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RESEARCH

A Novel Approach Using a Protective Desiccant Increases Shelf Life and Reduces Moisture in Oil Suspensions of Freeze-Dried *Limosilactobacillus reuteri*

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Abstract

To ensure efficacy of probiotic products, it is essential that adequate numbers of bacteria survive during the intended shelf life, a quality factor that is greatly challenged by exposure to moisture. This study aimed to investigate if the shelf life of freeze-dried *Limosilactobacillus reuteri* strains formulated in oil could be prolonged using a moisture-absorbing technology. We show that by incorporating a desiccant strip in the oil suspension, we effectively control the moisture content until the end of shelf life and consequently increase survival of *L. reuteri* DSM 17938 and *L. reuteri* BG-R46®. This enables a shelf life of oil-formulated *L. reuteri* of more than 24 months at 25 °C. Batches lacking the desiccant strip had a dramatic reduction in viable bacterial counts, falling below the specified quality limit after only 6 months of storage at 25 °C. The loss of viable bacteria in batches without the desiccant strip was significantly higher than batches supported by the desiccant strip at all time points after 2 months ($p = 0.0025$). There was a strong negative correlation between water content and CFU in batches lacking the desiccant strip, whereas no such correlation was observed in batches containing the desiccant strip. In conclusion, this paper demonstrates a novel method using a desiccant strip technology in probiotic oil suspensions (LongevityGuard®), which efficiently increases shelf life and protects freeze-dried probiotics from moisture-induced cell death and product spoilage.

Keywords Shelf life · Probiotics · Desiccant strip · *Limosilactobacillus reuteri* · DSM 17938 · BG-R46®

Introduction

During infancy, many pivotal processes and systems develop, including for example establishment of the microbiota and maturation of the immune system (Schoultz et al., 2025). Probiotics, defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (Hill et al., 2014), may positively affect human health (Uusitalo et al., 2016). However, the efficacy of a probiotic product relies on careful selection of bacterial strains, ensuring that they exhibit relevant host interactions, stress tolerance, and processability. Preceding the stress in the gastrointestinal tract are abiotic stressors that accompany cultivation, freeze-drying and storage, and the strain must have a robust growth profile, high viability after freeze-drying, as well as good storage stability. The aim of this study was to improve storage stability of probiotic supplements formulated in oil, which may facilitate effective delivery of probiotics to infants regardless of feeding regimen.

Stressors that are associated with storage stability include moisture, temperature fluctuations, and oxygen exposure. By having a solid process for strain selection, optimizing the production process, and ensuring stable storage conditions, the probability of success in developing probiotic products that deliver consistent health benefits increases (Wendel, 2021). To ensure that the beneficial effects of a probiotic product are maintained throughout its shelf life, special care in production and product handling is crucial for maintaining high viability and vitality until use. The industry standard for achieving

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this is through freeze-drying, which helps maintain the structural integrity of cell membranes and enzymes, thereby maintaining the viability of the bacteria. The reduction of free water also minimizes the risk of microbial degradation and chemical reactions. Additionally, the dry and stabilized form of probiotics simplifies its incorporation into various product formulations without the need for a continuous cold chain. However, moisture absorption of freeze-dried probiotic products is a major threat that negatively affects viability and accelerates various degradation processes that are detrimental to both product quality and efficacy. Exposure to ambient moisture during production and storage is very difficult to completely avoid, and effective measures must be taken to protect the freeze-dried bacteria from moisture uptake (Fenster et al., 2019). An example of this is the use of aluminum foils with a plastic layer on the exterior side of the product compartment, which reduces the exposure of the freeze-dried bacteria to both moisture and oxygen. Alternatively, in dry product formats like tablets and capsules, desiccants such as silica or molecular sieves that bind water molecules can be incorporated into the interior of the package, thereby removing moisture. Another way to protect freeze-dried probiotics is to use oil-based formulations, see for example U.S. Pat. No. 4,518,696 (Gehrman & Porubcan, 1985). However, a problem with oil-based formulations is that it is difficult to remove all moisture from the oil itself and to ensure that no water enters the product during packaging or storage.

To address this problem, we have evaluated the impact of a specific moisture-absorbing technology on the storage stability of two freeze-dried *Limosilactobacillus reuteri* strains: DSM 17938—the first probiotic product formulated as an oil formulation, commonly given to infants to alleviate colic, and one of the most extensively studied probiotic strains worldwide (Dinleyici et al., 2025; Mu et al., 2018), as well as the novel strain BG-R46®, both formulated in oil-based products.

Material and Methods

Strains

Industrially produced freeze-dried *Limosilactobacillus reuteri* DSM 17938 (commercial name *L. reuteri* Protectis®, trademark of BioGaia AB) (Rosander et al., 2008) and *L. reuteri* BG-R46® (trademark of BioGaia AB, also designated DSM 32846) (Ermann Lundberg et al., 2025; Pang et al., 2022) were used throughout this study.

Manufacturing of Probiotic Product with Freeze-Dried *L. reuteri* Formulated in Oil

The probiotic products were produced by mixing sunflower oil and medium-chain triglyceride oil with silicon dioxide (particle size < 0.02% on 325-mesh) and then adding the freeze-dried *L. reuteri* DSM 17938 (2% w/w) or by mixing sunflower oil with equal amounts of *L. reuteri* DSM 17938 (2% w/w) and *L. reuteri* BG-R46® (2% w/w).

The bacterial suspensions were held in a vessel until filling into 6 ml glass bottles (Figs. 1 and 2), or an EasyDropper tube, a packaging and dispensation system developed for liquid preparations (Fig. 3). Each glass bottle was equipped with a dropper insert, a tamper-evident screw cap and with or without a desiccant strip with a surface area of 1.6 cm^2 (M-0026 Activ-Film™, Aptar CSP Technologies, USA) resulting in a complete primary packaging with a moisture vapor transmission rate (MVTR) of 0.002 g/year at 25 °C/60% RH. The EasyDropper tube was also equipped with a dropper insert, a tamper-evident screw cap, and, with or without, a desiccant strip with a surface area of 1.6 cm^2 (M-0026 Activ-Film™, Aptar CSP Technologies, USA). All products with and without the desiccant strip were produced with vacuum dried oil.

Study of Storage Stability and Analysis of Water Content

Samples were transported in ambient conditions to the lab and stored at 5 °C until the start of the study. The samples were kept in cold storage after production, and stability studies were typically initiated within 14 days. Thereafter, the samples were stored in climate cabinets at 25 ± 2 °C/60 ± 5% RH. Sampling was carried out according to ICH guideline recommendations (ICH Expert Working Group, 2003) and samples were taken at 0, 2, 4, 6, 9, 12, 15, 18, 21, and 24 months. After incubation, the samples were analyzed with the viable count method described below. For the results described in Figs. 2 and 3, the water content was analyzed at each sample pull, in parallel with the viable count analysis.

Viable Count Analysis

Viable count analysis was performed by diluting each sample 1:10 with preheated (42 °C) MRS broth (Oxoid, Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 0.2% Tween 80 and 0.15% antifoam, in a sterile stomacher bag followed by homogenization in a Smasher/Stomacher for 60 s at normal speed (500 strokes per minute). Samples were incubated at room temperature

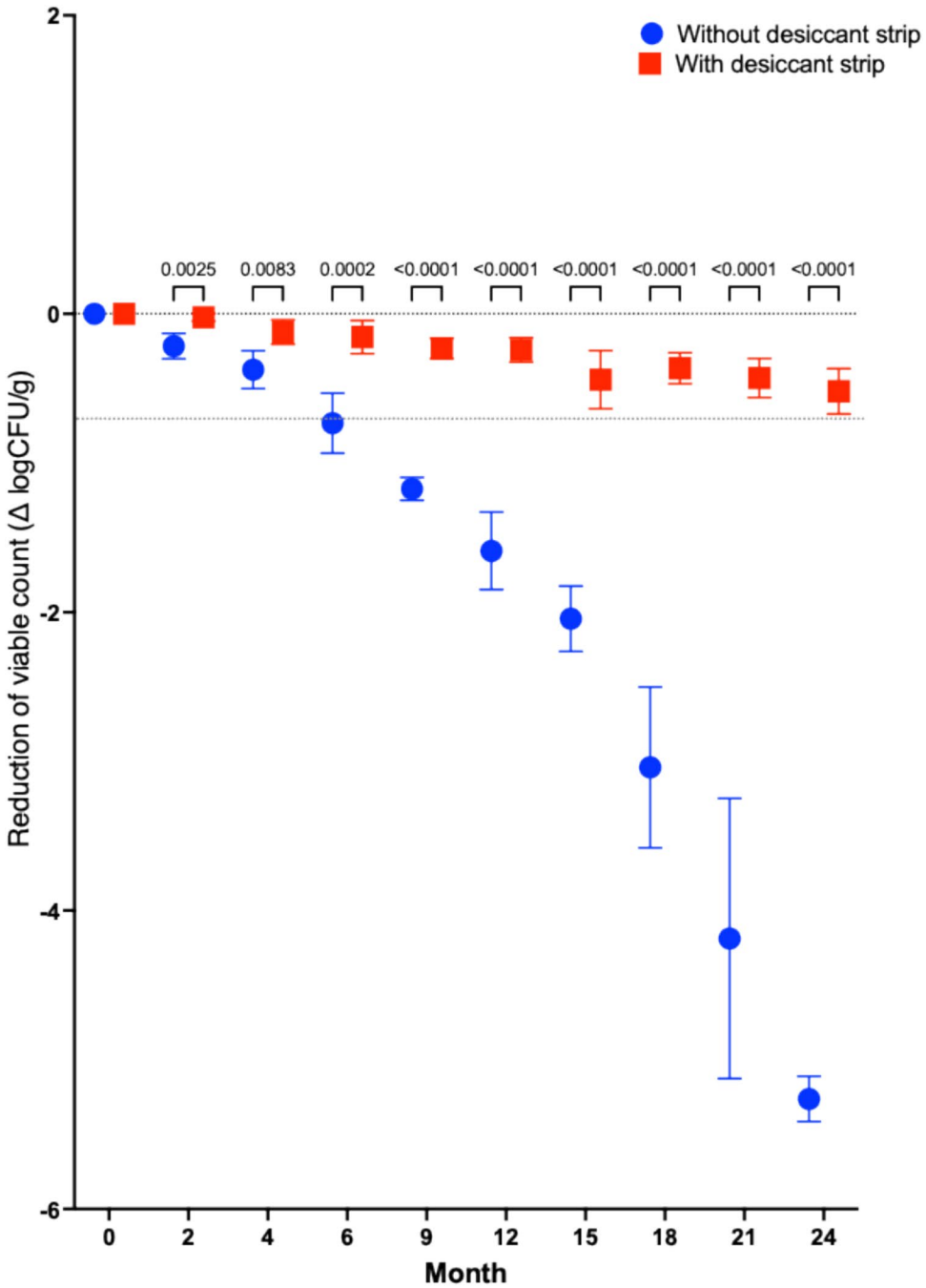

Fig. 1 Δ logarithmic loss of CFU in commercial batches of *L. reuteri* DSM 17938 formulated in oil, produced with or without the desiccant strip and stored at 25 °C. The loss of viability of the products is expressed as -Δlog(CFU/g). Batches with desiccant strips ($n=6$) are shown in red and batches without desiccant strips ($n=3$) in blue. Data are presented as mean values with error bars showing the standard deviations. The lower specification limit is shown in a dotted gray line. Unpaired *t*-tests followed by two-stage step-up false discovery rate procedure of Benjamini, Krieger, and Yekutieli were used in the statistical analysis and *q*-values are indicated above each time point

for 10–20 min prior to a second round of homogenization. Once completed, the samples were diluted by serial dilution, using 0.9% NaCl to reach a final dilution corresponding to 30–400 CFU/0.1 ml. Each sample was plated on MRS agar plates supplemented with cysteine (Mikrobiologen, Skåne University Hospital, Lund, Sweden) in triplicates and incubated anaerobically at 37 °C for 2–3 days. Results were calculated as the mean of CFU counted on six plates (two samples analyzed at each time point on triplicate plates) multiplied by the dilution factor.

Water Content

Water content was measured in the oil suspensions containing the freeze-dried bacteria using colorimetric automated Karl Fischer Titration using an *Excellence T7 titrator* from Mettler Toledo. The samples were heated in the oven autosampler at 80 °C. Dry nitrogen with a flow rate of 80 ml/min transported the water from the oven to the titrator cell. Hydranal Cuolomat AG-Oven was used as titrator reagent.

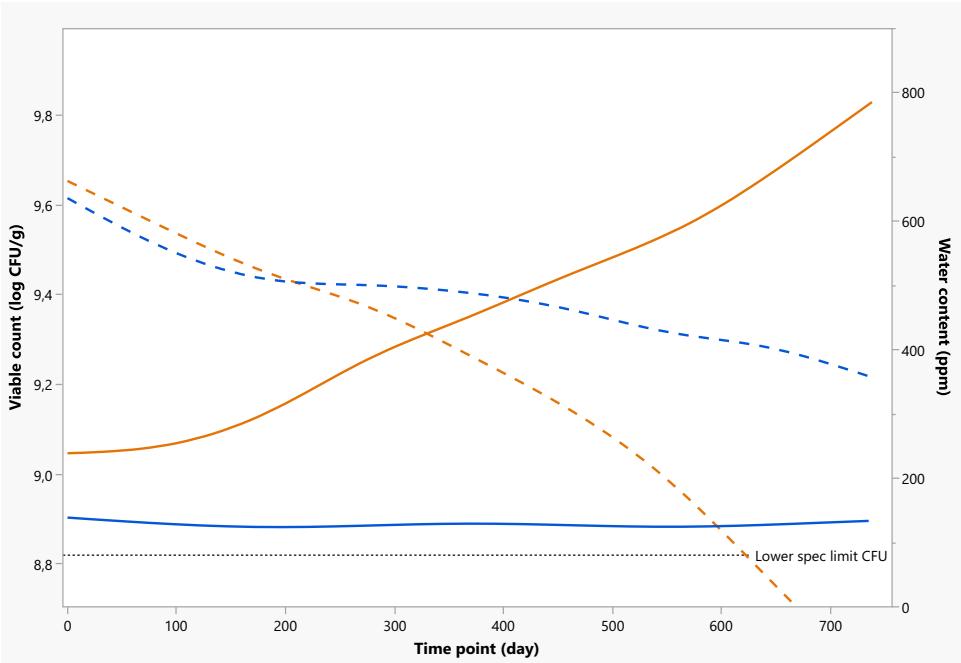

Fig. 2 Desiccant strips prevent an increase in water content and limit the loss of viability of freeze-dried *L. reuteri* DSM 17938 formulated in oil and packaged in glass bottles. Water content (solid lines) and viable counts (dashed lines) of product batches with (blue) or without (orange) desiccant strip during stability assessment for 24 months at 25 °C. The average logarithmic loss of CFU was 0.4 for samples with desiccant strip, and 1.1 for samples without desiccant

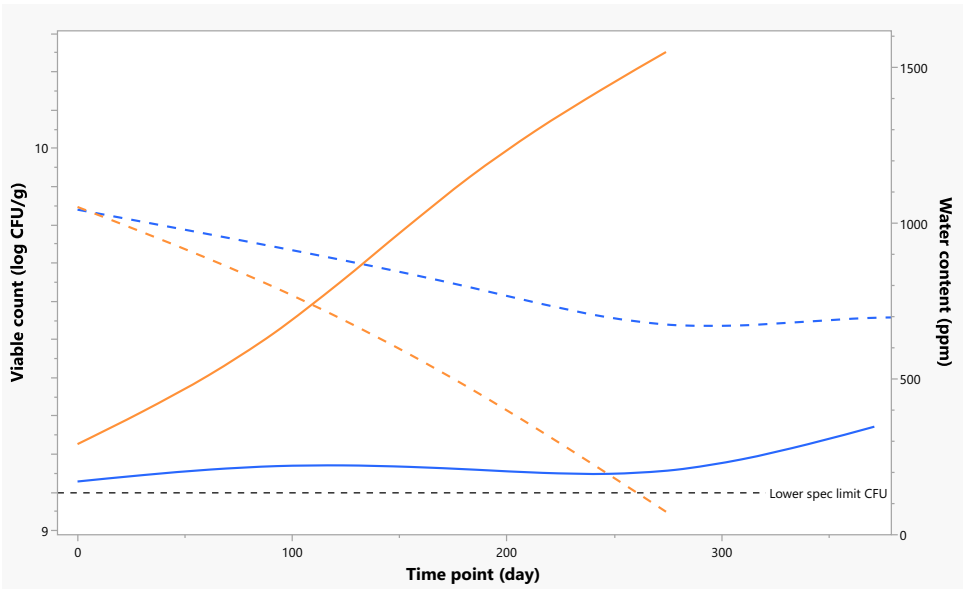

Fig. 3 Desiccant strips maintain low water content and limit the loss of viability of freeze-dried *L. reuteri* strains DSM 17938 and BG-R46® in oil-based EasyDropper products. Water content (solid lines) and viable counts (dashed lines) of product batches with (blue) or without (orange) desiccant strips during stability assessment for 9 months (without strips) and 12 months (with strips) at 25 °C. The average logarithmic loss of CFU was 0.2 for samples with desiccant strip, and 0.8 for samples without desiccant strip. Since the batch without desiccant strips did not pass the specification limit for CFU at $t=9$ months, the analysis of that batch was terminated after this time point

Statistical Analysis

Statistical analyses were performed in GraphPad Prism version 11.0.0 and the α-level of 0.05 was used for all statistical interpretations. The test for normal distribution of data was assessed using the Shapiro–Wilk method. The stability data in Fig. 1 was analyzed using multiple unpaired *t*-tests followed by two-stage step-up false

discovery rate procedure of Benjamini, Krieger, and Yekutieli to account for multiple testing across time points. Effect sizes (Hedges' g) were calculated on log-transformed delta values. This transformation reduces within-group variability and reflects cumulative declines over time. Consequently, standardized effect sizes are large and reflect high signal-to-noise ratios rather than unusually large absolute differences. Pearson's correlation analysis displayed in Figs. 2 and 3 was used to assess the correlation between water content and CFU.

Results

To increase the stability and quality of oil suspensions containing freeze-dried *L. reuteri* DSM 17938 throughout the stated shelf life, the use of vacuum-dried oil has been a proprietary process used by BioGaia AB in commercial production. However, vacuum drying of the oil is not sufficient to consistently produce batches that meet the room temperature shelf-life specification of 24 months (Fig. 1, blue dots). To minimize the water content and investigate whether the stability and quality of the product could be further improved, the product was provided with a desiccant strip submerged in the oil suspension. This novel method using desiccant strip technology in probiotic oil suspensions (LongevityGuard®) significantly reduced the decline in viable cell counts and ensured that the product achieved a shelf life of at least 24 months (Fig. 1, red dots). The difference between groups increased with each time point, and the effect size at all time points after 2 months was larger than 2 (Supplementary Table 1).

To investigate the correlation between water content and viability, product batches containing DSM 17938 were divided into two groups, one with and one without the desiccant strip, and monitored for 24 months at 25 °C (Fig. 2). The desiccant strip maintained the water content of the product at the initial low level, while the product without the desiccant strip had a strong increase in its water content (Fig. 2, solid lines). The increase in water content had a strong negative correlation with CFU in batches lacking LongevityGuard® (Pearson's $r(7) = -.9798$, $p < 0.0001$). However, in batches with LongevityGuard®, the correlation was diminished, Pearson's $r(3) = .6208$, $p = 0.2638$, Fig. 2, dashed lines).

Additionally, we evaluated the effect of LongevityGuard® in batches containing a mix of *L. reuteri* strains DSM 17938 and BG-R46® packaged in an Easy-Dropper tube. Similarly to the data presented in Fig. 2, a negative correlation was observed between moisture and CFU in batches lacking the desiccant strip, Pearson's $r(2) = -.9798$, with a $p = 0.0202$, but in batches with the desiccant strip the correlation diminished, Pearson's $r(3) = -.1811$, $p = .7706$ (Fig. 3).

Discussion

This article describes the use of a moisture-reducing technology, LongevityGuard®, that enables high retained viability of probiotic organisms in oil-based products over time. Given the definition of probiotics that emphasizes the need for live bacteria in adequate amounts, it is of utmost importance to maintain high viability throughout the product shelf life to ensure probiotic efficacy. Retention of viable bacteria after drying processes is a challenge in the industry, and many products actually do not meet the stated content in terms of colony-forming units on the label within the specified shelf life (Drago et al., 2010; Elliot & Teversham, 2004; Morovic et al., 2016; Temmerman et al., 2003). Furthermore, by applying the desiccant strip, the CFU content of the products is consistent both over time and between batches. This ensures that the correct dose is delivered with each unit ingested throughout the shelf life of the product or during clinical studies, directly contributing to the probiotic health benefit.

The effect of relatively small amounts of moisture on probiotic viability can possibly be explained by the moisture-absorbing capacity of the bacterial cells. As water is generated, e.g., through Maillard reactions, the bacteria act as reservoirs for this water due to their high hydrophilicity compared with the surrounding hydrophobic oil. Consequently, in products lacking the desiccant, most of the generated water is retained within the bacterial cells. In contrast, in LongevityGuard® products, the desiccant strip outcompete the bacterial water absorption, drawing water toward the desiccant strip, ultimately leading to a dryer state of the bacterial cells and extended shelf life.

As previously described, humidity is a challenge in dried material, and likely even more so in countries with humid climates. The desiccant strip enables transport to and within humid countries without the need for cold-chain transportation, while also reducing the risk of product defects. Furthermore, only about 50% of households in low- and middle-income countries with high humidity own a refrigerator (Karlsson & Subramanian, 2023), and food is often stored at ambient temperature. The use of the desiccant technology in probiotic oil suspensions allows infants and children in these regions to consume probiotics for health benefits without the need for a refrigerator. Thus, the desiccant strip therefore also serves as an environmentally friendly alternative for refrigeration of probiotic products. Finally, the time from production to distribution of freeze-dried probiotic products renders

desiccant strip devoid products severely disadvantaged and hardly feasible from a commercial perspective.

In summary, this article describes a novel method using desiccant strip technology in probiotic oil suspensions (LongevityGuard®) that efficiently delays CFU decline in freeze-dried *L. reuteri* DSM 17938 and BG-R46®, formulated in oil and packaged in both glass bottles and Easy-Dropper tubes.

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Declarations

Ethical Approval No ethical approval was required for the described work.

Competing Interest All authors are employees of BioGaia AB.

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