RESEARCH PAPER

COMPARATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF PIGMENTED AND CONVENTIONAL RICE VARIETIES BEFORE AND AFTER FERMENTATION: AN IN VITRO STUDY

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Abstract

Introduction: Fermented rice recently gained attention for its symbiotic and probiotic potential, which enhance gut and oral health. Pigmented rice varieties are known to possess antioxidant and antimicrobial properties; limited evidence exists on how fermentation influences their antimicrobial efficacy.

Aim and Objectives: The aim of the study was to compare the antimicrobial activity of pigmented and conventional rice varieties before and after fermentation.

Materials and Methods: Five rice varieties Raw rice (Pachha Arisi), Parboiled rice (Pulungal Arisi), Bridegroom rice (Mapillai Samba), Red rice (Matta) and Black rice (Kavuni Arisi) were analyzed. Rice water samples were collected before and after a 24-hour natural fermentation process and mixed with human saliva containing oral microbiota. Antimicrobial activity was tested against Streptococcus mutans, Fusobacterium nucleatum, Tannerella forsythia and Porphyromonas gingivalis using quantitative real-time PCR. Statistical analyses were performed using one-way ANOVA, post hoc Tukey's test, and paired t-tests, with $p < 0.05$ considered significant.

Results: All rice varieties showed significant enhancement in bacterial inhibition after fermentation ($p < 0.05$). Pigmented rice varieties exhibited stronger antimicrobial activity than conventional types, particularly against S. mutans and P. gingivalis. Mapillai Samba, Red rice, and Black rice achieved complete (100%) inhibition of S. mutans post-fermentation, The two types of rice Parboiled and Raw showed different levels of growth inhibition where Parboiled achieved 90% and Raw achieved 60% inhibition. The fermentation process increased antimicrobial activity of rice but especially improved it in pigmented rice varieties. The fermentation process produces bioactive phenolic compounds and probiotic metabolites which lead to enhanced activity.

Conclusion: The research findings show that fermented pigmented rice functions as a natural dietary supplement which helps people maintain their oral health by restoring their mouth bacterial balance and preventing dental diseases.

Keywords: Fermented rice; Pigmented rice; Antimicrobial activity; *Streptococcus mutans*; *Porphyromonas gingivalis*; Probiotics; Oral microbiome; Phenolic compounds

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1. Introduction

People around the world eat rice which belongs to the rice (Oryza sativa) species as a vital food source.[1] Rice varieties include both white rice and pigmented rice. The consumption of type of rice varies according to geographic location as several factors such as geology, land use, soil type, climatic conditions influence the type of rice production.[2] South Asians predominantly consume white rice. However, in late 2000s, there was a paradigm shift to consumption of pigmented rice varieties mainly due to their noted nutritional properties.[3] Few studies have proved the health benefits of pigmented rice.[3,4] It has been discussed that phenolic compounds and flavonoids present in pigmented rice promotes health by lowering cholesterol levels, blood glucose levels and also initiates anti-oxidant activities.[4]

The role of pigmented rice varieties on anti-microbial activity has been discussed frequently in literature. Thanawat et al., (2021) in their study proved the anti-microbial effect of purple rice which predominantly grows in Thailand.[5] Gemaima C et al., (2025) in their scoping review mentioned about the anti-microbial properties of pigmented rice.[6] Recently,Xiu Qian et al., (2025) in their in-vitro study investigated a darkest rice variety named Bajong (in dark purple colour) and confirmed its anti-microbial activity and nutraceutical effects.[7] Despite the proven effects, it must be understood that not all pigmented rice varieties were assessed for anti-microbial effects. Also, the anti-microbial properties tend to vary based on the geographic location in which the rice has been cultivated.

In recent times, the symbiotic effect of fermented rice is being advocated in scientific literature.[8] Fermentation process of rice promotes digestion by activating gut health microbes.[8] The role of fermented rice on gut health despite known[9,10], there is dearth in literature regarding anti-microbial effects of pigmented rice after undergoing fermentation process. This forms the basis for the present study where anti-microbial effects of different forms of south Indian pigmented rice namely Bridegroom rice (*Mapillai samba*), Black rice (*Kavuni arisi*), Red rice (*Matta*) was compared with conventional rice such as Parboiled rice (*Pulungal Arisi*) and raw rice (*Pachha arisi*) before and after fermentation process against *Streptococcus mutans*, *Fusobacterium nucleatum*, *Tannerella forsythia* and *Porphyromonas gingivalis.*

2. Methodology

2.1 Study Design and Ethical Approval

This *in vitro* experimental study was conducted in a private molecular biology laboratory. Ethical approval was obtained from the Institutional Ethical Committee of Meenakshi Ammal Dental College & Hospital (Approval number: MADC/IEC-I/01/2022). All experimental procedures adhered to institutional ethical standards for biological research and complied with the Declaration of Helsinki.[11]

2.2 Sample Collection and Identification of Rice Varieties

Five rice varieties were selected for the study, including two conventional types - Raw rice (*Pachha Arisi*), Parboiled rice (*Pulungal Arisi*), three pigmented varieties - Bridegroom rice (*Mapillai Samba*), Red rice (*Matta*), and Black rice (*Kavuni Arisi*). The rice samples were procured from a certified agricultural market and the identity of each variety was verified by an agricultural scientist based on morphological characteristics and local classification standards.[3,4]

2.3 Saliva collection

Unstimulated saliva samples were collected from a healthy adult volunteer aged between 20–30 years who was not under antibiotic or probiotic therapy for at least 30 days prior to sampling. The participant provided informed consent before collection. Saliva was collected in the morning (between 8:00–10:00 a.m.) to minimize diurnal variation. The participant was instructed to refrain from eating, drinking, or performing oral hygiene procedures for at least 1 hour before collection. Approximately 5 mL of saliva was collected by passive drooling into a sterile polypropylene container. The collected saliva was immediately transported on ice to the molecular biology laboratory and processed within 30 minutes of collection to preserve microbial viability. A portion of the sample was aliquoted for microbial enrichment, while another aliquot was used for screening the presence of oral pathogens[12] (*Streptococcus mutans*, *Fusobacterium nucleatum*, *Tannerella forsythia*, and *Porphyromonas gingivalis*) using polymerase chain reaction (PCR) prior to inoculation (Table 1- Primers used). All procedures were conducted under aseptic conditions following biosafety level 2 (BSL-2) laboratory guidelines.

2.4 Fermentation Process and Sample Preparation

Each rice sample was soaked in sterile distilled water, and rice water was collected before and after a 24-hour natural fermentation process conducted at room temperature (~28°C) under aseptic conditions. The fermentation period was determined based on previously standardized methods used in probiotic and symbiotic studies.[8,9]

2.5 Enrichment of Oral Bacteria: 100 µL of freshly collected saliva was inoculated into 5 mL of Mueller–Hinton Broth and incubated at 37 °C for 24 hours to allow enrichment of oral microorganisms.

Treatment Groups: After enrichment, samples were divided into three experimental groups:

- Group A (Fermented): Enriched broth + 100 µL fermented rice water
- Group B (Non-Fermented): Enriched broth + 100 µL non-fermented rice water
- Group C (Control): Enriched broth + 100 µL sterile water (negative control)

Incubation Conditions: All groups were incubated at 37 °C for 24 hours. To simulate microaerophilic conditions required for oral pathogens, the Candle Jar Method was employed. In this method, inoculated tubes were placed inside an airtight jar containing a lit candle. The candle was sealed within the jar and allowed to extinguish naturally, creating an atmosphere with approximately 5–10% oxygen and increased CO_2 concentration. The sealed jar was then incubated at 37 °C for 24 hours.[7] All experimental assays were performed in triplicate to ensure reproducibility and minimize analytical error.

2.6 DNA Extraction, PCR Analysis and Metagenomics Sequencing

After incubation of the inoculated samples, DNA extraction was performed following a modified phenol–chloroform method to ensure high-quality nucleic acid yield. Briefly, the microbial suspensions and saliva-derived samples were centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the resulting pellet was resuspended in 200 µL of lysis buffer (100 mM Tris-HCl, 1.0 mM EDTA, 1.0% Triton X-100, pH 7.8). The mixture was placed in a boiling water bath for 10 minutes, cooled to room temperature, and centrifuged again for 5 minutes at 10,000 rpm. The resulting supernatant was collected as the DNA template and stored at –70 °C until further use. For detection of target microorganisms, both 16S rRNA gene amplification and quantitative real-time PCR (qRT-PCR) analyses were performed. Each PCR reaction contained 10 µL of DNA template, 5 µL of 10× PCR buffer, 1.25 U Taq DNA polymerase (0.4 µL), 0.2 mM of each dNTP (1 µL), 1 µL of forward and reverse primers specific for each microorganism (Table 4), and 31.6 µL of nuclease-free water, making a total volume of 50 µL. PCR was carried out in a thermocycler (Applied Biosystems, USA) with the following temperature profile:

- Initial denaturation at 95 °C for 2 min,
- 36 cycles of: denaturation at 95 °C for 30 s, annealing at 60 °C for 1 min, extension at 72 °C for 1 min,
- Final extension at 72 °C for 2 min.

Amplified products (10 µL aliquots) were electrophoresed on 0.75% agarose gels containing 0.5 µg/mL ethidium bromide in 1× TAE buffer. Gels were visualized under UV illumination (300 nm) and photographed. A 100 bp DNA ladder (Bangalore Genei Pvt. Ltd.) served as the molecular weight marker. Selected PCR products were sequenced using an automated sequencer (Genetic Analyzer 3130; Applied Biosystems, USA), and the sequences were compared with GenBank database entries using BLAST to confirm species identification. Quantitative analysis of bacterial load was performed using a SYBR Green RT-PCR Kit (Lot No.: 1201416; Applied Biosystems, UK) on an ABI 7500 RT-PCR system (Applied Biosystems, USA). PCR amplification parameters were as follows: initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, and elongation at 60 °C for 30 seconds. Each reaction was run in duplicate, and average cycle threshold (Ct) values were used for analysis. A ubiquitous primer set served as a positive control to ensure successful amplification, and sterile water was included as a negative control. The method followed was adapted from Forssten et al. (2010) and Bhaskar et al. (2023).[10,13]

Fermented rice water sample of Mapillai Samba Rice (MP-1) and Black Rice (BR-2) were analyzed for Shotgun Metagenomics Sequencing. Fermented Sample of MP-1 and BR-2 DNA were extracted using Xploregen kit. PCR Amplification of 16s region were used by 16sF: - 5' CCTACGGGNGGCWGCAG3'and 16sR: - 5' GACTACHVGGGTATCTAATCC3'. 40ng of Extracted DNA is used for amplification along with 10pM of each primer. Initial Denaturation - 95 degree C, 25 Cycles of the following condition, Denaturation @ 95 degree Celsius for 15 sec, Annealing @ 60 degree Celsius for 15 sec, Elongation @ 72 degree Celsius for 2 mins Final Extension at 72 degrees Celsius for 10 mins and hold at 4 degrees Celsius**.** The amplified 16s PCR Product is purified and subjected to GEL Check and Nanodrop QC. The Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 is used to determine the DNA's quality. The amplicons from each sample were purified with Ampure beads to remove unused primers and an additional 8 cycles of PCR was performed using Illumina barcoded adapters to prepare the sequencing libraries. Libraries were purified using Ampure beads and quantitated using Qubit dsDNA High Sensitivity assay kit. Sequencing was performed using Illumina Miseq with 2x300PE v3-v4 sequencing kit. Bioinformatics Protocol includes the bcl data received from the sequencer is de-multiplexed into fastq raw data. The de-multiplexed data quality will be checked using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools. The QC passed samples are qualified for further analysis and we use our own pipeline for metagenomics (Biokart Pipeline) for 16s metagenomic. The workflow of the pipeline is as follows. Once the run is over we get the final raw OTU table from which we start visualization of the analysis.

2.7 Assessment of Antimicrobial Activity

The antimicrobial activity of each rice sample was evaluated against four oral pathogenic bacteria: *Streptococcus mutans*, *Fusobacterium nucleatum*, *Tannerella forsythia*, and *Porphyromonas gingivalis*.[14,15] The inhibition of bacterial growth was determined before and after the fermentation process to assess the enhancement of antimicrobial properties due to fermentation.

2.8 Statistical Analysis

All numerical data were entered into Microsoft Excel (Microsoft Corp., USA) and analyzed using the Statistical Package for the Social Sciences (SPSS). Experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to compare bacterial inhibition among the five rice groups, followed by post hoc Tukey's multiple comparison test where applicable. Paired *t*-tests were performed to assess differences before and after fermentation. A *p*-value < 0.05 was considered statistically significant.[16]

3. Results and Discussion

The 16S rRNA amplicon sequencing of fermented black rice water (BR-2) and Mapillai Samba fermented rice water (MP-1) generated approximately 0.115 million and 0.1 million high-quality reads with GC contents of 55.0% and 53.5%, respectively, indicating acceptable sequencing quality for downstream taxonomic profiling . Despite similar sequencing depth, the two samples exhibited markedly different microbial community structures, reflecting distinct fermentation dynamics influenced by substrate composition and environmental conditions.

The BR-2 sample demonstrated an extremely low-diversity microbial ecosystem dominated almost entirely by *Paenibacillus* spp. (91.35%), with a secondary contribution from *Heyndrickxia coagulans* (6.76%). All other taxa were present at negligible levels (<1%). This pattern indicates a highly selective fermentation environment in which spore-forming, enzyme-producing bacteria outcompete other microbial groups. Members of *Paenibacillus* are well documented for their ability to produce extracellular enzymes such as amylases and proteases, enabling efficient degradation of complex plant polysaccharides and proteins (Grady et al., 2016; Lal & Tabacchioni, 2009). The dominance of this genus strongly suggests that fermentation in black rice water is primarily driven by enzymatic hydrolysis rather than classical lactic acid fermentation. The limited presence of lactic acid bacteria, including *Lacticaseibacillus* and *Bifidobacterium* (<1%), further supports the conclusion that acidification plays a minimal role in shaping the microbial ecosystem of BR-2. The detection of minor levels of environmental and opportunistic bacteria such as *Aeromonas*, *Pseudomonas*, and *Serratia* (<0.5% combined) indicates low contamination without evidence of pathogenic dominance (Table - 5).

In contrast, the MP-1 sample exhibited a more diverse and functionally complex microbial community dominated by anaerobic, gut-associated, and short-chain fatty acid (SCFA)-producing bacteria. The most abundant taxa included *Segatella hominis* (20.78%), *Roseburia* spp. (18.75%), *Bifidobacterium* spp. (7.61%), *Segatella copri* (5.22%), and *Faecalibacterium longum* (4.69%), along with several other SCFA-producing and carbohydrate-fermenting organisms such as *Blautia*, *Anaerostipes hadrus*, and *Collinsella aerofaciens*. These taxa are typically associated with the human gut microbiome and are known for their roles in complex carbohydrate degradation and production of metabolites such as butyrate and propionate, which are important for intestinal health. The MP-1 fermentation system supports metabolic processes which produce short-chain fatty acids because it contains multiple butyrate-producing genera such as *Roseburia* and *Faecalibacterium* and *Anaerostipes*. Actual metabolomic data and functional gene data need to be analyzed through functional analysis which depends on established taxonomic features of the studied organisms. The MP-1 sample showed moderate levels of recognized probiotic genera which included Bifidobacterium longum Bifidobacterium breve and *Ligilactobacillus ruminis* but differed from BR-2 results. The presence of lactic acid bacteria at non-dominant levels shows that fermentation in MP-1 remains unaffected by acidification. The microbial community shows a mixed fermentation system which produces SCFA through carbohydrate fermentation. The fermentation process detects *Akkermansia muciniphila* and *Faecalibacterium* spp. as obligate anaerobes which do not normally occur during food fermentation. Scientists can investigate whether environmental microbes entered through handling. The active role of organisms in fermentation activities remains unknown because 16S rRNA sequencing in BR-2 does not differentiate between living and dead organisms. The MP-1 sample contained only a small number of dangerous bacteria which can also act as opportunistic pathogens. (0.45%) and *Vibrio* spp. (0.01%), although their overall abundance remained below 1%. The study found minimal pollution in the environment but detected waterborne bacteria which proved that environmental elements such as water conditions and sanitation practices affect the microbial composition in the study area. The researchers cannot determine microbiological safety because they lack results from virulence and toxin gene testing (Table – 6).

The two sample sets demonstrate their basic differences through their different fermentation ecology patterns. The BR-2 system which operates through enzyme-based processes exhibits low biological diversity because *Paenibacillus* bacteria control all fermentation activities which lead to macromolecule breakdown and this process constitutes their primary metabolic activity. The MP-1 system shows a microbial community that produces short-chain fatty acids and includes gut bacteria which break down carbohydrates thus establishing a more intricate biological process. Black rice and Mapillai Samba rice varieties create their distinct characteristics through their different biochemical compositions which include varying starch structures and phenolic compound contents and fiber content and their different fermentation parameters which include oxygen exposure and the selection of microbial inoculum sources.

The results show that fermented rice water systems develop unique microbial communities which depend on their specific substrate and environmental conditions as they produce either enzyme-based communities or diverse metabolic groups. While both systems contain bacteria with potential functional and probiotic relevance, these properties remain inferential and require further validation through targeted metabolomic, enzymatic, and in vivo studies.

When percentage inhibition of all five varieties was compared against the oral pathogens such as *Streptococcus mutans*, *Fusobacterium nucleatum*, *Tannerella forsythia*, and *Porphyromonas gingivalis* before fermentation ('0' hr), all the rice varieties showed inhibition percentage which was statistically significant against all microorganisms ($p < 0.05$). However, inhibition percentage was higher for *Streptococcus mutans* compared to other microorganisms ranging from 10 ± 3.6% in Mapillai samba to 70 ±0% in parboiled rice (Table 2). A significant percentage of inhibition was seen among all the rice varieties against all studied microorganism at '0' hr. (Table 2). The results are consistent with the study conducted by Anong Somsap et al in which it was found that non fermented rice water have small number of flavonoids and phenolic compounds.[20] After '24' hrs of fermentation where all rice varieties (study variable) were compared for inhibition percentage against all the studied microorganisms, they were found to be statistically significant ($p < 0.05$). However complete inhibition of *Streptococcus mutans was observed* in Mapillai Samba, red rice, and black rice. Parboiled and raw rice showed 90 ± 2.51% and 60 ± 13.11% inhibition, respectively (Table 3). On fermentation we observe that all the rice varieties significant inhibited all the microorganism under investigation. (Table 3). The marked increase in inhibition following fermentation may result from the breakdown of complex phenolic compounds into simpler, more bioavailable forms, thereby enhancing antimicrobial potency.[10] This aligns with findings by Thanawat et al. (2021), who demonstrated strong antibacterial effects in Thai purple rice varieties,[5] and Xiu Qian et al. (2025), who confirmed antimicrobial activity in the deep-purple Bajong rice.[7] Wijayanti et al. (2025) observed morphological disruption and growth inhibition in *E. coli*, *Candida albicans*, and *S. aureus* treated with fermented black rice extract.[19] When all the rice varieties were compared with the inhibition percentage of the studied microorganism between the two time points ('0' hr and '24' hr) they were found to be statistically significant ($p < 0.05$). However, no statistical significance was found for black rice against *Tannerella forsythia* (Table 4). Moon and Chang (2021) reported that fermentation of rice bran using *Lactiplantibacillus plantarum* enhanced both sensory and antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli*.[18] These results are consistent with earlier findings showing that fermentation enhances the bioactivity of cereal and rice-based substrates. [8],[9] Mapillai Samba, red rice, and black rice exhibited superior antibacterial activity both before and after fermentation compared to white rice varieties. This can be attributed to their higher concentrations of polyphenols, anthocyanins, and flavonoids, which are retained in the bran layer of minimally processed rice. These phytochemicals have been shown to disrupt bacterial cell membranes, interfere with quorum sensing, and inhibit biofilm formation. [17] The present study adds to this evidence by showing that locally cultivated pigmented rice varieties exhibit similar behavior and that fermentation further amplifies their antimicrobial effects. When all the rice varieties were compared from '0' to '24' hr against the studied oral pathogens, we found all rice varieties were able to show good percentage of inhibition. (Table 4). However, Mapillai samba, red rice and black rice showed higher inhibition, specifically for *Streptococcus mutans.* All rice varieties demonstrated measurable antimicrobial activity against *S. mutans*, *Fusobacterium nucleatum*, *T. forsythia*, and *Porphyromonas gingivalis*. However, the magnitude of inhibition varied significantly among varieties ($p < 0.001$). Pigmented rice varieties consistently displayed greater inhibitory activity than non-pigmented types, particularly after fermentation. Fermented rice naturally harbors beneficial microorganisms, including *Lactobacillus* species, which are known to produce bacteriocins capable of inhibiting cariogenic and periodontopathogenic bacteria.[13],[18],[19] The synergistic action of these bacteriocins, along with increased levels of phenolic compounds after fermentation, likely accounts for the observed antimicrobial enhancement.

Inhibition of *P. gingivalis* by fermented Mapillai Samba, red rice, and black rice may result from disruption of bacterial membranes and suppression of proteolytic enzymes such as gingipains, which play a key role in periodontal tissue destruction. [14] The dual effect of phenolic compounds and microbial metabolites generated during fermentation thus provides a plausible explanation for the pronounced inhibition observed. Differences in bacterial response to rice extracts were evident. While red rice showed strong inhibition against both *F. nucleatum* and *T. forsythia*, black rice demonstrated reduced activity against *T. forsythia*, with no statistically significant change after fermentation (p = 1.0). Such variability likely reflects differences in bacterial susceptibility to rice-derived phenolics and fermentation by-products.[15] This study was conducted under *in vitro* conditions, and therefore, the findings may not fully represent the complex interactions occurring within the oral cavity or the human body. The analysis was also limited to four major oral pathogens. Future studies should explore a broader spectrum of microorganisms and include *in vivo* models or clinical trials to validate these effects in real-world conditions. Moreover, the identification and quantification of specific antimicrobial compounds formed during fermentation would help elucidate the underlying biochemical mechanisms. Together, these results confirm that fermentation enhances the antimicrobial efficacy of rice, particularly pigmented varieties, likely through increased bioactive compound availability and generation of new antimicrobial metabolites. The bacterial strains tested *S. mutans*, *P. gingivalis*, *F. nucleatum*, and *T. forsythia*—are key contributors to dental caries and periodontal diseases. The strong inhibitory effects observed, particularly against *S. mutans* and *P. gingivalis*, indicate potential oral health benefits of fermented pigmented rice.

4. Conclusion

This *in vitro* study demonstrated that fermentation significantly enhances the antimicrobial properties of rice, particularly in pigmented varieties. All tested rice types exhibited increased bacterial inhibition following fermentation, with *Mapillai Samba*, red rice, and black rice showing the highest activity. The pigmented varieties achieved complete inhibition against Streptococcus mutans and Porphyromonas gingivalis which demonstrates their ability to provide oral health advantages. The two factors which produce increased antimicrobial effects include two factors which produce improved bioavailability of phenolic compounds and the creation of probiotic metabolites during fermentation. The test results demonstrate that fermented pigmented rice provides better biofunctional characteristics than standard white rice. Fermented pigmented rice functions as a natural dietary supplement that helps control oral bacteria which cause cavities and gum disease. The research requires future studies to use in vivo models and clinical trials which will validate the results and investigate the molecular mechanisms that control its antibacterial properties.

Table 1. Primer Set Used for PCR

S.No	Microorganisms	Primers
1	*Streptococcus mutans*	F – ACTACACTTTCGGGTGGCTTGG R – CAGTATAAGCGCCAGTTTCATC
2	*Fusobacterium nucleatum*	F – GATCCAGCAATTCTGTGTGC R – CTTGTAGTTCCGCYTACCTC
3	*Tannerella forsythia*	F - GCG TAT GTA ACC TGC CCG CA R -TGC TTC AGT GTC AGT TAT ACC
4	*Porphyromonas gingivalis*	F - AGG CAG CTT GCC ATA CTG C R - ACT GTT AGC AAC TAC CGA TGT

Table 2. Comparison of percentage inhibition produced by five different non-fermented rice varieties against four oral pathogens

Rice Variety	*S. mutans* (Mean ± SD)	*F. nucleatum* (Mean ± SD)	*T. forsythia* (Mean ± SD)	*P. gingivalis* (Mean ± SD)	p-value

Parboiled rice	70 ± 0.00 %	20 ± 0.00 %	43 ± 1.52 %	39 ± 3.51 %	< 0.001*
Raw rice	40 ± 8.54 %	10 ± 1.15 %	51 ± 1.52 %	50 ± 2.00 %	< 0.001*
Mapillai Samba	10 ± 3.60 %	40 ± 2.51 %	50 ± 1.15 %	67 ± 2.51 %	< 0.001*
Red rice	51 ± 0.00 %	30 ± 3.05 %	50 ± 1.52 %	60 ± 1.15 %	< 0.001*
Black rice	50 ± 5.29 %	15 ± 6.80 %	59 ± 1.15 %	55 ± 2.00 %	< 0.001*

Note: One-way ANOVA; *p* < 0.05 considered statistically significant.

Table 3. Comparison of percentage inhibition produced by five different fermented rice varieties against four oral pathogens

Rice Variety	***S. mutans* (Mean ± SD)**	***F. nucleatum* (Mean ± SD)**	***T. forsythia* (Mean ± SD)**	***P. gingivalis* (Mean ± SD)**	**p-value**
Parboiled rice	90 ± 2.51 %	30 ± 3.60 %	73 ± 2.51 %	81 ± 2.51 %	< 0.001*
Raw rice	60 ± 13.11 %	20 ± 7.23 %	76 ± 2.00 %	79 ± 1.15 %	< 0.001*
Mapillai Samba	100 ± 0.00 %	50 ± 14.18 %	89 ± 2.00 %	98 ± 1.75 %	< 0.001*
Red rice	100 ± 0.00 %	60 ± 19.28 %	90 ± 2.00 %	95 ± 2.08 %	< 0.001*
Black rice	100 ± 0.00 %	40 ± 2.51 %	41 ± 0.57 %	97 ± 2.51 %	< 0.001*

Note: One-way ANOVA; *p* < 0.05 considered statistically significant.

Table 4. Comparison of study variables with the various microorganisms between the two time points (0 & 24hrs)

Rice Variety	***S. mutans* MD / *p*-value**	***F. nucleatum* MD / *p*-value**	***T. forsythia* MD / *p*-value**	***P. gingivalis* MD / *p*-value**
Parboiled rice	20 / < 0.001*	10 / 0.01*	30 / < 0.001*	42 / < 0.001*
Raw rice	20 / < 0.001*	10 / 0.01*	25 / < 0.001*	29 / < 0.001*

Mapillai Samba	25 / < 0.001*	10 / 0.01*	39 / < 0.001*	31 / < 0.001*
Red rice	20 / < 0.001*	30 / < 0.001*	31 / 0.001*	35 / < 0.001*
Black rice	30 / < 0.001*	25 / < 0.001*	1 / 1.00 (NS)	42 / < 0.001*

Note: MD = Mean difference between pre- and post-fermentation; Paired *t*-test; $p < 0.05$ considered statistically significant; NS = Not significant.

Table 5. Metagenomic Analysis of Black Rice Fermented Water (BR-2)

Category (Probiotic / Non-Probiotic)	**Bacterial Genus / Species**	**Relative Abundance (%)**	**Functional Significance**	**Role in Food / Host System**
Probiotic / Beneficial Bacteria	*Paenibacillus sp.*	91.35	Enzyme-producing, spore-forming bacterium with probiotic potential	Enhances fermentation; produces amylases and proteases; some strains promote gut health
	Heyndrickxia coagulans (*Bacillus coagulans*)	6.76	Spore-forming lactic acid bacterium	Survives gastric acid; produces lactic acid; improves digestion and immune modulation
	Segatella copri	0.35	Commensal intestinal anaerobe	Aids carbohydrate metabolism; supports gut microbiome diversity
	Cetobacterium somerae	0.25	Anaerobic vitamin B12 producer	Supports nutrient synthesis; common in fermented foods
	Lacticaseibacillus sp.	0.18	Lactic acid bacterium	Produces lactic acid; enhances flavor; inhibits pathogens
	Ligilactobacillus ruminis	0.12	Heterofermentative LAB	Produces bacteriocins; supports gut homeostasis
	Bifidobacterium sp.	0.08	Key probiotic gut bacterium	Produces acetic and lactic acids; improves lactose digestion; enhances immunity
	Blautia sp.	0.07	Anaerobic SCFA producer	Maintains colonic health; exhibits anti-inflammatory effects

	Faecalibacterium sp.	0.05	Butyrate-producing commensal	Anti-inflammatory; maintains mucosal barrier integrity
	Roseburia sp.	0.04	SCFA-producing commensal	Regulates energy metabolism; supports colon health
	Collinsella aerofaciens	0.03	Gut commensal fermenter	Ferments carbohydrates; aids nutrient absorption
	Laceyella sp.	0.02	Thermophilic spore former	Contributes enzymes that enhance fermentation
Non-Probiotic / Potentially Harmful Bacteria	*Acinetobacter sp.*	0.11	Opportunistic environmental bacterium	May cause spoilage and nosocomial infections
	Aeromonas sp.	0.08	Waterborne pathogen	Causes gastroenteritis; indicates water contamination
	Pseudomonas sp.	0.07	Spoilage organism	Produces proteases and lipases; reduces shelf life
	Serratia sp.	0.05	Opportunistic pathogen	Associated with infections and pigment production
	Stenotrophomonas sp.	0.05	Multidrug-resistant opportunist	Causes infections in immunocompromised individuals
	Plesiomonas shigelloides	0.04	Enteric pathogen	Causes diarrhea; found in contaminated water
	Ralstonia insidiosa	0.03	Biofilm-forming contaminant	Water system contaminant; no probiotic role
	Desulfovibrio desulfuricans	0.02	Sulfate-reducing anaerobe	Produces hydrogen sulfide; contributes to odor and spoilage
	Nocardioides sp.	0.02	Soil bacterium	Environmental origin; non-pathogenic but non-probiotic
	Sphingomonas sp.	0.01	Environmental bacterium	Occasional contaminant; no functional benefit

Table 6. Metagenomic Analysis of Mapillai Samba Fermented Rice Water (MP-1)

Category (Probiotic / Non-Probiotic)	**Bacterial Genus / Species**	**Relative Abundance (%)**	**Functional Significance**	**Role in Food / Host System**
Probiotic / Beneficial Bacteria	*Segatella hominis*	20.78	Gut commensal with fermentative and SCFA-producing potential	Supports gut health and carbohydrate metabolism
	Roseburia sp.	18.75	Butyrate-producing anaerobe	Anti-inflammatory; improves gut barrier function
	Bifidobacterium sp.	7.61	Key probiotic genus	Enhances lactose digestion, immunity, and intestinal balance
	Segatella copri	5.22	Fiber-fermenting gut microbe	Contributes to carbohydrate breakdown and SCFA production
	Faecalibacterium longum	4.69	Butyrate-producing commensal	Reduces gut inflammation; supports mucosal integrity
	Bifidobacterium longum	4.65	Well-known probiotic strain	Regulates bowel function; enhances immunity
	Segatella sp.	4.62	Commensal anaerobe	Aids in digestion and SCFA formation
	Hallella multisaccharivorax	4.13	Carbohydrate-degrading bacterium	Participates in polysaccharide breakdown during fermentation
	Faecalibacterium sp.	3.15	Anaerobic probiotic	Produces butyrate; maintains intestinal health
	Ligilactobacillus ruminis	1.60	Lactic acid bacterium	Produces bacteriocins and lactic acid; enhances gut microflora
	Bifidobacterium breve	0.83	Gut probiotic	Improves digestion and modulates immune response
	Collinsella aerofaciens	0.83	Gut commensal	Involved in carbohydrate fermentation and bile acid metabolism

	Cetobacterium somerae	0.70	Anaerobic vitamin B12 producer	Supports nutrient synthesis and fermentation
	Blautia sp.	0.62	SCFA-producing commensal	Helps maintain colonic health and pH balance
	Anaerostipes hadrus	0.60	Butyrate producer	Contributes to gut anti-inflammatory activity
	Paenibacillus sp.	0.59	Enzyme-producing fermenter	Produces amylase and protease; enhances fermentation process
	Butyrivibrio crossotus	0.24	Butyrate-producing Firmicute	Involved in fiber digestion and energy metabolism
	Akkermansia muciniphila	0.29	Mucin-degrading probiotic	Improves gut barrier and metabolic balance
	Heyndrickxia coagulans (*Bacillus coagulans*)	0.02	Spore-forming lactic acid bacterium	Improves digestion and resists gastric conditions
Non-Probiotic / Potentially Harmful Bacteria	*Aeromonas sp.*	0.45	Waterborne pathogen	Causes gastroenteritis and foodborne infections
	Acinetobacter sp.	0.05	Opportunistic contaminant	Associated with spoilage or clinical infections
	Ralstonia insidiosa	0.03	Biofilm-forming bacterium	Environmental contaminant; non-probiotic
	Stenotrophomonas maltophilia	0.03	Opportunistic pathogen	Multidrug-resistant; causes infection in immunocompromised individuals
	Desulfovibrio desulfuricans	0.01	Sulfate-reducing anaerobe	Produces hydrogen sulfide; causes odor and spoilage
	Pseudaeromonas sharmana	0.05	Opportunistic water bacterium	Can cause spoilage; rarely pathogenic
	Shewanella xiamenensis	0.01	Marine-associated opportunist	Linked to spoilage and rare infections
	Vibrio sp.	0.01	Pathogenic marine bacterium	Causes gastroenteritis and foodborne illness

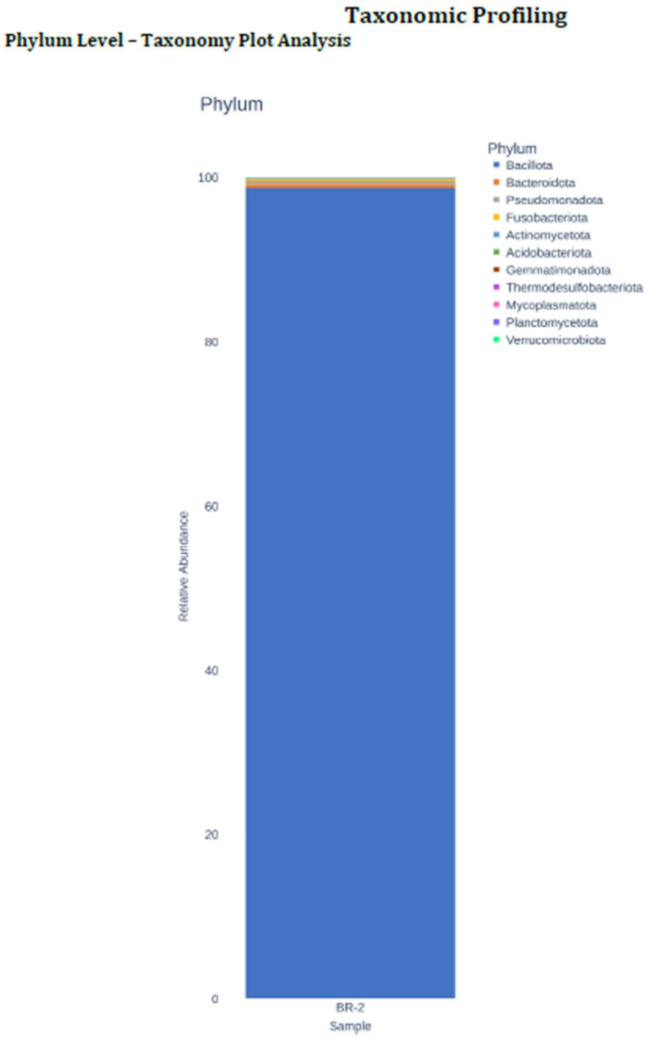

Figure 1: Phylum Level – Taxonomy Analysis of BR-2 sample

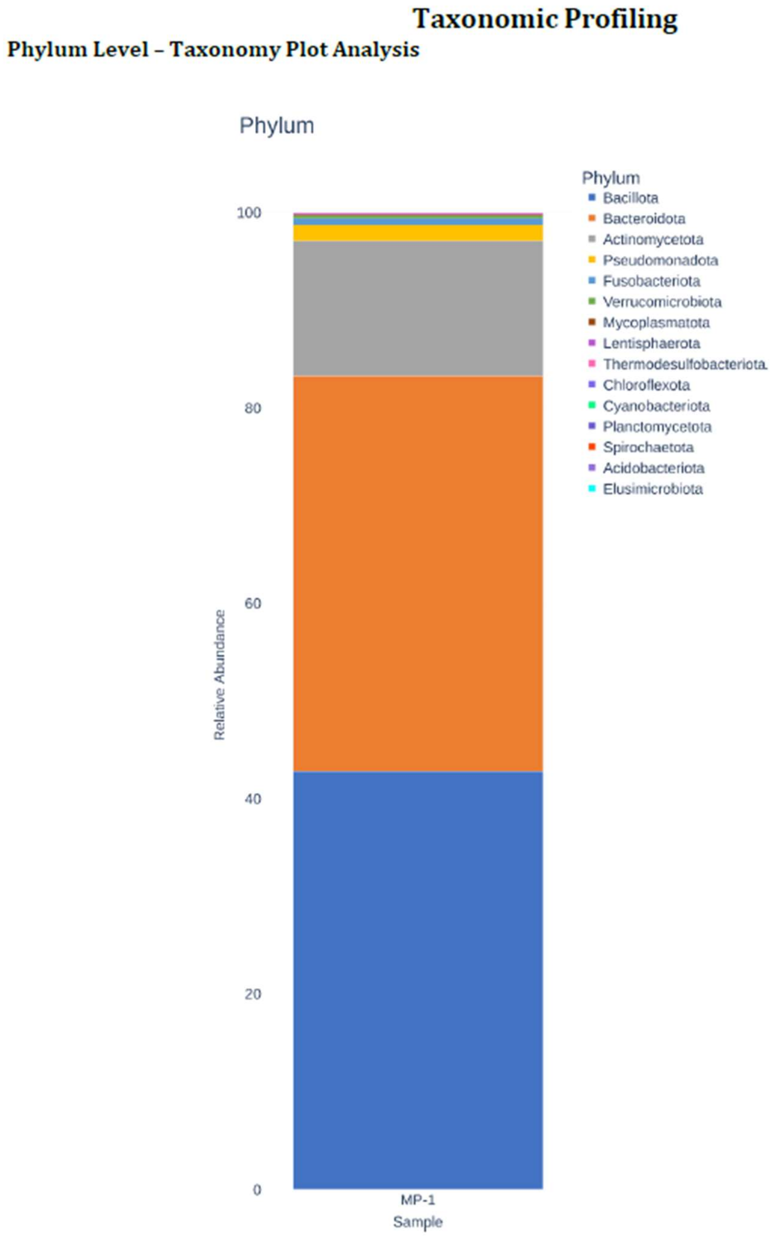

Figure 2: Phylum Level – Taxonomy Analysis of MP-1 sample

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Informed Consent: Not applicable, since it is an in-vitro study

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