Probiotic Potential of Fermented Rice Lactic Acid Bacteria in Regulating Gut Microbiota and Iron Bioavailability

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Abstract—**Background**
Fermented rice is a traditional staple food consumed by over one billion people across South and Southeast Asia. The fermentation process generates diverse communities of lactic acid bacteria (LAB) whose probiotic properties — particularly their capacity to modulate gut microbiota composition and enhance non-haem iron bioavailability — remain largely unexplored in the scientific literature. Given the dual burden of iron deficiency anaemia and intestinal dysbiosis in nutritionally vulnerable populations of the region, investigation of fermented rice LAB represents an important and underexplored area of nutritional science.
Objective
To isolate and characterise LAB strains from traditionally prepared fermented rice, evaluate their probiotic properties in vitro, and assess their capacity to enhance non-haem iron bioavailability and modulate gut microbiota diversity in a pilot human study.
Methods
LAB strains were isolated from five traditional fermented rice preparations (idli batter, ambali, panta bhat, ganji, and fermented rice water) and identified by 16S rRNA gene sequencing. Probiotic characterisation encompassed acid and bile tolerance, cell surface hydrophobicity, auto-aggregation, antimicrobial activity, and haemolytic assay. Iron bioavailability was evaluated using an in vitro digestion-Caco-2 cell ferritin formation assay, with phytate quantified by HPLC. Gut microbiota modulation was assessed in a four-week pilot study (n = 10) using 16S rRNA V3-V4 amplicon sequencing on the Illumina MiSeq platform.
Results
Five LAB strains were isolated, of which Lactobacillus plantarum FR-LP01 exhibited the highest probiotic indices: acid survival >92% (pH 2.0, 2 h), bile tolerance >88%, cell surface hydrophobicity 68.3%, and strong antimicrobial activity against common enteric pathogens. Fermentation for 24 hours reduced phytate content by 61.2% and increased in vitro iron bioavailability by 38.4% relative to unfermented controls ($p < 0.001$). Post-intervention gut microbiota analysis revealed significant increases in Lactobacillus spp. (+85%, $p = 0.003$), Bifidobacterium spp. (+88%, $p = 0.001$), and Akkermansia muciniphila (+112%, $p = 0.008$), accompanied by a 40% reduction in Proteobacteria. Clinical iron parameters improved significantly, with serum ferritin rising by 50% (18.4 to 27.6 ng/mL, $p = 0.004$) and haemoglobin improving from 12.1 to 13.2 g/dL ($p = 0.022$).
Conclusion
Fermented rice LAB — particularly L. plantarum FR-LP01 — demonstrate robust probiotic credentials and a biologically plausible capacity to improve iron nutrition through phytate degradation and beneficial gut microbiota restructuring. These findings support adequately powered randomised controlled trials to validate the utility of traditional fermented rice as a cost-effective, culturally acceptable nutritional intervention for iron deficiency in rice-consuming populations.

I. INTRODUCTION

1.1 Fermented Rice: A Traditional Functional Food

Fermented rice preparations — including idli batter, ambali, panta bhat, ganji, and fermented rice water — constitute dietary staples for over one billion people across South and Southeast Asia (Tamang et al., 2016).[11] Fermentation fundamentally alters the nutritional and microbiological profile of raw rice: it reduces anti-nutritional factors such as phytate and

tannins, increases B-vitamin content through microbial biosynthesis, and generates a diverse community of lactic acid bacteria (LAB) with established health-promoting properties (Nithya et al., 2012).[9]

Despite the antiquity and widespread prevalence of fermented rice consumption, the scientific characterisation of its resident LAB — and the mechanistic pathways through which these organisms may confer nutritional benefit — has received comparatively limited research attention. The majority of probiotic LAB research has focussed on dairy-derived strains; traditional cereal fermentation systems remain an underexplored reservoir of potentially beneficial microorganisms (Holzapfel & Wood, 2014).[5]

1.2 Lactic Acid Bacteria in Rice Fermentation

The dominant LAB species identified in fermented rice fermentations include Lactobacillus plantarum, L. fermentum, L. helveticus, Pediococcus acidilactici, and Leuconostoc mesenteroides (Nithya et al., 2012; Tamang et al., 2016). These organisms produce lactic and acetic acids, lower the pH of the fermentation substrate, and elaborate a range of bioactive metabolites including bacteriocins, exopolysaccharides, and phytase enzymes. The phytase activity of LAB — particularly that associated with L. plantarum — is of particular nutritional interest, as it degrades phytate (inositol hexaphosphate), the principal storage form of phosphorus in cereals and a potent inhibitor of non-haem iron, zinc, and calcium absorption (Hotz & Gibson, 2007).[6][9,11]

1.3 Iron Deficiency: A Global Nutritional Emergency

Iron deficiency anaemia (IDA) affects approximately 1.62 billion people globally and remains the most prevalent micronutrient deficiency worldwide (WHO, 2023). The condition is particularly prevalent in South and Southeast Asia, where rice-based diets high in phytate severely constrain the bioavailability of dietary non-haem iron. Current interventions — iron supplementation, fortification, and dietary diversification — face challenges of compliance, cost, and cultural acceptability. Fermentation-based strategies that enhance native iron bioavailability from traditional foods represent a potentially scalable and sustainable approach.[13]

1.4 Gut Microbiota and Iron Metabolism

Emerging evidence implicates the gut microbiome in the regulation of systemic iron status through multiple mechanisms, including competition for luminal iron, regulation of hepcidin (the master iron-regulatory hormone), and modulation of intestinal epithelial iron transport proteins (Caminero et al., 2019). Probiotic LAB supplementation has been associated with improved iron absorption in both animal models and human studies, though the mechanistic pathways remain incompletely characterised. The expansion of Akkermansia muciniphila — a mucolytic bacterium associated with enhanced mucosal integrity and reduced intestinal permeability — may play a particularly important role in facilitating mineral absorption.[1]

1.5 Research Gap and Study Rationale

Despite these converging lines of evidence, no published study has simultaneously characterised the probiotic properties of fermented rice LAB, quantified their phytase-mediated effects on iron bioavailability in validated cell models, and evaluated their impact on gut microbiota composition in a human intervention study. The present investigation was designed to address this gap, using a multi-method approach encompassing in vitro probiotic characterisation, Caco-2 cell bioavailability assay, and 16S rRNA amplicon sequencing in a pilot human cohort.

II. OBJECTIVES AND HYPOTHESES

2.1 Primary Objectives

1. To isolate and phenotypically and genotypically identify LAB strains from traditionally fermented rice samples collected from five regional sources.
2. To characterise the probiotic properties of isolated LAB strains, including acid and bile tolerance, cell surface hydrophobicity, auto-aggregation, mucin adhesion, antimicrobial activity, and haemolytic safety profile.
3. To evaluate in vitro iron bioavailability using a validated Caco-2 cell ferritin formation assay and to quantify phytate degradation as a function of fermentation duration.
4. To assess the effect of a four-week fermented rice intervention on gut microbiota composition in healthy adult volunteers using 16S rRNA amplicon sequencing.

5. To determine the correlation between fermentation duration, phytase activity, phytate reduction, and iron bioavailability indices.

2.2 Research Hypotheses

H_1: Fermented rice LAB will demonstrate tolerance to gastric acid (pH 2.0–3.0) and bile salts (0.3%) at levels sufficient for classification as probiotics under FAO/WHO (2002) criteria.

H_2: LAB fermentation will significantly reduce phytate content and increase in vitro non-haem iron bioavailability relative to unfermented controls.

H_3: Oral administration of fermented rice will significantly modulate gut microbiota composition, increasing beneficial taxa (Lactobacillus, Bifidobacterium, Akkermansia) and reducing pathobionts (Proteobacteria).

H_4: Iron bioavailability indices will positively correlate with LAB fermentation duration and phytase activity.

III. MATERIALS AND METHODS

3.1 Sample Collection

Traditionally fermented rice samples were collected from five regional sources representing the major fermented rice preparations of South and Southeast Asia: idli batter, ambali, panta bhat, ganji, and fermented rice water. Three replicate samples were collected from each source (n = 3 per type; n = 15 total). Samples were collected in sterile 50 mL polypropylene tubes, transported on ice, and processed within four hours of collection. All sampling was conducted under institutional research authorisation.

3.2 LAB Isolation and Identification

Serial decimal dilutions (10^{-1} to 10^{-8}) of each sample were prepared in sterile 0.85% saline. Appropriate dilutions were plated on de Man, Rogosa and Sharpe (MRS) agar (HiMedia, India) and incubated at 37°C for 48 h under anaerobic conditions (Anoxomat system). Morphologically distinct colonies were subcultured and subjected to Gram staining and catalase testing. Presumptive LAB
(Gram-positive, catalase-negative rods and cocci) were further characterised biochemically using the API 50 CHL identification kit (bioMérieux, France).
Definitive species identification was performed by 16S rRNA gene sequencing. Bacterial DNA was extracted using a commercial kit (Qiagen DNeasy), and the near-complete 16S rRNA gene was amplified using universal primers 27F and 1492R. PCR products were purified and sequenced by Sanger sequencing (Eurofins Genomics). Sequences were compared against the NCBI GenBank database using BLASTn, with species assignment at >99% sequence identity.

3.3 Probiotic Characterisation

Acid tolerance was evaluated by exposing log-phase cultures to MRS broth adjusted to pH 2.0, 2.5, and 3.0 (HCl) for 2 h at 37°C. Viable counts were determined before and after acid exposure, and survival rates calculated. Bile tolerance was assessed by incubation in MRS broth supplemented with 0.3% ox bile (Sigma-Aldrich) for 4 h at 37°C, with viability determined by viable plate count.
Cell surface hydrophobicity was measured by the microbial adhesion to hydrocarbons (MATH) assay using n-hexadecane. Auto-aggregation was quantified by measuring the decrease in optical density (OD600) of bacterial suspensions over 24 h. Mucin adhesion was assessed using porcine gastric mucin (Sigma-Aldrich) coated microtitre plates. Antimicrobial activity was evaluated by the agar well diffusion method against Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Salmonella typhimurium ATCC 14028, and Candida albicans ATCC 10231. Haemolytic activity was assessed on 5% sheep blood agar. Antibiotic susceptibility was determined by disc diffusion per EUCAST guidelines.

3.4 In Vitro Iron Bioavailability Assay

Iron bioavailability was assessed using the in vitro digestion-Caco-2 cell ferritin formation assay, a validated surrogate measure of iron absorption at the intestinal epithelium (Glahn et al., 1998). Fermented and unfermented rice samples (0, 12, and 24 h fermentation) were subjected to sequential in vitro digestion simulating gastric (pepsin, pH 2.0, 37°C, 1 h) and intestinal (pancreatin–bile extract, pH 6.7, 37°C, 2 h) phases. The digesta were ultrafiltered (10 kDa cut-off), and the filtrate applied to Caco-2 cell monolayers (passage 20–30; 14-day post-confluence). After 24 h incubation, cells were lysed and ferritin concentration quantified by ELISA (Ramco Laboratories, USA), expressed as ng ferritin per mg cell protein.[3]

Phytate content was quantified by high-performance liquid chromatography (HPLC) using an ion chromatography system with conductivity detection. Iron content was determined by atomic absorption spectrophotometry (AAS; PerkinElmer AAnalyst 200).

3.5 Human Pilot Study (n = 10)

A single-arm, pre–post intervention pilot study was conducted in 10 healthy undergraduate volunteers (18–30 years; 5 males, 5 female) recruited by convenience sampling from [Institution Name]. Inclusion criteria required omnivorous dietary pattern, no iron supplementation, and no antibiotic use within three months preceding enrolment. Exclusion criteria included gastrointestinal disorders, diagnosed anaemia, pregnancy, or lactation.

Following a two-week washout period during which participants abstained from fermented foods, probiotics, and fermented beverages, baseline assessments were conducted (T0) including: collection of a fresh stool sample, measurement of serum ferritin, serum iron, total iron-binding capacity (TIBC), transferrin saturation, and haemoglobin concentration, and a three-day dietary recall. Participants then consumed 200 g of traditionally prepared fermented rice daily for four weeks as part of their habitual diet (T0–T4). Post-intervention assessments (T4) were conducted at the end of the four-week period using identical methods.

3.6 Gut Microbiota Analysis

Stool DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (Qiagen). The 16S rRNA V3-V4 hypervariable region was amplified using primers 341F/806R and sequenced on the Illumina MiSeq platform (2 × 300 bp paired-end). Raw reads were quality-filtered using DADA2, denoised into amplicon sequence variants (ASVs), and taxonomically classified against the SILVA 138 database. Alpha-diversity was computed as Shannon diversity index; beta-diversity was assessed by Bray-Curtis dissimilarity with PERMANOVA.

3.7 Statistical Analysis

All data were analysed using IBM SPSS Statistics v26. Paired t-tests were used to assess within-group changes in clinical and microbiota parameters between T0 and T4. Pearson correlation coefficients were calculated to assess the relationship between phytate reduction and iron bioavailability. One-way ANOVA with Tukey post-hoc test was used to compare iron bioavailability across fermentation time points. Statistical significance was set at $p < 0.05$. Data are presented as mean ± standard deviation unless otherwise stated.

IV. RESULTS

4.1 LAB Isolation and Identification

A total of five LAB species were isolated and confirmed from the fermented rice samples. Table 1 presents the identified strains, their sources, relative isolation frequency, and key probiotic survival indices. L. plantarum (FR-LP01) and L. fermentum (FR-LF02) were the most frequently isolated species, consistent with published reports of the dominant LAB flora of South Asian fermented rice preparations (Nithya et al., 2012; Tamang et al., 2016).[9,11]

Table 1. Identified LAB Strains from Fermented Rice Samples with Probiotic Survival Indices

Species	Strain ID	Source	Isolation Frequency	Acid Survival (pH 2.0)	Bile Survival (0.3 %)
Lactobacillus plantarum	FR-LP01	Idli batter	High	92%	88%
Lactobacillus fermentum	FR-LF02	Panta bhat	High	87%	84%
Pediococcus acidilactici	FR-PA03	Ambali	Moderate	83%	79%
Leuconostoc mesenteroides	FR-LM04	Ganji	Moderate	78%	74%

Species	Strain ID	Source	Isolation Frequency	Acid Survival (pH 2.0)	Bile Survival (0.3 %)
Lactobacillus helveticus	FR-LH05	Fermented rice water	Low	85%	81%

L. plantarum FR-LP01 demonstrated the highest probiotic indices across all characterisation parameters and was selected as the lead candidate strain for subsequent in vitro and pilot human investigations.

4.2 Probiotic Characterisation

The probiotic characterisation results for the lead strain FR-LP01 are summarised in Table 2. All evaluated strains were haemolysis-negative, confirming safety for human use. FR-LP01 demonstrated superior acid and bile tolerance, high cell surface hydrophobicity, and strong antimicrobial activity against all tested pathogens.

Table 2. Probiotic Characterisation of FR-LP01 (L. plantarum) — Key Parameters

Parameter	Result	Benchmark / Interpretation
Acid survival (pH 2.0, 2 h)	>92%	>80% (FAO/WHO 2002) — Excellent
Bile tolerance (0.3%, 4 h)	>88%	>80% (FAO/WHO 2002) — Excellent
Auto-aggregation	74%	>70% — Strong self-adhesion
Cell surface hydrophobicity	68.3%	>60% — High colonisation potential
Mucin adhesion index	4.2 log CFU	Strong epithelial adhesion
Co-aggregation with E. coli	71.4%	Competitive exclusion capacity
Haemolytic activity	Negative (100%)	Safe for human use
Exopolysaccharide (EPS) production	Positive	Immunomodulatory potential
Antibiotic susceptibility	Sensitive	No transmissible resistance risk

Table 3. Antimicrobial Activity of FR-LP01 and FR-LF02 — Zones of Inhibition (mm)

Pathogen	FR-LP01 (mm)	FR-LF02 (mm)	Clinical Significance
E. coli ATCC 25922	18.4	15.2	Strong inhibition
Staphylococcus aureus ATCC 25923	16.8	14.0	Moderate-strong
Salmonella typhimurium ATCC 14028	19.2	16.5	Strong inhibition
Candida albicans ATCC 10231	14.5	12.8	Moderate inhibition

4.3 Iron Bioavailability and Phytate Reduction

Fermentation duration significantly reduced phytate content and enhanced in vitro iron bioavailability in a time-dependent manner. At 24 h fermentation, phytate content declined by 61.2% relative to the unfermented control (642.5 to 249.1 mg/100g, $p < 0.001$). Correspondingly, Caco-2 cell ferritin formation — the surrogate measure of iron absorption — increased by 38.4% relative to unfermented controls ($p < 0.001$). These changes were statistically significant by one-way ANOVA with Tukey post-hoc test ($p < 0.001$).

Table 4. Phytate Content and In Vitro Iron Bioavailability as a Function of Fermentation Duration

Fermentation Duration	Phytate Content (mg/100g)	Phytate Reduction (%)	Iron Bioavailability (ng ferritin/mg protein)	% Change vs. Control
0 h (Unfermented Control)	642.5 ± 18.4	—	Baseline	—
12 h	380.2 ± 14.1	40.8%	+21.6%	p = 0.012
24 h	249.1 ± 9.8	61.2%	+38.4%	p < 0.001

Pearson correlation analysis revealed a strong negative correlation between phytate content and iron bioavailability ($r = -0.94$, $p < 0.001$), and a strong positive correlation between fermentation duration and iron bioavailability ($r = +0.97$, $p < 0.001$), supporting H_4.

4.4 Gut Microbiota Modulation

16S rRNA amplicon sequencing of paired pre- and post-intervention stool samples from all 10 participants revealed significant shifts in gut microbiota composition following the four-week fermented rice intervention. At the taxonomic level, beneficial LAB genera increased markedly: Lactobacillus spp. increased by 85% ($p = 0.003$) and Bifidobacterium spp. by 88% ($p = 0.001$). Particularly notable was the 112% increase in Akkermansia muciniphila ($p = 0.008$), a next-generation probiotic candidate associated with mucosal integrity and reduced intestinal permeability.

Concurrently, Proteobacteria — a phylum enriched in potential pathogens and associated with intestinal dysbiosis — decreased by 40% ($p = 0.012$). Shannon diversity index increased by 0.48 units ($p = 0.021$), indicating improved alpha-diversity.

Table 5. Gut Microbiota Changes Post-Intervention (n = 10; T0 vs. T4)

Taxon / Index	Change (%)	Direction	p-value
Lactobacillus spp.	+85%	↑ Increase	0.003
Bifidobacterium spp.	+88%	↑ Increase	0.001
Akkermansia muciniphila	+112%	↑ Increase	0.008
Proteobacteria	−40%	↓ Decrease	0.012
Shannon Diversity Index	+0.48 units	↑ Increase	0.021

4.5 Clinical Iron Parameters

Significant improvements were observed across all measured clinical iron parameters over the four-week intervention period (Table 6). Serum ferritin increased by 50% (18.4 ± 3.2 to 27.6 ± 4.1 ng/mL, $p = 0.004$), indicating a meaningful increase in iron stores. Serum iron increased by 30% (72.5 to 94.1 µg/dL, $p = 0.002$), transferrin saturation improved from 20.1% to 29.6% ($p = 0.003$), and total iron-binding capacity decreased from 362 to 318 µg/dL ($p = 0.018$) — indicative of improved iron sufficiency. Haemoglobin concentration improved from 12.1 to 13.2 g/dL ($p = 0.022$).

Table 6. Clinical Iron Parameters Pre- and Post-Intervention (n = 10; Mean ± SD)

Parameter	T0 (Baseline)	T4 (Post-intervention)	% Change	p-value
Serum Ferritin (ng/mL)	18.4 ± 3.2	27.6 ± 4.1	+50.0%	0.004
Serum Iron (µg/dL)	72.5 ± 8.4	94.1 ± 9.2	+29.8%	0.002
TIBC (µg/dL)	362 ± 28	318 ± 24	−12.2%	0.018

Parameter	T0 (Baseline)	T4 (Post-intervention)	% Change	p-value
Transferrin Saturation (%)	20.1 ± 3.8	29.6 ± 4.5	+47.3 %	0.003
Haemoglobin (g/dL)	12.1 ± 0.9	13.2 ± 1.1	+9.1 %	0.022

V. DISCUSSION

5.1 Probiotic Properties of Fermented Rice LAB

The five LAB strains isolated in this study demonstrated probiotic characteristics consistent with — and in several cases exceeding — the minimum criteria specified in the FAO/WHO (2002) joint guidelines for the evaluation of probiotics.[2] The acid survival rates of FR-LP01 (>92% at pH 2.0, 2 h) and FR-LF02 (>87%) are broadly comparable with, and in some instances superior to, those reported for commercial probiotic strains including Lactobacillus rhamnosus GG and L. acidophilus NCFM (Marteau et al., 2004).[8] These findings confirm H_1 and align with prior characterisation of L. plantarum strains from South Asian traditional fermented foods (Nithya et al., 2012).[9]

The high cell surface hydrophobicity (68.3%) and mucin adhesion index (4.2 log CFU) of FR-LP01 suggest robust colonisation potential at the gut epithelial surface — a prerequisite for efficacious probiotic action. Co-aggregation with E. coli (71.4%) further indicates a capacity for competitive exclusion of enteric pathogens, a mechanism of particular clinical relevance in populations at risk of recurrent gastrointestinal infection.

The negative haemolysis result for all five strains confirms the absence of lytic toxin production and supports the safety of these isolates for human administration. The sensitivity of all strains to clinically relevant antibiotics mitigates concerns regarding transmissible antibiotic resistance genes.

5.2 Iron Bioavailability Enhancement via Phytate Degradation

The 61.2% reduction in phytate content and 38.4% increase in Caco-2 ferritin formation at 24 h fermentation represent clinically meaningful changes in iron bioaccessibility. These findings are mechanistically consistent with the established phytase activity of L. plantarum: the PhyA phytase enzyme of this species has been shown to degrade phytate to lower inositol phosphate forms that bind iron with substantially reduced affinity, releasing free ionic iron for absorption at the enterocyte apical surface (Hotz & Gibson, 2007). The magnitude of phytate reduction observed in the present study (61.2%) is comparable with values reported for fermented legumes and cereal-based complementary foods, where phytase-active LAB reduced phytate by 55–65% (Hotz & Gibson, 2007), confirming H_2.[6][6]

The strong inverse correlation between phytate content and iron bioavailability ($r = -0.94$) provides compelling mechanistic evidence that phytate degradation is the principal driver of improved iron bioavailability in fermented rice — consistent with the findings of Hurrell and colleagues (2003) who demonstrated that phytate reduction from >1000 to <60 mg/100g markedly improved iron absorption in cereal-based diets.[7]

5.3 Gut Microbiota Modulation

The eubiotic shift observed following the four-week fermented rice intervention — increases in Lactobacillus, Bifidobacterium, and Akkermansia muciniphila alongside a reduction in Proteobacteria — is consistent with the established microbiota-modulating effects of probiotic LAB supplementation (Hill et al., 2014). The 112% increase in Akkermansia muciniphila is a particularly noteworthy finding: this organism has emerged as a key mucosal commensal associated with enhanced intestinal barrier integrity, reduced endotoxaemia, and improved mineral absorption through mechanisms including upregulation of tight junction proteins and modulation of toll-like receptor signalling (Plovier et al., 2017).[10][4]

The 40% reduction in Proteobacteria is consistent with the antimicrobial activity of FR-LP01 observed in vitro, and may reflect the in vivo elaboration of bacteriocins and organic acids that suppress the growth of gram-negative pathobionts. The observed improvement in Shannon diversity index (+0.48 units, $p = 0.021$) is clinically relevant: reduced gut microbial diversity has been associated with a range of non-communicable diseases, metabolic disorders, and

impaired mineral absorption (Turnbaugh et al., 2006).[12]

5.4 Clinical Iron Parameter Improvement

The 50% increase in serum ferritin and 30% improvement in serum iron over four weeks represent clinically meaningful changes in iron nutritional status. The concurrent decrease in total iron-binding capacity (TIBC) is consistent with improved iron saturation of transferrin — a physiological response to increased iron availability. The haemoglobin improvement (12.1 to 13.2 g/dL, $p = 0.022$), while modest, is biologically plausible within a four-week timeframe and is consistent with the kinetics of erythropoiesis following improved iron supply.

These clinical findings, taken together with the in vitro bioavailability data and gut microbiota results, support a biologically coherent mechanistic pathway: fermented rice LAB phytase activity degrades dietary phytate → iron is released from phytate-iron chelates → free ionic iron is absorbed at the Caco-2/intestinal epithelial surface → gut microbiota restructuring (particularly Akkermansia expansion) enhances mucosal permeability and mineral transport → systemic iron status improves.

5.5 Limitations

The principal limitation of this study is the small pilot sample size ($n = 10$), which limits statistical power and precludes generalisation of the clinical findings. The absence of a concurrent control group means that observed changes cannot be attributed with certainty to the fermented rice intervention rather than to seasonal, dietary, or other temporal confounders. Dietary intake during the intervention was assessed by self-report (three-day dietary recall) and was subject to recall bias; biochemical phytase activity was not directly quantified in participants. These limitations are acknowledged explicitly and do not diminish the novelty or mechanistic significance of the findings; they do, however, emphasise the need for adequately powered controlled trials before clinical recommendations can be made.

VI. CONCLUSIONS

Fermented rice lactic acid bacteria — in particular L. plantarum FR-LP01 isolated from idli batter — demonstrate robust probiotic credentials that exceed minimum FAO/WHO benchmarks for acid and bile tolerance, and exhibit strong intestinal adhesion, antimicrobial activity, and exopolysaccharide production. Fermentation for 24 hours reduced phytate content by 61.2% and enhanced in vitro non-haem iron bioavailability by 38.4%, with a strong correlation between phytate degradation and iron absorption ($r = -0.94$).

In a four-week pilot human study, daily consumption of 200 g fermented rice produced a statistically significant and clinically meaningful eubiotic shift in gut microbiota composition, with increases in Lactobacillus, Bifidobacterium, and Akkermansia muciniphila and a reduction in Proteobacteria. These microbiota changes were accompanied by a 50% increase in serum ferritin, 30% improvement in serum iron, and significant improvements in transferrin saturation and haemoglobin concentration.

These findings collectively support the mechanistic hypothesis that fermented rice LAB improve systemic iron nutrition through dual complementary pathways: (i) direct enhancement of luminal iron bioaccessibility via phytase-mediated phytate degradation, and (ii) optimisation of iron absorption capacity through beneficial gut microbiota restructuring.

Fermented rice represents a cost-effective, culturally accepted, and widely available functional food with significant untapped potential as a dietary strategy for reducing iron deficiency in rice-consuming populations. Adequately powered randomised controlled trials with matched control arms, extended intervention periods (8–12 weeks), and comprehensive mechanistic sub-studies are warranted to confirm and quantify the clinical utility of this approach.

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