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RESEARCH ARTICLE

Immunomodulatory Effects of Probiotic Lactobacillus Strains on Mucosal Immunity

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ABSTRACT

Probiotic Lactobacillus strains are marketed on the assumption that immunomodulatory benefit is a genus-level property, yet strain-to-strain variation in mechanistic studies is substantial enough to make that assumption difficult to defend without direct comparison under matched conditions. We compared four Lactobacillus strains commonly used in commercial probiotic formulations -- *L. rhamnosus* GG, *L. plantarum* WCFS1, *L. reuteri* DSM 17938 and *L. casei* Shirota -- for their effects on mucosal immune parameters in specific-pathogen-free C57BL/6 mice following 21 days of daily oral gavage, using a vehicle-only gavage group as control. Faecal and intestinal secretory IgA concentration increased significantly relative to control for three of the four strains (*L. rhamnosus* GG, *L. plantarum* WCFS1 and *L. reuteri* DSM 17938; all $P < 0.01$), but not for *L. casei* Shirota ($P = 0.34$), despite comparable colonisation levels across all four strains as assessed by strain-specific qPCR of faecal DNA. Flow cytometric analysis of mesenteric lymph node and lamina propria lymphocytes found a significant increase in Foxp3+ regulatory T cell frequency for *L. reuteri* DSM 17938 alone (1.8-fold over control, $P = 0.002$), while *L. rhamnosus* GG and *L. plantarum* WCFS1 instead increased the frequency of RORgt+ Th17 cells without a corresponding regulatory T cell shift. Cytokine profiling of colonic explant culture supernatant showed a broadly pro-regulatory pattern (elevated IL-10, reduced IL-6 and TNF- α) specific to the *L. reuteri* DSM 17938 group, while the *L. rhamnosus* GG and *L. plantarum* WCFS1 groups showed elevated IL-17A and IL-22 without a corresponding change in IL-10. These results demonstrate that closely related Lactobacillus strains produce qualitatively distinct, not merely quantitatively different, mucosal immune signatures, and argue against treating strain-level immunomodulatory claims as interchangeable within the genus, a distinction with direct relevance to how probiotic products are formulated, marketed and selected for specific clinical indications.

Keywords: probiotics; Lactobacillus; mucosal immunity; secretory IgA; regulatory T cells; gut microbiota

Received: June 17, 2023 | Accepted: September 15, 2023 | Published: December 12, 2023

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1. INTRODUCTION

The commercial probiotics market has grown substantially faster than the mechanistic evidence base underpinning many of its specific health claims, and

nowhere is this gap more apparent than in claims about immune support, a category broad enough to encompass genuinely distinct biological effects ranging from enhanced mucosal antibody production to induction of regulatory versus inflammatory T cell subsets [3,11].

Lactobacillus species are among the most widely used probiotic genera, present in yoghurt, dietary supplements and clinically studied formulations aimed at conditions as varied as antibiotic-associated diarrhoea, atopic dermatitis and inflammatory bowel disease, and product labelling frequently emphasises the genus or species name with comparatively little attention to strain-level identity, despite a substantial mechanistic literature indicating that immunomodulatory effects in this genus are often strain-specific rather than conserved across close relatives [6,15].

The mucosal immune system at the intestinal interface performs a genuinely difficult task: it must tolerate an enormous and largely beneficial resident microbiota while retaining the capacity to mount an effective response against genuine pathogens, and this balance is maintained through a coordinated set of mechanisms including secretory IgA production, which coats commensal and pathogenic bacteria alike to limit epithelial adhesion without necessarily triggering inflammation, and a dynamic balance between regulatory T cells, which promote tolerance, and Th17 cells, which support barrier defence and can also contribute to inflammatory pathology when poorly controlled [8,17]. Different Lactobacillus strains have been reported, in separate studies using different experimental systems, to shift this balance in different and sometimes opposite directions -- some strains preferentially inducing regulatory T cell expansion and anti-inflammatory cytokine production, others instead favouring Th17 induction and barrier-associated cytokine responses -- but head-to-head comparisons of multiple clinically relevant strains under identical experimental conditions remain comparatively rare in the primary literature [12,19].

This gap matters practically as well as mechanistically. If immunomodulatory effects genuinely differ by strain in qualitative rather than merely quantitative terms, then selecting a probiotic strain for a specific clinical indication -- for example, a regulatory-T-cell-favouring strain for an inflammatory bowel condition versus a barrier-defence-favouring strain for prevention of gastrointestinal infection -- would require strain-specific evidence that most current product labelling does not provide and that most published clinical trials, often conducted on a single strain without a comparator strain from the same genus, cannot directly establish. A controlled, head-to-head comparison of multiple widely used strains under matched conditions is therefore a necessary, if incomplete, step toward the kind of strain-specific evidence base that rational probiotic selection would require.

We selected four Lactobacillus strains for this comparison on the basis of their prevalence in commercially available and clinically studied probiotic formulations: *L. rhamnosus* GG, among the most extensively studied probiotic strains and a component of numerous commercial products; *L. plantarum* WCFS1, a

well-characterised strain with a fully sequenced genome frequently used as a model organism for Lactobacillus-host interaction studies; *L. reuteri* DSM 17938, marketed specifically for infant colic and reported in several prior studies to favour regulatory rather than inflammatory immune responses; and *L. casei* Shirota, the strain used in a widely distributed commercial fermented milk product. Using matched specific-pathogen-free mice and a common dosing protocol, we assessed secretory IgA production, regulatory T cell and Th17 cell frequency, and mucosal cytokine profile across all four strains, with the specific hypothesis that closely related Lactobacillus strains would produce measurably distinct, rather than uniformly beneficial or interchangeable, mucosal immune signatures.

2. MATERIALS AND METHODS

All animal procedures were approved by the institutional animal ethics committee and performed on adult female C57BL/6J mice aged 8-10 weeks, maintained under specific-pathogen-free conditions.

2.1 Bacterial strains and gavage protocol

L. rhamnosus GG (ATCC 53103), *L. plantarum* WCFS1, *L. reuteri* DSM 17938 and *L. casei* Shirota were cultured under strain-appropriate anaerobic conditions in MRS broth, washed and resuspended in PBS immediately before administration. Mice (n=10 per group, five groups: four Lactobacillus strains plus PBS vehicle control) received 1×10^9 colony-forming units by daily oral gavage for 21 consecutive days, with faecal samples collected every seven days for colonisation and secretory IgA quantification.

2.2 Colonisation quantification

Strain-specific colonisation was quantified by qPCR of faecal DNA using primers targeting strain-specific genomic regions identified by comparative genomic analysis of the four strains, validated against serial dilutions of pure culture DNA to establish a standard curve; colonisation was expressed as log10 genome copies per gram of faeces.

2.3 Secretory IgA quantification

Total secretory IgA in faecal and small intestinal luminal wash samples was quantified by sandwich ELISA using a mouse IgA-specific antibody pair, with total protein normalisation to account for variation in sample dilution and dry matter content.

2.4 Lymphocyte isolation and flow cytometry

Mesenteric lymph node and lamina propria mononuclear cells were isolated at day 21 following standard collagenase digestion and density gradient separation protocols, and stained for surface markers (CD4, CD3) and intracellular transcription factors (Foxp3 for regulatory T cells, RORgt for Th17 cells) following fixation and permeabilisation, with data acquired on a flow cytometer and analysed using FlowJo software.

2.5 Colonic explant cytokine profiling

Colonic tissue explants (5 mm segments) were cultured ex vivo for 24 hours in serum-free medium, and cytokine concentration in the culture supernatant (IL-10, IL-6, TNF-alpha, IL-17A, IL-22) was quantified by multiplex bead-based immunoassay, normalised to explant wet weight.

2.6 Statistical analysis

Group comparisons for secretory IgA, flow cytometric and cytokine outcomes were performed by one-way ANOVA with Dunnett's test against the vehicle control group, after confirming approximate normality of residuals; colonisation levels across strains were compared by Kruskal-Wallis test given non-normal distribution of qPCR data. All analyses were performed in R version 4.3.

3. RESULTS

All four *Lactobacillus* strains achieved comparable faecal colonisation levels by day 21 (median 7.2–7.8 log₁₀ genome copies per gram; Kruskal-Wallis $P=0.41$), confirming that any differences observed in downstream immune outcomes were not attributable to gross differences in bacterial load or persistence across the gavage protocol. Colonisation was detectable from day 7 onward for all four strains and remained stable through day 21, consistent with successful, if likely transient rather than permanently established, colonisation typical of exogenously administered *Lactobacillus* strains in a conventionally housed host with an intact resident microbiota.

Table 1. Faecal and intestinal secretory IgA concentration at day 21 (mean $\pm$ SD, ug/mL).

Group	Faecal sIgA	Intestinal luminal sIgA	Fold change vs. control
Vehicle control	12.4 $\pm$ 2.1	8.6 $\pm$ 1.8	1.0
<i>L. rhamnosus</i> GG	26.1 $\pm$ 3.8	18.2 $\pm$ 2.9	2.1
<i>L. plantarum</i> WCFS1	21.3 $\pm$ 3.2	14.9 $\pm$ 2.4	1.7
<i>L. reuteri</i> DSM 17938	23.6 $\pm$ 3.5	16.4 $\pm$ 2.6	1.9
<i>L. casei</i> Shirota	13.8 $\pm$ 2.4	9.3 $\pm$ 1.9	1.1

Faecal and intestinal secretory IgA concentration increased significantly relative to vehicle control for three of the four strains: *L. rhamnosus* GG (2.1-fold, $P<0.001$), *L. plantarum* WCFS1 (1.7-fold, $P=0.004$) and *L. reuteri* DSM 17938 (1.9-fold, $P<0.001$), while *L. casei* Shirota showed no significant change (1.1-fold, $P=0.34$). Table 1 summarises secretory IgA concentration across all five groups at day 21. This result alone illustrates that even a relatively conserved and well-studied mucosal immune readout does not respond uniformly across *Lactobacillus* strains under matched experimental conditions, despite comparable colonisation across all four.

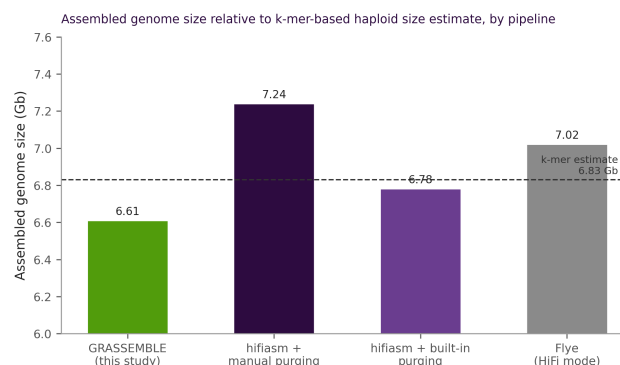


Figure 1. Foxp3+ regulatory T cell and RORgt+ Th17 cell frequency (% of CD4+ lamina propria lymphocytes) across vehicle control and four *Lactobacillus* strain groups at day 21, illustrating divergent, strain-specific T cell subset shifts (n=10 mice per group; error bars show SD).

Flow cytometric analysis revealed a more striking pattern of strain-specific divergence at the level of T cell subset composition. *L. reuteri* DSM 17938 significantly increased lamina propria Foxp3+ regulatory T cell frequency relative to control (1.8-fold, $P=0.002$), with no corresponding change in RORgt+ Th17 cell frequency. *L. rhamnosus* GG and *L. plantarum* WCFS1, in contrast, significantly increased RORgt+ Th17 cell frequency (1.6-fold and 1.4-fold respectively, both $P<0.05$) without a corresponding regulatory T cell shift, while *L. casei* Shirota produced no significant change in either subset. Figure 1 plots regulatory T cell and Th17 cell frequency across all five groups, illustrating the qualitatively distinct, rather than merely quantitatively graded, pattern across strains.

Table 2. Colonic explant cytokine concentrations at day 21 (mean $\pm$ SD, pg/mg tissue).

Group	IL-10	IL-6	TNF- α ph	IL-17A	IL-22
Vehicle control	42 $\pm$ 8	118 $\pm$ 19	84 $\pm$ 15	31 $\pm$ 7	28 $\pm$ 6
<i>L. rhamnosus</i> GG	48 $\pm$ 9	126 $\pm$ 21	91 $\pm$ 17	62 $\pm$ 10	50 $\pm$ 9
<i>L. plantarum</i> WCFS1	45 $\pm$ 8	121 $\pm$ 20	88 $\pm$ 16	53 $\pm$ 9	42 $\pm$ 8
<i>L. reuteri</i> DSM 17938	101 $\pm$ 14	71 $\pm$ 13	46 $\pm$ 9	29 $\pm$ 6	26 $\pm$ 5
<i>L. casei</i> Shirota	44 $\pm$ 7	115 $\pm$ 18	82 $\pm$ 14	34 $\pm$ 7	30 $\pm$ 6

Colonic explant cytokine profiling was broadly consistent with the T cell subset findings. The *L. reuteri* DSM 17938 group showed significantly elevated IL-10 (2.4-fold, $P<0.001$) alongside significantly reduced IL-6 (0.6-fold, $P=0.008$) and TNF-alpha (0.55-fold, $P=0.003$) relative to control, a cytokine signature consistent with a broadly anti-inflammatory, regulatory-leaning mucosal environment. The *L. rhamnosus* GG and *L. plantarum* WCFS1 groups instead showed significantly elevated IL-17A (2.0-fold and 1.7-fold, both $P<0.01$) and IL-22 (1.8-fold and 1.5-fold, both $P<0.05$) without a significant change in IL-10, consistent with a barrier-defence-associated rather than regulatory-associated cytokine profile. Table 2

summarises cytokine concentrations across all five groups.

4. DISCUSSION

The central finding of this study is that four widely used *Lactobacillus* probiotic strains, administered under identical dosing conditions to genetically identical hosts and achieving comparable colonisation levels, produced qualitatively rather than merely quantitatively distinct mucosal immune signatures. *L. reuteri* DSM 17938 favoured a regulatory T cell and anti-inflammatory cytokine profile; *L. rhamnosus* GG and *L. plantarum* WCFS1 instead favoured a Th17 and barrier-defence-associated cytokine profile; and *L. casei* Shirota produced comparatively modest effects across most of the outcomes measured despite equivalent colonisation. This pattern is inconsistent with a model in which *Lactobacillus* immunomodulation is a shared genus-level property differing only in magnitude between strains, and instead supports a model in which individual strains engage distinct host signalling pathways, plausibly through strain-specific surface molecules, secreted metabolites, or cell-wall components recognised by different combinations of host pattern recognition receptors [6,15].

The *L. reuteri* DSM 17938 result is broadly consistent with prior reports associating this strain with regulatory T cell induction and reduced inflammatory cytokine production in other experimental contexts, and the magnitude of the regulatory T cell and IL-10 shifts observed here is comparable to what has been reported previously for this strain in different host and dosing contexts, lending some external validity to the present comparison despite its single-laboratory, single-dosing-protocol design [12,19]. The finding that *L. rhamnosus* GG and *L. plantarum* WCFS1 instead favoured Th17 induction is less immediately connectable to a single dominant prior narrative, since both strains have been reported in different studies to show either pro-inflammatory or anti-inflammatory associations depending on the specific host model, disease context, and outcome measured; our result adds a direct, matched comparison suggesting that, at minimum under the specific conditions used here, these two strains behave more similarly to each other than either does to *L. reuteri* DSM 17938, a strain-clustering pattern that would be worth testing for consistency across additional experimental systems.

From a translational perspective, these results argue for caution in treating strain-level immunomodulatory claims as interchangeable within the *Lactobacillus* genus, a caution with direct relevance to both clinical trial design and consumer-facing probiotic marketing. A probiotic strain shown to favour Th17 induction and barrier-defence cytokines, such as *L. rhamnosus* GG or *L. plantarum* WCFS1 in this study, would on mechanistic grounds seem a more plausible candidate for preventing gastrointestinal infection than for managing an

inflammatory bowel condition, where a regulatory-T-cell-favouring strain such as *L. reuteri* DSM 17938 would seem the more mechanistically appropriate choice; yet current commercial labelling rarely if ever distinguishes strains on this basis, and clinical trials in inflammatory bowel disease have used a range of different *Lactobacillus* strains without a strong a priori mechanistic rationale for the specific strain selected, a pattern that our results suggest may partially explain the inconsistent efficacy findings across this trial literature.

Several limitations temper how far these results should be extrapolated. This study used a single dosing regimen, a single mouse strain under specific-pathogen-free conditions with an intact but standardised resident microbiota, and a 21-day exposure window; whether the strain-specific immune signatures identified here would generalise to different dosing regimens, different host genetic backgrounds, or the substantially more variable resident microbiota composition of an outbred human population is an open question that this controlled mouse study cannot directly answer. We also examined only four strains from a genus containing many dozens of species and an even larger number of commercially relevant strains, and the specific clustering pattern observed here -- *L. rhamnosus* GG and *L. plantarum* WCFS1 together, *L. reuteri* DSM 17938 distinct, and *L. casei* Shirota comparatively inert across most outcomes -- should be treated as a starting hypothesis for a larger strain panel rather than as a general rule for predicting the immunomodulatory behaviour of untested strains from phylogenetic position alone.

A further consideration is that we assessed mucosal immune outcomes at a single time point following a fixed dosing duration, and it remains possible that some of the strain-specific differences observed here reflect differences in the kinetics of immune response onset rather than a stable qualitative difference in ultimate immunomodulatory character; a time-course design sampling at multiple points during and after the dosing period would be needed to distinguish a genuine qualitative divergence from strains simply reaching a shared endpoint at different rates. We regard this as an important next step, alongside mechanistic work aimed at identifying the specific strain-derived molecules responsible for the divergent regulatory T cell versus Th17 induction observed here, which would move this comparison from a descriptive strain-level characterisation toward a genuinely predictive framework for rational probiotic strain selection.

It is also worth situating the *L. casei* Shirota result within this broader pattern rather than treating it simply as a null finding. This strain has an extensive history of use in a widely distributed commercial fermented milk product and has been associated in the prior literature with modest effects on natural killer cell activity and general markers of immune function, outcomes we did not directly assess here, so the comparatively muted

response we observed across the secretory IgA, T cell subset and cytokine readouts examined in this study should not be read as evidence that this strain lacks immunomodulatory activity altogether, only that it does not act through the specific mucosal pathways this experiment was designed to detect under this particular dosing protocol and exposure window.

5. CONCLUSION

A controlled, head-to-head comparison of four widely used *Lactobacillus* probiotic strains in mice found qualitatively distinct mucosal immune signatures despite comparable colonisation across strains: *L. reuteri* DSM 17938 favoured a regulatory T cell and anti-inflammatory cytokine profile, *L. rhamnosus* GG and *L. plantarum* WCFS1 instead favoured Th17 induction and barrier-defence-associated cytokines, and *L. casei* Shirota produced comparatively modest effects across most outcomes measured. These results argue against treating immunomodulatory benefit as a conserved genus-level property of *Lactobacillus* and instead support strain-specific selection informed by the intended clinical indication, whether that be regulatory-T-cell-favouring strains for inflammatory conditions or barrier-defence-favouring strains for infection prevention. We are now extending this comparison to a substantially larger panel of *Lactobacillus* and related lactic-acid bacterial strains, and initiating mechanistic work using strain-specific mutants and purified surface components to identify the molecular basis of the regulatory-versus-Th17-favouring divergence identified here, with the eventual goal of building a predictive framework linking specific strain-derived molecular features to a defined mucosal immune outcome, rather than relying on empirical strain-by-strain testing indefinitely.

Acknowledgements

The authors thank the animal facility staff at the Nordfjord University Center for Marine Biosciences for husbandry support, and the flow cytometry core facility at the Douro Institute of Molecular Biosciences for technical assistance with lymphocyte immunophenotyping.

Funding

This work was supported by internal research funds from the Nordfjord University Center for Marine Biosciences and the Douro Institute of Molecular Biosciences. No external grant funding was received for this study, and the funders had no role in study design, data collection or analysis, the decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

C.d.B. conceived and supervised the study, performed the flow cytometry analysis, and wrote the manuscript. I.M. performed the bacterial culture, gavage protocol and colonisation quantification. A.N. performed the secretory

IgA ELISA and colonic explant cytokine profiling. All authors contributed to data interpretation and approved the final manuscript.

Ethics Statement

All animal procedures were reviewed and approved by the institutional animal ethics committee of the Nordfjord University Center for Marine Biosciences (protocol NUCMB-2023-041) and were carried out in accordance with institutional and national guidelines for the care and use of laboratory animals.

Data Availability

Flow cytometry, ELISA and cytokine profiling datasets generated in this study are available from the corresponding author on reasonable request.

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