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Research Article

Probiotic and metabolite profiling of poongar rice fermentation matrix: A zebrafish-based intervention study for gut dysbiosis

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Abstract

Background: Traditional rice-based fermented foods, prevalent across Asia particularly India, constitute an unexplored reservoir of probiotic microorganisms with high commercial potential for functional foods and synbiotics.

Aims: This study investigates the probiotic properties and therapeutic efficacy of a fermented matrix derived from *Oryza sativa* (Poongar rice) against antibiotic-induced gut dysbiosis.

Methods: Fermented Poongar rice water underwent microbial isolation, biochemical characterization, and 16S rRNA sequencing, which confirmed the presence of *Lactococcus lactis* with 96.7% homology. The isolated strain was assessed for its tolerance to acidic pH, bile salts, and salinity. Gas chromatography–mass spectrometry analysis was employed to characterize its bioactive metabolite profile, including short-chain fatty acids (SCFAs). Nutritional composition analysis and exopolysaccharides (EPS) quantification were also performed. Zebrafish (*Danio rerio*) were utilized to evaluate acute toxicity (LC_{50} = ~2,138 ppm) and to examine gut recovery following erythromycin-induced dysbiosis.

Results: The isolated strain exhibited high survivability under simulated gastrointestinal conditions and demonstrated the production of SCFAs and EPS, both of which contribute to gut health. Zebrafish administered the probiotic matrix after antibiotic exposure displayed restored swimming behavior and significant improvement in intestinal histoarchitecture, characterized by reduced goblet cell hyperplasia and diminished villi damage.

Özet

Dayanak: Özellikle Hindistan olmak üzere Asya›da yaygın olan geleneksel pirinç bazlı fermente gıdalar, fonksiyonel gıdalar ve sinbiyotikler için yüksek ticari potansiyele sahip, keşfedilmemiş bir probiyotik mikroorganizma rezervuarı oluşturmaktadır.

Amaçlar: Bu çalışma, *Oryza sativa* (Poongar pirinci) kaynaklı fermente matrisin antibiyotik kaynaklı bağırsak disbiyozuna karşı probiyotik özelliklerini ve terapötik etkinliğini araştırmaktadır.

Yöntemler: Fermente Poongar pirinç suyu, mikrobiyal izolasyon, biyokimyasal karakterizasyon ve 16S rRNA dizileme işlemlerinden geçirilmiş ve %96,7 homoloji ile *Lactococcus lactis* varlığı doğrulanmıştır. İzole edilen suş, asidik pH, safra tuzları ve tuzluluğa karşı toleransı açısından değerlendirilmiştir. Gaz kromatografisi-kütle spektrometrisi analizi, kısa zincirli yağ asitleri (SCFAs) dahil olmak üzere biyoaktif metabolit profilini karakterize etmek için kullanılmıştır. Besin bileşimi analizi ve ekzopolisakkarit (EPS) miktarının belirlenmesi de gerçekleştirildi. Zebra balığı (*Danio rerio*) akut toksisiteyi (LC_{50} = ~2.138 ppm) değerlendirmek ve eritromisin kaynaklı disbiyozis sonrası bağırsakların iyileşmesini incelemek için kullanıldı.

Bulgular: İzole edilen suş, simüle edilmiş gastrointestinal koşullar altında yüksek hayatta kalma oranı sergilemiş ve bağırsak sağlığına katkıda bulunan SCFAs ve EPS üretimi göstermiştir. Antibiyotik maruziyetinden sonra probiyotik matris uygulanan zebra balıkları, yüzme davranışlarının düzeldiğini ve bağırsak histoarkitektüründe, goblet hücre hiperplazisinin azalması ve villus hasarının azalması ile karakterize edilen önemli bir iyileşme göstermiştir.

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Conclusion: Fermented Poongar rice contains a functionally robust strain of *L. lactis* capable of restoring gut integrity *in vivo*. The findings supports its potential development as a culturally rooted, cost-effective probiotic candidate for gut health applications.

Sonuç: Fermente Poongar pirinci, *in vivo* bağırsak bütünlüğünü geri kazanabilen, işlevsel olarak güçlü bir *L. lactis* suşu içerir. Bulgular, bağırsak sağlığı uygulamaları için kültürel kökenli, uygun maliyetli bir probiyotik adayı olarak potansiyel gelişimini desteklemektedir.

Keywords: Short-chain fatty acids, intestinal microbiota modulation, host–microbe interaction, functional fermented foods, aquatic toxicology model, gut-brain axis signaling

Introduction

The human gastrointestinal tract harbors trillions of microorganisms that collectively form a dynamic and highly complex ecosystem referred to as the gut microbiota. This microbial community plays a crucial role in digestion, immune modulation, nutrient metabolism, and the maintenance of intestinal barrier integrity (Hills et al., 2019). Disruptions in this delicate balance, known as gut dysbiosis, have been associated with a wide range of health disorders, including metabolic dysfunctions, gastrointestinal inflammation, and neuropsychiatric diseases (Ogunrinola et al., 2020). Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts have gained considerable attention as therapeutic agents capable of restoring microbial homeostasis (Nagpal et al., 2012). Commonly employed probiotic genera, including *Lactobacillus*, *Bifidobacterium*, and *Lactococcus*, have demonstrated significant efficacy in improving gut health, reducing pathogen load, and modulating immune responses (Kechagia et al., 2013). However, the effectiveness of probiotics is highly strain-specific and largely dependent on their capacity to withstand gastrointestinal stressors such as acidic pH, bile salts, and digestive enzymes (Binda et al., 2020). Traditional fermented foods represent a natural and rich reservoir of beneficial microorganisms. In this context, indigenous rice varieties such as Poongar rice present promising potential for probiotic development. Poongar rice is a red-husked, unpolished grain cultivated in Tamil Nadu's Cauvery Delta and is known for its high content of micronutrients, antioxidants, and dietary fiber. Historically utilized in Ayurvedic medicine, it is reputed for its benefits in supporting reproductive health, regulating blood glucose levels, and improving skin and digestive health (Radha et al., 2022; Rathna Priya et al., 2019). Despite these traditional claims, scientific validation of the microbiota-enhancing properties of Poongar rice remains limited, with few studies like fermented rice water screening (*Leuconostoc lactis*, *Weissella cibaria*; >80% gastric survival) and general synbiotic effects of fermented rice beverages (*Pediococcus pentosaceus* isolation) providing preliminary evidence but lacking Poongar-specific microbiota modulation data (Chavan et al., 2022). The present study investigates the probiotic potential of a fermented Poongar rice matrix through the isolation and characterization of native microbial strains. The study further evaluates the ability of these strains to endure simulated gastrointestinal conditions and identifies key functional metabolites, including short-chain fatty acids (SCFAs) and exopolysaccharides (EPS). To validate the *in vivo* efficacy and safety of the fermented matrix, a Zebrafish (*Danio rerio*) gut dysbiosis model is employed, leveraging its genetic homology with humans and its transparent gut morphology, which facilitates microbiome assessment (Lu et al., 2021).

This research integrates traditional food knowledge with contemporary microbial science to advance the development of culturally rooted probiotic interventions aimed at restoring gut microbial balance.

Materials and Methods

Sample Collection and Fermentation of Poongar Rice

Traditional Poongar rice grains were procured from Rengachipatti, Thalinji village in Tamil Nadu. The rice (100 g) was thoroughly washed and boiled for one hour. The resulting cooking water was allowed to cool to room temperature, transferred into sterile glass containers, and loosely sealed to permit limited air exchange. Fermentation was carried out at ambient temperature (approximately 28 $\pm$ 2 °C) for three days under semi-aerobic conditions. To minimize microbial contamination, all vessels and utensils were sterilized prior to use, and the entire setup was maintained under clean environmental conditions. The fermented rice water thus obtained was used as the base medium for probiotic screening, following the method of Fuloria et al. (2022).

Isolation and Morphological Analysis of Probiotic Strain

To isolate potential probiotic candidates, the fermented rice water was subjected to serial dilution and spread onto de Man, Rogosa, and Sharpe (MRS) agar plates. The plates were incubated at 30 °C for 24 hours. Morphologically distinct colonies were selected and subcultured repeatedly to obtain pure isolates. Microscopic examination, including Gram staining and the hanging drop technique, were performed to determine cellular morphology and motility.

Biochemical Profiling

The purified isolate was subjected to comprehensive biochemical characterization using standard assays. These included the IMViC tests and enzymatic activity assays. The tests evaluated indole production, methyl red, Voges-Proskauer reaction, citrate utilization, catalase and oxidase activities, triple sugar iron utilization, and starch hydrolysis.

Molecular Confirmation Using 16S rRNA Gene Sequencing

Genomic DNA was extracted using the HiPurA® purification kit. The 16S rRNA gene was amplified by polymerase chain reaction using universal primers 27f and 1492r. The amplified products

were confirmed by agarose gel electrophoresis. The obtained sequences were analyzed using the BLAST tool, and phylogenetic relationships were inferred using MEGA11 software to support taxonomic identification.

Evaluation of Probiotic Survivability Under Stress Conditions

The robustness of the isolate was assessed by evaluating its tolerance to acidic pH, bile salts, and elevated sodium chloride concentrations. Acid tolerance was tested at pH 3.0. Salt tolerance was examined using nutrient broth supplemented with varying NaCl concentrations, while bile salt resistance was evaluated using agar infused containing bile salts.

Gas Chromatography–Mass Spectrometry (GC–MS)-Based Metabolite Profiling

An aqueous extract of the fermented Poongar rice matrix was prepared and analyzed using GC–MS to identify the major metabolic compounds produced during fermentation.

Quantification of EPS Production

EPS was quantified using the Anthrone reagent method. Polysaccharide concentration was determined by comparing absorbance values against a glucose standard calibration curve, enabling estimation of EPS content in the fermented matrix.

Nutritional Composition Analysis

The fermented substrate was analyzed for its macronutrient composition. Total carbohydrate content was estimated using the Anthrone assay. Lipids were quantified using the Bligh and Dyer extraction method, and protein concentration was determined by the Bradford assay.

Zebrafish Toxicity Assay and Maintenance Protocol

Ethical approval for zebrafish maintenance and handling was obtained from the Saveetha Dental College & Hospital Institutional Human Ethical Committee (SDC-IHEC) (reference number: 24/BIOCHEM/049, dated: 28.05.2024). Adult *Danio rerio* were randomly allocated into six groups comprising one control and five treatment groups, with 10 fish per group and a total of 60 individuals. The fish were maintained in aquaria under controlled laboratory conditions at 28.5 °C with a 14-hour light and 10-hour dark photoperiod. Freeze-dried probiotic matrix was administered at concentrations of 0, 400, 800, 1600, 3200, and 6,400 ppm. These concentrations were selected to cover the complete dose–response spectrum, enabling the assessment of both sub-lethal and lethal effects for accurate LC_{50} determination. Each concentration was tested in triplicate to perform acute toxicity evaluation, and the median LC_{50} was calculated using probit regression analysis.

Induction of Gut Dysbiosis and Probiotic Remediation Strategy

Gut Dysbiosis was experimentally induced by exposing zebrafish to erythromycin at a concentration of 0.6 mg/mL for a duration of 96 hours. Following antibiotic exposure, a subset of fish received the probiotic formulation. Behavioral parameters were monitored after treatment to assess recovery and physiological normalization.

Histological Examination of Intestinal Tissue

After treatment, zebrafish intestinal tissues were excised and fixed in 4% formalin. Standard paraffin embedding procedures were followed, and tissue sections were prepared. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically for histopathological changes related to mucosal and structural integrity.

Statistical Analysis

All experimental assays were performed in triplicate. The fish were exposed to the fermented Poongar matrix for 96 hours under static conditions. Mortality was confirmed when no visible movement, including opercular motion, was observed and when stimulation of the caudal peduncle elicited no response. Mortalities and visible abnormalities in appearance and behavior were recorded. The concentrations required to cause 50% mortality (LC_{50}) was determined (Singleman & Holtzman, 2014). Acute toxicity over 96 h was calculated as LC_{50} and subjected to probit analyses using Finney's method (Finney, 1971) (Figure 5). Results with $p < 0.05$ were considered statistically significant. The LC_{50} value was obtained both arithmetically using regression equations and graphically by plotting logarithmic concentration values against probit mortality. Differences among treatment groups were analyzed using regression analysis and one-way analysis of variance (ANOVA) with a 95% confidence interval. Toxicological data were further modeled using residual and probability output metrics to assess goodness of fit and predictive accuracy. All statistical analyses were performed using Microsoft (MS) Excel 2007 with the Analysis Toolpak enabled for data validation and advanced statistical computation (Praskova et al., 2011).

Results

Fermentation of Poongar rice led to the emergence of distinct microbial colonies after 72 hours. Following serial dilution and plating on MRS agar, the colonies exhibited a cocci morphology upon microscopic observation and were identified as Gram-positive. Hanging drop analysis further confirmed that the isolates were non-motile. These morphological characteristics are typical of lactic acid bacteria, which are commonly associated with probiotic properties.

The isolated strain was subsequently subjected to a series of biochemical assays for further characterization. The strain tested positive for methyl red and triple sugar fermentation, while it was negative for indole production, Voges-Proskauer reaction, citrate utilization, catalase activity, oxidase activity, and motility. Additionally, the presence of a clear zone in the starch hydrolysis assay confirmed the organism's ability to produce extracellular amylase. These biochemical features are consistent with the established metabolic profile of *Lactococcus* spp., which are known for efficient carbohydrate fermentation and growth under acidic conditions.

Genomic DNA extracted from the isolate was of high quality, as confirmed by agarose gel electrophoresis (Figure 1). Amplification of the 16S rRNA gene using universal primers produced a distinct and specific band. Sequence analysis using BLAST revealed a 96.7% identity with *Lactococcus lactis*.

Phylogenetic tree analysis further validated the molecular identification by clustering the isolate within the *Lactococcus* genus, a taxonomic group widely recognized for its probiotic applications in food systems (Figure 2).

Acid tolerance studies demonstrated the robust survival of the isolate at pH 3.0, thereby simulating gastric conditions. Likewise, the strain tolerated NaCl concentrations of up to 8%, confirming its ability to persist in high salt environments such as the human gastrointestinal tract or preserved food matrices. Bile salt tolerance was further confirmed by visible colony growth on bile agar plates after 24 hours of incubation. Collectively these traits highlight the isolate's potential to survive in gastrointestinal conditions - an essential requirement for probiotics intended for oral administration (Figure 3).

GC–MS Analysis of SCFAs

The aqueous extract of the fermented Poongar rice matrix was analyzed using GC–MS, which revealed the presence of various SCFAs and other bioactive compounds (Figure 4 and Table 1). Among the detected metabolites, beta-D-glucopyranoside tetraacetate and 2-ketobutyric acid were identified as the predominant components. These compounds are known to be associated with enhanced intestinal barrier function and anti-inflammatory activity. Their detection in the fermented matrix contributes to the functional significance of the rice-based probiotic formulation.

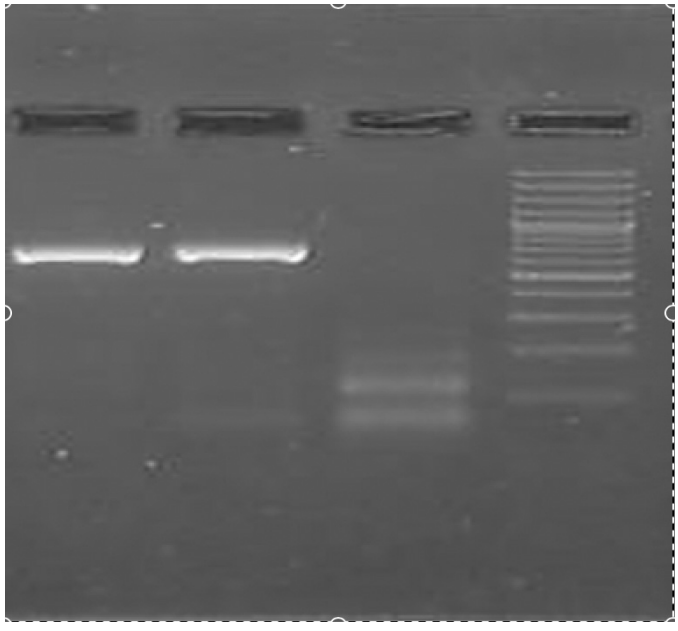

Figure 1. Agarose gel electrophoresis showing the genomic DNA of the isolated strain.

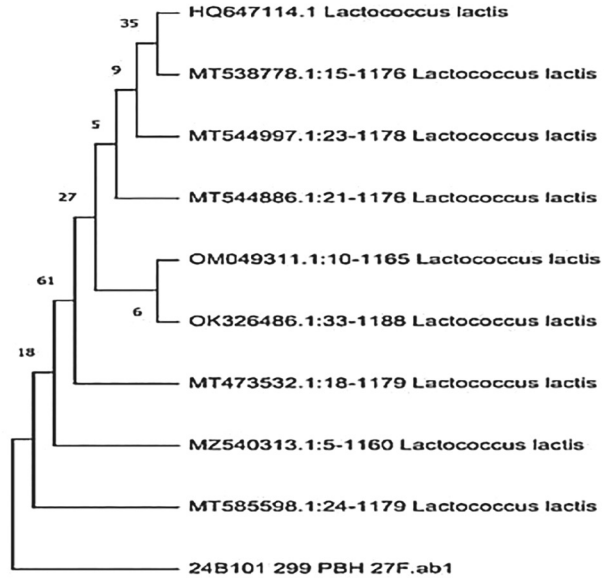

Figure 2. Phylogenetic tree showing the highest percentage of sequence similarity, constructed using MEGA11 software.

The Anthrone assay revealed that the EPS content of the fermented matrix was 0.506 g per 100 g of rice powder. EPS s are known to enhance microbial adhesion, modulate immune responses, and contribute to the prebiotic potential of probiotic formulations (Table 2). The relatively high EPS concentration further confirms the isolate's ability to support gut health through both effective colonization and metabolic activity. Biochemical profiling also indicated that the fermented rice matrix is nutritionally rich, with the lipid content estimated at 40% using the Bligh and Dyer method. The carbohydrate and protein contents were calculated as 0.089 g and 0.055 g per 100 g of rice powder, respectively. These macronutrients may serve as essential energy sources that support the viability and metabolic performance of probiotic strains during gastrointestinal transit and subsequent colonization in the gut.

To evaluate safety, zebrafish were exposed to a concentration range of 400–3,600 ppm of the lyophilized rice-probiotic matrix. This range was selected to encompass sub-therapeutic to elevated doses, thereby capturing a comprehensive toxicity profile. This chosen concentrations included levels both below and above the anticipated LC_{50}, allowing for robust determination of toxicity thresholds and ensuring the detection of adverse effects at higher doses. Statistical analyses using ANOVA and regression revealed a significant treatment effect on zebrafish toxicity ($f = 12.58$, $p < 0.039$), with the regression model indicating that toxicity increased by approximately 4.62 units per unit increase in concentration ($p < 0.04$). The LC_{50} value of 2,138 ppm, derived using MS Excel 2007 Probit analysis, reflects moderate toxicity. The LC_{50} value determined using nonlinear regression analysis in GraphPad Prism was 1,828 ppm, while the 95% confidence interval ranged from 1,294 to 2,487 ppm, which closely aligns with the value

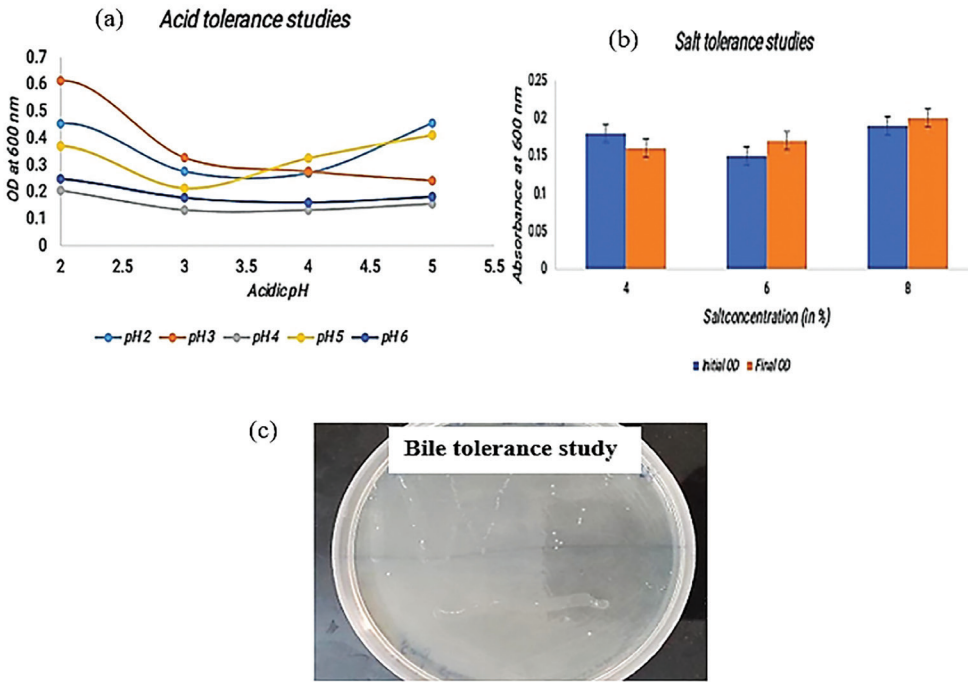

Figure 3. Probiotic endurance studies showing (a) bacterial growth under different pH conditions ranging from acidic to basic; (b) bacterial growth under varying salt concentrations; (c) growth of the probiotic strain on bile salt agar medium.

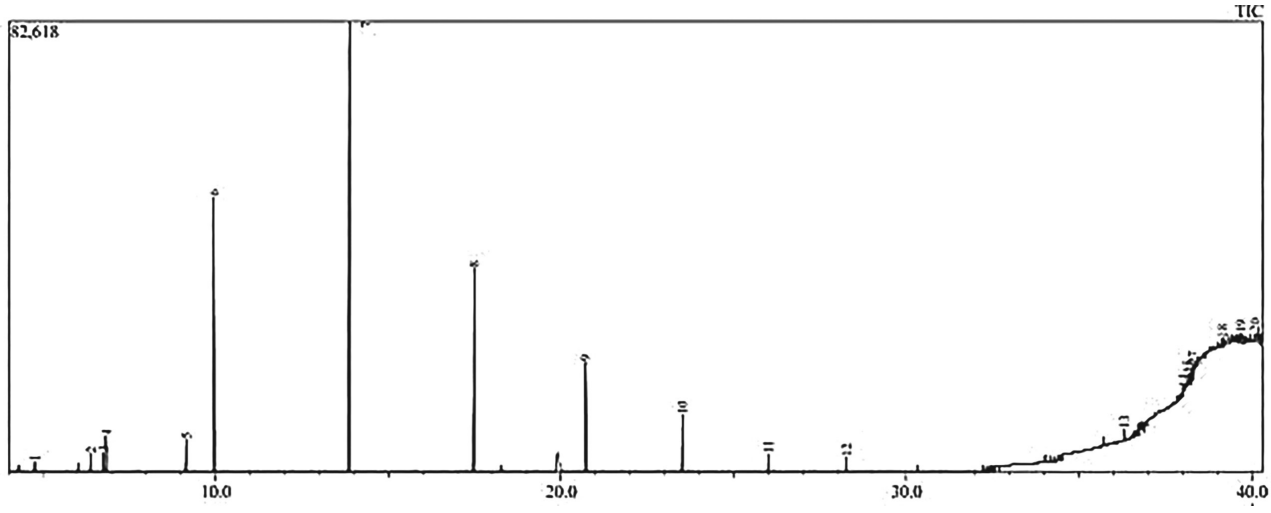

Figure 4. GC–MS spectrum of fermented Poongar rice.

obtained from the MS Excel regression-based data validation. Probit modeling further confirmed that concentrations below this threshold did not induce significant mortality or adverse effects, thereby supporting the safety of the formulations at therapeutically relevant doses. Zebrafish exposed to sub-lethal concentrations maintained normal shoaling, swimming, and exploratory behaviors, without no observable stress-related responses. Imaging and visual assessments also revealed no developmental abnormalities, organ damage, or morphological alterations below the LC_{50} level (Figures 5 and 6; Tables 3–5).

Antibiotic-induced gut dysbiosis was established by treating zebrafish with erythromycin (0.6 mg/mL) for a period of 96 hours. The treated fish exhibited reduced motility, loss of appetite, and increased mortality. In contrast, fish that received the probiotic matrix following antibiotic exposure demonstrated marked recovery in swimming behavior and body weight. These observations suggest that the formulation supported effective gut recolonization and promoted systemic physiological recovery.

Table 1. GC–MS-identified compounds of fermented Poongar rice.

Number	Name	Retention	Peak area %
1	**2-Keto-butyric-acid****	4.767	0.80
2	(2.Alpha.,3.alpha.,4a.beta.,5.beta.,8a.alpha.)-(+-)-3,4,4a,5,6,7,8,8a-octahydra- 4a,7,7-trimethyl-5-[[(1,1- dimethyl)dimethy	6.391	1.53
3	2-(2-oxo-2-phenyl-ethyl)-1,3-dioxolane	6.745	1.29
4	Dodecane,1,1-difluro-	6.823	2.72
5	Dodecane,1,1-difluro-	9.142	1.99
6	Cyclopentasiloxane, decamethyl-	9.949	20.79
7	Cyclohexasiloxane, dodecamethyl-	13.869	35.54
8	Cyclohexasiloxane, tetradecamethyl-	17.48	15.23
9	**Benzoic acid,2,4-bis(trimethylsiloxy0-,trimethyl ester****	20.714	8.45
10	Phosphonous dibromide,[2,2,2-(trifluoromethyl)-1-[(trimethylsilyl)oxy]ethyl]-	23.511	3.59
11	1,3-diphenyl-1-((trimethylsily)oxy)-1(z) heptane	25.998	1.12
12	Tri-o-trimethylsilyl,n-pentafluoropropionyl derivative of terbutaline	28.267	0.72
13	**Beta.-d-glucopyranoside,ethyl,tetra acetate****	36.316	0.92
14	n-(t-butyl)-2-benzoylbenzamide	38.021	0.33
15	n-(t-butyl)-2-benzoylbenzamide	38.155	0.67
16	n-(t-butyl)-2-benzoylbenzamide	38.193	0.73
17	2-fluro-5-trifluromethylbenzoic acid,pentyl ester	38.286	0.65
18	Trans-6-(hydroxmethyl)-5- (trimethylsilyl) bicycol[4.4.0]-ene	39.145	1.06
19	Ethanone,1-[4([1,1- dimethylethoxy)methyl]phenyl]-	39.687	0.88
20	Cesium trimethylfluro)aluminate	40.292	0.95

**The SCFAs and benzoic acid derivatives essential for gut health.

Table 2. Nutritional and EPS profile of the fermented rice matrix.

Component	Content (g/100 g of rice powder)	Method used
EPS	0.506	Anthrone assay
Lipid	40	Bligh and dyer
Carbohydrate	0.089	Anthrone assay
Protein	0.055	Bradford assay

Histological examination of zebrafish intestinal tissue provided further confirmation of the therapeutic efficacy of the Poongar rice matrix (Figure 7). Fish exposed to erythromycin showed pronounced mucosal erosion, villus destruction, and goblet cell hyperplasia—classic indicators of intestinal tissue damage. Conversely, probiotic-treated fish exhibited restoration of villus architecture, reduced inflammatory infiltration, and normalization of mucosal integrity. These histological improvements reinforce the role of the formulation in modulating gut health and restoring epithelial function.

Discussion

L. lactis was isolated from fermented Poongar rice, underscoring the potential of traditional rice-based diets to function as reservoirs of beneficial microorganisms (Radha et al., 2022). The demonstrated tolerance to acidic pH (as low as 3.0), bile salts, and elevated salt concentrations reflects the physiological resilience required for survival and functionality within the gastrointestinal tract. Recent studies have further emphasized the probiotic potential of *L. lactis* strains, documenting their capacity to endure gastrointestinal stresses while exhibiting antibacterial activity (Paul et al., 2025). The *L. lactis* strain isolated from fermented Poongar rice exhibits notable probiotic potential through the activation of immune signaling pathways such as the mitogen-activated protein kinase cascade, involving phosphorylation of c-Jun N-terminal kinase and extracellular signal-regulated kinase. These molecular events regulate cytokine production and immune responses, thereby supporting gut homeostasis. Additionally, the strain produces antimicrobial substances that inhibit pathogenic bacteria and promote a balanced gut microbiota. The secretion of bioactive molecules such as α-mannosidase further influences immune signaling and contributes to the protection of gut epithelial cells.

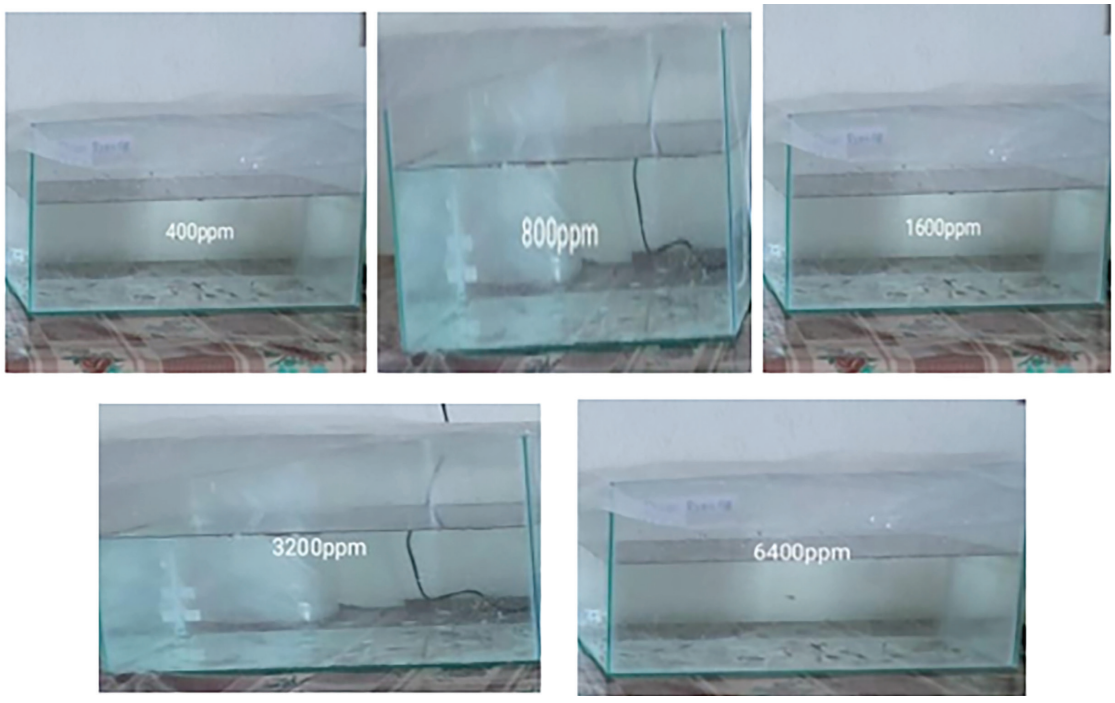

Figure 5. Experimental grouping of zebrafish treatment sets.

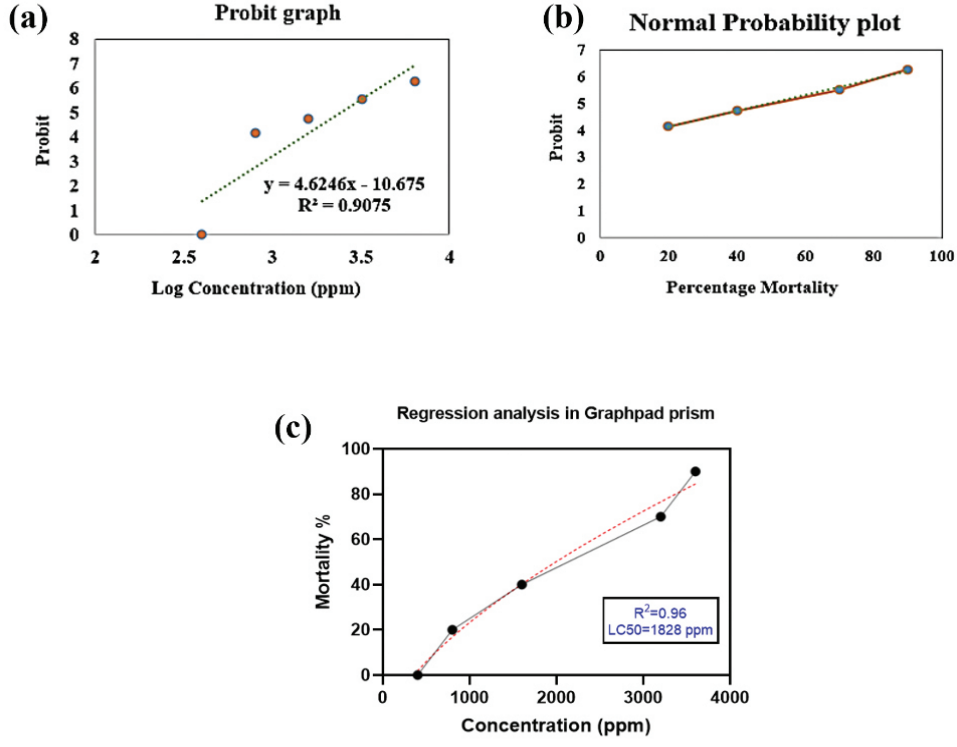

Figure 6. Probit plot generated using MS Excel 2007-Analysis Toolpak (a); normal probability plot of regression analysis (MS Excel 2007) (b); regression analysis performed using GraphPad Prism (c).

Table 3. Probit analysis of zebrafish mortality using Finney's method.

Conc. (ppm)	$\log_{10}$(Conc.)	Zebrafish (n = 10)	Number of death after 96 h exposure	Percentage mortality	Probit value
400	2.602	10	0	0	0
800	2.903	10	2	20	4.16
1,600	3.204	10	4	40	4.75
3,200	3.505	10	7	70	5.52
6,400	3.806	10	9	90	6.28

Conc. = concentration.

Table 4. ANOVA and regression analysis performed using MS Excel 2007.

Source	df	SS	MS	f	Significance *f*			
Regression	1	19.37664	19.37664	12.58376	0.038163			
Residual	3	4.61944	1.539813					
Total	4	23.99608	t stat					
Term	**Coefficients**	**SE**	**-2.53347**	***p*-value**	**Lower 95%**	**Upper 95%**	**Lower 95.0%**	**Upper 95.0%**
Intercept	-10.6752	4.213661	3.547359	0.085165	-24.0849	2.734579	-24.0849	2.734579
X variable 1	4.624585	1.30367		0.038163	0.475726	8.773443	0.475726	8.773443

Df = degrees of freedom; SS = sum of squares; SE = standard error; MS = mean square.

Table 5. Nonlinear regression analysis performed using GraphPad Prism.

[Inhibitor] vs. normalized response – variable slope	
Best-fit values	
IC_{50}	**1.828**
HillSlope	2.140
$\log_{IC50}$	3.262
95% confidence interval (profile likelihood)	
IC_{50}	1.294–2.487
HillSlope	1.159–4.251
$\log_{IC50}$	3.112–3.396
Goodness of fit	
Degrees of Freedom	3
R squared	0.9663
Sum of Squares	179.3
p-value	<0.05

GC–MS analysis of the fermented Poongar rice matrix identified key SCFAs and related bioactive metabolites, including 2-ketobutyric acid, benzoic acid derivatives, and beta-D-glucopyranoside ethyl tetra acetate at notable concentrations. These compounds are closely associated with the probiotic activity of *L. lactis*. SCFAs such as 2-ketobutyric acid play essential roles in reinforcing gut barrier integrity by stimulating tight junction protein expression and enhancing mucus production, thereby preventing pathogenic invasion. Benzoic acid and its derivatives are shown to support gut health by modulating microbiota composition, exerting mild antimicrobial effects, and influencing immune cell responses (Bui et al., 2025). Beta-D-glucopyranoside compounds provide prebiotic-like support by promoting the growth and colonization of beneficial microbes. Collectively, these molecular signatures identified through GC–MS correlate directly with *L. lactis*-mediated immune regulation, stimulation of T-regulatory cells, modulation of cytokine production, and maintenance of gut homeostasis. The presence of these metabolites also implies potential influences on neurotransmitter synthesis, indicating a possible gut-brain axis effect and aligning biochemical output with the observed physiological benefits of probiotic fermentation (Falcinelli et al., 2015).

The EPS content measured in this study (0.506 g per 100 g of fermented matrix) aligns with previous reports demonstrating that *Lactococcus*-derived EPS contributes significantly to colonic health and mucosal immunity. EPS synthesized by lactic acid bacteria has been shown to exert immunomodulatory effects, enhance adherence to the intestinal mucosa, and resist enzymatic degradation, thereby improving digestive persistence (Salazar et al., 2016). Moreover, EPS can function as prebiotic substrate, selectively promoting the growth and metabolic activity of beneficial gut microbiota and therefore improving overall intestinal health (Monteagudo-Mera et al., 2019).

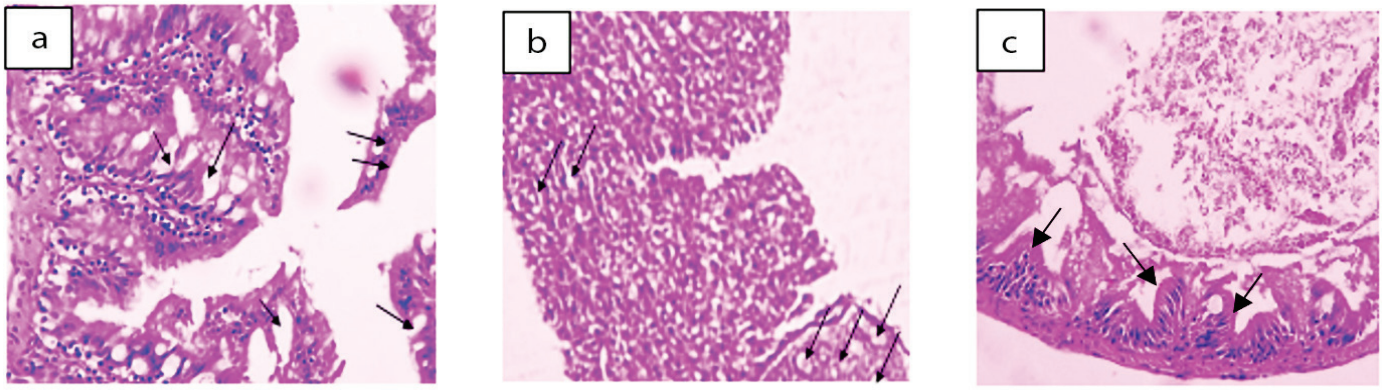

Figure 7. Histological analysis of zebrafish intestinal tissue under different treatment conditions using H&E staining. Normal control with no antibiotic exposure, showing intact villi structure with goblet cells indicated by arrows (a); negative control treated with antibiotic only, showing goblet cell proliferation and disrupted villi morphology (arrows) (b); positive control treated with both antibiotics and fermented rice matrix, showing blood cell accumulation at the villus margins, suggesting the initiation of tissue repair (arrows) (c).

The *in vivo* zebrafish model proved to be an effective platform for evaluating both probiotic safety and therapeutic efficacy. Zebrafish (*Danio rerio*) have emerged as an powerful model for investigating gut microbiota in relation to human diseases such as hypertension and cardiovascular disorders (Brugman, 2016). In the present study, zebrafish exposed to erythromycin exhibited marked behavioral disturbances and histological gastrointestinal damage. However, administration of the fermented Poongar matrix facilitated restoration of intestinal villi architecture, reduction of goblet cell hyperplasia, and normalization of behavior, closely mirroring patterns reported in earlier probiotic rescue studies (Falcinelli et al., 2015).

The safety of the formulation is supported by its low toxicity, with an LC_{50} value as high as 2,138 ppm. Comparable outcomes from oral toxicity studies of conventional lactic acid bacteria validate their Generally Recognized As Safe status and suitability for functional food applications (Sanders et al., 2010). The rice-probiotic formulation maintained gut integrity and did not induce immune stress markers, consistent with literature demonstrating that cereal-based prebiotics and probiotics generally support vertebrate health and physiological tolerance. Statistical analyses comprehensively tested the research hypothesis and revealed significant differences among treatment groups through regression and probit modeling, indicating strong dose-dependent toxicity and robust model fitting (Chakraborty et al., 2022; Preskova et al., 2011). These findings are consistent with prior zebrafish toxicity studies in which probit analysis is recognized for reliable LC_{50} determination and regression analysis confirms exposure-response relationships (Khan et al., 2025). The application of MS Excel 2007 with the Analysis Toolpak enabled systematic data validation and reproducible statistical workflows, aligning with best practices in experimental aquatic toxicology (Ahmed et al., 2015).

Collectively, the findings demonstrate that fermented Poongar rice represents a feasible probiotic formulation, offering a low-cost, culturally relevant alternative to commercial probiotic products. Future integration of advanced omics approaches, including metabolomics and transcriptomics, could substantially enhance mechanistic understanding while facilitating scale-up for clinical translation and nutritional interventions.

Conclusion

The present study highlights the strong probiotic potential of a fermented matrix derived from Poongar rice, an indigenous variety native to the Cauvery Delta region. *L. lactis* was successfully isolated and identified from the fermented rice matrix, and its remarkable tolerance to acidic pH, bile salts, and high salt tolerance supports its suitability as a robust probiotic candidate. In addition to its nutritional value, the matrix was found to produce substantial quantities of bioactive scompounds, including SCFAs and EPSs. Importantly, *in vivo* validation using a zebrafish model confirmed that the formulation is both non-toxic and effective in restoring gut integrity following antibiotic-induced dysbiosis. The histological recovery of the gastrointestinal lining, along with the normalization of behavioral patterns, clearly demonstrates its therapeutic efficacy. Collectively, these findings establish the fermented Poongar rice matrix as a culturally relevant, safe, and functional platform for gut health interventions. Future studies should focus on optimizing the delivery format, conducting comprehensive omics-level investigations, and extending validation to mammalian models and human clinical trials in order to fully realize its potential in the development of customized probiotic or functional food-based therapeutics.

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Ethics

Ethics Committee Approval: Ethical approval for zebrafish maintenance and handling was obtained from the Saveetha Dental College & Hospital Institutional Human Ethical Committee (SDC-IHEC) (reference number: 24/BIOCHEM/049, dated: 28.05.2024).

Data Sharing Statement: All data are available within the study.

Footnotes

Conflict of Interest: The author(s) have no conflicts of interest to declare.

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AI Use Declaration: The authors declare that artificial intelligence (AI) tools were used in the preparation of this manuscript solely for language editing, grammar refinement, and improvement of readability. During the preparation of this work, the author(s) used ChatGPT, Grammarly, and Paperpal for text editing and language correction. After using these tools, the author(s) carefully reviewed and edited the content as required and take(s) full responsibility for the accuracy and integrity of the final publication.

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