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Amomum aromaticum prebiotic-like polysaccharides enhance yeast fermentation and antioxidant profile of traditional rice beer - '*kiad*'

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Traditional rice-based fermented beverages contain plant additives such as *Amomum aromaticum*, which provide bioactive polysaccharides that function as prebiotics and enhance beverage properties. Understanding *the interaction between A. aromaticum polysaccharides and* yeast during fermentation is vital for developing health-promoting foods. The rice beer samples were collected from Meghalaya brewer. Prebiotic-like polysaccharides from *Amomum aromaticum* underwent acid and enzymatic digestion. FTIR analysis was used to characterize the functional groups. Yeasts were identified through ITS sequencing, and their growth in the presence of the prebiotic-like polysaccharides was assessed using resazurin and crystal violet assays. Biochemical, antioxidant, and GC-MS analyses of rice beer samples were performed. Prebiotic-like polysaccharides yielded 2.7% with 13.00±1.07 µg/mL of total carbohydrates. Post treatment, 28.5 µg/mL total sugars and 103.78±1.58 µg/mL reducing sugars. FTIR confirmed the presence of polysaccharide groups. Rice beer, Sohra (RB23), showed higher acidity (pH ~4.0, 0.50% lactic acid) than rice beer, Tyrsad (RB16) (pH ~4.3, 0.30%). RB16 had higher protein and carbohydrate (40.75 and 63.71 µg/mL) than RB23 (15.76 and 62.64 µg/mL, respectively). RB23 exhibited the highest DPPH radical scavenging activity (94.88%), followed by RB16 (86.38%) and PLP extract (86.33%).GC-MS showed that RB23 contained ethyl acetate, benzeneacetaldehyde, and ethyl hydrogen succinate, while RB16 contained 2-thiazolamine. The yeast was identified as *Saccharomyces cerevisiae* (GenBank: MK942688). Prebiotic-like polysaccharides enhanced yeast biofilm formation by 17.07% at 6 µg/mL and 8.87% at 0.06 µg/mL demonstrating a concentration-dependent stimulatory effect. *Amomum aromaticum* polysaccharides enhanced yeast activity, improved nutritional and antioxidant properties, contributed to flavor profiles, and supported *S. cerevisiae* growth, suggesting its potential as a functional beverage additive.

Keywords: *Amomum aromaticum*, Antioxidant activity, Fermentation, Prebiotic polysaccharides, Rice beer, *Saccharomyces cerevisiae*

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Traditionally, fermented foods are rich sources of probiotics and prebiotics, offering health advantages that are just beginning to be understood[1]. In Northeast India, traditional rice beers crafted from *Amomum aromaticum* (Zingiberaceae)[2] are believed to have digestive and therapeutic benefits[3]. Locally known as Khawiang, *A. aromaticum* alleviates dyspepsia, diarrhea, and respiratory issues[4]. Although its anti-inflammatory and antimicrobial properties are well-documented[5,6], its role as a prebiotic enhancer in symbiotic fermentation systems has not yet been explored. *Amomum tsao-ko* extracts have demonstrated bifidogenic activity *in vitro*, indicating their potential as prebiotics[7,8]. As significant as *A. aromaticum* is ethnobotanically used in traditional fermentations, the biochemical mechanisms through which its polysaccharides regulate *S. cerevisiae* metabolism, specifically biofilm formation, and its impact on antioxidant synthesis and volatile compound production in beer, is unknown. This disconnection between ethnobotanical knowledge and mechanistic validation hinders the development of functional food products. Indigenous species such as *A. aromaticum* could help bridge this gap while preserving traditional wisdom. Given the emerging need for pathogen control strategies, such as probiotic biofilms enhanced by prebiotics for pathogen clearance, understanding how *A. aromaticum* polysaccharides fortify yeast biofilms offers timely biochemical insights[9]. This work interprets the biochemical interaction of *A. aromaticum*-derived prebiotic-like polysaccharides and their plausible utilization by *S. cerevisiae* during

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fermentation to improve the functional outputs in the final rice beer produced. Prebiotics and probiotics work together to improve gut health by balancing microbial populations. Fermented traditional foods such as rice beer from Meghalaya are sources of both.

Amomum aromaticum, a member of the ginger family, is used in local fermentation[10]; however, its prebiotic potential remains largely unexplored. Khasi and Jaintia communities produce rice beer *'kiad'* using plant-based starters, *Thiat*, including *A. aromaticum* and various herbs[3,11]. These traditional recipes provide both flavor and health benefits. Alcoholic fermented rice-based beverages are enriched with medicinal benefits due to their microflora and medicinal plants, emphasizing the importance of starter plants in fermentation[12]. Despite their significance, systematic compositional studies have been scarce. Research on Assam rice beers has revealed notable antioxidant and phenolic contents (DPPH scavenging ~2–4 mg ascorbate-equiv/mL, phenolics ~2–5.4 mg gallic-acid-equiv/mL)[13], and GC-MS metabolomics has identified sugars, acids, alcohols, and nutraceuticals[14].

We compared rice beer from Sohra and Tyrsad to explore how local practices affect its chemistry, while investigating the "prebiotic-like" effects of *A. aromaticum* on yeast. Plant materials provide microbial inocula and bioactive compounds that can influence microbial growth and beverage production. We also examined the chemical characteristics of '*kiad'* from two locations and the influence of *A. aromaticum* extracts on the growth of *Saccharomyces cerevisiae* isolated from *kiad*. To bridge this knowledge gap, we performed FTIR-based functional group analysis of polysaccharides, DPPH scavenging tests, and yeast biofilm quantitation to elucidate structure-function relationships in rice beer matrices. This allowed us to investigate the influence of *A. aromaticum*-derived prebiotics on the behavior and growth of *S. cerevisiae* (Fig. 1). Understanding *A. aromaticum*'s biochemical function in promoting yeast biofilms and functional metabolites, this study offers a template for leveraging traditional knowledge in the design of enzymatically optimized, health-enhancing

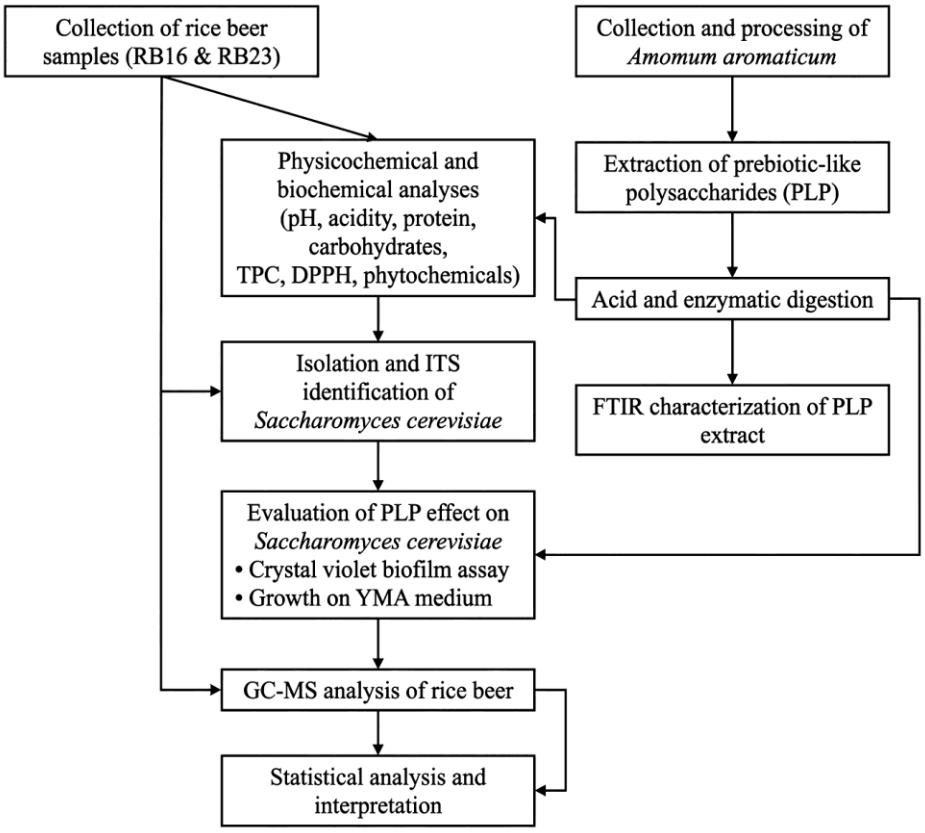

Fig. 1 — Schematic workflow of the experimental design in the present study for extraction and characterization of *Amomum aromaticum* prebiotic-like polysaccharides (PLP), characterization of traditional rice beer, evaluation of the effect of PLP on *Saccharomyces cerevisiae*, and downstream analyses

fermented beverages. Further studies are required to classify the extract as a prebiotic.

Materials and Methods

Sample collection

Two traditional rice beer samples were obtained from local brewers: one from Sohra (East Khasi Hills; 25.286°N, 91.721°E) and the other from Tyrsad (West Jaintia Hills; 25.409°N, 91.673°E). Each sample (500 mL) was collected aseptically after 4-5 days fermentation in earthen pots. The beers were clarified by sedimentation and stored at 4°C until further analysis. Fresh *Amomum aromaticum* plant material (leaves and rhizomes) was collected from wild plants near Tyrsad, and washed. The plant material was air-dried at room temperature (20-25°C) for 10-20 days, pulverized to a fine powder, and stored in airtight containers. All samples were assigned unique identification codes and labeled with collection dates.

Extraction of prebiotic-like polysaccharides (PLP)

A 20 g of the plant powder was combined with 200 mL of 80% ethanol. The mixture was continuously stirred for 5 h using a magnetic stirrer on a ceramic heating plate, followed by extraction in a hot water bath at 90°C for 3 h. The extract was then filtered through four layers of cotton cheesecloth. The filtrate was then concentrated to 1/50 of its original volume using a rotary evaporator at 60°C. The insoluble materials were removed by centrifugation at 8,000 rpm for 10 min. The digested extract was transferred into regenerated seamless cellulose dialysis tubing (HiMedia, LA387-5MT; molecular weight cut-off (MWCO) 12–14 kDa; pore size 2.4 nm) and dialyzed against distilled water at 4°C for 24 h. Prebiotic-like polysaccharides (PLP) were isolated from the dialyzed samples through ethanol precipitation. The resulting precipitate was oven dried at 50°C until it reached a constant weight. The dried extract was stored in a crucible disc or a dark bottle for further analysis[15]. The plant extract yield was calculated using the following formula:

$$Yield(\%) = \left(\frac{Weight\ of\ dried\ PLP\ extract\ (g)}{Weight\ of\ dried\ plant\ powder\ (g)}\right) * 100$$

Resistance to acidity and enzymatic digestion of the prebiotic-like polysaccharides

Aqueous solutions (10% w/v) were prepared from the PLP using distilled water. Acidic digestion was performed by incubating the solutions at 37°C in HCl buffer (pH 1) for 4 h following previously reported protocol[16,17] with modifications. The reaction was terminated using 1N NaOH. Subsequently, enzymatic digestion was conducted by incubating the acid-digested solutions at 37°C with α-amylase (SRL Pvt. Ltd., 95114; activity: 5 units/mg) was used at 2U/mL in a 0.1M phosphate buffer (pH 6.9) for 6 h. The enzymatic digestion was halted by heating at 80°C for 10 min. All digestions were performed in triplicates. To quantify the indigestible polysaccharides, the extracts were analyzed for reducing sugar content (mg/g) using a modified dinitrosalicylic acid method[17]. The total sugar content (mg/g) of the digests was determined using a modified phenol sulfuric method[18]. Indigestible polysaccharide content (mg/g dry extract) was calculated as follows: Indigestible polysaccharides (mg/g) = Total sugar after acid-enzyme digestions (mg/g) – Reducing sugar before digestions (mg/g)

FTIR-analysis of the prebiotic-like polysaccharides

An aliquot of dried PLP was combined with potassium bromide (KBr) and compressed into a pellet for Fourier Transform Infrared (FTIR) spectroscopic analysis. Spectra were recorded using a Bruker FTIR spectrometer over a range of 4000–400 cm^{-1}. Major absorption bands were identified based on existing literature. The sample preparation involved encapsulating 10 mg of dried PLP powder in a 100 mg KBr pellet to create translucent discs. FTIR spectroscopy was set to a scan range of 400–4000 cm−1 with a resolution of 4 cm^{-1}. Spectra were baseline-corrected and normalized using OPUS 7.5 software. Peak assignments followed published polysaccharide signatures[19].

Physicochemical analyses of rice beer

Physicochemical parameters were measured in triplicate for each beer sample. The pH and temperature of rice beer samples were measured at 25°C using a calibrated digital pH meter. For the measurements, 10 mL of each sample was placed in a clean beaker. Titratable acidity was determined using potentiometric titration. A 10 mL aliquot of the sample was titrated against 0.05 N sodium hydroxide (NaOH) with phenolphthalein indicator. The volume of NaOH needed to reach pH 8.2 was recorded using a pH meter. Titratable acidity was expressed as grams of lactic acid per litre of beer.

Protein content

The protein content of the rice beers and PLP were quantified using a modified Bradford assay. Bradford reagent was added to 1 mL of each sample until color change was observed. The absorbance was measured spectrophotometrically at 595 nm. Protein concentrations were expressed as BSA equivalents (mg/mL), using BSA as a standard[20].

Carbohydrate content

The carbohydrate content was determined using the anthrone method[21]. Rice beers (1:1000 dilution) and PLP (1 µg/mL) were mixed with anthrone reagent (1:4 ratio), incubated in boiling water for 10 min, and cooled. The absorbance was measured at 620 nm. The results were expressed as glucose equivalents (mg/mL).

Total phenolic content

Folin-Ciocalteu reagent was used to determine the total phenolic content (TPC), as described in published literature[22]. The beer samples were mixed with Folin-Ciocalteu reagent, and the aqueous sodium absorbance was read at 650 nm. TPC was expressed as mg/mL of gallic acid, using gallic acid as the standard equivalent. The absorbance was measured at 650 nm.

Antioxidant activity by DPPH

The DPPH free radical scavenging activity was determined spectrophotometrically. Rice beers were mixed with 2.9 mL ethanol DPPH solution, and 2.75 mL ethanol DPPH solution was mixed with the PLP. The mixtures were incubated in the dark at room temperature for 30 min, and the absorbance was measured at 515 nm. Relative antioxidant activity was calculated as follows:

$$RSA(\%) = ((ADPPH - Asample)/ADPPH)\ ) * 100$$

Where A_{DPPH} is DPPH solution absorbance and A_{sample} is absorbance of rice beer and the PLP. All measurements were performed in triplicate and the results are presented as mean values[22]. Results expressed as % scavenging relative to ascorbic acid standard (IC_{50} in µg ascorbic acid equivalents (AAE)/mL).

Reducing sugar content

Reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNSA) method, as described in published literature[17]. A 0.4 mL aliquot of the PLP (1 µg/mL) was combined with 2 mL of DNSA reagent and thoroughly mixed at room temperature for 10 min. The mixture was incubated in a water bath at 85°C for 5 min. Subsequently, 1 mL of distilled water was added and the absorbance was measured spectrophotometrically at 575 nm. The reducing sugar content was expressed as glucose equivalents (mg/mL) with glucose serving as the standard.

Qualitative phytochemical analyses

Phytochemical analysis was conducted on the rice beers, and the PLP was dissolved in ethanol, following standard procedures to identify the constituents[23]. Tests for flavonoids, carbohydrates, reducing sugars, saponins, tannins, alkaloids, terpenoids, coumarins, and phlobatannins were also performed.

GC-MS analysis of rice beers

GC-MS analysis was performed using an Agilent 597x single quadrupole mass spectrometer with a gas chromatograph and an ALS. Separation was performed on an Agilent HP-5ms capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness; temperature range: −60°C to 325°C). The GC oven temperature was set at 50°C, ramped at 10°C/min to 200°C (2-min hold), and then 10°C/min to 280°C (2-min hold). Run time was 27 min, with 60°C post-run temperature and 1-min equilibration. The inlet was operated in pulsed split mode (10:1) at 250°C, with a 25-psi injection pulse pressure for 0.5 min. Helium carrier gas flowed at 1 mL/min (column pressure: 7.6522 psi). The septum purge and total flow rates were 3 mL/min and 14 mL/min, respectively. The mass spectrometer was operated in electron ionization mode at 70 eV, with source and quadrupole temperatures of 230°C and 150°C, respectively. Mass spectral data were acquired in full scan mode (40–550 m/z) at 1,562 u/sec with a 150-count threshold and an electron multiplier voltage of 1200 V (gain factor 1).Volatiles were identified using NIST 2020 library.

Isolation and identification of yeast from rice beers

The rice beer samples were processed for yeast isolation by serial dilution and spread plating. Dilutions were prepared in sterile 0.8% saline-distilled water and plated on YMA and SDA media. Plates were incubated at 25–28°C for 1–2 days, with daily monitoring. Distinct colonies were selected and sub-cultured for purity by quadrant streaking. Isolates were maintained on YMA slants at 4°C. Distinct morphological colonies were stained with lactophenol

cotton blue and examined under a 100× oil immersion microscope. Genomic DNA was extracted using the phenol-chloroform protocol, and the internal transcribed spacer (ITS) region was amplified using universal fungal primers. Sequences were analysed using BLAST against NCBI GenBank for taxonomic identification, and confirmed sequences were submitted to obtain accession numbers.

Effect of prebiotic-like polysaccharides on yeast

Biofilm formation was assessed to evaluate prebiotic-driven adhesion as a key determinant of fermentation efficiency[24]. Dilutions of the prebiotics were prepared in Saboraud Dextrose broth in microtiter plate wells, corresponding to 6 and 0.06 µg/mL with 200 µL per well. Yeast culture (20 µL) was added to each well and the plates were incubated overnight at 37°C. After incubation, the spent medium was decanted and the wells were washed with sterile PBS (pH 7.4). The wells were stained with 1% crystal violet solution for 10 min and washed to remove the unbound dye. After drying at room temperature for 2-3 h, 30% glacial acetic acid was added, and the absorbance was measured at 575 nm using spectrometry[25]. The value of CV retained in the wells is indicative of the amount of biofilm produced by the yeast. Spread plate technique was also used to evaluate the utilization of prebiotic-like polysaccharides by yeast. Two YMA plate types were prepared: YMA alone and YMA with 1 mL of prebiotic-like polysaccharides per 30 mL of medium. The prebiotic medium was mixed and cooled before use. For both types, 100 µL yeast broth was spread on each plate. YMA plates without inoculation served as controls. Plates were incubated at room temperature for 24 h, then growth variations on the agar surface were observed.

Statistics analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analyses were performed using the Data Analysis ToolPak in Microsoft Excel. Comparisons among three groups (RB16, RB23, and PLP) were analysed using one-way analysis of variance (ANOVA), whereas comparisons between two groups (RB16 and RB23) were analysed using Welch's two-sample *t*-test. A p-value of < 0.05 was considered statistically significant.

Results

Biochemical analyses of the prebiotic-like polysaccharides

Following the extraction of *Amomum aromaticum* with 80% ethanol, the yield of the dried extract was 2.7% from 20 g of plant material. The total carbohydrate content of the crude extract prior to treatment was 12.997±1.070 µg/mL, as determined by the anthrone reagent assay. Antioxidant activity, assessed by the DPPH assay before acid and enzyme digestion, showed a maximum radical scavenging activity of 74.13±9.49% in PLP extract. The extract was then subjected to acid and enzymatic digestion, followed by dialysis against distilled water at 4°C for 24 h using regenerated cellulose dialysis tubing (12-14 kDA MWCO). Post-treatment, reducing sugar content determined by the DNSA method was found to be 103.777±1.582 µg/mL at extract concentrations of 60 µg/mL, based on the glucose standard curve (Table 1).

FTIR analysis of the prebiotic-like polysaccharides

The FTIR spectrum of the PLP of *Amomum aromaticum* exhibited prominent absorption bands at 3363.79 cm^{-1}, corresponding to O–H or N–H stretching vibrations; 2936.01 cm^{-1}, indicative of C–H stretching in aliphatic compounds; 1606.95 cm^{-1}, associated with C=C or aromatic ring stretching; 1408.41 cm^{-1}, related to C–H bending or aromatic skeletal vibrations; 1077.35 cm^{-1}, assigned to C–O stretching vibrations; and 625.94 cm^{-1}, attributed to out-of-plane bending modes (Fig. 2).

Table 1 — Biochemical report of the rice beer samples (RB16 and RB23) collected from Meghalaya and the prebiotic-like polysaccharides (PLP) extracted from *Amomum aromaticum*

Samples	Protein content[a] (µg/mL)	Total carbohydrate content[a] (µg/mL)	Reducing sugar content (µg/ml)	Total phenolic content[b] (mg GAE/mL)	Maximum DPPH radical scavenging activity[a] (%)
RB16	40.751±3.540	63.709±1.137	na	0.127±0.006	86.38±1.86
RB23	15.764±0.809	62.639±0.669	na	0.122±0.003	94.88±1.63
PLP	59.566±10.419	12.997±1.070	103.777±1.582	na	74.13±9.49

Note- Values are presented as mean ± SD (n = 3).
[a] Compared using one-way ANOVA.
[b] Compared using Welch's two-sample *t*-test.
na = Not assessed.

Physicochemical analyses of rice beer

Physicochemical analysis of the rice beer samples revealed variations in acidity and temperature stability (Table 2). The rice beer samples exhibited characteristics typical of fermented beverages. Rice beer from Sohra (RB23) demonstrated higher acidity with pH 3.82±0.02 compared to the rice beer from Tyrsad (RB16) with pH 4.045±0.04. RB23 demonstrated higher titratable acidity (0.943±0.013%) compared to RB16 (0.303±0.013%), which aligns with the observed pH difference. Titratable acidity measurements, conducted using 0.05 N NaOH, varied significantly, with RB23 requiring 10.5 mL, RB16 requiring 3.4 mL.

Protein and carbohydrate content

The protein content of the samples RB16, RB23, and PLP, as determined by the Bradford assay at 595 nm, was 40.75±3.54 µg/mL, 15.76±0.81 µg/mL, and 59.57±10.42 µg/mL, respectively. The protein content differed significantly among the three groups indicated by one-way ANOVA (F = 35.69, p = 0.000466). Total carbohydrate content, measured by the anthrone method, was found to be 63.71±1.14 µg/mL for RB16, and 62.64±0.67 µg/mL for RB23 and 12.99±1.07 µg/mL for the PLP extract. One-way ANOVA revealed a significant difference in total carbohydrate content among the three groups (F = 2617.17, p< 0.001) (Table 1).

Table 2 — The physiochemical attributes of the rice beer samples from Meghalaya

Physiochemical attributes	Rice beer Sohra (RB23)	Rice beer Tyrsad (RB16)
pH	3.82±0.02	4.045±0.04
Titratable acidity (% lactic acid)	0.943±0.013	0.303±0.013

Phytochemical content and antioxidant activity

Phytochemical screening of RB16 and RB23 revealed the presence of flavonoids, carbohydrates, reducing sugars, saponins, and tannins, whereas alkaloids were detected only by the Wagner reagent in both samples. Terpenoids, coumarins, and phlobatannins were absent from both RB16 and RB23. The total phenolic content, expressed as gallic acid equivalents (GAE), was found to be 0.127±0.006 mg GAE/mL for RB16 and 0.122±0.003 mg GAE/mL for RB23. The difference was not statistically significant (Welch's t-test, p = 0.334). In the DPPH free radical scavenging assay, both rice beer samples exhibited concentration-dependent antioxidant activity, with maximum radical scavenging activity observed at 100 µL. RB23 exhibited the highest radical scavenging activity (94.88±1.63%), followed by RB16 (86.38±1.86%). PLP extract showed 74.13±9.49% maximum radical scavenging activity (Table 1). One-way ANOVA demonstrated a significant difference in the antioxidant activity among the three groups (F = 10.15, p = 0.0119).

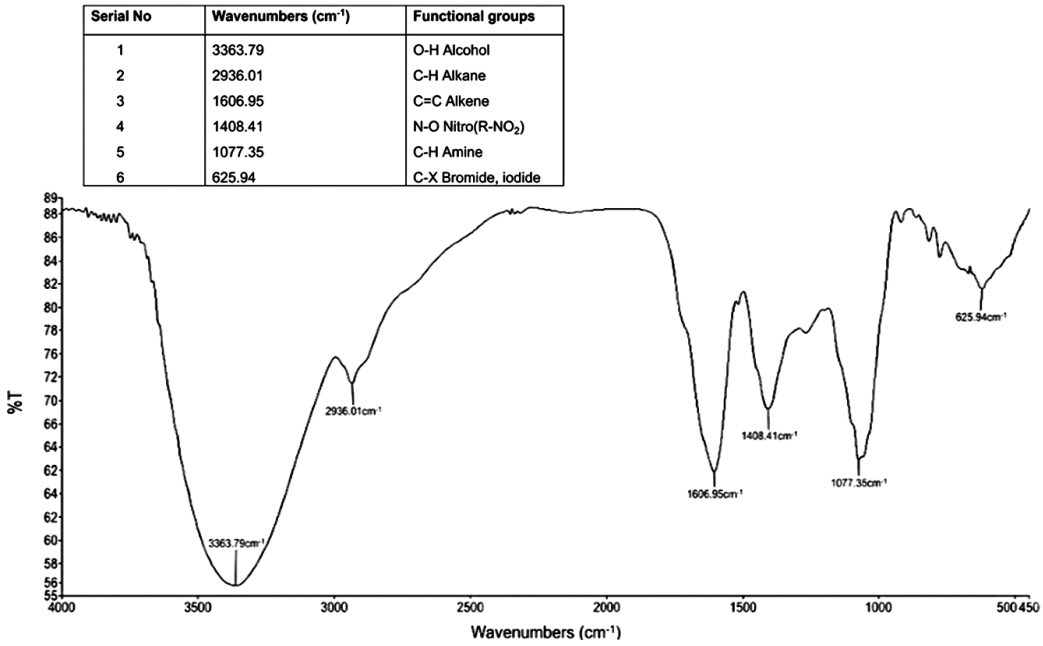

Fig. 2 — The FTIR spectra of the prebiotic-like polysaccharides extract from *Amomum aromaticum*. Table in the image indicates the plausible functional groups corresponding to the wave numbers

GCMS analyses of the rice beers

Gas chromatography-mass spectrometry (GC-MS) analysis of crude rice beer samples RB23 and RB16 revealed distinct volatile compound profiles (Table 3). In sample RB23, five major compounds were identified: dimethyl ether (C_2H_6O) with a retention time (RT) of 1.111 min and a mass-to-charge ratio (m/z) of 45.1, ethyl acetate ($C_4H_8O_2$) at 1.650 min (m/z 43), benzeneacetaldehyde (C_8H_8O) at 7.796 min (m/z 91), and ethyl hydrogen succinate ($C_6H_{10}O_4$) at 9.847 min (m/z 101). Conversely, sample RB16 exhibited two prominent compounds: 2-thiazolamine, 4-(4-pyridinyl)- ($C_8H_7N_3S$) detected at 0.975 min (m/z 177), and dimethyl ether (C_2H_6O), observed at two retention times of 1.131 and 1.198 min (m/z 45.1). Notably, ethyl acetate, benzeneacetaldehyde, and ethyl hydrogen succinate were absent in RB16, while 2-thiazolamine, 4-(4-pyridinyl)-, was unique to this sample. Dimethyl ether was present in both RB23 and RB16, although with slightly different retention times.

Table 3 — GCMS identified compounds in the rice beers from Meghalaya

Samples	Name of compound	Retention time	m/z
Rice beer Sohra (RB23)	Dimethyl ether	1.111	45.1
	Ethyl Acetate	1.65	43
	Benzeneacetaldehyde	7.796	91
	Ethyl hydrogen succinate	9.847	101
Rice beer Tyrsad (RB16)	4-(4-pyridinyl)-2-Thiazolamine	0.975	177
	Dimethyl ether	1.131	45.1

These analyses were conducted without fractionation, indicating the natural occurrence of these compounds in unprocessed rice beer samples.

Isolation and identification of yeast

Yeast colonies isolated from fermented rice beer samples (RB16 and RB23) grown on Yeast Malt Agar (YMA) exhibited large, round, white, opaque, raised, and dry colonies with a distinct odor. Microscopic examination using lactophenol cotton blue (LCB) staining revealed large ovoid cells with multiple budding forms present within a single microscopic field. Colony-forming units (CFU) were calculated using serial dilution and plate counting methods. The CFU/mL in RB16 was 2.14×10^5 at the 10^{-3} dilution, and a similar value of 2.14×10^5 was recorded in RB23 at the 10^{-2} dilution. A representative isolate was selected for molecular identification because of the consistent colony morphology across both samples. Amplification and sequencing of the Internal Transcribed Spacer regions ITS1 and ITS2 produced a 675 bp product, which was submitted to GenBank under the accession number MK942688 and identified as *Saccharomyces cerevisiae* (Fig. 3).

Effect of prebiotic-like polysaccharides on yeast

Quantification of biofilm formation using the crystal violet (CV) assay demonstrated enhanced biomass accumulation in the media supplemented with PLP compared to media alone. At concentration of 6 µg/mL, absorbance at 575 nm increased from

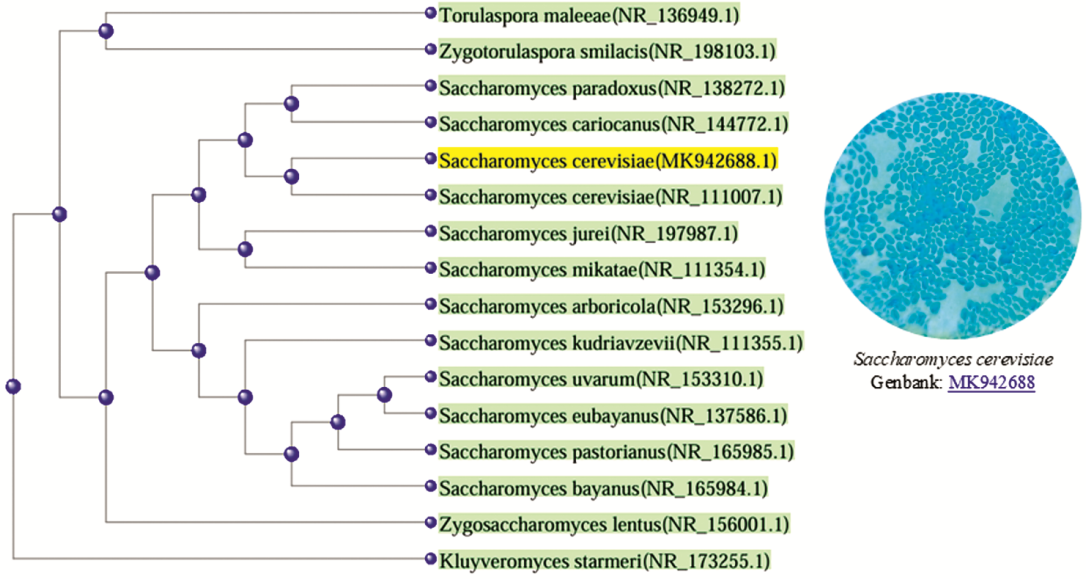

Fig.3 — Phylogenetic tree of *Saccharomyces cerevisiae* (MK942688) isolated and identified in rice beer, and its microscopic slide at 40X magnification after lactophenol cotton blue staining

0.150±0.025 to 0.176±0.074, corresponding to a 17.07% increase in biofilm formation. However, one-way ANOVA indicated that these differences were not statistically significant (p = 0.593) (Fig. 4). At lower concentration of 0.06 µg/mL, absorbance was 0.137±0.010, corresponding to an 8.86% increase in biomass accumulation in the presence of PLP. This indicates a concentration-dependent stimulatory effect on *S. cerevisiae* biofilm formation relative to the control. In the YMA-based spread plate assay (Fig. 5), the negative control (YMA agar without yeast inoculation) showed no growth, thus confirming sterility. Plates containing medium alone with yeast exhibited uniform surface growth, but colonies were relatively sparse and translucent. In contrast, YMA plates supplemented with the PLP and inoculated with yeast displayed luxuriant dense growth across the agar surface, with colonies appearing opaque and more prominent.

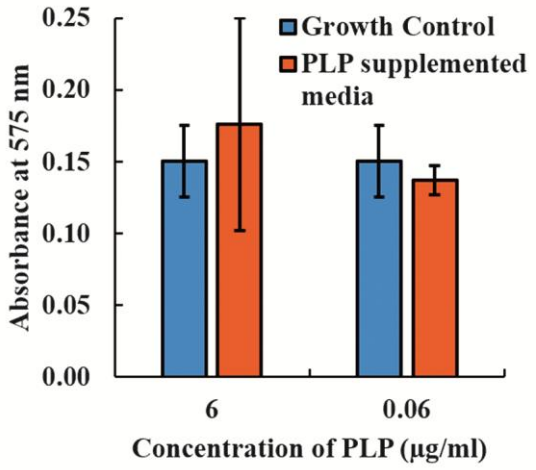

Fig. 4 — Bar graph representing OD values at 575 nm indicative of biofilm formation by *Saccharomyces cerevisiae* (MK942688) with and without PLP in the media

Discussion

This study indicates that *Amomum aromaticum* polysaccharides biochemically enhance *Saccharomyces cerevisiae* performance during the fermentation of rice beer (*kiad*). The plant extract demonstrates prebiotic-like properties that facilitate yeast growth and metabolic activity, thereby enhancing the functional potential of the final product. The traditional preparation of the starter culture involves cutting *A. aromaticum* into small pieces, drying it away from sunlight for 5 to 7 days, and mixing it with finely ground rice powder to form dough, which was subsequently smoked for one week. For rice beer fermentation, cooked rice was cooled on a mat, thoroughly mixed with the prepared starter culture, and incubated at 24°C for 1-2 days. Thereafter, water was added to completely submerge the rice for an additional 2-3 days before filtering and tasting, in accordance with the traditional fermentation process practiced in Meghalaya. The 80% ethanol extract of *A. aromaticum* yielded 2.7% (from 20 g) dried extract, which contained substantial carbohydrate content (12.99 µg/mL pre-digestion). Following acid and enzymatic digestion, the extract released significantly higher levels of reducing sugars (103.78 µg/mL at 60 µg/mL extract), indicating the presence of fermentable oligosaccharides or polysaccharides that act as prebiotic substrates; we used the term

Fig. 5 — YMA-based spread plate assay of the effect of prebiotic-like polysaccharides (PLP) on yeast growth. First plate shows no growth control; second plate show growth of yeast without supplementation of PLP; third plate shows growth of yeast in media supplemented with PLP

prebiotic-like polysaccharides (PLP). FTIR spectra corroborated this finding, revealing characteristic carbohydrate functional groups (notably a broad O–H stretch at 3363.79 cm^{-1} and a C–O stretch at 1077.35 cm^{-1}), which likely contribute to microbial stimulation. FTIR-determined PLP structural motifs especially the O–H stretch (3363 cm^{-1}) and C–O stretch (1077 cm^{-1}) validating the existence of β-glycosidic bonds characteristic of microbial-stimulating polysaccharides. The crystal violet assay demonstrated that supplementation with PLP enhanced yeast biofilm formation in a concentration-dependent manner. An enhancement of 17.07% was observed at 6 µg/mL of PLP, whereas at 0.06 µg/mL of PLP supplementation the biofilm formation increased by 8.87% relative to the growth control. The differences were not statistically significant (p = 0.593). This may be attributable to the limited number of experimental replicates (n = 3) and the variability observed among measurements. Yet, this increase suggests that PLP promoted biomass accumulation and surface colonization by *S. cerevisiae*. Enhanced biofilm formation may facilitate yeast persistence during fermentation, although the underlying mechanisms were not investigated in the present study. The yeast isolated from both rice beer samples (RB16 and RB23) was identified as *Saccharomyces cerevisiae* (GenBank MK942688), a well-established fermentative yeast. Colony-forming units remained consistently high (~2.14×10^5 CFU/mL), indicating stable yeast populations supported by the starter culture. Luxuriant yeast growth on YMA plates supplemented with PLP, compared with sparse growth on medium alone, further substantiates the prebiotic effect. Physicochemical characterization of the rice beer samples revealed notable variations consistent with fermentation dynamics influenced by microbial activity and substrate composition. Rice beer from Sohra exhibited a lower pH (~4.0) and higher titratable acidity (~0.50% lactic acid) than rice beer from Tyrsad (pH ~4.3, acidity ~0.30%), highlighting the differential microbial metabolism potentially driven by starter culture variability and substrate availability. Protein content differed significantly among RB16, RB23 and PLP ($p<0.001$). PLP exhibited highest protein content 59.57±10.42 µg/mL relative to rice beer samples RB16 (40.75±3.45 µg/mL) and RB23 (15.76±0.81 µg/mL).The total carbohydrate levels differed significantly among RB23 (62.64±0.67 µg/mL), RB16 (63.71±1.14 µg/mL) and PLP (12.99±1.07 µg/mL), which is consistent with carbohydrate transformation during the fermentation process. Phytochemical screening identified flavonoids, saponins, tannins, and reducing sugars in the beers. Both rice beer samples exhibited strong antioxidant activity via DPPH assay, with RB23 demonstrating 94.88±1.63% radical scavenging at 100 µL followed by RB16 (86.38±1.86% radical scavenging). The PLP extract also demonstrated strong antioxidant activity (74.13±9.49%).One-way ANOVA indicated that antioxidant activity differed significantly among the three groups (p = 0.0119). RB16 exhibited a slightly higher mean total phenolic content than RB23. However, the difference was not statistically significant (Welch's t-test, p = 0.334). This suggests that both rice beer samples contained comparable levels of phenolic compounds, which may contribute to their antioxidant properties. This enhanced antioxidant potential likely arises from the synergistic action of yeast metabolism and bioactive plant compounds like phenolics, which contribute to the functional food attributes of rice beer. GC-MS analysis revealed distinct volatile profiles, with RB23 containing ethyl acetate, benzeneacetaldehyde, and ethyl hydrogen succinate; compounds associated with fruity and floral aroma were absent in RB16. Conversely, RB16 contained 2-thiazolamine, 4-(4-pyridinyl)-, a nitrogenous compound that can influence flavour complexity. Dimethyl ether was common to both, underscoring shared fermentation metabolites. Overall, these results underscore the pivotal role of *A. aromaticum* starter culture in providing prebiotic substrates that enhance *Saccharomyces cerevisiae* growth and activity. This microbial-plant interaction facilitates efficient carbohydrate breakdown, flavour compound generation, and enhanced antioxidant properties, thereby supporting the classification of rice beer as a functional fermented beverage. The observed increases in biomass, metabolic activity, and phenolic content reinforce the potential application of traditional fermentation systems in developing novel functional foods with probiotic-prebiotic synergy. The observations confirm the established practices of Khasi and Jaintia, where *A. aromaticum* in *Thiat* starters is responsible for the therapeutic status of *Kiad*. However, the different metabolite profiles of RB23 and RB16 highlight the effects of terroir and processing differences on functional effects. Future

research will aim to determine the monosaccharide composition of PLP to identify bioactive motifs, *S. cerevisiae* transcriptome profile under PLP induction to define adhesion pathways, and assess *in vivo* activities against the gut microbiota.

Conclusion

This study elucidated the synergistic interactions between plant-derived prebiotic-like polysaccharides and yeast in traditional fermentation processes. The principal findings indicate that the incorporation of *Amomum aromaticum* polysaccharides into rice beer supports the activity of *Saccharomyces cerevisiae* and enhances the antioxidant potential of the final product. Specifically, *A. aromaticum* prebiotic-like polysaccharides functioned as substrates, resulting in a more intricate array of esters and phenolics, and a markedly improved antioxidant capacity. These findings have significant implications for the development of functional foods, and by integrating bioactive plant ingredients into fermentation processes, producers can create traditional beverages with customized nutritional benefits. These findings provide a basis for future studies on the development of functional beverages by integrating traditional knowledge with biochemical characterization.

Author Contributions

Conceptualization - DH, BR, VVB; Formal analysis- VVB; Investigation - DH, BR, VVB; Resources - DH, BR; Supervision- VVB; Writing - original draft - DH, BR; Writing - review & editing - VVB.

Conflict of Interest

The authors declare no conflict of interest in relation to the present research paper.

Use of Artificial Intelligence (AI)

During the preparation and revision of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, improvement of grammar, and organization of the manuscript. All scientific content, interpretation of the results, data verification, and final manuscript preparation were performed and verified by the authors, who take full responsibility for the content of this article.

Data Availability

The data used to support the findings of this study is included in the article.

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