

Article

Preserving Probiotic Viability to Support Microbiota-SCFA Function: A Low-Water-Activity Five-Strain Synbiotic Formulation

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Abstract: A fermentation-oriented synbiotic has two immediate formulation requirements: the probiotic cells must remain viable during storage, and enough cells must survive gastrointestinal exposure to encounter fermentable substrate. We characterized a 3.0-g BALIMONT five-strain powder produced by separate premixing and low-humidity post-blending. Water activity was 0.21, moisture 4.3% and 12-month viable-cell retention 69.8%. Simulated-digestion survival was 60.5%, compared with 46.2% after direct blending and 44.6% after premature contact with sensitive components. In the *in vitro* fermentation model, relative SCFA production was normalized to 1.00 for the optimized powder and measured 0.66 and 0.68 for the two comparators. Human trials of inulin-type fructans, FOS, resistant fermentable substrates and *L. plantarum*-containing interventions show reproducible changes in selected microbiota features, whereas fecal SCFA concentrations and metabolic outcomes vary with substrate and host phenotype. Multispecies synbiotic trials demonstrate that human SCFA endpoints can change under defined conditions. The formulation data therefore establish a delivery-controlled platform for direct studies that measure strain persistence, community composition, fecal and circulating SCFAs and related host responses together.

Keywords: *gut-liver axis; probiotics; prebiotics; bile acids; short-chain fatty acids; choline; low water activity*

1. Introduction

Acetate, propionate and butyrate are products of microbial fermentation, but a stool SCFA concentration is not a direct production-rate measurement. It reflects synthesis, microbial cross-feeding, intestinal absorption and transit at the time of sampling. Human interpretation is strongest when SCFA measurements are paired with the substrate that was consumed, microbiota composition and host metabolic context.

The formulation has to deliver an ecological starting population before any fermentation question can be asked. Probiotic cells that lose viability during shelf life or gastric/intestinal exposure have less opportunity to interact with resident organisms and prebiotic carbohydrates. Water activity, moisture and component contact are therefore biological exposure variables as well as manufacturing variables.

BALIMONT combines *L. fermentum*, *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* and *B. pseudocatenulatum* with resistant dextrin, fructooligosaccharides and/or inulin. Choline, taurine, inositol and antioxidant nutrients are handled in a separate premix before final low-humidity blending. We examined whether that architecture preserved viable cells, improved simulated-digestion survival and maintained a stronger SCFA-related fermentation signal than less controlled process conditions.

2. Materials and Methods

2.1 Formulation architecture

Each 3.0-g unit contained approximately 650 mg of the five-strain probiotic blend, with 2.0×10^{10} CFU added during preparation. The viable-cell proportions were 24% *L. fermentum*, 24% *L. plantarum*, 18% *L. delbrueckii* subsp. *bulgaricus*, 16% *S. thermophilus* and 18% *B. pseudocatenulatum*. Choline tartrate (150 mg), taurine (150 mg), inositol (100 mg), vitamin C (50 mg), vitamin E (10 mg α -TE equivalent) and selenium (30 μ g elemental equivalent) were combined with approximately 1,850 mg of resistant dextrin/FOS/inulin carrier. Milk-thistle seed extract (50 mg) and silicon dioxide (10 mg) completed the representative composition.

Table 1. Representative 3.0-g unit-dose composition.

Component	Amount	Role
Five-strain probiotic blend	≈ 650 mg; 2.0×10^{10} CFU added during preparation	<i>L. fermentum</i> 24%; <i>L. plantarum</i> 24%; <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> 18%; <i>S. thermophilus</i> 16%; <i>B. pseudocatenulatum</i> 18%
Choline tartrate	150 mg	Metabolic nutrient module
Taurine	150 mg	Metabolic nutrient module
Inositol	100 mg	Metabolic nutrient module
Vitamin C	50 mg	Antioxidant nutrient module
Vitamin E	10 mg α -TE equivalent	Antioxidant nutrient module
Selenium	30 μ g elemental equivalent	Trace antioxidant nutrient
Resistant dextrin / FOS / inulin	$\approx 1,850$ mg; q.s. to 3.0 g	Prebiotic carrier
Milk thistle seed extract	50 mg	Optional botanical auxiliary
Silicon dioxide	10 mg	Flowability auxiliary

2.2 Manufacturing and comparator conditions

Viable-cell retention was measured after 12 months of sealed storage at 25°C/60% relative humidity. Separate samples underwent sequential simulated gastric and intestinal exposure. Fermentation was assessed with a normalized relative endpoint for the combined major SCFAs. The optimized preparation was compared with direct one-step blending and with premature probiotic contact with choline, vitamin C and botanical components. No unreported inferential statistics were added.

2.3 Performance assessments

Human evidence was selected from randomized or controlled trials of inulin-type fructans, FOS, other fermentable prebiotic substrates, *L. plantarum* and multispecies synbiotics. Studies were retained when they measured microbiota composition, SCFAs or closely linked metabolic outcomes. Clinical results from other strains or substrate systems were used to define mechanisms and endpoint behavior, not as BALIMONT-specific outcomes.

3. Results

3.1 Formulation-level observations

Viable-cell retention after 12 months was 69.8% for the optimized formulation, 47.9% after direct blending, 49.2% after premature contact and 43.8% under the high-humidity process condition. After simulated digestion, survival was 60.5%, 46.2% and 44.6% for the optimized, direct-blending and premature-contact preparations. The process that protected shelf-life viability therefore also retained a larger viable fraction through the simulated gastrointestinal sequence.

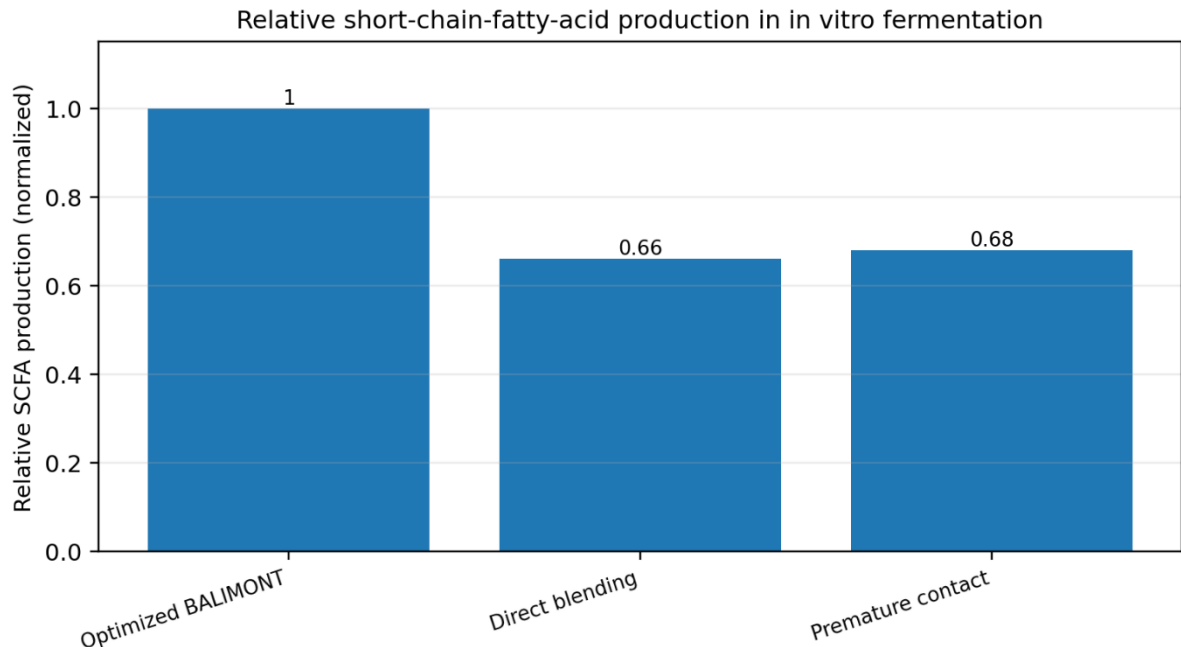


Figure 1. Relative SCFA production in the optimized BALIMONT formulation and process comparators (optimized formulation normalized to 1.00).

Table 2. Comparative functional observations.

Condition		Bile-acid binding (%)	Digestion survival (%)	Relative SCFA
Optimized formulation	BALIMONT	28.6	60.5	1.00
Direct-blending comparator		17.9	46.2	0.66
Premature-contact comparator		18.7	44.6	0.68

3.2 Biological interpretation of the formulation module

Relative SCFA production was set to 1.00 for the optimized formulation and measured 0.66 after direct blending and 0.68 after premature contact. The endpoint is normalized rather than an absolute concentration, so it describes comparative fermentation performance within this assay. Individual acetate, propionate and butyrate concentrations, microbial sources and host absorption remain separate questions for human work.

4. Human Evidence and Mechanistic Interpretation

Birkeland et al. used a randomized crossover design in people with type 2 diabetes and found that inulin-type fructans produced a clear bifidogenic response and altered fecal microbial composition; fecal SCFA changes were not uniform across all measured acids [1]. Rahayu et al. tested *L. plantarum* Dad-13 in overweight adults and reported changes in gut-microbiota composition and intestinal-health-related measures [2]. The Dad-13 strain differs from the *L. plantarum* used in BALIMONT, but the study establishes a human microbiota endpoint for this species.

Salamat et al. provided a functional SCFA example: in a randomized double-blind trial in men with dyslipidemia, a multispecies synbiotic increased fecal SCFAs and IL-10 [3]. Li et al. then compared inulin with FOS in normal-weight and overweight/obese adults; the two substrates produced different microbiota and metabolic response patterns [4]. The carrier fraction in a synbiotic should therefore be treated as an active

experimental variable rather than as an interchangeable filler.

Costabile et al. evaluated *L. plantarum* ECGC 13110402 for 12 weeks in normal-to-mildly hypercholesterolemic adults. Lipid effects appeared in selected groups, but the whole cohort did not show a major global fecal-microbiome shift [5]. Function and broad taxonomic composition can therefore diverge, which argues for measuring metabolites alongside sequencing.

The clinical literature also makes clear that a microbiota change and an SCFA change need not be proportional. That distinction is important for the normalized fermentation signal reported here and motivates a broader human endpoint panel.

Dose-response trials help define the substrate side of that panel. Bouhnik et al. found dose-dependent increases in fecal bifidobacteria with short-chain FOS in healthy adults [6]. A later study by the same group showed that bifidogenic effects were detectable at relatively low FOS intakes [7]. Tandon et al. extended this work with repeated sampling across several FOS doses and documented changes in *Bifidobacterium*, *Lactobacillus* and butyrate-associated taxa over time [8].

Metabolic studies show that microbial and host endpoints can separate. Dehghan et al. reported changes in inflammatory and metabolic-endotoxemia markers during oligofructose-enriched inulin supplementation in women with type 2 diabetes [9]. In obese women, Dewulf et al. found inulin-type fructans altered the gut microbiota and host metabolism [10]. Salazar et al. linked inulin-type fructans to bifidobacterial changes and fecal SCFA-related outcomes in obese women [11].

Other fermentable substrates provide useful functional context. Vulevic et al. studied a bifidogenic galacto-oligosaccharide mixture in older adults and reported microbiota and immune/metabolic changes [12]. Chambers et al. used targeted colonic propionate delivery and observed effects on weight maintenance and adiposity-related measures [13]. Direct colonic SCFA infusion by Canfora et al. altered substrate and energy metabolism in overweight/obese men [14]. A recent randomized study of resistant dextrin also reported enrichment of beneficial fecal bacteria, providing a carrier-relevant context for the resistant-dextrin component of the formulation [15].

Table 4. Selected primary human studies relevant to the formulation pathway.

Study	Design/population	Intervention	Key observation	Relevance
Birkeland et al., 2020 [1]	Randomized crossover; T2D; 6-week intervention periods	Inulin-type fructans vs maltodextrin	Clear bifidogenic/microbiota effects; fecal SCFA response not uniform	Prebiotic ecology and SCFA interpretation
Rahayu et al., 2021 [2]	Randomized double-blind placebo-controlled; overweight adults	<i>L. plantarum</i> Dad-13 powder	Gut-microbiota and intestinal-health-related changes	<i>L. plantarum</i> human microbiota context
Salamat et al., 2024 [3]	Randomized double-blind; men with dyslipidemia	Multispecies synbiotic	Fecal SCFAs and IL-10 increased	Functional human SCFA bridge
Li et al., 2025 [4]	Randomized double-blind; 4 weeks; normal-weight and overweight/obese adults	15 g/day inulin vs FOS vs maltodextrin	Microbiota and metabolic responses differed by substrate and host phenotype	Carrier-specific prebiotic context
Costabile et al., 2017 [5]	Double-blind randomized; 12 weeks	<i>L. plantarum</i> ECGC 13110402	Lipid effects in selected groups; no major global microbiome shift	Demonstrates function-composition

5. Discussion

Three formulation observations point in the same direction: 69.8% viable-cell retention at 12 months, 60.5% survival after simulated digestion and a normalized SCFA-production value of 1.00. Direct blending and premature contact performed worse on all three. The most useful interpretation is not that the formulation guarantees a human SCFA increase, but that it preserves the starting conditions needed to ask that question under controlled exposure.

The five strains occupy different ecological roles. *L. plantarum* and *L. fermentum* can interact with fermentable carbohydrates and resident organisms; *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* add a fermented-food-associated module; *B. pseudocatenulatum* supplies a bifidobacterial component. Future strain-resolved assays can determine which organisms persist and whether that persistence tracks with specific metabolites rather than assuming equal contribution from all five.

Human studies should measure composition and function in parallel. Shotgun or targeted sequencing can track strain persistence and community change. Targeted chromatography or mass spectrometry can quantify acetate, propionate and butyrate in stool and plasma, while transit measures help interpret fecal concentrations. The prebiotic dose and identity should be recorded explicitly because FOS, inulin and resistant dextrin do not produce identical ecological responses [4], [15].

6. Research Scope and Future Directions

A randomized human study can start with the same manufacturing specifications used here and then follow strain persistence, stool community structure, fecal and circulating SCFAs, bowel transit and selected inflammatory/metabolic markers. A factorial or parallel comparison of prebiotic-carrier configurations would help determine whether FOS, inulin and resistant dextrin produce distinct signatures when the probiotic blend is held constant. Repeated stool sampling is preferable to a single pre/post comparison because several prebiotic studies show time-dependent adaptation.

7. Conclusion

The optimized BALIMONT powder retained 69.8% viable cells after 12 months and 60.5% after simulated gastrointestinal exposure. Its normalized SCFA-production value was 1.00, compared with 0.66 and 0.68 for direct-blending and premature-contact comparators. Human prebiotic and probiotic studies show reproducible microbiota responses but more variable SCFA and metabolic outcomes. The formulation is therefore ready for a direct exposure-controlled test in which strain persistence, substrate use, SCFAs and host endpoints are measured together.

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