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Selenium-enriched *Bacillus siamensis* and *Staphylococcus arlettae* isolated from fermented rice

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ABSTRACT

Selenium-enriched probiotics offer a promising strategy to combat oxidative stress and enhance gut health due to their antioxidant and immune-modulating properties. However, limited studies have explored selenium biofortification in non-conventional strains isolated from traditional Indian fermented foods. To address the research gap, this study aimed to isolate and characterize selenium-enriched probiotic strains from koozh, a naturally fermented rice-based product widely consumed in southern India. Bacterial strains were isolated from three fermented rice samples, enriched with selenium, and identified using 16S ribosomal RNA gene sequencing. Selenium uptake was quantified through atomic absorption spectroscopy, and probiotic potential was assessed based on tolerance to acidic pH, bile salts, and carbohydrate fermentation capacity. Among 20 isolates screened, *Bacillus siamensis* and *Staphylococcus arlettae* exhibited strong survival under gastrointestinal conditions and selenium-enrichment ability. Notably, *S. arlettae* showed the highest selenium uptake efficiency, marking it as a strong candidate for functional food development. These findings highlight the potential of selenium-enriched probiotics derived from traditional fermented foods as novel bioactive ingredients in fortification strategies aimed at promoting gut health and antioxidant defense.

1. INTRODUCTION

Selenium is an essential trace element that plays a crucial role in various biological functions in both humans and animals. Though required in small amounts, it is indispensable for maintaining overall health and preventing disease. Selenium is incorporated into selenoproteins, vital for antioxidant defense, regulation of redox processes, and metabolism of thyroid hormones. These functions underscore the importance of selenium in biological systems and highlight its significance as a micronutrient essential for optimal health [1,2]. Its biological significance is highlighted through its role in crucial enzymes like glutathione peroxidase, which shields cells from oxidative harm by lowering hydrogen peroxide and organic hydroperoxides. Furthermore, selenium plays a pivotal role in thioredoxin reductase, aiding in the preservation of the cell's reducing environment and participating in DNA synthesis and repair processes [3,4]. Selenium deficiency poses significant health risks, including the development of Keshan disease, a cardiomyopathy prevalent in regions low in selenium, and Kashin-Beck disease, characterized by osteoarthropathy that affects bone and joints. These conditions underscore the critical role of selenium in maintaining overall health and preventing specific diseases associated with its deficiency [5]. However, selenium's role as an antioxidant has spurred investigations into its potential to mitigate the risk of chronic conditions such as cancer, cardiovascular disease, and thyroid dysfunction [6]. Selenoproteins, including glutathione peroxidase and thioredoxin reductase, play crucial roles in cellular antioxidant defense, safeguarding cells from oxidative stress and ensuring cellular balance [7]. These enzymes are involved in detoxification of reactive oxygen species and modulation of thyroid hormone metabolism, indicating selenium's role in metabolic regulation [1].

Selenium-enriched probiotics can thus bolster body's antioxidant defense, reducing oxidative stress and its associated risks, such as chronic inflammation and cancer. The synergy of selenium and probiotics can therefore provide a robust boost to the immune system, potentially lowering the risk of infections and autoimmune diseases [8]. Probiotics are extensively used to promote gastrointestinal health by balancing the gut microbiota, preventing the growth of harmful bacteria, and enhancing the gut barrier function. Selenium-enriched probiotics can further support gastrointestinal health by reducing inflammation and oxidative damage in the gut, thereby improving overall gut function and preventing gastrointestinal diseases [9]. The combination of selenium and probiotics can thus provide synergistic benefits, enhancing immune function more effectively than either component alone. Selenium-enriched probiotics have been shown to improve the immune response in both animal models and human

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studies, indicating their potential in boosting immune health and reducing infection risk [10].

Spore-forming probiotics, particularly from *Bacillus*, are commonly isolated from fermented cereals. Studies in isolated *Bacillus subtilis* and *Bacillus coagulants* from various fermented foods have noted their high resilience and ability to survive harsh conditions such as those in the gastrointestinal tract. These strains are identified through advanced techniques such as high-throughput sequencing and metagenomics, which help in understanding their composition and functional potential [11,12]. Isolated strains from fermented cereals exhibit various probiotic properties, including antimicrobial activity, the ability to modulate the gut microbiota, and immune-enhancing effects. Recent research evidences prove that *Bacillus* strains isolated from fermented cereals have shown potential in improving gut health and protecting against gastrointestinal pathogens [13]. In India, the consumption of naturally fermented foods varies across regions and communities, reflecting diverse cultural practices. Fermentation, a traditional global practice, enhances taste and nutritional value, offers health benefits, and extends food shelf life by acting as a natural preservative [14-16]. Koozh is a delicious, naturally fermented cereal-based beverage consumed regularly in the rural areas of Tamil Nadu, India. It is a significant staple meal and offers nourishment and energy to millions of people in India with low and medium incomes. It is made by cooking presoaked millet or rice, fermenting it overnight, and then blending with or without curd for breakfast [14]. Research indicates that selenium-enriched probiotics can improve metabolic health by modulating lipid metabolism and enhancing insulin sensitivity. This is particularly beneficial for conditions such as non-alcoholic fatty liver disease and obesity induced by high-fat diets [17,18].

Despite their potential, there are challenges in the consistent isolation and cultivation of these probiotics. Factors such as the medium composition, sporulation conditions, and recovery techniques are critical for optimizing the yield and viability of spore-forming probiotics. Future research is directed toward optimizing these conditions and exploring the synergistic effects of different probiotic strains in fermented cereal matrices [19]. Selenium-enriched bacterial strains can biotransform inorganic selenium into organic selenium. Thus, it has been demonstrated that selenium-enriched strains can meet the recommended dietary allowance of populations, prevent diseases, and improve immune function and gastrointestinal health [20].

The bioavailability of selenium (Se) is affected by its chemical form and the microbial carrier. Probiotic bacteria, including *Lactobacillus* and *Bifidobacterium*, have been identified to transform inorganic selenium (e.g., selenite) into organic forms such as selenomethionine and selenium-enriched proteins, which exhibit greater bioavailability and reduced toxicity to humans [21]. Furthermore, selenium-enriched probiotics provide dual benefits by supplying essential trace elements and exhibiting probiotic properties. A comparative study revealed that various bacterial species exhibit considerable differences in their capacity to accumulate and convert selenium into bioavailable forms, highlighting the significance of strain selection in selenium enrichment strategies [22]. Recent investigations indicate that selenium-enriched probiotics maintain their functional properties throughout the processing and storage in food applications. A study demonstrated that selenium-enriched Lactobacillus plantarum integrated into yogurt preserved selenium stability and probiotic viability for 21 days under cold storage, suggesting its applicability in commercial food products [23].

While extensive research exists on the bioaccumulation and biotransformation capabilities of selenium-enriched species, studies focusing on the probiotic potential of selenium-enriched strains isolated from fermented South Indian rice samples remain limited. This study aims to isolate probiotic strains from various traditionally fermented rice samples. The objectives include optimizing isolation techniques to enhance selenium uptake by probiotics, characterizing the isolated strains for their selenium content, evaluating their probiotic potential, and exploring their application in developing selenium-enriched functional foods. This study is one of the few recent investigations into the possibility of selenium-enriched probiotics to enhance immune function. It highlights a promising approach in improving gut health and combating oxidative stress by isolating and optimizing bacterial strains from fermented cereal-based products.

2. MATERIALS AND METHODS

The study focused on preparing koozh using two different cereals: Bamboo Rice and Karuppu Kavuni Rice. The methodology involved the following: 500 g of each grain was weighed, thoroughly rinsed with deionized water, and soaked in deionized water for 12 h at room temperature. After soaking, the grains were cooked in boiling water for 30 min and then drained and allowed to cool to room temperature. The cooled grains were transferred to containers covered with breathable fabric to facilitate natural fermentation. The samples were left to ferment at ambient temperature (25–30°C) for 5–7 days, during which signs of fermentation–such as bubbling and development of a sour odor–were monitored.

The mixture was homogenized using a stomacher or blender for 2 min to release the microorganisms. Serial dilutions of the homogenized sample were performed in sterile saline solution (e.g., 10^{-1}–10^{-6}). A known volume of 0.1 mL of each dilution was plated onto de Man, Rogosa, and Sharpe (MRS) agar plates to isolate lactic acid bacteria, and the plates were incubated anaerobically at 37°C for 48–72 h. The plates were then examined for distinct colonies exhibiting probiotic characteristics such as smooth, white, or creamy colonies. Representative colonies were selected and streaked onto fresh MRS agar plates to obtain pure cultures, which were incubated anaerobically at 37°C for 24–48 h [24].

2.1. Strain Identification by 16S ribosomal RNA (16S rRNA) Sequencing

Genomic DNA was isolated using the Qiagen DNeasy Blood and Tissue Kit, according to the manufacturer's protocol, with slight modifications to enhance bacterial DNA yield. The quality and quantity of the DNA were assessed using a Nanodrop spectrophotometer. The V3–V4 region of the 16S rRNA gene, commonly used for identifying lactic acid bacteria, was amplified with the primer pair 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). Polymerase chain reaction (PCR) amplification was carried out in a 25 µL reaction mixture, consisting of 12.5 µL of 2× KAPA HiFi HotStart ReadyMix, 0.2 µM of each primer, and approximately 10 ng of template DNA. The thermal cycling conditions were as follows: initial denaturation at 95°C for 3 min, 25 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. PCR products were purified using AMPure XP beads as per the manufacturer's guidelines. The concentration and purity of the products were verified through gel electrophoresis and a Qubit

fluorometer. Sequencing libraries were created using the Nextera XT DNA Library Preparation Kit and sequenced on an Illumina MiSeq platform with 2 × 300 bp paired-end reads. Raw sequencing reads underwent quality control with Trimmomatic [13]. High-quality reads were further analyzed using the DADA2 pipeline in QIIME2 [17] for sequence denoising, chimera removal, and identification of amplicon sequence variants. Thereafter, the phylogenetic tree was constructed by sequencing outcome of amplicon by the neighbor-joining method using MEGA software.

2.2. Carbohydrate Fermentation Test

A series of carbohydrate broths, each containing a specific carbohydrate (glucose, lactose, sucrose, or mannitol), were prepared with phenol red as a pH indicator. A Durham tube was placed in each broth to capture the gas produced during fermentation. Pure cultures of the probiotic candidates were inoculated into these sterile broths and incubated at 37°C for 24–48 h. During incubation, bacterial metabolism of the carbohydrates produces organic acids and/or gases. The production of acids lowered the pH, causing the phenol red to change from red to yellow. Gas production was indicated by bubbles in the Durham tube. After incubation, results were interpreted by observing color changes and gas production [25].

2.3. Evaluation of Tolerance to Artificial Gastric and Intestinal Fluids

Initially, 1 mL of bacterial suspension of each strain was mixed with 9 mL of artificial gastric fluid, comprising 0.2 g sodium chloride and 1 g pepsin dissolved in 100 mL of HCl solution adjusted to pH 2.0. These samples were incubated statically at 37°C for 0, 30, 60, 90, and 120 min. At each time point, samples were withdrawn to determine the viable bacterial count in CFU/mL. Subsequently, 1 mL of the bacterial suspension in artificial gastric fluid was mixed with 9 mL of artificial intestinal fluid, containing 0.68 g potassium dihydrogen phosphate and 1.0 g trypsin dissolved in 100 mL distilled water, adjusted to pH 7.0 using NaOH solution. This mixture was similarly incubated at 37°C for 150, 180, 210, and 240 min, with samples taken at each interval for CFU/mL determination. The survival rates were calculated based on the initial and final viable bacterial counts, providing insights into the ability of the probiotic strains to withstand the harsh conditions of the stomach and intestine. The survival rate of the bacteria was calculated using the formula: Survival rate (%) = (Final CFU/mL/Initial CFU/mL) × 100. Statistical analysis was conducted to assess the reliability of the results across experimental replicates. This methodology enabled the evaluation of probiotic strains' ability to withstand gastrointestinal conditions, crucial for assessing their potential health benefits and viability as probiotic supplements.

2.4. Determination of Bile Salt Tolerance

Bacterial suspensions of each strain were prepared, and their initial viable counts (CFU/mL) were determined by plating serial dilutions on agar plates. Subsequently, the suspensions were separately inoculated into media containing different concentrations of oxgall: 0% (control), 0.3%, and 0.5% (w/v). The cultures were then incubated at 37°C for 24 h. After incubation, final bacterial counts were determined by plating and counting CFUs. The results were analyzed to assess the tolerance of each strain to bile salts at varying concentrations. For each strain, the survival rate (%) was calculated using the formula: Final CFU/mL after exposure/Initial CFU/mL × 100. This calculation yielded the percentage of viable bacteria that survived exposure to bile salts at each concentration.

2.5. Selenium-enriched Bacterial Cultivation and Selenium Quantification

Isolated pure cultures were utilized for preparing selenium-enriched MRS broth by adding a selenium solution, specifically sodium selenite, to attain a final concentration of 0.5 mg/L. The pure cultures were initially streaked on MRS agar plates to confirm purity and were then transferred to fresh MRS broth. A 1% (v/v) inoculum of these pure cultures was introduced into the selenium-enriched MRS broth. The inoculated broth was subsequently incubated under anaerobic conditions at a constant temperature of 37°C for 24–48 h to facilitate optimal bacterial growth and selenium assimilation. Following the incubation period, the cultures were subjected to centrifugation at 5000 rpm for 10 min to separate the bacterial cells from the growth medium. The resulting bacterial pellet was carefully collected and washed twice with sterile saline solution (0.85% NaCl) to eliminate any residual selenium that might remain in the medium. Subsequently, the strains were analyzed for their capacity to withstand selenium.

The concentrations of selenium in bacterial biomasses were measured using ultraviolet (UV)–vis molecular absorption spectroscopy [18]. For the calibration curve, aliquots ranging from 0.025 to 0.250 mg/L of Se were taken from a stock solution and placed into a series of 10-mL volumetric flasks. Each flask received 1 mL of 2% (mass/vol.) KI and 1 mL of 2 M HCl. The mixture was gently stirred until a yellow color appeared, indicating the release of iodine. Then, 200 µL of 1% (mass/vol.) starch solution was added, and the final volume was brought up with ultrapure water [19]. The absorbance of the solutions was measured with a UV–vis molecular absorption spectrophotometer at 589 nm. All samples underwent the same procedure, and the measurements were performed in triplicate. Statistical analysis was conducted using t test, and all analyses were performed using SPSS version 23.0.

3. RESULTS AND DISCUSSION

3.1. Colony Morphology

The colony morphology of probiotics isolated from fermented samples was characterized and shown in Table 1. On MRS media, colonies appeared with various characteristics: irregular shapes with a rough surface and convex elevation, creamy white, and size ranging from 2 mm to 4 mm. Other colonies were circular, smooth, and raised, appearing creamy white and measuring 1–2 mm. Some colonies were also circular and raised, showing a creamy white to pale yellow color, approximately 1–2 mm in diameter. In addition, colonies displayed irregular shapes with a rough surface, often with a flat to slightly raised elevation, creamy white, and typically sized between 3 mm and 5 mm. Furthermore, the total bacterial count for isolates 1 and 2 was 1.8×10^8 CFU/mL and 1.7×10^8 CFU/mL, respectively.

Table 1: Colony morphology of the selenium-enriched probiotics.

Isolate number	Colony shape	Surface texture	Elevation	Color	Size (mm)	Total bacterial count (CFU/mL)
Bacillus siamensis	Circular	Smooth	Raised	Creamy white	1–2 mm	1.8×10^8
Staphylococcus arlettae	Circular	Smooth	Raised	Creamy white to pale yellow	1–2 mm	1.7×10^8

3.2. Strain Identification by 16S rRNA Sequencing

The 16S rRNA sequencing analysis of selenium-enriched strains isolated from fermented samples revealed the taxonomic composition of the microbial community. Of the 20 strains isolated, only 2 were capable of withstanding selenium uptake. Each sample analysis identified a single bacterial strain, and the findings are summarized in Table 2. Three morphologically distinct colonies subjected to 16S rRNA sequencing were identified as representatives of the genera *Bacillus* and *Staphylococcus*. These results underscore the diverse microbial population present in the fermentation ecosystem of the samples.

Table 2: Identification of samples from the fermented samples.

Type of cereals	Code	Identified bacterial strains
Bamboo rice	BR	*Bacillus siamensis*
Purple rice	PR	*Staphylococcus arlettae*

The isolates were identified as *Bacillus siamensis* and *Staphylococcus arlettae*, showing 100% sequence homology. The sequencing results of these bacterial strains are shown in Figures 1 and 2. Comprehensive characterization tests were performed for both the bacterial isolates, *B. siamensis* and *S. arlettae*.

3.3. Carbohydrate Fermentation Test

The carbohydrate fermentation test was employed to identify probiotic strains by assessing their ability to ferment various sugars, which

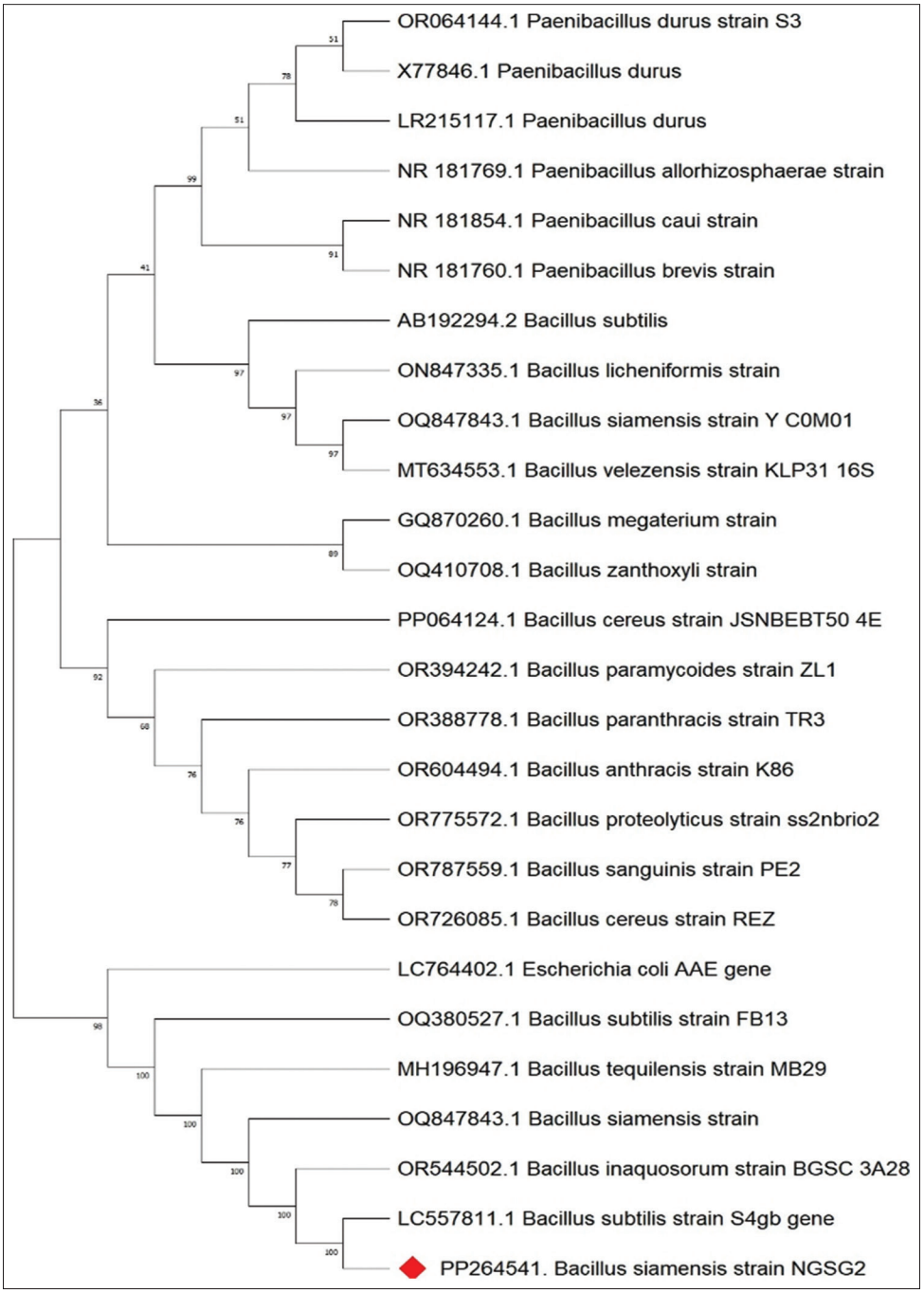

Figure 1: 16S ribosomal RNA sequencing result of *Bacillus siamensis*.

supports their metabolic and functional potential in gut health. The results of the carbohydrate fermentation tests conducted on the isolates from fermented samples are summarized in Table 3. Positive results, indicated by a color change from red to yellow in the phenol red broth, were observed for all tested carbohydrates across isolates from isolated samples. This indicates the robust carbohydrate fermentation capabilities of the isolates across various carbohydrate substrates commonly found in fermented cereals. These results demonstrate the carbohydrate fermentation abilities of probiotics isolated from the fermented samples, highlighting their capacity to utilize various carbohydrates for metabolic activity.

3.4. Assessment of Resistance to Gastric and Intestinal Fluids

The assessment of resistance to gastric and intestinal fluids evaluates a probiotic strain's ability to survive harsh gastrointestinal conditions, including acidic pH and digestive enzymes. This test is crucial for determining the strain's potential efficacy in reaching the gut alive, where it can exert its beneficial effects. The bacteria were assessed for their tolerance to artificial gastric and intestinal fluids at intervals of 0, 30, 60, 90, and 120 min. Initial observations showed varied responses among the strains. For 0–30 min, these strains maintained stability, with minimal changes noted in their viability. At 60 min, one strain began to exhibit decreased viability in the gastric fluid environment, while the other remained resilient. This trend continued at 90 and 120 min, where significant reductions in viability were observed, particularly in the acidic gastric environment. The evaluation of tolerance to artificial gastric and intestinal fluids is shown in Table 4.

For the survival rates based on the data, initial counts of bacterial populations were determined as 1.5×10^8 CFU/mL in both artificial gastric and intestinal fluids. For *B. siamensis* after 120 min of exposure, the final counts in gastric fluid were measured at 5.8×10^7 CFU/mL, while in intestinal fluid, they were 6.2×10^7 CFU/mL. However, for *S. arlettae*, the counts were 3.2×10^7 CFU/mL and 4.5×10^7 CFU/mL in intestinal fluid.

Table 3: Carbohydrate fermentation test.

Bacteria	Glucose	Lactose	Sucrose	Mannitol	Maltose
Bacillus siamensis	+	+	-	+	+
Staphylococcus arlettae	+	+	-	-	-

Table 4: Assessment of resistance to gastric and intestinal fluids.

Colony	Time (min)	CFU/mL in gastric fluid	CFU/mL in intestinal fluid	*P*-value
Bacillus siamensis	0	1.5×10^8	1.5×10^8	0.08
	30	1.4×10^8	1.5×10^8	
	60	1.2×10^8	1.4×10^8	
	90	1.0×10^8	1.3×10^8	
	120	5.8×10^7	6.2×10^7	
Staphylococcus arlettae	0	1.5×10^8	1.5×10^8	0.31
	30	1.3×10^8	1.4×10^8	
	60	1.0×10^8	1.2×10^8	
	90	8.0×10^7	1.0×10^8	
	120	3.2×10^7	4.5×10^7	

3.5. Bile Salt Tolerance Determination

The survival and growth of strains were evaluated after 24 h in media containing varying concentrations of oxgall (0%, 0.3%, and 0.5%). Initial colony-forming units per milliliter (CFU/mL) for each strain were consistent across all conditions: *B. siamensis* started at 1.5×10^8 CFU/mL, but *S. arlettae* at 8.5×10^8 CFU/mL. After exposure to 0% oxgall, the final CFU/mL remained unchanged for all strains. In 0.3% oxgall, Strain A reduced to 1.0×10^8 CFU/mL, but Strain B to 6.5×10^7 CFU/mL. The highest concentration tested, 0.5% oxgall, further showed 7.3×10^7 CFU/mL for Strain A and 4.8×10^7 CFU/mL for Strain B. These results demonstrate varying degrees of susceptibility among the strains to oxgall, with higher concentrations correlating with decreased bacterial survival and growth after 24 h. The overall results of the bile tolerance test are shown in Table 5. The bile tolerance test assesses a probiotic strain's ability to withstand bile salts, simulating the conditions of the small intestine, which is essential for determining its survival and potential for gut colonization. The outcome of this study demonstrated that these three isolated organisms indicate their ability to survive in the small intestine. The *P*-value suggested that no statistical significance was found in bacterial counts between gastric fluid and intestinal fluid among two isolates, indicating our isolated culture can survive under different harsh environmental conditions. Therefore, these isolates are more suitable for selenium enrichment.

3.6. Selenium Quantification

The quantification of selenium in bacteria isolated from fermented samples highlights significant variations in selenium uptake across different species. *B. siamensis* exhibited a selenium concentration of 200 µg/g dry weight and an absorbance of 0.600 AU, suggesting a greater capability for selenium uptake. The most efficient selenium uptake was observed in *S. arlettae,* which recorded the highest selenium concentration at 220 µg/g dry weight and absorbance of 0.660 AU. The results of the selenium utilization are shown in Table 6.

This study isolated and identified selenium-enriched strains of *B. siamensis* and *S. arlettae* from fermented cereals consumed by the South Indian population, highlighting their potential in enhancing the nutritional value of foods through selenium bioaccumulation. Using selective culture techniques and 16S rRNA gene sequencing, the researchers accurately identified these bacterial species. The strains demonstrated significant selenium uptake in selenium-supplemented

Table 5: Bile tolerance determination of isolated probiotics.

Strain	Initial CFU/mL	Final CFU/mL after 24 h in 0% oxgall	Final CFU/mL after 24 h in 0.3% oxgall	Final CFU/mL after 24 h in 0.5% oxgall
Bacillus siamensis	1.5×10^8	1.5×10^8	1.0×10^8	7.3×10^7
Staphylococcus arlettae	8.5×10^7	8.5×10^7	6.5×10^7	4.8×10^7

Table 6: Selenium uptake by bacteria.

Bacterial species	Absorbance (AU, Mean±SEM)	Selenium concentration in biomass (µg/g dry weight)
Bacillus siamensis	0.598±0.007	200
Staphylococcus arlettae	0.605±0.004	220

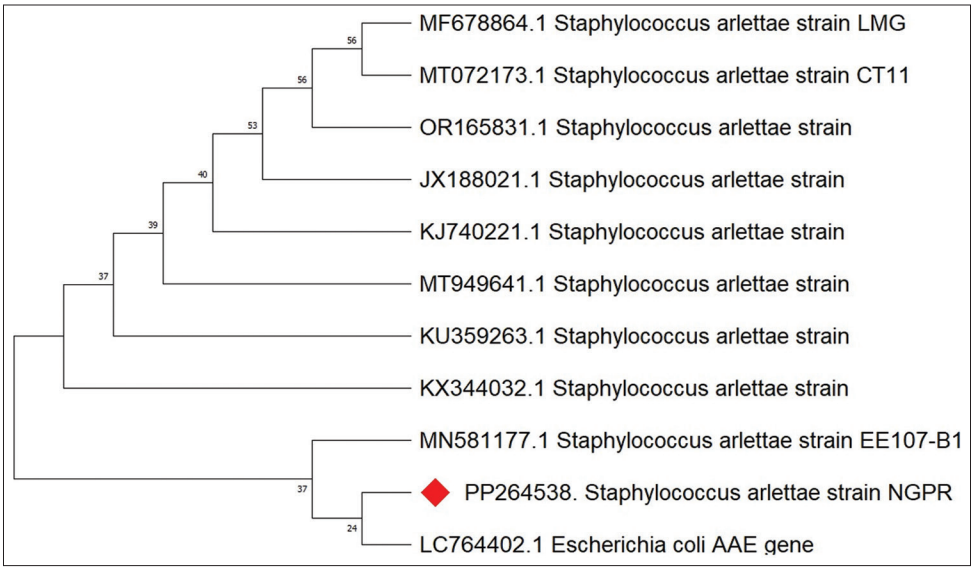

Figure 2: 16S ribosomal RNA sequencing result of *Staphylococcus arlettae*.

media, indicating their bioaccumulation capabilities. *B. siamensis* is robust and has potential in agricultural and environmental applications, while *S. arlettae*, found in fermented foods, also showed notable selenium accumulation. The selenium enrichment suggests promising applications in producing selenium-fortified fermented foods, addressing selenium deficiency, and providing antioxidant benefits. This study opens new avenues for using these bacterial strains to create functional foods aimed at improving nutritional health and dietary supplementation strategies.

The research conducted by Dong *et al*. demonstrated the isolation of *B. siamensis* from various fermented food products, highlighting its significant presence in such environments [26]. In addition, Heo *et al*. identified *B. siamensis* in fermented kimchi, further corroborating its prevalence in fermented foods [27]. These studies collectively underscore the ubiquitous nature of *B. siamensis* in fermented food matrices, suggesting its integral role in the fermentation process and its potential probiotic applications. This study on the isolation of probiotics from fermented Bamboo Rice koozh revealed the presence of *B. siamensis*, consistent with the previous researches by Dong *et al*. [26] and Heo *et al*. [27]. These consistent findings across different studies reinforce the established presence of *B. siamensis* in fermented foods.

The study conducted by Fowoyo and Ogunbanwo specifically isolated probiotic strains, emphasizing the prevalence of *Staphylococcus* species within fermented foods [28]. This research sheds light on the potential probiotic properties of *Staphylococcus* strains found in traditional fermented food sources. The research conducted by Jia *et al* unveiled the isolation of probiotic strains, with a particular emphasis on *Staphylococcus* species derived from fermented foods [29]. This work on isolating probiotics from fermented *Kavuni* Rice koozh revealed the presence of *S. arlettae*. This observation corresponds with prior research emphasizing the presence of *Staphylococcus* species in fermented foods, indicating their potential as probiotics within these traditional food contexts.

Research on the carbohydrate fermentation tests of probiotics such as *Staphylococcus* and *Bacillus* species has demonstrated varied abilities to ferment different carbohydrates. A study on *Bacillus* species by Simon *et al*. revealed its capability to ferment 11 out of 49 tested carbohydrates, including D-ribose, L-arabinose, D-xylose, D-glucose, D-fructose, and D-mannitol. This was confirmed through *in vitro* genomic analysis identifying related metabolic genes [30]. Similarly, the research by Nithya *et al*. revealed that the fermentation abilities of *Bacillus* species isolated from different food sources, demonstrating their potential probiotic characteristics. These species showed significant fermentation activities, which are critical for their probiotic functionality and contribution to gut health [31]. The results of this investigation corroborate that probiotics isolated from fermented cereals possess the ability to ferment a diverse array of carbohydrates. These results are consistent with the previous studies conducted on probiotics derived from other fermented foods, which have also demonstrated similar carbohydrate fermentation capabilities.

Research has shown that probiotics such as *Staphylococcus* and *Bacillus* species exhibit resistance to gastric and intestinal fluids, making them effective in surviving the harsh conditions of the gastrointestinal tract. According to several research evidences by Khatri *et al*. and Lee *et al*., probiotics possess mechanisms that enable them to withstand the acidic conditions of the stomach and the bile salts in the intestines [32,33]. This includes their ability to form spores, which are highly resistant to extreme conditions. These research results also confirm that the probiotics isolated from fermented cereals exhibit significant resistance to gastric and intestinal fluids. This observation aligns with the existing literature indicating that probiotics must withstand the acidic conditions of the stomach and the presence of bile salts in the intestines to be effective.

Research evidence indicates that probiotics such as *Staphylococcus* and *Bacillus* species exhibit notable tolerance to bile salts, a critical trait for their survival and functionality within the gastrointestinal tract. *Bacillus* species isolated from various food sources were tested for bile tolerance by assessing their growth at different bile concentrations and determining the time lag to reach the logarithmic phase [34,35]. This study confirms that probiotics isolated from fermented cereals have significant resistance to bile salts. This finding underscores their robust survivability under conditions mimicking the gastrointestinal environment, which is crucial for their potential application as probiotic supplements or functional foods.

The quantification of selenium in bacteria isolated from fermented cereals reveals notable differences in selenium uptake among the species studied, with *B. siamensis* and *S. arlettae* displaying varying efficiencies in selenium assimilation. Previous research supports these findings, highlighting the potential for certain bacterial species to accumulate selenium. For instance, Mörschbächer *et al*. demonstrated that bacterial strains *Enterococcus faecalis*, *Lactobacillus parabuchneri*, *Lactobacillus paracasei*, and *L. plantarum* could utilize the selenite to give elemental selenium, accumulating significant amounts of selenium within the bacterial cells [36]. This study underscores the ability of certain bacteria to transform selenium into less toxic forms and store it intracellularly, which aligns with the high selenium concentrations observed in our study. Selenium is a key trace element known to influence microbial metabolism and stress resistance when incorporated in bioavailable forms. In the context of probiotics, selenium enrichment has been reported to enhance not only bacterial survivability under oxidative and gastrointestinal stress, but also to boost health-promoting functions such as antioxidant activity and host adhesion [37,38]. Although *S. arlettae* showed the highest selenium accumulation, further validation is needed to confirm its functional benefits. Future studies will include antioxidant (DPPH/ABTS), aggregation, and adhesion assays using intestinal cell lines (e.g., Caco-2) to assess the probiotic potential. In addition, selenium-related enzyme activity and gene expression (selD, selA, selB) will be analyzed to clarify the mechanisms of selenium assimilation and its impact on probiotic efficacy. Though the selenium intake method in *S. arlettae* is not completely elucidated yet, few studies have proposed the selenium uptake mechanism in *Staphylococcus* spp., which entails the movement of selenium ions, usually as selenite or selenate, via membrane-associated transporter proteins. Upon entering the cell, selenium either gets reduced to its elemental state or integrated into selenoproteins by enzymatic activities [39-41].

This research intended to isolate selenium-enriched probiotics from fermented cereals, specifically targeting *Staphylococcus* and *Bacillus* species. 16S rRNA analysis, carbohydrate fermentation assays, and assessments of resistance to stomach and intestinal fluids, as well as bile salt tolerance, completely support the presence of selenium enrichment in the isolated strains. Genetic analysis identified them as *Staphylococcus* and *Bacillus* species. These probiotics demonstrated robust carbohydrate fermentation capabilities and significant tolerance to gastrointestinal conditions, highlighting their potential health benefits. However, further research is needed to assess their long-term efficacy and safety as probiotic supplements or functional food ingredients in promoting health. The future direction of this study involves exploring the long-term effects and safety of selenium-enriched probiotics in human health, particularly their role in immune function and oxidative stress reduction. This research lies in the identification of specific *Staphylococcus* and *Bacillus* species from fermented cereals, offering a unique approach to developing functional foods with enhanced antioxidant and probiotic benefits.

4. CONCLUSION

The present study effectively isolated and characterized selenium-enriched *Bacillus siamensis* and *Staphylococcus arlettae* from *koozh,* emphasizing their probiotic potential and selenium absorption. Their resistance under gastrointestinal circumstances renders them a potential option for the production of functional foods. Furthermore, subsequent investigations should examine their *in vivo* efficacy and safety to facilitate extensive implementation in food fortification..

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

The data supporting the findings of this study are available with all the authors and will be provided upon reasonable request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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