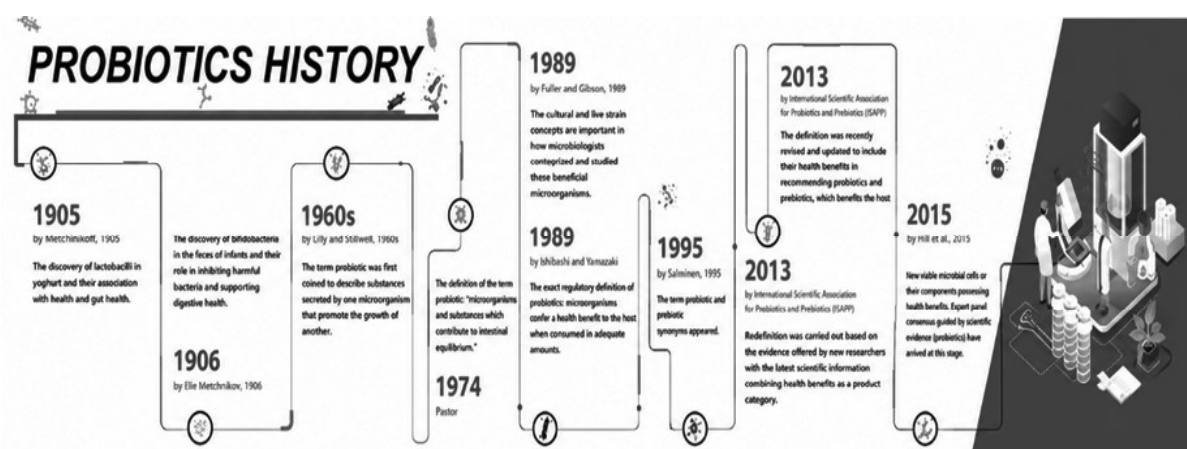
For the purpose of enhancing product quality, the dairy industry currently uses the greatest variety and quantity of probiotics of any food industry. There have been two significant developments in the use of probiotics in dairy products in recent years: (1)

the fermentation of dairy products using either traditional strains in conjunction with probiotics or select probiotics; and (2) the inclusion of probiotic strains into food matrices.

Next-generation probiotics (NGPs), non-LAB (Lactic Acid Bacteria) probiotics, and LAB-based classical probiotics are the three categories of probiotics. The different kinds of probiotics and their commercial applications are categorized in detail in Figure 3.

LAB are anaerobic, which have been used in food and dairy fermentations for thousands of years, with plant materials and fermented foods being the largest reservoirs. Non-LAB probiotics include *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Bacillus*, *Clostridium*, and probiotic yeasts, with *Bifidobacterium* containing the most probiotics, *Bacillus* used in supplementation and in food industry. Conventional probiotics are beneficial to both human and animal health. As our understanding of the gut microbiota grows, next generation-probiotics (NGPs) such as *Faecalibacteriumprausnitzii*, *xylanisolvens*, *fragilis*, *Akkermansiamuciniphila*, and *Bacteroides ovatus* have been identified. These bacteria may have health benefits if given in sufficient quantities.

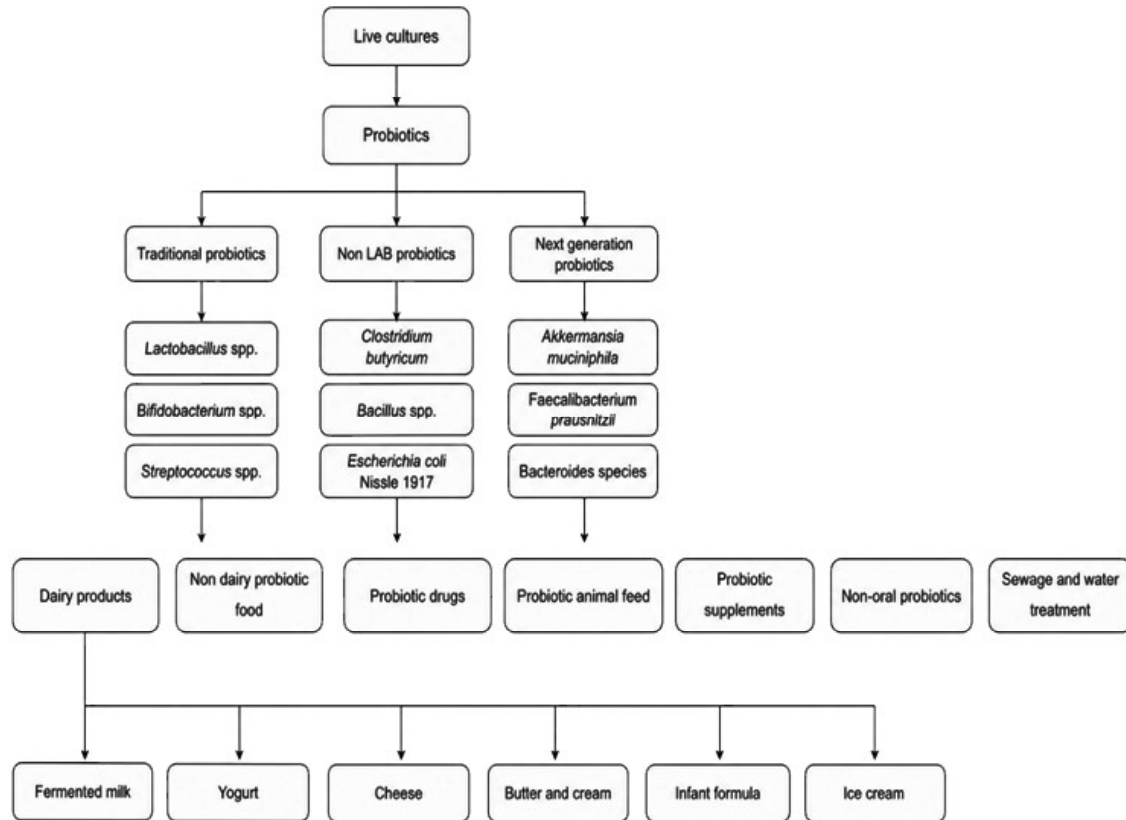
Probiotic-based fermentation or the incorporation of probiotic strains into fermented or nonfermented food items are the two most recent trends in the development of probiotic dairy products or functional meals. One can utilize probiotics either by themselves or in conjunction with conventional starter cultures. The application of probiotics as fermentative cultures in dairy products is discussed in the following sections.



Gao *et al.*, 2021

Figure.1 Evolution of Probiotics, Prebiotics, Symbiotics and Postbiotics (1905–Present)

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**Figure.2.** Probiotics are widely used in fermented dairy products like yogurt, cheese, and infant formula.

### Fermented milk:

In the dairy sector, fermented milk is the main source of probiotics. Probiotic strains have been utilized successfully in milk fermentation to improve organoleptic and textural properties, as well as promote health. Milk-based probiotic products are the most often utilized globally due to their high nutritional content and availability. **Table. 1** lists popular probiotic-based fermented milk products worldwide. Kefir and Koumiss are widely used naturally fermented milks globally (Gao *et al.*, 2019; Gao *et al.*, 2012).

Research suggests that adding probiotic strains to fermented milks can improve their health benefits. In order to improve the taste and physiological characteristics of fermented milk and increase its acceptability, probiotic bacteria are used in conjunction with conventional starter cultures during milk fermentation (Kakisu *et al.*, 2011). Along with yogurt starter cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, fermentation by *Lactobacillus plantarum* P-8 enhanced the yoghurt's flavor profile by generating 3-methylbutanal, acetoin, onanal, 2

**Table.1** Microbes categorized as probiotics

| Bifidobacterium species | Lactobacillus species                       | Non lactic acid bacteria                | Other lactic acid bacteria   |
|-------------------------|---------------------------------------------|-----------------------------------------|------------------------------|
| <i>B.longum</i>         | <i>L.casei</i>                              | <i>Saccharomyces boulardii</i>          | <i>Lactococcus lactis</i>    |
| <i>B.bifidum</i>        | <i>L. bulgaricus</i> and <i>delbreuckii</i> | <i>Bacillus cereus</i> var. <i>toyo</i> | <i>Enterococcus faecalis</i> |
| <i>B.animalis</i>       | <i>L.Acidophilus</i>                        | <i>Propionibacterium freudenreichii</i> |                              |
| <i>B. Breve</i>         | <i>L.amylovorus</i>                         |                                         |                              |
| <i>B.lactis</i>         | <i>L.crispatus</i>                          |                                         |                              |

heptanone, hexanal, (E)-2-octenal, and 2-nonanone in comparison to the control (Dan *et al.*, 2019). Comparing milk fermented by *L. casei* DN-114001 to the control, the latter showed greater levels of acetic acid, acetoin, 2-butanone, caproic acid, butyric acid, and 2-pentanone (Zareba *et al.*, 2014). It is difficult for dairies to produce fermented milk because milk is not the best medium for probiotic bacteria growth and metabolism (Ostlie *et al.*, 2005). Maintaining a probiotic level of 10<sup>6</sup>-10<sup>7</sup>CFU/g or CFU/mL in the food is essential to ensuring probiotic survival. The growth and metabolism of probiotic bacteria varies during milk fermentation, and choosing the right strains for milk fermentation is essential because of their varied and heterogeneous mechanisms of action (Ostlie *et al.*, 2003, Li *et al.*, 2017 and Plaza Diaz *et al.*, 2019). While magnesium salts encourage the growth of *B. animalis* subsp. *lactis* Bb-12 and enhance its physicochemical and textural characteristics, *Lactococcus lactis* lowers oxygen damage and increases the survival of *B. longum* BB536 in fermented milk. *B. bifidum* survival in fermented milk can be increased by altering the redox potential of the milk without influencing the development of other strains or the kinetics of fermentation (Gao *et al.*, 2021).

#### Yoghurt:

Remarkably, some probiotics, such *Lactobacillus acidophilus* and *Lactobacillus plantarum*, may significantly reduce the amount of bisphenol A, an estrogenic substance, in yogurt (Taghizadeh Moghaddam *et al.*, 2020). It has been noted that probiotic-based yogurt fermentation (*L. bulgaricus* and *L. reuteri* or *L. acidophilus*) increases the hardness and adhesiveness of yogurt (Mani-Lopez *et al.*, 2014). In comparison to ordinary yogurt, yogurt produced using *L. plantarum* alone or in combination with *L. casei* had significantly higher levels of calcium, phosphorous, protein, and ash with low syneresis (Soni *et al.*, 2020). The physicochemical, rheological, and sensory characteristics of yogurt made by *E. faecium* and *E. durans* (as adjunct starter cultures) could also be compared to those of regular yogurt (Akpinar *et al.*, 2020). When combined with standard starter cultures, *L. casei* Zhang's yogurt demonstrated a significantly lower pH (4.12) and syneresis (22% after 7 days of

storage) than the control yogurt, while the starter cultures' growth remained unaffected. Probiotic supplementation in yogurts can improve texture and flavor by adding functional ingredients like sodium caseinate, polymerized whey protein, and sodium calcium caseinate. These additions can enhance the yogurt's texture and produce more aldehydes, alcohols, and nitrogenous compounds. However, the addition of *L. helveticus* H9 during fermentation can also lead to increased production of these compounds over a 28-day storage period (Zhou *et al.*, 2019).

#### Cheese:

Cheese is a superior carrier of probiotics due to its low water activity, low storage temperature, higher pH, and high fat content. However, adding probiotics to cheese requires ensuring their survival during salting, ripening, and storage (Cas tro *et al.*, 2015), and preventing sensory abnormalities. Probiotics are typically mixed with traditional cheese starter cultures during fermentation in milk in a defined ratio to ensure their safety. When manufacturing cheese, aerobic conditions are not ideal for probiotics, especially *Bifidobacterium*. Probiotics development is impacted by metabolically active LAB, the cooking and cheddaring processes, which hinders their growth. As a result, adding probiotics to cheese is more suited for the later phases of cheesemaking (salting and milling) (Dinkar and Mistry, 1994). When administered at the salting stage, probiotic *B. longum* is more stable and viable over time than when injected in milk either before or after renneting (Fortin *et al.*, 2011). Numerous studies have documented detrimental effects of probiotics on cheese's sensory qualities. In several trials, encapsulated probiotics have been added to cheese, which may help limit the metabolic activity of the bacteria in the cheese. However, because of their limited metabolic role, this could not be beneficial for probiotics that give cheese beneficial physiological properties. Probiotic *Lactobacillus salivarius* strains and two *Lactobacillus paracasei* strains were employed as the dairy starter adjunct in the production of cheddar cheese (Gardiner *et al.*, 1998). During the cheese's 6-month ripening process, *Lactobacillus paracasei* strains developed effectively and maintained high viability (Ganesan *et al.*, 2014). When

*L. casei* subsp. *casei* L2A was added to milk before rennet was added during the production of Cheddar cheese, it improved maturation and produced a 40% improvement in flavor quality when compared to the control cheese samples (Trépanier *et al.*, 2010). More free fatty acids and soluble proteins are produced by *Bifidobacterium bifidum* than by the control, making it a good adjunct culture for making probiotic Edam cheese. In comparison to the control cream, probiotic cream has been shown to have higher levels of linoleic and  $\alpha$ -linolenic acids (Yilmaz-Ersan, 2013).

### Butter and cream:

Cheese, yogurt, and fermented milk are not the only items that contain probiotic strains; due to their many advantages, probiotics are also used in a variety of other foods, including butter. Despite its high fat content, butter has a number of health advantages (Pimpin *et al.*, 2016). According to reports, certain probiotic bacteria (*Lactobacillus maltaromicus* AC 3<sup>64</sup> 16 and *L. casei* subsp. *casei* AB16<sup>65</sup>) lower the cholesterol level in butter and cream (Alođlu & Öner, 2006). In another investigation, fermentation with *B. lactis* resulted in higher levels of linoleic and  $\alpha$ -linolenic acids in probiotic cream as compared to the control cream (Yilmaz-Ersan, 2013). Non-dairy-based butters, such as peanut butter and butters made from sunflower, coconut, and flaxseed oils, are more frequently used to contain probiotics.

### Ice cream:

Ice creams are also significant probiotic carriers, and adding probiotics to ice cream has several benefits. Not only does it make ice cream a functional, healthful food, but it also improves the viability of probiotics in ice cream during production and storage, which is superior to many other dairy products. Probiotic viability is still impacted by a number of elements during product production and storage, thus it's important to find the best ways to combine probiotic viability with the physicochemical characteristics of ice creams. Probiotic viability in ice creams is influenced by a number of parameters, such as the fermentation process (if the ice cream is fermented), freezing and churning procedures, overrun procedures, frozen storage duration, and product melting and thawing (Mohammadi *et al.*, 2011).

Probiotic-infused ice cream has been suggested to ferment, which is a superior approach than adding probiotics to the ice cream mixture without any further fermentation. Compared to ice cream that had probiotics added after fermentation, the ice cream mix fermented with *L. acidophilus* ATCC 4356 reached the requisite count (106 CFU/g) of the probiotic strain after 90 days of storage at 20 °C (Arslan *et al.*, 2016). In fermented ice creams, low pH during fermentation reduces the amount of many probiotic bacteria. For optimal probiotic viability, the ice cream mix should be fermented under controlled conditions and allowed to freeze at a pH of about 5.6 (Davidson *et al.*, 2000). There is no evidence that the fat level of ice cream has an impact on the probiotics' ability to survive (Haynes & Playne, 2002). Following 40 days of storage at -16°C, the survivability of *Lactobacillus delbrueckii* UFV H2b2 was tested in low-fat, high-fat, and fat-free ice creams. No discernible changes in its count were observed in any of the ice cream samples (Leandro *et al.*, 2013). The ice cream mix had a greater total solid (42%), titratable acidity (2.2%), and proteins (16.5%) content when *L. acidophilus* La5 was added before fermentation (Senanayake *et al.*, 2013).

### Milk powder and infant mixes:

Probiotics are added to baby formulae to alter intestinal microbiota and support the growth of the immune system. According to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition, formulas containing probiotics or prebiotics do not pose a risk to growth. In neonatology, *bifidobacteria* and *lactobacilli* strains are more frequently utilized. The probiotics in skim milk can be effectively preserved by spray drying it (Braegger *et al.*, 2011, Watkinson *et al.*, 2018). Gardiner *et al.* preserved human-derived *L. salivarius* UCC 118 and *L. paracasei* NFBC 338 by spray drying. While the UCC 118 count dropped with storage, the *lactobacilli* content of powdered milk particles stayed constant. Probiotic viability during spray drying was also positively affected by growth phase and prebiotics. The durability of probiotics depends on the encapsulation material, which includes whey proteins, gums, maltodextrin, starches, and reconstituted skim milk. There is no evidence that adding polydextrose

or inulin to skim milk improves viability. According to studies, sodium caseinate, skim milk powder, and whey protein concentrate can increase the viability of *L. acidophilus* cells.

### Role of probiotics in biotransformation of nutrients:

Dairy products frequently contain probiotics *Lactobacillus* and *Bifidobacterium*, whose interactions with the matrix vary based on the addition and fermentation process. In order to actively participate in fermentation, one must take starters and media composition into account. Bacteria are a possible fortifying agent since they can create lactic acid or restrict growth. They are an important tool for food fortification because they can also obtain metals, vitamins, and peptides through biosynthesis. Current research attempts to identify probiotic bacteria from

different sources in order to investigate their potential for biotransformation, the creation of vitamins and peptides, and the optimization of dairy product production methods in order to attain prescribed amounts of micronutrients (Urrutia *et al.*, 2024).

Dairy products' Se enrichment is influenced by pH; fermentation lowers pH by producing lactic acid. Se enrichment capability can be decreased by hydrogen ion accumulation. Research has investigated minerals such as zinc in dairy products by increasing ion permeation with ZnSO<sub>4</sub> pulsed electric field (PEF) and *Lactobacillus rhamnosus* B 442. pH is not impacted by the addition of PEF or zinc ions, but it can be by fermentation. 0.962 mg/L to 1.81 mg/L was the accumulation recorded by PEF technology, which is a low dose that is advised for everyday use.

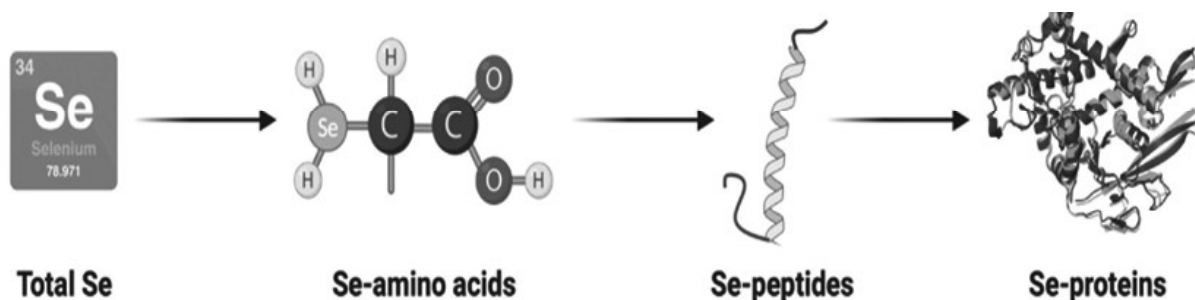
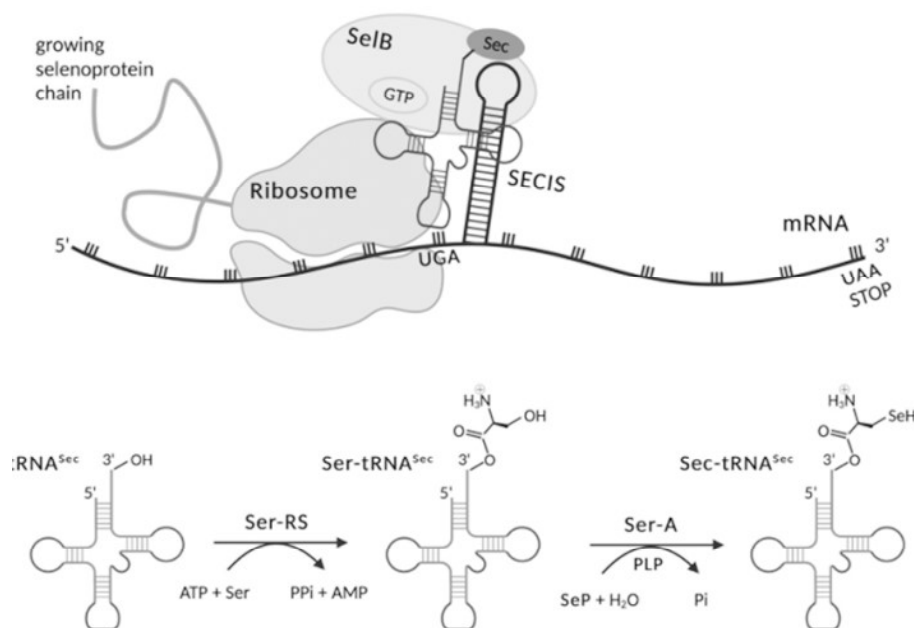


Figure.3 Selenium's general integration into a protein



(Metanis and Hilvert, 2014)

Figure. 4 Mechanism of bacterial selenoprotein production

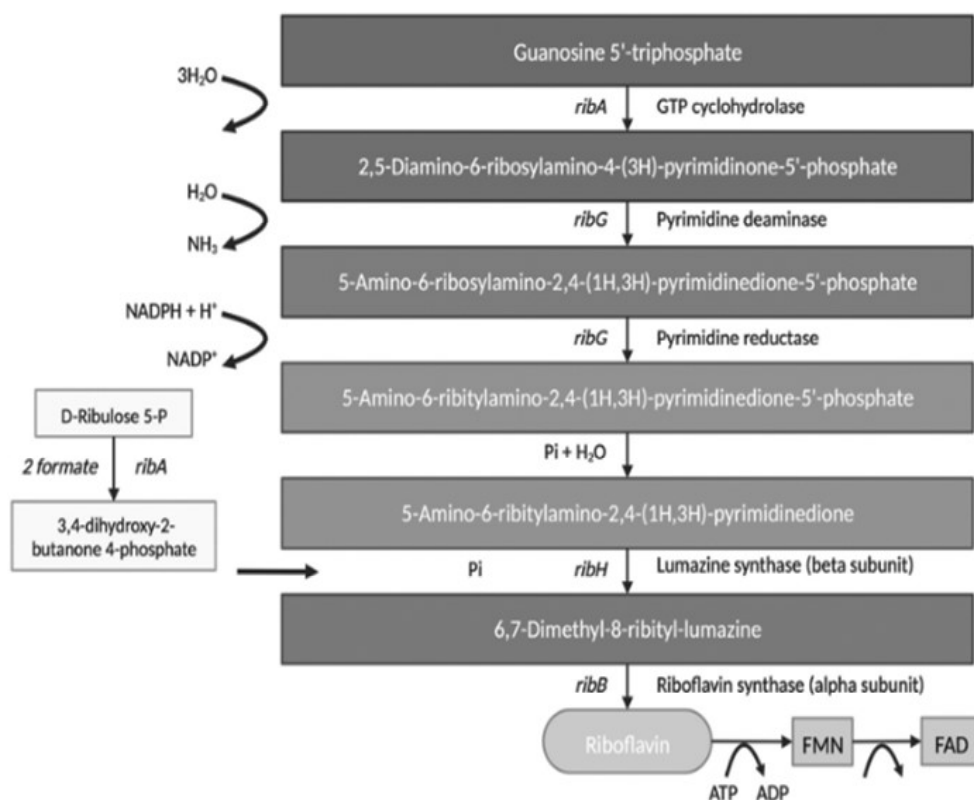
Through fermentation, probiotic bacteria can produce vitamins, especially those from the B complex. LAB bacteria, for instance, can produce riboflavin on-site, which can serve as a source of vitamins for human hosts. The mononucleotide RFN, which codes for additional biosynthesis and transport proteins, is one of the alternative RNA structures formed during the production of riboflavin. Several catalytic enzymatic activities of guanosine triphosphate (GTP) and ribulose-5-phosphate, which are encoded by four genes (*ribG*, *ribB*, *ribA*, and *ribH*), drive the synthesis forward.

According to a suggested method, probiotics can also manufacture vitamin B9. *Streptococcus* and *Lactobacillus* are the two microorganisms most frequently employed to generate this vitamin; their concentrations range from 173 to 700 ng/ml. Because of the *Lactobacillus plantarum* CRL 725 bacteria, soy milk had a higher riboflavin content, while *S. thermophilus* bacteria did not substantially boost it. In terms of folate synthesis, *Streptococcus thermophilus* is unable to produce vitamin B6, whereas *Streptococcus lutetiensis* and

*Streptococcus infantarius* were able to produce less than 71 ng/mL of the vitamin. Since a more watery medium was required and lactic acid was more readily available, cheese concentrations were lower than those of other substances. In the absence of a matrix, *Lactobacillus plantarum* exhibited greater quantities for vitamin B12 synthesis, suggesting that a dry environment lowers its ability for synthesis

### Scientific Barriers to the Probiotics' Future Viability in Milk and Milk Products

Maintaining microbial viability is crucial to the high effectiveness of probiotic products. Throughout food processing, storage, and the gastrointestinal transit, probiotics are subjected to a variety of stressful situations (Mbye *et al.*, 2020). Fermented milk must contain a minimum of 106 CFU/g of *B. bifidum* and 107 CFU/g of *L. acidophilus* according to numerous international regulations. The Fermented Milk and Lactic Acid Beverages Association in Japan states that a probiotic fermented milk must have a minimum of 107 CFU/mL of live bifidobacteria when it is consumed (Lourens-Hattingh & Viljoen, 2001). Since



(Thakur *et al.*, 2015)

Figure.5 Bacterial riboflavin (vitamin B2) production mechanism

the majority of probiotic strains are oxygen-sensitive, the presence of oxygen in dairy products has an impact on their survival. One significant technological obstacle in the creation of probiotic fermented milk and yogurt is the toxicity of oxygen in dairy products. When probiotics are exposed to dissolved oxygen, harmful oxygenic metabolites such superoxide anion build up inside the cells, ultimately decreasing the survival of the organisms (Talwalkar & Kailasapathy, 2004). Probiotic viability issues can be addressed through technological advancements in a number of ways, such as choosing oxygen-resistant probiotic strains, removing molecular oxygen with oxygen-scavenging compounds (such as the synthetic antioxidants butylated hydroxyanisole and butylated hydroxytoluene and the natural antioxidant tocopherols), using passive barriers (such as multilayer structures with aluminium foils), and using oxygen-impermeable packaging materials. According to Mohammadi *et al.* (2011), probiotic viability can also be increased by encapsulating and adding nutrients and prebiotics to dairy products.

## CONCLUSION:

Numerous industries, including food and animal feed, nutritional supplements, pharmaceuticals, and natural medicinal agents, use probiotics-micro organisms that have health-promoting properties. When it comes to probiotic applications, the dairy industry is the biggest and fastest-growing sector. Probiotic goods such as fermented milk, cheese, yogurt, butter, powdered milk, and ice cream are accessible all over the world. Low pH and storage conditions present difficulties for the incorporation of probiotics into fermented dairy products. Probiotics in dairy products can have their stability and survival increased through technological means. Until the product's shelf life is through, dairy producers should make sure that the number of probiotic cells in it stays within the suggested range listed on the food label. Exploiting probiotics in dairy products requires research on the microbial ecology of fermented dairy products, with a particular emphasis on strain-level characterization of probiotic bacteria. Cryoprotectants, prebiotics, and encapsulation are among methods that can assist stable probiotics in a variety of processing and storage scenarios. Since their stability and functionality are not a major problem, paraprobiotics and postbiotics can be great health-promoting ingredients for dairy products.

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