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Solid-State Fermentation of Rice Bran Using Different Commercial Products

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Abstract

This study evaluated solid-state fermentation (SSF) to improve the nutritional profile and microbial composition of rice bran using three commercial products with different microbial/enzimatic complexity. The experiment was conducted in triplicate at LCM-UFSC and assessed 0, 24, and 48 hours fermentations by measuring pH, proximate composition, amino acids, organic carbon, and metagenomes (16S rRNA HTS for bacteria; ITS for fungi). Rice bran (250 µm sieve) was autoclaved, mixed with deionized water and products (water:bran:product ratio 1.5:1:0.025), and incubated at 30˚C. Post-fermentation samples were frozen (−20˚C/−80˚C), lyophilized, and analyzed. The products contained probiotics (*Bacillus*, *Lactobacillus*), *Saccharomyces cerevisiae*, and enzymes. SSF reduce pH and organic carbon while increasing protein, fiber, ash, and essential amino acids such as methionine and lysine. Bacterial metagenomics showed Firmicutes dominance, particularly *Bacillus* and *Lysinibacillus*, while fungal profiles shifted toward *S. cerevisiae*. These results suggest that SSF may help valorize rice bran as an agro-industrial byproduct for future aquafeed applications.

Keywords

Aquaculture, Antinutrient, Metagenomics, Chemical Composition, Circular Economy, Feed

1. Introduction

Aquaculture provides over 50% of global seafood for human consumption, yet sustainability challenges persist due to high feed costs representing 70% - 80% of operational expenses [1]. Alternative ingredients from agroindustrial by-products offer cost-effective, environmentally friendly solutions aligned with circular econ-

omy principles [2] [3].

Aquaculture faces feed challenges such as fishmeal shortages and price volatility, which necessitate plant-based alternatives [4]. However, many contain antinutritional factors (ANFs) that limit digestibility [5]. Fermentation has been proposed as a strategy to reduce antinutritional factors, including phytates, and thus may improve the nutritional quality of rice bran [6]. Rice bran, generated at 60 - 80 kg per ton of milled rice, provides 12% - 15% protein, lipids, vitamins, and bioactive compounds (γ-oryzanol, phytosterols) at low cost [5] [7]. Yet, high fiber (8% - 12%), phytic acid, and spoilage fungi (*Aspergillus*, *Fusarium*, *Penicillium*) restrict inclusion above 20% in aquafeeds [8].

Solid-state fermentation (SSF) enhances agro-byproducts by reducing ANFs, improving protein/amino acid bioavailability, and modulating microbial communities through microbial/enzymatic action [9]. Unlike submerged fermentation (95% water), SSF uses < 40% moisture, optimal for *Bacillus* and *Saccharomyces cerevisiae* on solid substrates [10]. Previous studies report SSF rice bran increases protein (+25%), essential amino acids (methionine, lysine +30–50%), and reduces phytate (40% - 70%) [11] [12].

Brazil produces 11 million tons of rice annually, yielding ~800,000 tons of bran ideal for feeds targeting dominant species like tilapia and shrimp (*Litopenaeus vannamei*; 70.9% pisciculture, 19.3% shrimp production) [13] [14]. This study addresses this gap by evaluating commercial microbial blends (probiotics + enzymes) via SSF on rice bran with comprehensive metagenomic analysis (16S rRNA for bacteria; ITS for fungi) under controlled conditions, providing insights for sustainable aquafeeds.

2. Materials and Methods

2.1. Rice Bran Substrate

Rice bran (Sucesso Agroindustrial e Consultoria LTDA, Santa Catarina, Brazil), a by-product of polished rice, was used as the substrate. According to Garofalo *et al.* [15], it contained 15.70% crude protein, 1.70% crude ether extract, 3740 kcal/kg gross energy, 13.70% crude fiber, 16.70% ash, 0.45% available phosphorus, 0.13% total calcium, and 1.10 mg/kg folic acid.

2.2. Microbial Inoculants

Three commercial products containing *Saccharomyces cerevisiae* were used for rice bran fermentation. According to the manufacturers' labels, Product 1 (complex) contained *Bacillus subtilis*, *Enterococcus faecium*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae*, together with phytase, protease, and mineral/nutritional additives, including calcium, cobalt, copper, iron, magnesium, manganese, potassium, selenium, zinc, lecithin, glyceryl ricinoleate, and mannan oligosaccharides. Product 2 (simple) contained *Saccharomyces cerevisiae* microspheres at 1×10^{10} CFU/g. Product 3 (+complex) contained *Enterococcus faecium*, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, dried/lyophilized yeasts, algae

(*Schizochytrium* sp. and *Chlorella vulgaris*), cellulase, phytase, protease, *β*-glucans, mannan oligosaccharides, vitamin C, copper, selenium, and zinc.

2.3. Experimental Design

A completely randomized design was used. The experimental units were 3.5-L plastic containers (253 × 174 × 122 mm). Rice bran and deionized water were autoclaved before use. The control consisted of dry rice bran without water or commercial product. The fermented treatments consisted of rice bran mixed with deionized water and one of three commercial products at a ratio of 1.5:1:0.025 (water:rice bran:product), corresponding to 750 mL of deionized water, 500 g of rice bran, and 12.5 g of product per container. The mixtures were covered with parafilm and incubated at 30˚C for 48 h.

The experiment included four treatments: (i) dry rice bran control without water or product; (ii) rice bran fermented with Product 1; (iii) rice bran fermented with Product 2; and (iv) rice bran fermented with Product 3. Each treatment was performed in triplicate, totaling 12 experimental units. The same experimental units were sampled non-destructively at 0, 24, and 48 h.

The 3.5-L container served as the experimental unit. pH was measured independently in each replicate at 0, 24, and 48 h, and proximate composition was determined from replicate samples collected from each unit. In contrast, amino acid, organic carbon, and metagenomic analyses were performed on pooled composite samples prepared by combining material from the corresponding treatment and sampling time; accordingly, these results are descriptive and not intended for formal replicated treatment-level statistical inference.

2.4. pH Measurement

At each sampling time (0, 24, and 48 h), 10 g of substrate from each experimental unit was mixed with 10 mL of deionized water, and pH was measured using a TEC-11/EL-M pH meter [16].

2.5. Bromatological Analysis

Proximate composition was determined on wet samples (100 g) collected from each experimental unit. At each sampling time (0, 24, and 48 h), material from all 10 containers (dry rice bran plus three replicate containers per treatment) was frozen at −20˚C, freeze-dried, vacuum-sealed, and sent individually to Análises Laboratoriais Ltda. CBO (Valinhos, SP, Brazil). Analyses followed the *Compêndio Brasileiro de Alimentação Animal* (2017) and AOCS methods, including: moisture/volatiles (Method 53), crude protein (Method 45, Dumas), ether extract (AOCS Am 5-04/ANKOM 12), crude fiber (ANKOM 200, Method 18), and mineral matter (Method 5, modified) [17].

2.6. Amino Acid Analysis

For amino acid analysis, material from the three replicate containers was pooled

for each selected treatment and sampling time to form composite samples (n = 7 conditions: dry bran, Products 1 - 3 at 24 h, and Products 1 - 3 at 48 h). The pooled samples (≈50 g wet) were freeze-dried and analyzed by HPLC after acid hydrolysis [18]-[21]. Because these analyses were performed on pooled composites rather than independent biological replicates, amino acid results are presented descriptively and are not intended for formal treatment-level statistical inference. Cystine/methionine was determined after oxidation, and taurine was co-analyzed using methods MA-001 R5 and MA-009 R0.

2.7. Organic Carbon Determination

Organic carbon was determined on the same pooled composite samples used for amino acid analysis (≈50 g wet per condition). The pooled samples were dried at 65˚C for 48 h, sieved to 0.250 mm, and analyzed at Laboratório Agronômico Unithal using the Walkley-Black method [22] [23]. As with amino acids, these values are descriptive and reflect treatment × time composites rather than replicated experimental units; consequently, they do not support formal treatment-level statistical inference.

2.8. Bacterial Metagenomics

For bacterial metagenomic analysis, samples from the same treatment and sampling time were pooled before sequencing. A total of seven pooled composite samples were prepared from dry rice bran and Products 1 - 3 at 24 h and 48 h (1 g wet; n = 7 conditions). Total DNA was extracted from each pooled sample, and bacterial community profiling targeted the V3-V4 region of the 16S rRNA gene using the 314F/806R primers [24] [25].

Sequencing was performed on an Illumina MiSeq platform using 300 bp single-end reads, and raw reads were processed with the Sentinel pipeline [26]. Quality control included FastQC, primer removal, trimming of bases with Phred scores below 20 using Trimmomatic v0.36, and removal of clusters with fewer than 5 reads [27] [28]. VSEARCH was used to remove chimeras and cluster reads into OTUs at 97% identity [29]. Taxonomic assignment was performed by BLASTn against the proprietary reference database used by the platform; the database incorporated GenBank and SILVA v132, with a 90% identity threshold for classification [30]-[32]. Because the samples were pooled, the community profiles are reported descriptively and not used for formal statistical inference.

2.9. Fungal Metagenomics

For fungal metagenomic analysis, samples from the same treatment and sampling time were pooled before sequencing. Pooled composite samples (1 g wet; n = 7 conditions: dry rice bran and Products 1 - 3 at 24 h and 48 h) were analyzed by high-throughput sequencing (HTS) of the ITS1 region using the ITS1 (GAACCWGCGGARGGATCA) and ITS2 (GCTGCGTTCTTCATCGATGC) primers [33]. Sequencing was performed on an Illumina MiSeq platform using the Senti-

nel/Neobiome workflow, with a minimum alignment length of 300 bp and 100,000 reads per sample [34]. Quality control included FastQC, trimming of primer sequences and bases with Phred scores below 20, and removal of clusters with fewer than 5 reads to reduce low-quality and potentially chimeric signals [27] [29]. Taxonomic assignment was performed by BLASTn v2.6.0+ against the NeoRefDB proprietary database, with species-level classification defined at 97% identity [35] [36]. Raw-read accession numbers will be provided once deposited in a public repository. Shannon and dominance indices were calculated using PAST 4.04 [37] [38].

Because the analyses were based on pooled composite samples, the fungal community patterns are interpreted descriptively and not used for formal treatment-level statistical inference.

2.10. Statistical Analysis

Data were first checked for normality and homogeneity of variances using Shapiro-Wilk and Levene tests. For variables measured repeatedly in time on the same experimental units (*e.g.*, pH), a two-way repeated-measures ANOVA was applied, followed by Tukey's post-hoc test when appropriate (α = 0.05; Jamovi 2.3.21). Multivariate analyses of bacterial community data were performed in PAST 4.04 [39]. Amino acid, organic carbon, and metagenomic results from pooled composite samples were treated as descriptive and were not subjected to formal replicated inferential statistics.

3. Results

3.1. Temperature and pH

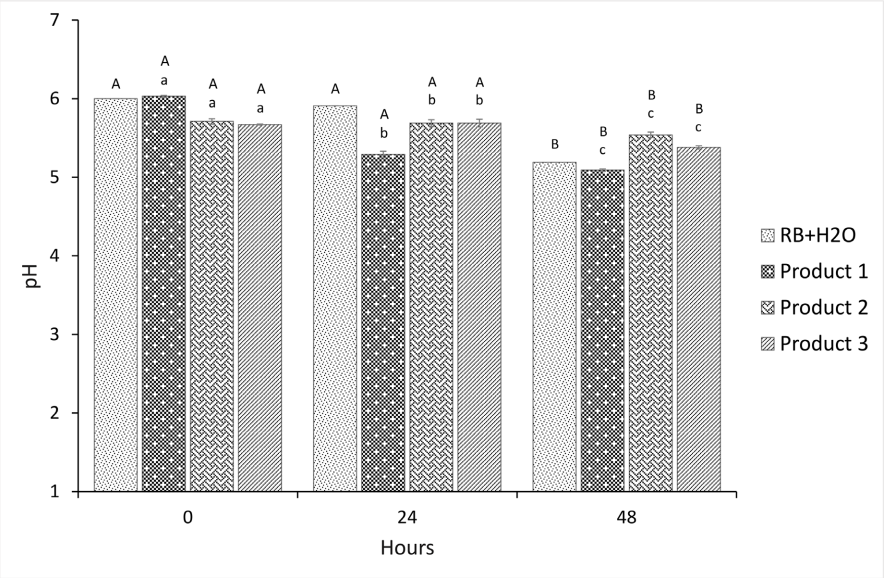
Temperature remained stable throughout fermentation (30˚C). pH decreased significantly over time (repeated-measures ANOVA, $p < 0.001$; **Figure 1**). The dry rice bran control showed pH values of 6.00 (0 h), 5.91 (24 h), 5.19 (48 h). Product 3 showed the greatest initial drop at 0 h (5.67 ± 0.01; −5.5% vs. control). At 24 h, Product 1 acidified most (5.29 ± 0.04; -10.5% vs. control); at 48 h, Product 1 reached 5.09 ± 0.02 (−1.9% vs. control). Post-hoc Tukey tests indicated no difference between 0 and 24 h ($p = 0.136$, NS), but significant differences between 0 and 48 h ($p = 0.008$) and between 24 and 48 h ($p = 0.011$). No product effect on pH ($p = 0.51$).

3.2. Bromatological Composition

Compositions (n = 10 experimental units per sampling time; **Figure 2**) varied by product and time (ANOVA, $p < 0.05$ unless otherwise noted).

Moisture/volatiles showed no significant differences among treatments ($p = 0.147$). The greatest reductions were observed for Product 2 at 0 h (2.36 ± 0.43%; −44% vs. dry bran control) and at 24 h, and for Product 1 at 48 h (2.70 ± 0.37%).

Crude protein differed significantly among treatments ($p < 0.001$). Product 2



Temporal variation of pH in experimental treatments. Data represent means ± standard deviation from repeated measures analysis. Different uppercase letters indicate significant differences over time ($p < 0.05$). Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Figure 1. pH of three microbial blends before fermentation (0 h), after 24 h and 48 h fermentation, and FA with water (control).

showed increases of approximately 13.9% at 24 and 48 h (14.39% ± 0.16% and 14.93% ± 0.20%, respectively), whereas Product 1 at 0 h showed a slight decrease of 1.3% relative to the control.

Ether extract also differed significantly ($p < 0.001$). Initial reductions were observed for Product 2 at 0 h (14.49% ± 1.08%; −9.8% vs. control), while post-fermentation increases were detected for Product 3 at 24 h (+28%) and for Product 2 at 48 h (+39.8%).

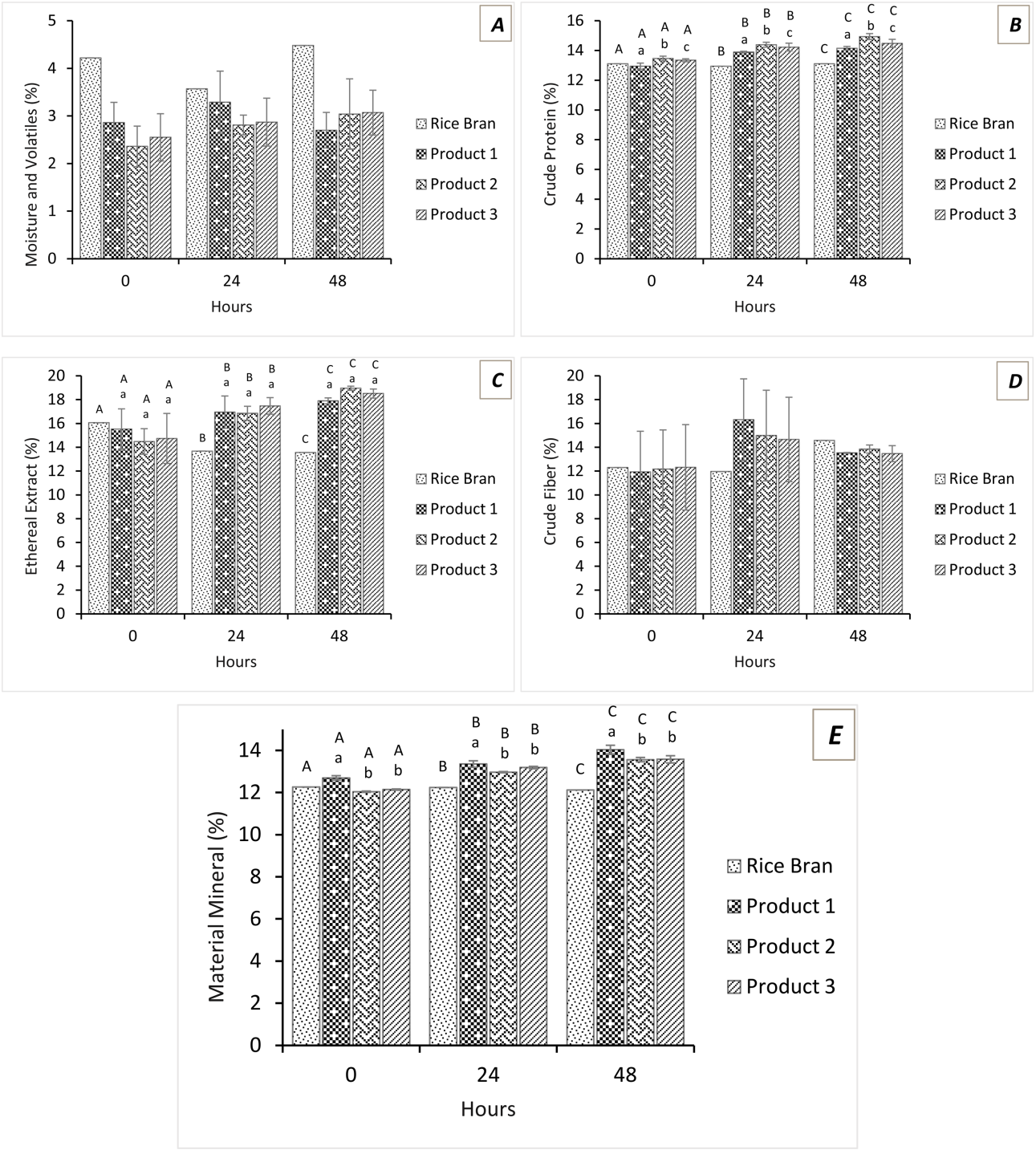
Crude fiber showed no significant treatment or time effect ($p = 0.561$), although Product 3 exhibited a 7.7% decrease at 48 h.

Mineral matter (ash) differed significantly ($p < 0.001$), with Product 1 showing a 15.8% increase at 48 h. These patterns likely reflect both fermentation-driven changes and direct nutrient contributions from the commercial inoculants, consistent with their label composition [40] [41].

3.3. Amino Acid Profile

Fermented samples (n = 7 pooled composite conditions; **Table 1** and **Table 2**) showed descriptively higher amino acid contents than dry rice bran. Patterns in essential amino acids suggest increases in the fermented treatments; for example, lysine was 82% higher in Product 1 at 48 h, and methionine was 71.4% higher in Product 2 at 48 h, relative to dry bran. Non-essential amino acids appeared highest in Product 3 at 48 h, with aspartic acid showing an increase of 13.7% relative to dry bran. Because these measurements are based on pooled composite samples for each

condition rather than independent biological replicates, amino acid data are interpreted descriptively and are not used for formal treatment-level statistical inference.



Panels A and D show no significant differences. Data in panels B, C, and E represent means ± standard deviation from repeated measures analysis. Different uppercase letters indicate significant differences over time ($p < 0.05$). Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Figure 2. Proximate composition (% dry matter) of dry rice bran (control) and solid-state fermented rice bran with commercial products at 0, 24, and 48 h.

Table 1. Guaranteed composition levels of three commercial microbial products.

Composition of commercial products		
Product 1	**Product 2**	**Product 3**
Bacillus subtilis[1]	*Saccharomyces cerevisiae*[1]	Beta-glucans[1]
Calcium[2]		Cellulase[2]
Cobalt[3]		Copper[3]
Copper[4]		*Enterococcus faecium*[4]
Enterococcus faecium[5]		Phytase[5]
Iron[6]		*Lactobacillus acidophilus*[6]
Phytase[7]		Mannan-oligosaccharides[7]
Lactobacillus acidophilus[8]		Protease[8]
Lecithin[9]		*Saccharomyces cerevisiae*[9]
Magnesium[10]		Selenium[10]
Mannan-oligosaccharides[11]		Vitamin C[11]
Manganese[12]		Zinc[12]
Potassium[13]		
Protease[14]		
Glyceryl ricinoleate[15]		
Saccharomyces cerevisiae[16]		
Selenium[17]		
Zinc[18]		

Values in UFC/g (microbial counts), U/g (enzymes), mg/kg or g/kg (minerals/nutrients). Product 1: [1]20,000 × 109 UFC/g^{-1}; [2]70,000 g/kg; [3]16,720 mg/kg; [4]3910,018 mg/kg; [5]1850 × 106 UFC/g^{-1}; [6]3,483,417 mg/kg; [7]2520 U/G; [8]1850 × 106 UFC/g^{-1}; [9]3000,000 mg/kg; [10]2004,363 mg/kg; [11]500,000 mg/kg; [12]2,786,730 mg/kg; [13]10,450 g/kg; [14]2540 U/G; [15]3000,000 mg/kg; [16]6850 × 107 UFC/g^{-1}; [17]42,867 mg/kg; [18]361,269 mg/kg. Produto 2: [1]1 × 10^{10} UFC/g^{-1}. Produto 3: (1) 17.9 mg/kg; (2) 0.55 u*2/g; (3) 2133 mg/kg; (4) 4 × 106 UFC/g^{-1}; (5) 4 u*3/g; (6) 4 × 106 UFC/g^{-1}; (7) 2500 mg/kg; (8) 250 u*1/g; (9) 8 × 107 UFC/g-1; (10) 75 mg/kg; (11) 2470 mg/kg; (12) 1249 mg/kg.

Table 2. Amino acid profile (% of total protein) of fermented rice bran versus dry control.

Aminoácidos	**FAes**	**Product1 - 24 h**	**Product1 - 48 h**	**Product2 - 24 h**	**Product2 - 48h**	**Product3 - 24 h**	**Product3 - 48 h**
Aspartic acid	1.01	1.01	1.03	1.14	1.10	1.07	1.17
Glutamic acid	1.63	1.57	1.69	1.85	1.87	1.84	1.77
Serine	0.57	0.53	0.58	0.66	0.62	0.63	0.58
Glycine	0.70	0.67	0.69	0.72	0.72	0.70	0.78
Histidine*	0.30	0.29	0.30	0.32	0.34	0.33	0.32
Taurine	0.01	0.03	0.04	0.04	0.01	0.01	0.01

Continued

Arginine*	0.81	0.86	0.86	0.96	0.79	0.88	0.90
Threonine*	0.50	0.45	0.50	0.58	0.53	0.53	0.54
Alanine	0.92	0.92	0.90	0.92	0.90	0.86	1.01
Proline	0.60	0.59	0.62	0.64	0.65	0.65	0.63
Tyrosine	0.27	0.34	0.37	0.41	0.44	0.44	0.41
Valine*	0.70	0.65	0.67	0.70	0.72	0.71	0.78
Methionine*	0.04	0.12	0.12	0.13	0.14	0.13	0.11
Cystine*	0.12	0.15	0.16	0.16	0.20	0.20	0.15
Isoleucine*	0.43	0.42	0.44	0.45	0.46	0.45	0.49
Leucine*	0.89	0.90	0.94	0.99	0.99	0.97	0.98
Phenylalanine*	0.57	0.51	0.56	0.60	0.61	0.60	0.62
Lysine*	0.55	2.39	3.06	0.82	0.68	0.66	0.67
Hydroxyproline	0.04	0.09	0.07	0.08	0.10	0.10	0.05

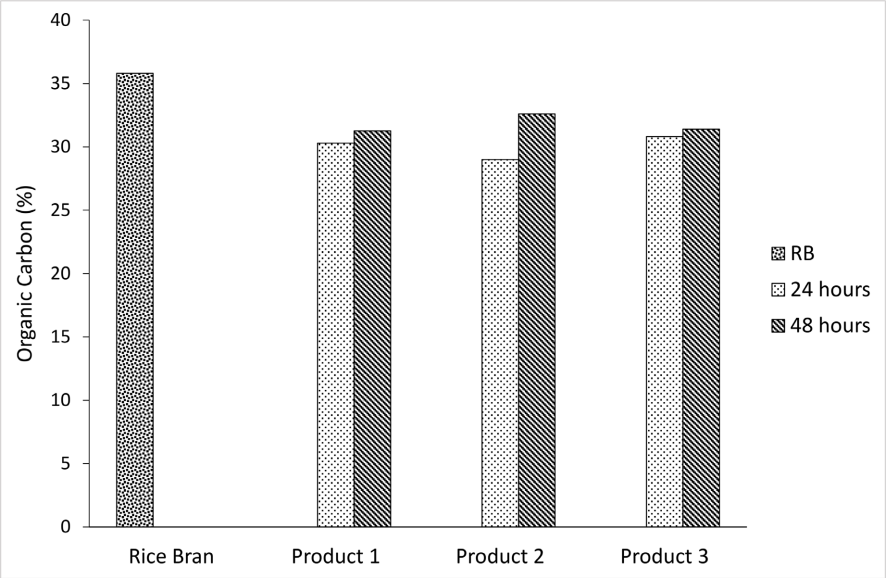
Amino acid profile (% of total protein) of rice bran SSF with commercial microbial products 1 - 3 at 24 and 48 h versus dry control (FA; n=7 *pools* per treatment). Essential amino acids marked (*) meet *Penaeus vannamei* requirements (lysine: 1.6% diet; Hardy et al., 2011). Greatest improvements: lysine +457% (Product 1 - 48 h), methionine +250% (Product 2 - 48 h).

3.4. Organic Carbon

Fermented samples showed descriptively lower organic carbon than dry rice bran (e.g., Product 2 at 24 h: −19%; Figure 3). Between 24 and 48 h, organic carbon in Product 2 increased by approximately 11%, suggesting partial recovery of carbon content over time. The distribution of organic carbon values did not meet normality assumptions (Shapiro-Wilk test), and because measurements were obtained from pooled composite samples for each condition, these patterns are interpreted descriptively and are not used for formal treatment-level statistical inference.

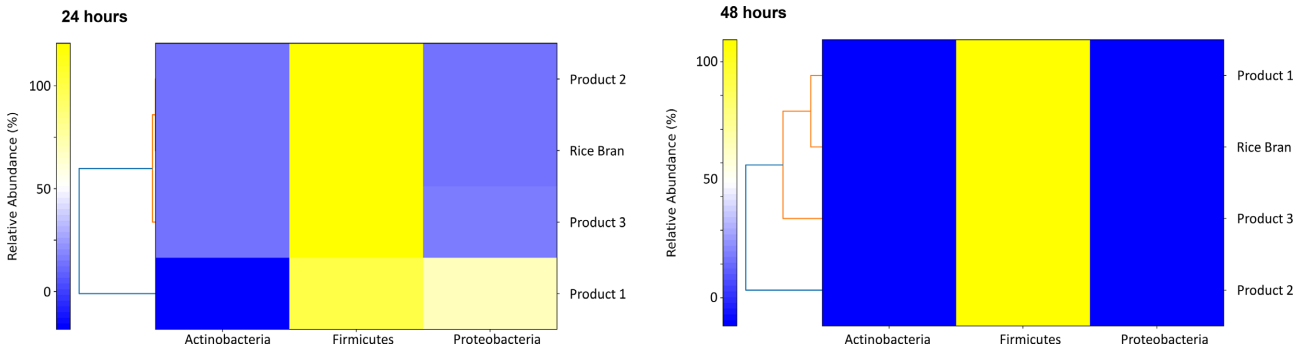
3.5. Bacterial Microbiome

A total of 276,966 sequences corresponding to 76 species were identified in the bacterial microbiome analysis (Figure 4). The community was dominated by Firmicutes across all samples, representing 99.84% in the control and approximately 98% - 99.8% in Products 1 - 3, whereas Proteobacteria showed a transient increase in Product 1 at 24 h, reaching 42.5%. Actinobacteria remained below 0.03% in all conditions. The most abundant species were *Rummeliibacillus pycnus* (33.86%; 92,970 sequences), *Clostridium butyricum* (26.18%), and *Pediococcus acidilactici* (22.20%). Principal coordinate analysis explained 68.88% of the variation in PC1 and separated the samples into two main groups: Group A, comprising Product 1 at 48 h and Product 3 at 24 h, and Group B, comprising the control



Organic carbon content (*pool*, %) in rice bran fermented with commercial products 1 - 3 at 24 and 48 h versus dry rice bran control. Reductions occurred at 24 h (maximum 19% for product 2), with increases from 24 to 48 h (3% - 11%).

Figure 3. Organic carbon content (*pool*, %) of fermented rice bran at 24 and 48 h compared to dry rice bran control.



Relative abundance (%) of major bacterial phyla (Actinobacteria, Firmicutes, Proteobacteria) at 24 h (left panels) and 48 h (right panels) fermentation across three commercial products (P1, P2, P3) and dry rice bran control. Data derived from 16S rRNA sequencing analysis.

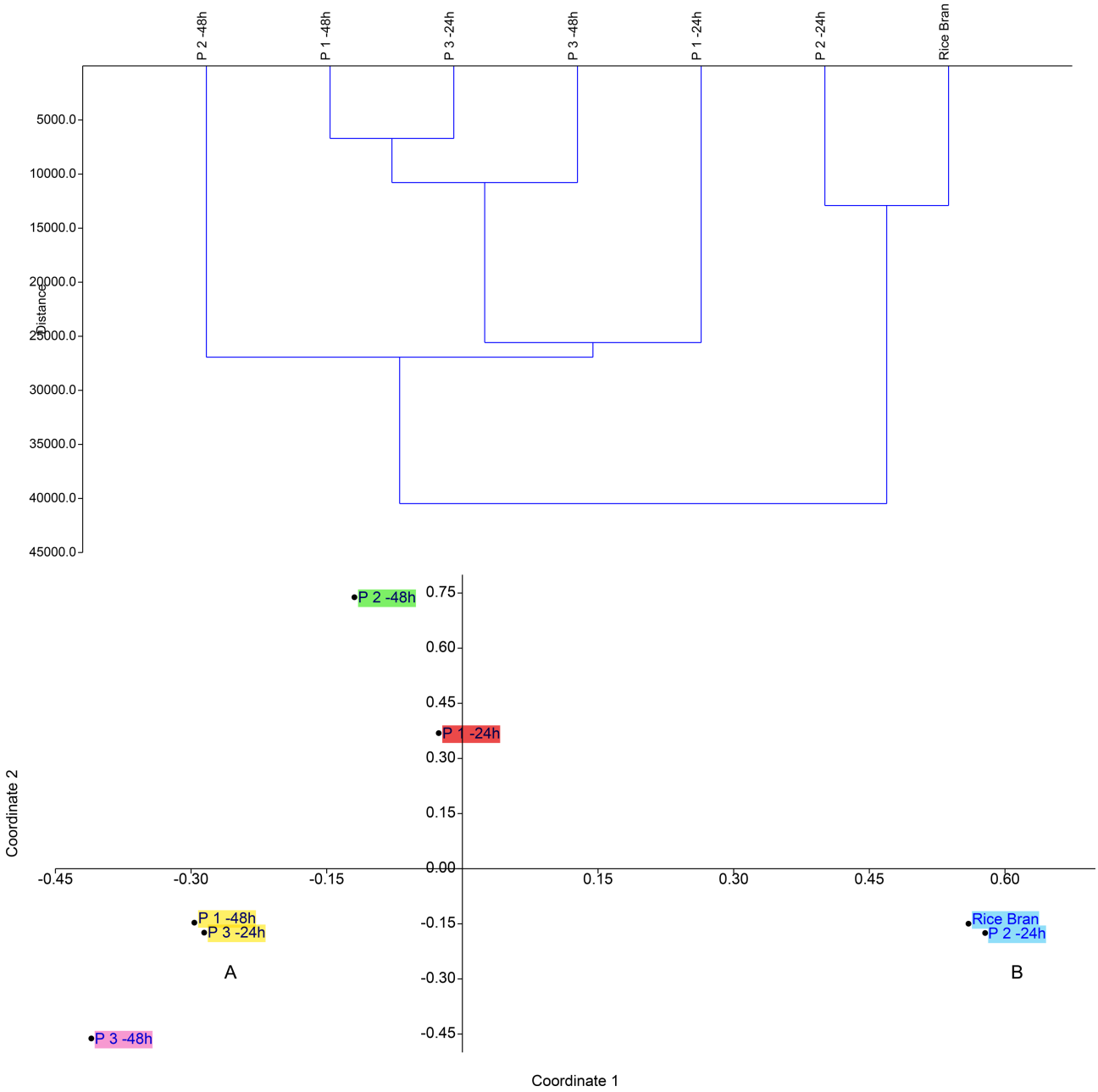
Figure 4. Relative abundance (%) of major bacterial phyla at two fermentation times (24 and 48 h) across three commercial products and dry rice bran control.

and Product 2 at 24 h (Figure 5). Venn analysis showed that Product 1 at 24 h contained the highest number of taxa, with 55 species detected (Figure 6). Because these microbiome profiles were derived from pooled composite samples, the results are reported descriptively and interpreted accordingly.

3.6. Fungal Microbiome

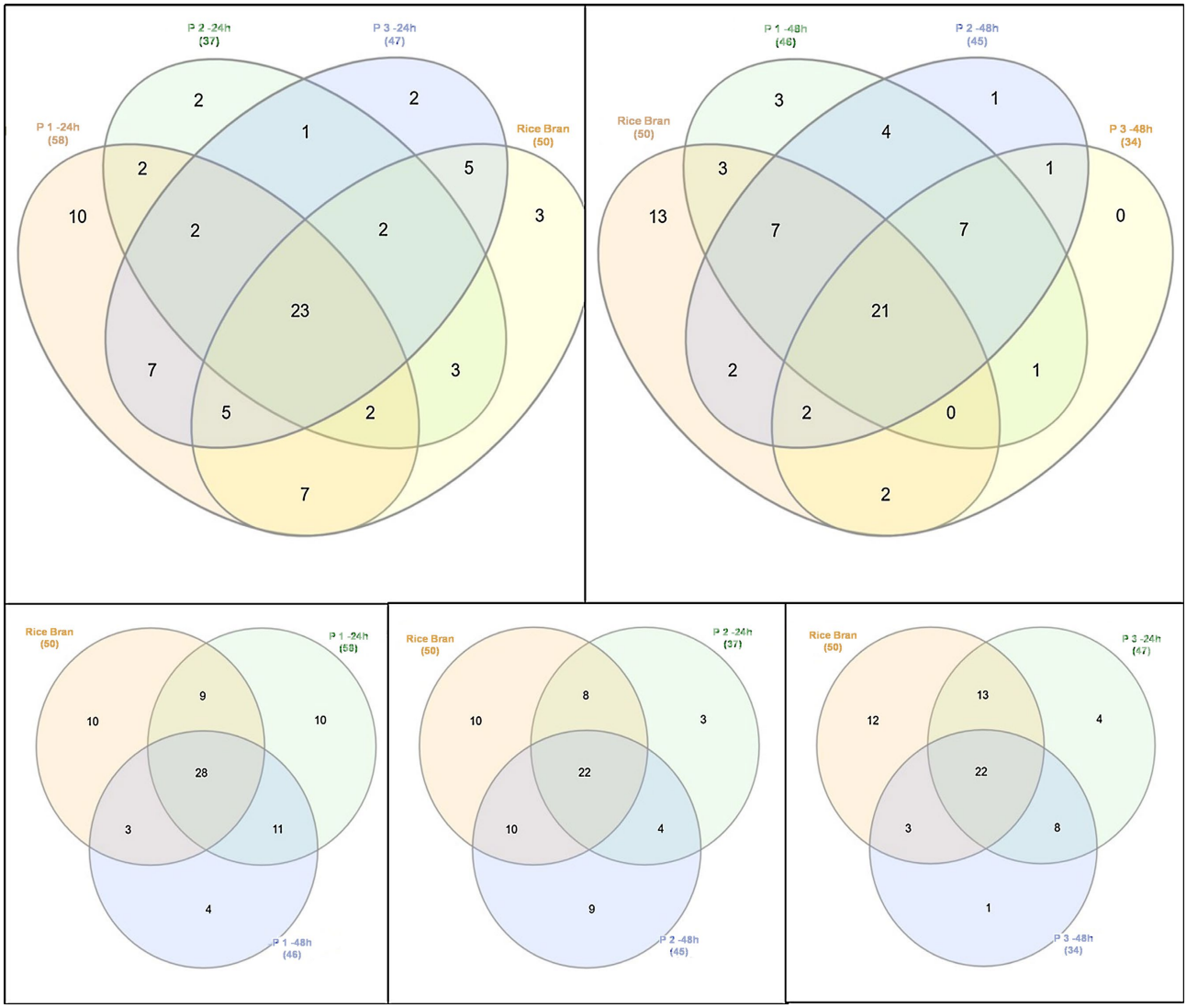
750,732 sequences (24 genera; Figure 7). Dominant: *Ascomycota* (69.47%), *Mucoromycota* (30.51%). *Saccharomyces cerevisiae* 65.38% (490,885 seq.). PCoA: 72.84% PC1; 48 h clustering (Figure 8). Venn: P1 - 24 h 29 taxa; P1 - 48 h 47 taxa

(Figure 9). As with the bacterial data, these fungal community results derive from pooled composite samples and are interpreted descriptively rather than used for formal treatment-level statistical inference.



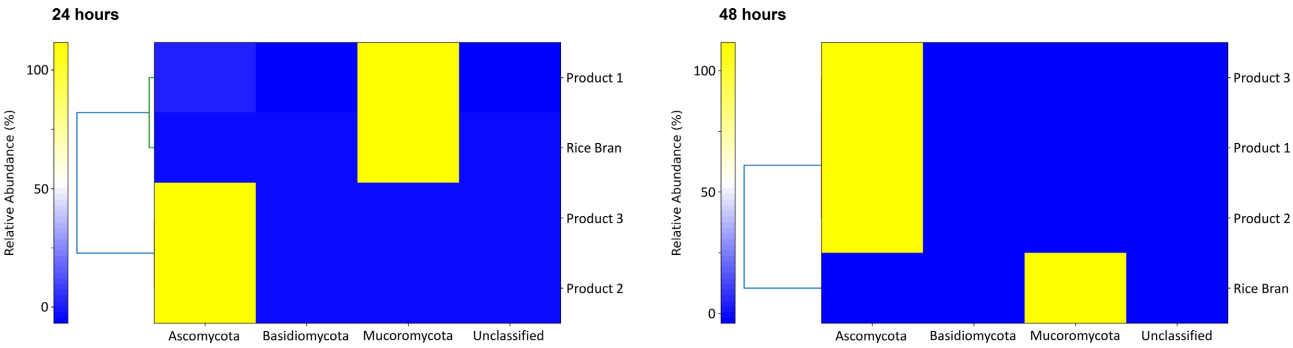
PCoA of bacterial communities in fermented rice bran (products 1 - 3 at 24 and 48 h) versus dry control. Clusters: A (product 1 - 48 h, product 3 - 24 h); B (dry bran, product 2 - 24 h).

Figure 5. Principal coordinates analysis (PCoA) of bacterial communities showing relationships between commercial products and fermentation times (24 and 48 h).



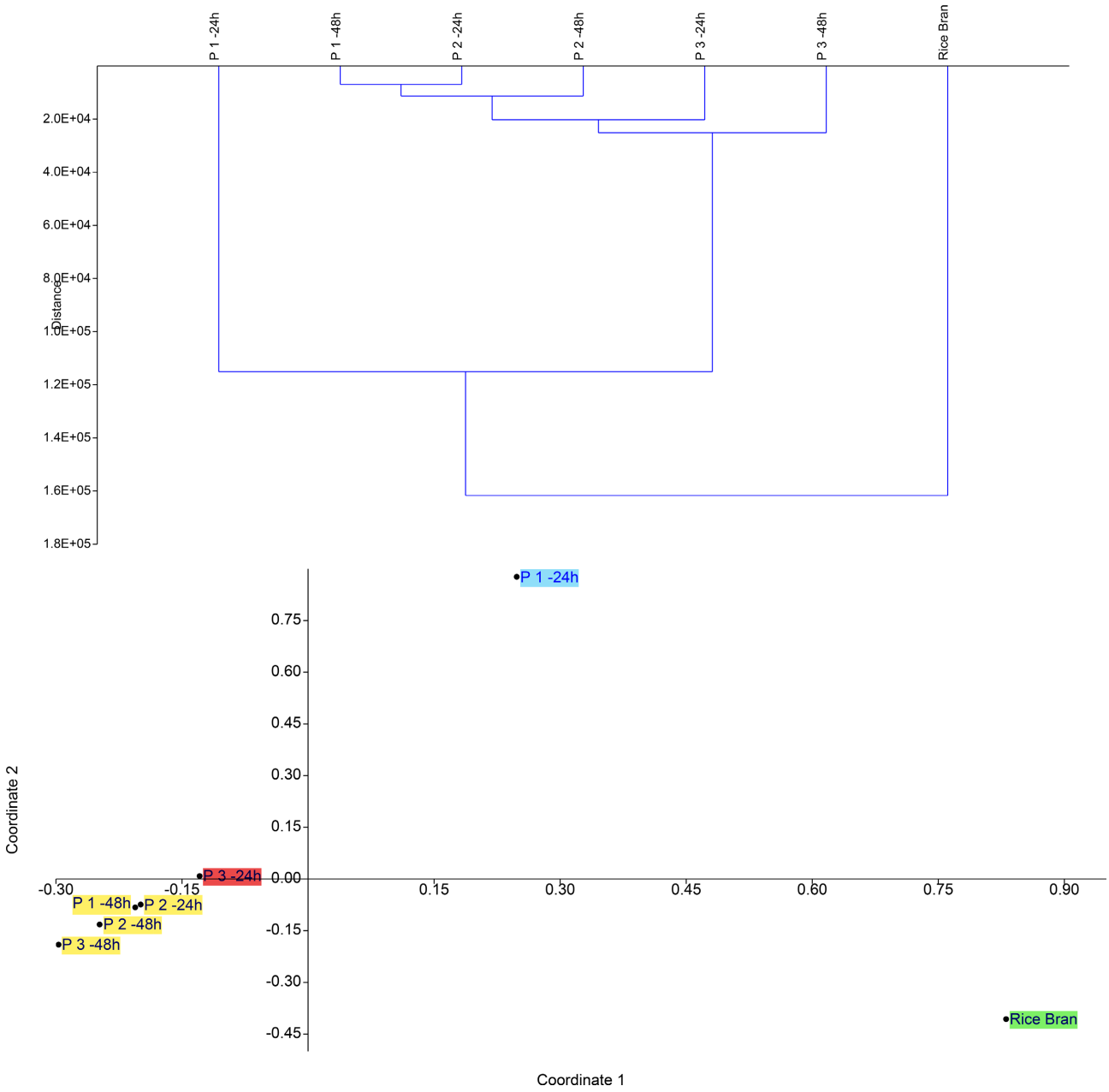
Venn diagram of shared bacterial taxa in rice bran fermented with commercial products 1 - 3 at 24 h (highest: product 1, 55 taxa) and 48 h (highest: dry bran, 50 taxa) versus dry rice bran control.

Figure 6. Venn diagram showing shared bacterial taxa between fermentation times (24 and 48 h) and microbial blends in solid-state rice bran fermentation.



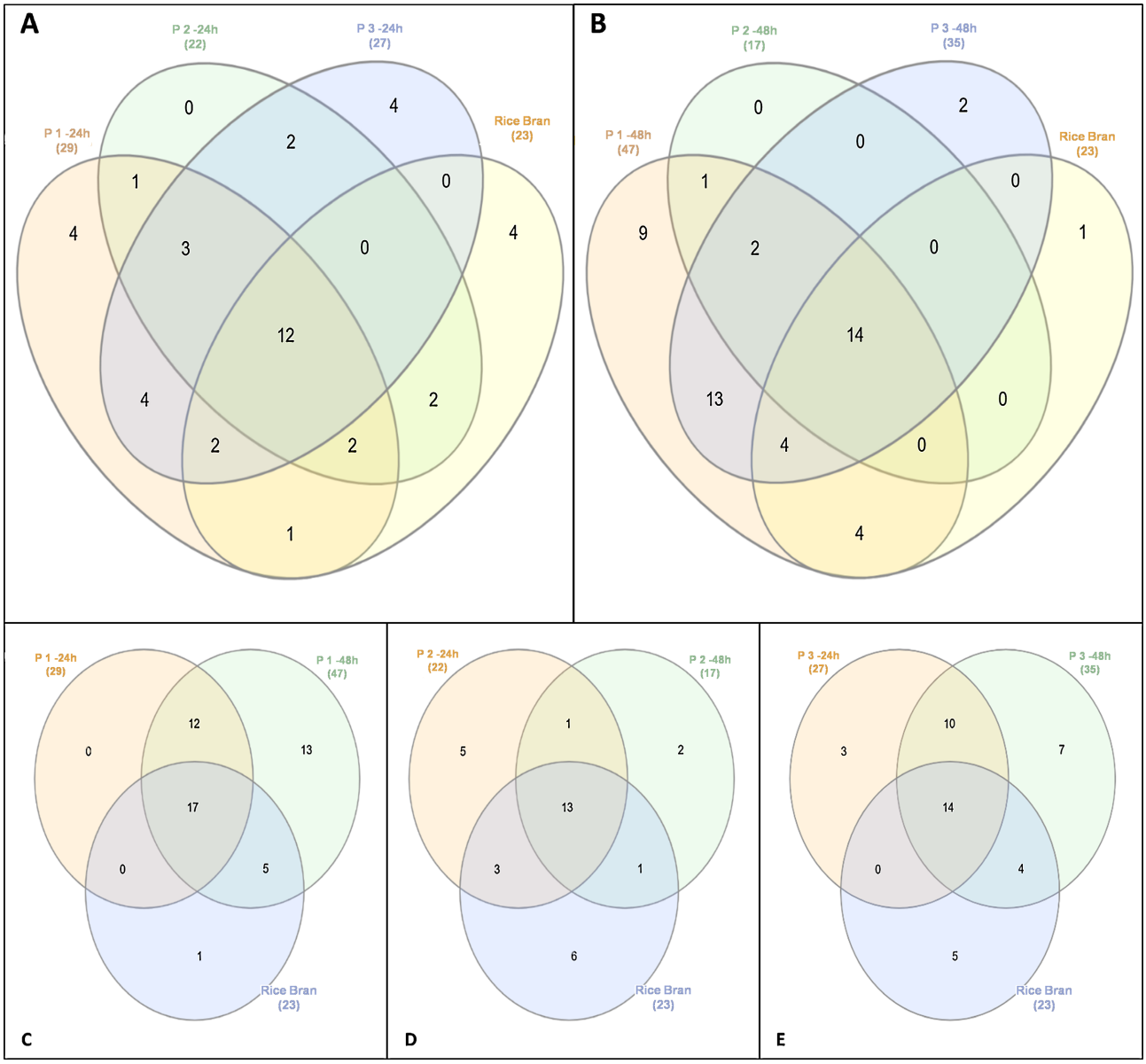
Relative abundance (%) of major fungal phyla (Ascomycota 69.5%, Mucoromycota 30.5%) in rice bran fermented with commercial products 1 - 3 at 24 and 48 h compared with the dry rice bran control.

Figure 7. Relative abundance (%) of fungal phyla in rice bran fermented with three commercial microbial products at 24 and 48 h.



PCoA of fungal communities in rice bran fermented with products 1 - 3 at 24/48 h versus dry control. 48 h treatments cluster together; product 1 - 24 h and dry bran separate.

Figure 8. Principal coordinates analysis (PCoA) of fungal communities showing relationships between commercial microbial products and fermentation times (24 and 48 h).



Venn diagrams of shared fungal taxa in rice bran fermented with products 1 - 3 at 24 h (A; highest: product 1) and 48 h (B; highest: product 1) versus other treatments.

Figure 9. Venn diagram showing shared fungal taxa between fermentation times (24 and 48 h) and microbial blends in solid-state rice bran fermentation.

4. Discussion

The three commercial products evaluated differ systematically in microbial complexity and biochemical composition. Product 1 features a complex formulation with probiotic consortia (*Bacillus subtilis*, *Enterococcus faecium*, *Lactobacillus acidophilus*), exogenous enzymes (protease, phytase), and minerals, which may have influenced the observed fermentation responses [40]. This contrasts with Product 2 (second-generation microencapsulated *Saccharomyces cerevisiae*), which prioritizes simplicity and prolonged stability [42], and Product 3, which

contains a diverse microbiota including multiple yeasts, algae, and DHA-rich *Schizochytrium* [41]. Because the commercial inoculants contained microorganisms, enzymes, minerals, vitamins, algae, and other additives, some of the observed changes may reflect both product composition and fermentation dynamics rather than fermentation alone.

Progressive pH decline (**Figure 1**) is consistent with established *S. cerevisiae* rice bran SSF patterns and may be associated with organic acid production (lactic/acetic) and CO_2 release [43] [44]. A pH range of 4.5 - 5.0 is generally considered favorable for yeast activity and saccharification, while very acidic conditions may reduce performance [45]. Complex inoculants (P1/P3) may have contributed to faster initial acidification, possibly through enzymatic pre-digestion of bran macromolecules by *Bacillus*-associated activities [46].

SSF-mediated increases in protein, fiber, and ash, together with reductions in moisture and lipid content, are consistent with microbial proteolysis and biomass accumulation during fermentation, although these mechanisms were not directly quantified in the present study [47]. The initial crude protein decline observed in Product 1 may indicate exogenous protease activity and early protein hydrolysis, as reported for *Rhizopus*-based rice bran SSF systems [48]. Product 2 exhibited the highest final protein levels, which may be associated with maintained viability of microencapsulated *S. cerevisiae*; however, direct comparisons with bacterial consortia should be interpreted cautiously [49].

The observed, non-significant increase in crude fiber may reflect the contribution of microbial biomass components, such as bacterial cell wall polysaccharides and yeast chitin/glucan, as previously reported in SSF systems [50] [51]. Given the lack of statistical significance, this interpretation remains tentative.

The observed descriptive organic carbon depletion (**Figure 3**) may indicate microbial assimilation associated with biomass and metabolite synthesis, including amino-acid production [52].

SSF-treated rice bran descriptively showed higher levels of amino acids that are considered limiting for *Penaeus vannamei* diets, notably lysine [53], and the lysine increases observed in this study were greater than those reported by Vieira [54]. Patterns in methionine and other amino acids (**Figure 2**) suggest that SSF rice bran may have potential as a partial fishmeal alternative; however, this potential must be confirmed in feeding trials and performance studies [43]. Importantly, amino acid, organic carbon, and metagenomic data were obtained from pooled composite samples; therefore, these results are presented descriptively and are not suited for formal treatment-level inferential statistics.

A limitation of the present study is that antinutritional factors (e.g., phytates) were not directly measured; consequently, any reduction in ANFs should be interpreted as a potential benefit inferred from previous studies rather than as an experimentally demonstrated outcome of this work.

Post-SSF, Firmicutes remained the dominant phylum across the fermented treatments, which is consistent with the composition of the commercial inocu-

lants, as they contained Firmicutes-associated taxa such as *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, and *Rummeliibacillus* [55]. The observed transient increase in Proteobacteria in Product 1 at 24 h (42.5%) likely reflects an early stage of community adjustment during fermentation, whereas the return to near-complete *Firmicutes* dominance at 48 h suggests a later stabilization of the bacterial community. Thus, the Proteobacteria peak should be interpreted as a treatment- and time-specific shift rather than as a contradiction of the overall Firmicutes-dominated profile.

Probiotic acidogenesis may have contributed to conditions less favorable for *Clostridium* while favoring the relative abundance of *Enterococcus*, *Pediococcus*, and *Rummeliibacillus*, which is consistent with moisture- and nutrient-dependent growth patterns reported in previous studies [56] [57].

Ascomycota/*Saccharomyces* succession may be associated with a reduction in the relative abundance of native *Rhizopus*, possibly due to shifts in pH and moisture that favor ascomycete growth and enzyme activity [8]. *S. cerevisiae* reached near-monodominance in P2 and P3 (**Figure 7**), which may reflect its high competitiveness under the fermentation conditions.

These microbial dynamics suggest that SSF rice bran may have promise as an aquaculture ingredient and may potentially contribute to partial fishmeal replacement, but these applications require confirmation in feeding trials. Product 2 may offer practical advantages for protein enrichment at scale, whereas Product 1 may be more favorable for improving amino acid content under the conditions tested. Further feeding trials and safety evaluations are needed to confirm industrial applicability.

5. Conclusions

Commercial inoculants of differing complexity appeared to influence rice bran SSF, as reflected in changes in pH, proximate composition, amino acid profiles, and microbial community structure. Product 3 showed broader compositional complexity, whereas Product 2 may offer practical advantages in formulation simplicity and stability. Further studies, including feeding trials and challenge assays, are needed to evaluate the potential of SSF rice bran as a sustainable ingredient for *Penaeus vannamei* diets.

Author Contributions

Álvaro Carlos Díaz Chacho: Conceptualization, methodology, investigation, data curation, formal analysis, writing—original draft, writing—review and editing, and project administration. Maria Helena Araújo: Methodology, data curation, and writing—review and editing. Mateus Aranha Martins: Investigation, formal analysis, data curation, and writing—review and editing. Scheila Pereira Dutra: Methodology, supervision, validation, and writing—review and editing. Felipe Boéchat Vieira: Conceptualization, supervision, project administration, resources, and writing—review and editing. All authors read and approved the final

version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Lima, A.F., Silva-Filho, J.D., *et al.* (2024) Manual de piscicultura familiar em viveiros escavados. Embrapa, 156 p.

[2] Chary, K., van Riel, A.-J., Muscat, A., *et al.* (2024) Transforming Sustainable Aquaculture by Applying Circularity Principles. *Reviews in Aquaculture*, **16**, 656-673.

[3] Gil, L.M.P. (2024) Abordagens sustentáveis na aquicultura: Um exame da cadeia produtiva em santo inácio, piauí. Dissertação, Universidade Federal do Ceará, 129 p.

[4] Fernandes, S.S. and Latorres, J.M. (2022) Aproveitamento de resíduos para a alimentação humana. Ed. da FURG, 122 p.

[5] Soares, K.J.A., Ribeiro, F.B., Bomfim, M.A.D. and Marchão, R.S. (2017) Valor nutricional de alimentos alternativos para tambaqui (Colossoma macropomum). *Archivos de Zootecnia*, **66**, 491-497. https://doi.org/10.21071/az.v66i256.2764

[6] Reis, S.R.D. (2022) Análise dos sistemas industriais de produção de ovos: Um comparativo dos custos de produção entre métodos convencional e alternativos. Mestre em Administração, Universidade Estadual Paulista, 86.

[7] Fernandes, I.M. (2021) Extração de produtos e valorização do farelo de arroz com tecnologias mais verdes. Tese, Instituto Superior de Engenharia de Lisboa, 120 p.

[8] Kawski, V.L. (2015) Avaliação de estratégias de estabilização oxidativa do farelo de arroz integral. Dissertação, Universidade Federal de Santa Maria, 93 p.

[9] Kuila, A. and Sharma, V. (2018) Principles and Applications of Fermentation Technology. John Wiley & Sons. https://doi.org/10.1002/9781119460381.

[10] Santos, S.F.D.M., Macedo, G.R.D., Silva, F.L.H.D., Souza, R.L.A.D. and Pinto, G.A.S. (2008) Aplicação da metodologia de superfície de resposta no estudo da produção e extração da poligalacturonase. *Química Nova*, **31**, 1973-1978. https://doi.org/10.1590/s0100-40422008000800010

[11] Silveira, C.M.D. and Furlong, E.B. (2007) Caracterização de compostos nitrogenados presentes em farelos fermentados em estado sólido. *Ciência e Tecnologia de Alimentos*, **27**, 805-811. https://doi.org/10.1590/s0101-20612007000400021

[12] Samtiya, M., Aluko, R.E., Puniya, A.K. and Dhewa, T. (2021) Enhancing Micronutrients Bioavailability through Fermentation of Plant-Based Foods: A Concise Review.

Fermentation, **7**, Article 63.
https://doi.org/10.3390/fermentation7020063

[13] Siqueira, T.V. (2018) Aquicultura: A nova fronteira para produção de alimentos de forma sustentável. *Revista do BNDES*, **25**, 119-170.

[14] Igarashi, M.A. (2019) Aspectos do potencial econômico da piscicultura, contribuição e perspectivas da atividade para o desenvolvimento sustentável no Brasil. *Revista Unimar Ciências*, **28**, 18-25.

[15] Fraterrigo Garofalo, S., Tommasi, T. and Fino, D. (2021) A Short Review of Green Extraction Technologies for Rice Bran Oil. *Biomass Conversion and Biorefinery*, **11**, 569-587. https://doi.org/10.1007/s13399-020-00846-3

[16] Dai, C., Hou, Y., Xu, H., Umego, E.C., Huang, L., He, R., *et al.* (2021) Identification of a Thermophilic Protease-Producing Strain and Its Application in Solid-State Fermentation of Soybean Meal. *Journal of the Science of Food and Agriculture*, **102**, 2359-2370. https://doi.org/10.1002/jsfa.11574

[17] Wlazło, Ł., Kwiecień, M., Bis-Wencel, H., Łopuszyński, W., Buszewicz, G., Karpińska, K., *et al.* (2023) Assessment of Health Safety of Pigs Taking Natural Sorbents with Feed. *BMC Veterinary Research*, **19**, Article No. 3.
https://doi.org/10.1186/s12917-022-03563-3

[18] Lucas, B. and Sotelo, A. (1980) Effect of Different Alkalies, Temperature, and Hydrolysis Times on Tryptophan Determination of Pure Proteins and of Foods. *Analytical Biochemistry*, **109**, 192-197. https://doi.org/10.1016/0003-2697(80)90028-7

[19] White, J.A., Hart, R.J. and Fry, J.C. (1986) An Evaluation of the Waters Pico-Tag System for the Amino-Acid Analysis of Food Materials. *Journal of Analytical Methods in Chemistry*, **8**, 170-177. https://doi.org/10.1155/s1463924686000330

[20] Hagen, S.R., Frost, B. and Augustin, J. (1989) Precolumn Phenylisothiocyanate Derivatization and Liquid Chromatography of Amino Acids in Food. *Journal of AOAC International*, **72**, 912-916. https://doi.org/10.1093/jaoac/72.6.912

[21] Dias, L.T.S. (2022) Otimização e validação de metodologia quantitativa para determinação de fenilalanina em farinha de trigo empregando cromatografia líquida de alta eficiência com detector UV-VIS. Dissertação, Universidade Federal de Minas Gerais, 82 p.

[22] Walkley, A. and Black, I.A. (1934) An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Science*, **37**, 29-38.
https://doi.org/10.1097/00010694-193401000-00003

[23] Aller-Rojas, O., Moreno, B., Aponte, H. and Zavala, J. (2020) Carbon Storage Estimation of *Lessonia trabeculata* Kelp Beds in Southern Peru: An Analysis from the San Juan De Marcona Region. *Carbon Management*, **11**, 525-532.
https://doi.org/10.1080/17583004.2020.1808765

[24] Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010) QIIME Allows Analysis of High-Throughput Community Sequencing Data. *Nature Methods*, **7**, 335-336. https://doi.org/10.1038/nmeth.f.303

[25] Martins, M.A., Mouriño, J.L.P., Seiffert, W.Q. and do Nascimento Vieira, F. (2022) Predictive Functional Profiling of Water Bacterial Community in the Integrated Culture of Pacific White Shrimp and Nile Tilapia under Heterotrophic and Mature Biofloc Systems. *Aquaculture Research*, **53**, 5428-5433.
https://doi.org/10.1111/are.16000

[26] Tawfik, S.A., Azab, M.M., Ahmed, A.A.A. and Fayyad, D.M. (2018) Illumina MiSeq

Secuen-ciación para análisis preliminar del microbioma que causa infecciones endodónticas primarias en Egipto. *International Journal of Microbiology*, **2018**, 1-15. https://doi.org/10.1155/2018/2837328

[27] Andrews, S. (2010) FastQC: A Quality Control Tool for High Throughput Sequence Data. Babraham Bioinformatics.

[28] Bolger, A.M., Lohse, M. and Usadel, B. (2014) Trimmomatic: A Flexible Trimmer for Illumina Sequence Data. *Bioinformatics*, **30**, 2114-2120. https://doi.org/10.1093/bioinformatics/btu170

[29] Rognes, T., Flouri, T., Nichols, B., Quince, C. and Mahé, F. (2016) VSEARCH: A Versatile Open Source Tool for Metagenomics. *PeerJ*, **4**, e2584. https://doi.org/10.7717/peerj.2584

[30] Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., *et al.* (2009) BLAST+: Architecture and Applications. *BMC Bioinformatics*, **10**, Article No. 421. https://doi.org/10.1186/1471-2105-10-421

[31] Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., *et al.* (2012) The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Research*, **41**, D590-D596. https://doi.org/10.1093/nar/gks1219

[32] Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Ostell, J., Pruitt, K.D., *et al.* (2017) GenBank. *Nucleic Acids Research*, **46**, D41-D47. https://doi.org/10.1093/nar/gkx1094

[33] Heather, J.M. and Chain, B. (2016) The Sequence of Sequencers: The History of Sequencing DNA. *Genomics*, **107**, 1-8. https://doi.org/10.1016/j.ygeno.2015.11.003

[34] Ravi, R.K., Walton, K. and Khosroheidari, M. (2018) MiSeq: A Next Generation Sequencing Platform for Genomic Analysis. In: DiStefano, J., Ed., *Methods in Molecular Biology*, Springer, 223-232. https://doi.org/10.1007/978-1-4939-7471-9_12

[35] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic Local Alignment Search Tool. *Journal of Molecular Biology*, **215**, 403-410. https://doi.org/10.1016/s0022-2836(05)80360-2

[36] Sayers, E.W., Bolton, E.E., Brister, J.R., Canese, K., Chan, J., Comeau, D.C., *et al.* (2021) Database Resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, **50**, D20-D26. https://doi.org/10.1093/nar/gkab1112

[37] Cock, P.J.A., Antao, T., Chang, J.T., Chapman, B.A., Cox, C.J., Dalke, A., *et al.* (2009) Biopython: Freely Available Python Tools for Computational Molecular Biology and Bioinformatics. *Bioinformatics*, **25**, 1422-1423. https://doi.org/10.1093/bioinformatics/btp163

[38] Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G. and Neufeld, J.D. (2012) PAN-DAseq: Paired-End Assembler for Illumina Sequences. *BMC Bioinformatics*, **13**, Article No. 31. https://doi.org/10.1186/1471-2105-13-31

[39] The Jamovi Project (2022) Jamovi (Version 2.3) [Software]. https://www.jamovi.org

[40] Zimmermann Aqua Solutions (2019) Fermented Aquafeed Premix. https://www.fermentaqua.com/en/

[41] Alltech (2022) Alltech Agri-Food Outlook 2022: Global Feed Production Survey and Industry Trends. Perspectives of the Agrifood Sector. https://www.alltech.com/agri-food-outlook

[42] Ribeiro, A.C., Graça, C.D.S. and Chiattoni, L.M. (2017) Fermentation Process in the Availability of Nutrients in Rice Bran. *Journal of Microbiology and Biotechnology*, **6**, 45-52.

[43] Feddern, V., Furlong, E.B. and Soares, L.A.D.S. (2007) Efeitos da fermentação nas propriedades físico-químicas e nutricionais do farelo de arroz. *Ciência e Tecnologia de Alimentos*, **27**, 800-804. https://doi.org/10.1590/s0101-20612007000400020

[44] Sousa, D.D.S., Baptista, J.A.A. and Zan, R.A. (2018) Produção e avaliação físico-química dos vinhos (fermentados) seco e suave a partir do araçá-boi (Eugenia Stipitata McVaugh). *Multi-Science Journal*, **1**, 27-29. https://doi.org/10.33837/msj.v1i13.594

[45] White, C. and Zainasheff, J. (2020) Levedura: Guia prático para a fermentação de cerveja. 1st Edition, Editora Krater, 328 p.

[46] Bakker, C.M.C.N. (2017) Avaliação da produção e aplicação de enzimas utilizando resíduo farelo de trigo como substrato por fermentação em estado sólido. Tese, Universidade Federal do Rio Grande do Norte, 143 p.

[47] Lacerda, D.B.C.L., Soares Júnior, M.S., Bassinello, P.Z., Castro, M.V.L.D., Silva-Lobo, V.L., Campos, M.R.H., *et al.* (2010) Qualidade de farelos de arroz cru, extrusado e parboilizado. *Pesquisa Agropecuária Tropical*, **40**, 521-530. https://doi.org/10.1590/s1983-40632010000400004

[48] Magalhães, A.P.L. and Teixeira, A.Z.A. (2020) Organoleptic and Protein Analysis of Rice Bran Fermentation with Saccharomyces Cerevisiae Yeast. *Revista Iberoamericana de Humanidades, Ciências e Educação*, **6**, 90-95.

[49] Célia, J.A. (2015) Influência do tratamento térmico nas características físi-co-químicas e reológicas de iogurtes naturais. Dissertação, Instituto Federal de Educação, Ciência e Tecnologia Goiano, 58 p.

[50] Oliveira, M.D.S. (2009) Disponibilização de compostos funcionais em farelo de arroz fermentado em estado sólido. Tese, Universidade Federal do Rio Grande, 132 p.

[51] Bouzon, Z.L., Gargioni, R. and Ouriques, L. (2010) Biologia Celular. 2nd Edition, BIOLOGIA/EAD/UFSC, 238 p.

[52] Liñan-Vidriales, M.A., Peña-Rodríguez, A., Tovar-Ramírez, D., Elizondo-González, R., Barajas-Sandoval, D.R., Ponce-Gracía, E.I., *et al.* (2021) Effect of Rice Bran Fermented with Bacillus and Lysinibacillus Species on Dynamic Microbial Activity of Pacific White Shrimp (*Penaeus vannamei*). *Aquaculture*, **531**, Article 735958. https://doi.org/10.1016/j.aquaculture.2020.735958

[53] Hardy, R.W., *et al.* (2011) Nutrient Requirements of Fish and Shrimp. National Academies Press, 624-6242.

[54] Vieira, A.D. (2023) Influência de combo microbiano na caracterização do farelo de arroz fermentado. Dissertação, Universidade Federal de Santa Catarina, 63 p.

[55] Rabelo, C.A. (2018) Otimização da produção de hidrogênio e ácidos orgânicos em reator em batelada a partir de consórcio de bactérias autóctones e alóctones do bagaço de cana-de-açúcar. Tese, Universidade de São Paulo, 156 p.

[56] Wendling, L.K. and Weschenfelder, S. (2013) Probióticos e alimentos lácteos fermentados—Uma revisão. *Revista do Instituto de Laticínios Cândido Tostes*, **68**, 49-57. https://doi.org/10.5935/2238-6416.20130048

[57] Manfredini, P.G. (2020) Produção concomitante de proteases e peptídeos bioativos por fermentação em estado sólido. Dissertação, Universidade de Passo Fundo, 171 p.