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Research article

Isolation of high-amylase *Rhizopus arrhizus* from Vietnamese '*Banh men*' for efficient hydrolysis of glutinous rice (*Oryza sativa* var. *glutinosa*)

Phuong-Mai Vo[a,b], Duy-Tan Nguyen[a,b,†], Huu-Thanh Nguyen[a,b,†], Phu-Tho Nguyen[a,b,†,*]

[a] *Department of Biotechnology, Faculty of Agriculture and Natural Resources, An Giang University, An Giang 90000, Vietnam*

[b] *Vietnam National University (VNU-HCM), Ho Chi Minh City 70000, Vietnam*

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Abstract

Importance of the work: Traditional fermented starters, such as '*Banh men'*, used in the production of Vietnamese sweet-fermented rice are valuable sources of microbial diversity with industrial potential. Identifying efficient amylase-producing molds from these starters can enhance starch-based bioprocessing, while preserving traditional fermentation knowledge.

Objectives: To isolate and characterize amylase-producing molds from Vietnamese *Banh men*, assess their enzymatic activity and investigate their ability to hydrolyze glutinous rice into fermentable sugars.

Materials and Methods: A sample of 14 mold strains was isolated from *Banh men* and screened for amylase activity on starch agar. Top producers were identified morphologically and *via* internal transcribed spacer sequencing. Selected strains were cultivated in glutinous rice, with hydrolysis monitored based on the extract yield, Brix value and sugar content (dinitrosalicylic acid method).

Results: Dominant isolates belonged to the *Mucor* and *Rhizopus* genera (70% prevalence). The strains BMTS1 and BMCP2.3 (both *Rhizopus arrhizus*) had the highest amylase activity (2.94 U/mL and 2.60 U/mL, respectively) and starch degradation indices (>95%). During rice hydrolysis, both strains produced approximately 100 mL of extract after 72 hr, with reducing sugars increasing from 6.38 mg/mL to 37.49 mg/mL and polysaccharides declining by 89%.

Main finding: *R. arrhizus* BMTS1 and BMCP2.3 from *Banh men* are potent starch-hydrolyzing agents, offering a sustainable solution for converting glutinous rice into sugar-rich substrates for food and beverage applications.

† Equal contribution.

* Corresponding author.
E-mail address: nptho@agu.edu.vn (P.T. Nguyen)

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Introduction

Sweet '*Banh men'*, a traditional dried starter used in the production of Vietnamese sweet fermented rice (known as '*Com Ruou'*), represent a rich source of microorganisms capable of enzymatic activity, particularly those involved in starch degradation (Taechavasonyoo, 2013). The presence of diverse mold species in these cakes not only contributes to the fermentation process but also enhances the overall quality of the final product. Research has shown that genera, such as *Aspergillus*, *Mucor* and *Rhizopus*, are commonly associated with starch fermentation due to their robust growth characteristics and ability to produce large amounts of amylases (Chay et al., 2017). The isolation and characterization of these molds will aid in identifying strains with superior enzyme-producing capabilities that can be effectively utilized in fermentation processes.

Glutinous rice (*Oryza sativa* var. *glutinosa*) is important alongside conventional white rice in many countries worldwide, particularly in East and Southeast Asia due to its high content of phenolics, anthocyanins and antioxidants and its availability in various colors, including black, purple, red and brown (Sompong et al., 2011). This diversity has garnered considerable interest as a raw material for the development of commercial health food products and dietary supplements (Phonsakhan and Kong-Ngern, 2015). Consequently, the production of maltodextrin-rich products, such as beverages, from starch sources, such as glutinous rice, is not only a matter of flavor but also of nutritional value. Maltodextrins, which are intermediate products of starch hydrolysis, are known for their potential health benefits, including improved digestibility and lower caloric content compared to simple sugars (Hofman et al., 2016). The ability to produce beverages that are not only rich in maltodextrins but also retain desirable sensory attributes has become increasingly important in the context of growing consumer interest in functional foods (Ravelo et al., 2023). In particular, short-chain dextrins have been reported to have considerable potential for development in the food industry and have attracted much attention due to their minimal impact on the sensory characteristics of foods and the digestive system (Li et al., 2023). Therefore, exploring the potential of isolated amylase-producing molds to enhance the production of such beverages from starch-rich substrates would promote bridging traditional practices with modern health trends.

The fermentation of starch-rich substrates is a critical process in various food and beverage industries, particularly in the production of bio-products such as alcohols, organic acids, microbial oils and hydrocarbons, enzymes, biosurfactants and biopolymers (Mohanty et al., 2021). In addition, maltodextrin-rich products may have potential for new product development due to their unique flavor and potential health benefits (Aliasgharzadeh et al., 2015, Delzenne et al., 2020). Typically, these products are produced through the enzymatic breakdown of starch into maltodextrins and simpler sugars (Triyono et al., 2017). The utilization of molds that produce amylases is essential in this process, as they can facilitate the efficient conversion of starches into sugars.

Although *Rhizopus arrhizus* is a well-documented amylase producer (Wan et al., 2025), the novelty of the current study was in the isolation of highly active strains directly from Vietnamese traditional fermentation starters (*Banh men*), a culturally specific and scientifically underexplored microbial niche. In addition, the study went beyond conventional screening by linking microbial diversity to functional application, specifically evaluating these isolates in the hydrolysis of glutinous rice—a starchy substrate of growing interest in the development of functional and culturally adapted food products. This study focused on the isolation of high amylase-producing molds from sweet *Banh men* for their possible application in the production of maltodextrin-rich products. The findings are expected to pave the way for innovative product development that honors traditional fermentation methods while meeting contemporary consumer demands for healthful and flavorful options.

Material and Methods

Sample collection and mold isolation

Samples of sweet *Banh men* (Fig. 1) were purchased from local markets in An Giang province, Vietnam, (in Chau Thanh, Chau Phu, Tan Chau, Chau Doc, Thoai Son, Cho Moi and Tri Ton districts). These regions represent major traditional fermentation hubs in the province. The samples were transported directly to the laboratory and stored in sterile plastic bags at 4°C. Samples of sweet *Banh men* were suspended in 10 mL of sterilized distilled water, followed by serial dilution into four different flasks. Next, a loopful of each diluted sample was streaked onto solidified potato dextrose agar (PDA) plates and incubated at 37°C for 48 hr to facilitate mold growth. The pure cultures were identified based on their morphology and colony characteristics and subsequently subcultured. The organisms were maintained on PDA slants and stored at 4°C for further study.

Fig. 1 Images of sweet *Banh men* (dried starter) used in Vietnamese sweet fermented rice production (*Com Ruou*) collected from An Giang province, Vietnam in: (A) Thoai Son district; (B) Tri Ton district; (C) Chau Doc city; (D) Chau Phu district. All images share the same scale, where scale bar = 1 cm.

Microscopic observation

The isolates were identified based on their morphological characteristics using the taxonomic key provided by Constantine et al. (2007). Both macroscopic and microscopic characteristics of the mold isolates were observed. For microscopic identification, the spores were suspended in a lactose phenol blue solution on a slide and the spore arrangement was examined under a microscope. The macroscopic examination recorded the color, texture and shape of the colonies.

Primary screening of amylase-producing molds

The primary screening for amylase production from the isolated molds was conducted using starch agar medium in conjunction with Gram's iodine solution (0.4% KI + 0.2% I_2). The mold isolates were inoculated onto 1% starch PDA and incubated at 28 ± 2°C for 24 hr, after which the plates were flooded with iodine solution. The presence of a clear zone around the mold colony indicated amylase production. The degradation efficiency was assessed based on the diameter of the clear zone surrounding the colony that was measured using a transparent plastic ruler (Deli G02512; Deli Group Co., Ltd.; China) with 1 mm precision (Ominyi et al., 2013).

Screening of isolates for amylase production

The isolates were quantitatively screened for amylase production in a starch broth medium composed of: Soluble starch, 20 g/L; KH_2PO_4, 14 g/L; NH_4NO_3, 10 g/L; KCl, 0.5 g/L; $MgSO_4 \cdot 7H_2O$, 0.1 g/L; and $FeSO_4 \cdot 7H_2O$, 0.01 g/L. The pH of the medium was adjusted to 6.9 and sterilized using autoclaving at 121°C for 15 min. Molds were cultivated on PDA at 30°C for 7 d. A 0.1% Tween 80 solution was used to disperse and suspend the spores. Then, 10 mL of the spore suspension (1×10^6 spores/mL) was transferred into 90 mL of starch broth and agitated at 200 revolutions per minute (rpm) and 30°C for 3 d. Subsequently, the mold cultures were centrifuged at 5,000 rpm and 4°C for 15 min, after which the supernatants were analyzed for amylase activity (Roongrojmongkhon et al., 2020).

Measurement of amylase activity

The activity of amylase was evaluated using two methods. The first method involved assessing the reduction in blue color intensity, which occurs as a result of enzyme-mediated hydrolysis of starch (Al-Qodah, 2006; Omemu et al., 2015). A sample of 10 mL of a 2% starch substrate (in a pH 6.5 buffer solution) was mixed with 2 mL of each crude enzyme solution and incubated at 55°C for 10 min in a water bath. The reaction was terminated by adding 5 mL of 5 N HCl solution, followed by stirring for 10 min. Then, the reaction mixture was neutralized with 2 mL of 4 N NaOH and the volume was adjusted to 100 mL with distilled water. Aliquots of 5 mL were withdrawn and combined with 5 mL of a 0.2% I_2-2% (weight per volume, w/v) KI solution to form a starch-iodine complex. The absorbance was measured at 680 nm using an ultraviolet-visible light spectrophotometer (V730ST; Jasco Inc.; Japan). A control was prepared using the same procedure, substituting 2 mL of phosphate buffer for the crude enzyme solution. The amylase activity was calculated using Equation 1:

$$\text{Amylase activity (\%)} = 100 \times (B - A) / B \quad (1)$$

where B is the absorbance of the blank sample and A is the absorbance of the test sample.

For the second method, a substrate solution was prepared by mixing 1 mL of 2% w/v starch (pH 6.5) with 1 mL of each crude enzyme solution, which was then incubated at 55°C for 10 min in a water bath. The reaction was terminated by adding 6 mL of 3,5-dinitrosalicylic acid. Subsequently, the tubes were immersed in boiling water (100°C) for 5 min. The absorbance of the solution was measured at 540 nm using the spectrophotometer and the amount of glucose liberated was quantified using a standard glucose curve. One unit of activity was defined as the micromoles of reducing sugar (expressed as glucose equivalents) produced per minute under the assay conditions (Al-Qodah, 2006; Omemu et al., 2015).

Testing ability to degrade glutinous rice

Glutinous rice was cooked with a rice-to-water ratio of 1:1.5 (g/mL) and cooled to room temperature (28–30°C). Subsequently, mold spores were inoculated into the cooked rice

(1×10^6 spores/g), which was incubated at 30°C for 24 hr, 48 hr and 72 hr. After the designated incubation periods, 100 g of the sample was taken and supplemented with 100 mL of water and the mixture was blended to achieve homogeneity. The resulting mixture was hydrolyzed at 50°C for 1 hr in a water bath. Following hydrolysis, the mixture was centrifuged at 6,000 rpm at 4°C for 10 min to determine the volume of the extract obtained. The concentration of soluble solids in the extract was measured using a digital Brix refractometer (PAL-1; Atago Co., Ltd.; Japan; precision ±0.2°Bx). The amount of polysaccharides in the extract was determined based on precipitation with ethanol to achieve a concentration of 80% (volume per volume), according to Yan (2017) and allowing it to stand at 4°C for 48 hr. Then, the mixture was centrifuged at 6,000 rpm for 10 min to collect the precipitate, which was dried at 50°C and weighed for quantification.

Molecular identification of isolate with greatest potential

Genomic DNA was extracted using a method reported by Zhang et al. (2010). Mycelia grown on PDA for 5 d were harvested and resuspended in 100 µL of sterile water. Then, the suspension was centrifuged at 10,000 rpm for 1 min and the supernatant was discarded. Subsequently, 100 µL of a lysis solution (50 mmol/L sodium phosphate at pH 7.4, 1 mmol/L EDTA and 5% glycerol) was added and the mixture was heated at 85°C for 30 min. The extracted genomic DNA was stored at 4°C until further use.

For polymerase chain reaction (PCR) amplification, 1 µL of genomic DNA was used as the template. Molecular identification used universal fungal primers targeting the internal transcribed spacer (ITS) region, specifically ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'), according to Schoch et al. (2012). The PCR cycling conditions were: an initial denaturation at 95°C for 5 min, followed by 30 cycles consisting of denaturation at 95°C for 30 s; annealing at 55°C for 30 s; extension at 72°C for 1 min; and a final extension at 72°C for 5 min. The amplified products were purified using a FavorPrep™ GEL/PCR Purification Kit (Favorgen Biotech Corp., Taiwan) and analyzed on a 1.2% agarose gel (Roongrojmongkhon et al., 2020). The resulting DNA sequences of the identified molds were compared with reference sequences available in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/blast). A phylogenetic tree based on the ITS regions was constructed using the NCBI online tool (NCBI Tree Viewer).

Statistical analysis

All experiments were conducted in triplicate. Statistical significance was assessed using one-way ANOVA followed by Tukey's honestly significant difference post hoc test to determine differences among mean values. All tests were considered significant at $p < 0.05$. Analyses were performed using the Statgraphics 18 software (Statgraphics Technologies, Inc.; USA). Data were presented as mean ± standard deviation (SD) values.

Results

Characterization of mold isolates

A summary of the results from both the microscopic and macroscopic examinations for the identification of the mold isolates is presented in Table 1. The isolated mold strains were suspected to belong to the genera *Aspergillus*, *Mucor*, *Rhizopus* and *Penicillium*. The two dominant groups of molds identified in the samples of sweet yeast cakes were in the *Mucor* and *Rhizopus* genera, collectively accounting for over 70% of the isolated strains.

Table 1 Macroscopic and microscopic characteristics of fungal isolates from various samples, with suspected genus-level identification based on colony morphology and spore structure.

Isolate	Macroscopy	Microscopy	Fungus suspected
BMTC2, BMTT1	White filamentous growth that transitions to a black powdery appearance upon sporulation	Long septate hyphae with conidiophores that bear brown spores	*Aspergillus* spp.
BMCT1.1, BMTS2, BMCM1, BMCĐ1, BMCĐ2, BMCT1.2	White, whorled aerial mycelial growth that subsequently darkens as it undergoes sporulation	Non-septate hyphae with straight sporangiophores bearing numerous spherical spores	*Mucor* spp.
BMCP 2.1, BMTS1, BMCM2, BMCP 2.3,	White, elongated hyphal growth that darkens as it undergoes sporulation within 2 d	Non-septate, long-branched mycelium with terminally shaped sporangia	*Rhizopus* spp.
BMTC1, BMTT2,	White to pale at the margins and greyish in the center	Conidiophores are at the end of each branch accompanied by conidia with green, spherical to nearly spherical in shape and possess smooth walls	*Penicillium* spp.

Amylase production of mold isolates

The primary screening of the mold isolates for amylase production on starch agar indicated that all 14 isolates had starch-hydrolyzing activity. The diameters of the zone of hydrolysis were in the range 6.5–55 mm (Table 2, Fig. 2). Based on these results, the strains with high starch-degrading capabilities were BMCT1.1, BMCP2.1, BMCP2.3 and BMCM1, having zones of hydrolysis greater than 50 mm. Furthermore, while the strains BMCT1.2, BMCĐ1 and BMTT1 produced rapid mycelial growth, their starch-degrading abilities were lower. However, the strains BMCP2.3, BMTS1 and BMCM2 had high starch degrading index values (reflecting the ability to degrade starch) exceeding 95%. Based on this index, these isolates were selected as the most efficient starch degrading isolates. Correlation analysis revealed a significant positive relationship between mycelial growth and clear zone diameter (Pearson's correlation coefficient, $r = 0.67$, $p = 0.0092$),as shown in Fig. 3, suggesting that faster-growing strains tend to produce larger hydrolysis zones. However, mycelial growth was not significantly correlated with the starch degrading index ($r = –0.34$, $p = 0.2284$), indicating that olony expansion did not necessarily reflect enzymatic efficiency.

Table 2 Mycelial growth, clear zone diameter and starch degrading index of mold strains isolated from sweet *Banh men*.

Isolate	Mycelial growth (mm)	Clear zone (mm)	Starch degrading index (%)
BMCT1.1	65.15 ± 0.15^{c}	55.25 ± 0.25^{a}	84.80
BMCT1.2	80.50 ± 0.71^{a}	45.50 ± 0.50^{b}	56.52
BMCP2.1	60.50 ± 0.50^{d}	55.40 ± 0.50^{a}	91.57
BMCP2.3	54.50 ± 0.56^{e}	54.30 ± 0.30^{a}	99.63
BMTC1	10.50 ± 0.52^{k}	6.50 ± 0.62^{f}	61.90
BMTC2	40.50 ± 0.50^{h}	30.50 ± 0.51^{d}	75.31
BMCĐ1	81.15 ± 1.15^{a}	31.00 ± 1.00^{d}	38.20
BMCĐ2	51.00 ± 1.00^{f}	45.50 ± 0.50^{b}	89.22
BMTS1	45.50 ± 0.50^{g}	43.83 ± 1.26^{bc}	96.34
BMTS2	45.40 ± 0.40^{g}	43.12 ± 0.73^{c}	94.97
BMCM1	60.35 ± 0.35^{d}	56.00 ± 1.00^{a}	92.79
BMCM2	31.00 ± 1.00^{i}	30.17 ± 1.26^{d}	97.31
BMTT1	70.50 ± 0.55^{b}	46.00 ± 1.11^{b}	65.25
BMTT2	31.55 ± 1.55^{i}	25.65 ± 0.65^{e}	81.30

Values within the same column with different lowercase superscripts indicate significant differences ($p < 0.05$, Tukey's honestly significant difference test). Starch degrading index = ratio of total diameter of clear zone and colony diameter.

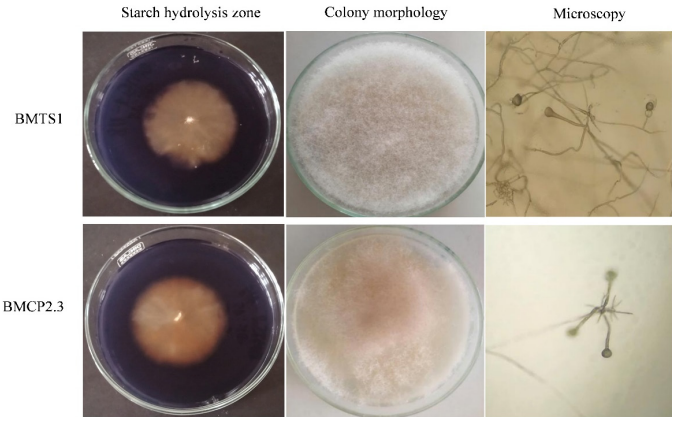

Fig. 2 Morphological and enzymatic characteristics of two superior amylase-producing fungal isolates: Left: starch hydrolysis zones formed on starch agar supplemented with iodine after incubation at 30°C for 24 hr; Center: colony morphology on potato dextrose agar after incubation at 30°C for 3 d; Right: characteristics observed under light microscope.

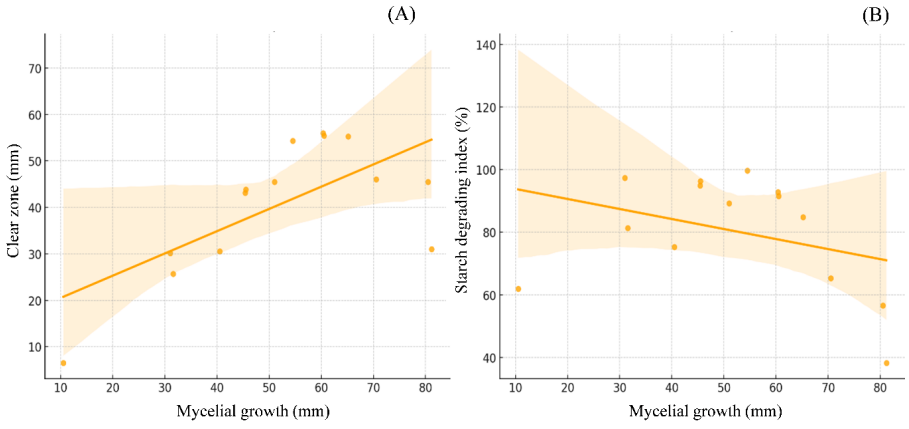

Fig. 3 Correlation analysis between mycelial growth and enzymatic performance of mold isolates: (A) significant positive correlation between mycelial growth and starch hydrolysis zone diameter (Pearson's correlation coefficient, $r = 0.67$, $p = 0.0092$), suggesting that larger colonies tend to produce wider clear zones; (B) no significant correlation between mycelial growth and starch degrading index ($r = –0.34$, $p = 0.2284$), indicating that growth rate alone is not a reliable indicator of enzymatic efficiency.

Based on the results, most of the isolated strains were capable of producing amylase (Fig. 4), with the highest amylase activity recorded for strain BMTS1 (58.63%), followed by strains BMCP2.3 (54.17%), BMCP2.1 (51.98%), BMTT1 (51.22%) and BMCT1.1 (50.65%), as shown in Fig. 4A. In contrast, strain BMCT1 had the lowest amylase production among the isolates, with an activity of only 9.41%. Amylase activity was further quantified by measuring the amount of reducing sugar released using the dinitrosalicylic acid method. Again strain BMTS1 had the highest activity (2.94 U/mL), followed by BMCP2.3 (2.60 U/mL), BMCĐ2 (2.46 U/mL) and BMCP2.1 (2.35 U/mL). The strains BMTC1, BMCT2 and BMTS2 had the lowest levels of amylase activity, in the range 0.57–0.99 U/mL (Fig. 4B).

Molecular identification of high amylase-producing isolates

The two most potent amylase-producing isolates were further confirmed using molecular analysis based on the ITS region. Isolate BMTS1 had a close relationship with *R. arrhizus* strain BS17 (OR098605.1) and *R. delemar* strain DTO 466-C7 (OQ550014.1), displaying a high similarity of 100% (Fig. 5A). On the other hand, isolate BMCP2.3 showed 100% similarity with *R. arrhizus* strain VT83 (OR625098.1) and 99.89% similarity with *R. delemar* strain DTO 466-C7 (OQ550014.1), as shown in Fig. 5B. Based on the phylogenetic classification, *R. arrhizus* corresponded closely to *R. oryzae* and *R. delemar*. Consequently, the two isolates, BMTS1 and BMCP2.3, were identified as *R. arrhizus*.

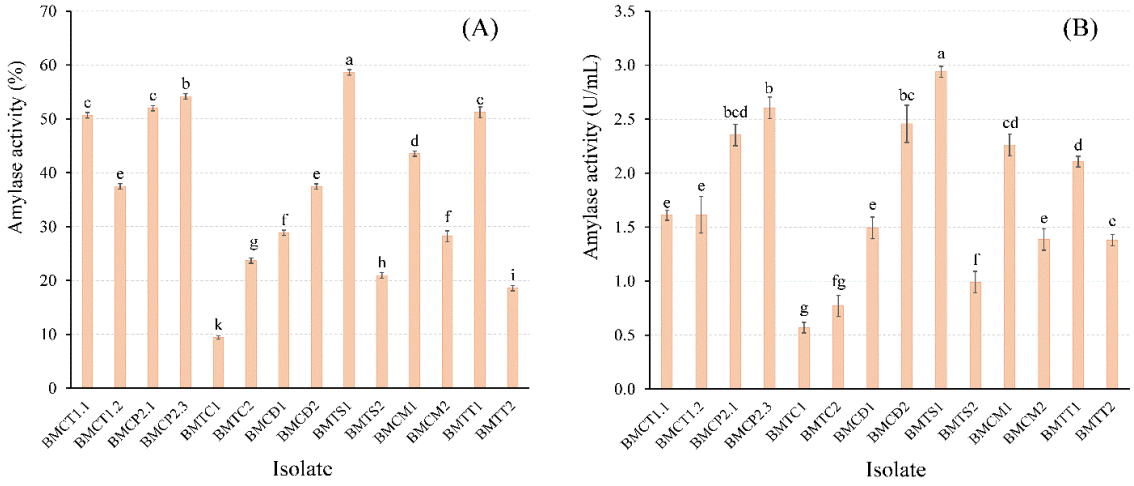

Fig. 4 Amylase production of mold strains isolated from sweet *Banh men*: (A) starch hydrolysis based on the reduction in blue color intensity of the starch-iodine complex; (B) measurement of amount of reducing sugar released via dinitrosalicylic acid method. Different lowercase letters above bars indicate significant differences ($p < 0.05$, Tukey's honestly significant difference test) among isolates.

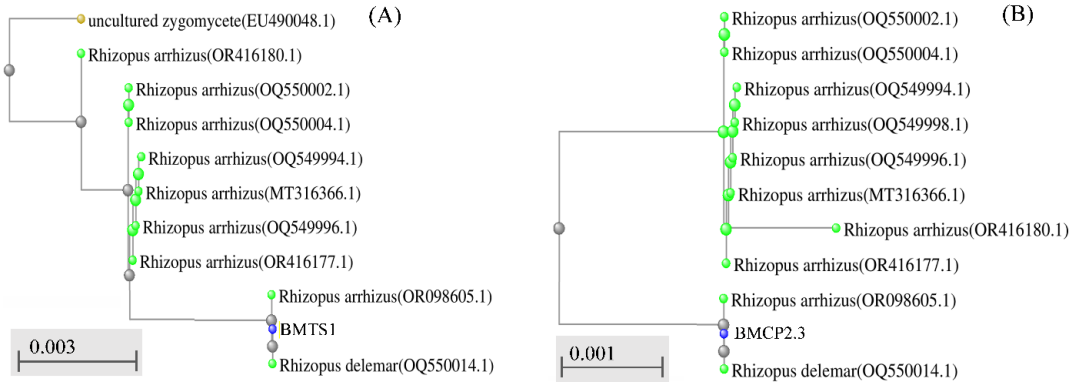

Fig. 5 Phylogenetic trees of: (A) BMTS1; (B) BMCP2.3 based on isolates constructed using a dataset derived from internal transcribed spacer gene sequences.

Ability to degrade glutinous rice

The two top-performing strains, BMTS1 and BMCP2.3, were selected for glutinous rice hydrolysis experiments. Both strains showed time-dependent increases in extract volume, with BMTS1 producing 102.9 ± 2.5 mL after 72 hr compared to 59.9 ± 1.5 mL at 24 hr (Table 3). The Brix values plateaued at 48 hr (15.3−15.9°Bx), suggesting near-complete starch conversion. Most notably, the reducing sugar content increased considerably over time, from 6.38 ± 0.10 mg/mL to 37.49 ± 1.85 mg/mL for BMTS1, compared to the inverse relation ship for the polysaccharide content, decreasing from 133.35 ± 3.00 mg/mL to 14.25 ± 1.00 mg/mL after 72 hr ($p < 0.05$). Similar trends were observed for BMCP2.3, confirming the robust hydrolytic capacity of these isolates (Table 3).

Discussion

The morphological identification revealed that the mold isolates belonged to four genera (*Aspergillus, Mucor, Rhizopus* and *Penicillium*) with *Mucor* and *Rhizopus* collectively accounting for over 70% of the isolates. Daroonpunt et al. (2016) also found that *Amylomyces* sp., *Rhizopus* sp., *Mucor* sp. and *Penicillium* sp. were prevalent in '*look-paeng-khao-mak*', a traditional starter culture used in the production of '*khao-mak*' (a Thai sweet rice). This dominance was consistent with findings based on fermented food environments, where *Rhizopus* spp. have been reported as prevalent due to their adaptability and strong amylolytic activity (Benabda et al., 2019). The frequent occurrence of *Rhizopus* in sweet yeast cakes suggested its ecological advantage in starch-rich substrates, possibly due to its ability to rapidly colonize and secrete hydrolytic enzymes.

All the isolated molds had the ability to hydrolyze starch, with hydrolysis zones ranging substantially among strains. The large halo diameters observed in BMCT1.1, BMCP2.1, BMCP2.3 and BMCM1 suggested robust amylase secretion. Notably, despite the rapid mycelial growth, some isolates (BMCĐ1 and BMCT1.2) produced smaller hydrolysis zones, indicating that rapid biomass development did not necessarily translate into high enzymatic activity. This observation was supported by the Pearson correlation analysis, which revealed a significant positive correlation between mycelial growth and clear zone diameter ($r = 0.67$, $p = 0.0092$), yet no significant correlation between mycelial growth and the starch degrading index ($r = –0.34$, $p = 0.2284$). These findings confirmed that while colony expansion may contribute to hydrolysis zone formation, it is not a reliable predictor of enzymatic efficiency. The SDI, which normalizes hydrolysis performance relative to colony size, proved valuable in identifying efficient strains, such as BMTS1 and BMCP2.3, with both having SDI values exceeding 95%. This dual assessment—combining qualitative observation with semi-quantitative indices—was consistent with Mawa et al. (2022) and enhanced the reliability of screening for high-performance amylase producers.

Quantitative assays confirmed BMTS1 and BMCP2.3 as top amylase producers, with enzymatic activities reaching 2.94 U/mL and 2.60 U/mL, respectively. These values are comparable to those reported by Ghosh and Ray (2011), where *R. oryzae* strains had activities in the range 2.5–3.2 U/mL under optimized conditions. These high levels of enzyme production suggest potential for industrial application, particularly in food and bioethanol sectors. Molecular identification based on ITS sequencing revealed both isolates as *R. arrhizus*, sharing complete sequence similarity with known strains. These results corroborated another report identifying *R. arrhizus* as a prolific amylase producer (Lopes et al., 2024), reinforcing its relevance for enzyme biotechnology.

Table 3 Hydrolysate yield, °Brix, reducing sugar and polysaccharide concentrations at different incubation times for two fungal isolates from sweet *Banh men*.

Isolate	Incubation time (h)	Hydrolysate yield (mL)	Brix (°Bx)	Reducing sugar (mg/mL)	Polysaccharides (mg/mL)
BMTS1	24	59.90 ± 1.50^{c}	14.7 ± 0.15^{b}	6.38 ± 0.10^{c}	133.35 ± 3.00^{a}
	48	90.20 ± 1.00^{b}	15.3 ± 0.15^{a}	23.38 ± 0.50^{b}	83.72 ± 2.50^{b}
	72	102.90 ± 2.50^{a}	15.9 ± 0.15^{a}	37.49 ± 1.85^{a}	14.25 ± 1.00^{c}
BMCP2.3	24	47.09 ± 1.50^{c}	15.4 ± 0.15^{b}	7.20 ± 0.15^{c}	134.04 ± 5.43^{a}
	48	67.06 ± 1.50^{b}	15.8 ± 0.10^{a}	16.91 ± 1.00^{b}	98.19 ± 1.00^{b}
	72	98.58 ± 2.29^{a}	15.9 ± 0.10^{a}	34.13 ± 2.08^{a}	15.48 ± 2.50^{c}

Mean values in the same column with different lowercase superscripts are significantly different ($p < 0.05$, Tukey's honestly significant difference test). The hydrolysate volume was measured using a 100 mL graduated cylinder (Duran Group; Germany).

In the glutinous rice hydrolysis assays, both BMTS1 and BMCP2.3 had significant time-dependent increases in reducing sugar levels, along with a sharp decline in polysaccharide content. The increase in Brix values until 48 hr, along with the continuous rise in reducing sugars and the sharp decline in polysaccharides, suggested that starch was efficiently broken down into simpler sugars. This indicated that the soluble solids were largely sourced from fermentable sugars, highlighting the enzymatic effectiveness of the isolates. These results aligned with another study showing *Rhizopus* spp. could efficiently saccharify cooked rice and other starch-based substrates (Wan et al., 2025). Although both strains had strong hydrolytic performance, BMTS1 consistently outperformed BMCP2.3 in extract volume and reducing sugar production. This may reflect superior enzyme secretion, substrate utilization efficiency or more rapid colonization by BMTS1. The marked performance of BMTS1, producing 37.49 mg/mL of reducing sugars, indicated its potential as a bio-catalyst in traditional fermentation processes or as a sugar source for microbial fermentation. This highlighted the feasibility of using indigenous mold isolates for sustainable and cost-effective bioconversion of starchy biomass. The isolated *R. arrhizus* strains not only reaffirmed their enzymatic potential but also highlighted the unexplored microbial wealth within traditional Vietnamese fermentative ecosystems. Their demonstrated ability to efficiently hydrolyze glutinous rice suggests potential applications in localized enzyme production, traditional beverage fermentation or even modern bioprocesses aiming to valorize native starch sources.

While this study successfully identified high-performing amylase producers from traditional starters, several limitations should be acknowledged. First, the enzyme characterization was conducted under controlled laboratory conditions, which may not fully replicate the complex microbial interactions occurring in traditional fermentation environments. Second, the evaluation of hydrolytic capacity was limited to glutinous rice, leaving open questions about strain performance on other economically important starchy substrates. Future research should: 1) investigate the synergistic effects between these *Rhizopus* strains and companion microbes (yeasts) in co-culture systems to better simulate natural fermentation (Mikai et al., 2015); 2) examine enzyme stability and productivity under scaled-up conditions relevant to industrial applications; and 3) explore the potential for solid-state fermentation approaches, which could enhance enzyme yields while reducing production costs (González et al., 2003). Additionally, comprehensive profiling of the hydrolysate composition, including oligosaccharide distribution and potential bioactive compounds, would provide deeper insight into the nutritional and functional properties of the fermented products.

Conclusion

Amylase-producing molds were successfully isolated and characterized from traditional Vietnamese *Banh men*, with the *R. Arrhizus* strains BMTS1 and BMCP2.3 demonstrating superior starch-hydrolyzing capabilities. Morphological and molecular analyses confirmed the dominance of *Rhizopus* and *Mucor* species in these starter cultures, aligning with their known ecological role in starch-rich fermentations. Quantitative assays revealed exceptional amylase activity in BMTS1 (2.94 U/mL) and BMCP2.3 (2.60 U/mL), while hydrolysis experiments with glutinous rice showed their practical efficacy, yielding significant increases in reducing sugars (up to 37.49 mg/mL) and extract volumes (approximately100 mL/100 g rice) within 72 hr. These findings highlight the untapped potential of traditional fermentation microbes for modern biotechnological applications.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Al-Qodah, Z. 2006. Production and characterization of thermostable alpha-amylase by thermophilic *Geobacillus stearothermophilus*. Biotechnol. J. 1: 850–857. doi.org/10.1002/biot.200600033

Aliasgharzadeh, A., Dehghan, P., Gargari, B.P., Asghari-Jafarabadi, M. 2015. Resistant dextrin, as a prebiotic, improves insulin resistance and inflammation in women with type 2 diabetes: a randomised controlled clinical trial. Br. J. Nutr. 113: 321–330. doi.org/10.1017/S0007114514003675

Alexopoulos, C.J., Mims, C.W., Blackwell, M.M. 2007. Introductory mycology, 4th ed. Wiley, New Delhi, India.

Benabda, O., Mhir, S., Kasmi, M., Mnif, W., Hamdi, M. 2019. Optimization of protease and amylase production by *Rhizopus oryzae* cultivated on bread waste using solid-state fermentation. J. Chem. 2019: 1–9. doi.org/10.1155/2019/3738181

Chay, C., Dizon, E., Elegado, F., Norng, C., Hurtada, W.I., Raymundo, L.C. 2017. Isolation and identification of mold and yeast in medombae, a rice wine starter culture from Kompong Cham Province, Cambodia. Food Res. 1: 213–220. doi.org/10.26656/fr.2017.6.101

Daroonpunt, R., Tanasupawat, S., Keeratipibul, S. 2016. Characterization and amylolytic activity of yeast and mold strains from Thai sweet rice. Malays. J. Microbiol. 12: 121−131. doi.org/10.21161/mjm.73915

Delzenne, N.M., Olivares, M., Neyrinck, A.M., et al. 2020. Nutritional interest of dietary fiber and prebiotics in obesity: lessons from the MyNewGut consortium. Clin. Nutr. 39: 414–424. doi.org/10.1016/j.clnu.2019.03.002

Hofman, D.L., Van Buul, V.J., Brouns, F.J.P.H. 2016. Nutrition, health and regulatory aspects of digestible maltodextrins. Crit. Rev. Food Sci. Nutr. 56: 2091–2100. doi.org/10.1080/10408398.2014.940415

Li, F., Muhmood, A., Akhter, M., et al. 2023. Characterization, health benefits and food applications of enzymatic digestion-resistant dextrin: A review. Int. J. Biol. Macromol. 253: 126970. doi.org/10.1016/j.ijbiomac.2023.126970

Lopes, P.H.S., Pasin. T.M., Benassi, V.M., Nelson, D., Oliveira, T. 2024. Standardization of the cultivation of *Rhizopus arrhizus* using agroindustrial residues: high production of amylases in pineapple peel. Braz. Arch. Biol. Technol. 67: 2024. doi.org/10.1590/1678-4324-2024240293

Mawa, J., Momo, M., Sultana, R. 2022. Isolation of starch degrading bacteria from rhizospheric soil of Mymensingh, Bangladesh. Stamford J. Microbiol. 12: 21–24. doi.org/10.3329/sjm.v12i1.63339

Mikai, S., Nanako, I., Konomi, J., Sato, Y., Era, M., Ninomiya, J., Morita, H. 2015. Simultaneous increase of glucoamylase and α-amylase production in submerged co-culture of *Aspergillus* and *Rhizopus* strains. Jpn. J. Food Eng. 16: 111–121. doi.org/10.11301/jsfe.16.111

Mohanty, S.S., Koul, Y., Varjani, S., Pandey, A., Ngo, H.H., Chang, J.S., Wong, J.W.C., Bui, X.T. 2021. A critical review on various feedstocks as sustainable substrates for biosurfactants production: a way towards cleaner production. Microb. Cell Factories 20: 120. doi.org/10.1186/s12934-021-01613-3

Ravelo, R.C.M., Gastl, M., Becker, T. 2023. Influence of dextrins and β-glucans on palate fullness and mouthfeel of beer. Eur. Food. Res. Technol. 250: 1–15. doi.org/10.1007/s00217-023-04394-8

Omemu, A., Bamigbade, G., Obadina, A., Obuotor, T. 2015. Isolation and screening of amylase from moulds associated with the spoilage of some fermented cereal foods. Br. Microbiol. Res. J. 5: 359–367. doi.org/10.9734/BMRJ/2015/11190

Ominyi, M.C., Ogbonna, J.C., Nwoba, E.G., Nwagu, K.E., Uche, R. 2013. Isolation of alpha amylase and glucoamylase producing fungi and their application in bioethanol production. Int. J. Sci. Nat. 4: 44–50.

Phonsakhan, W., Kong-Ngern, K. 2015. A comparative proteomic study of white and black glutinous rice leaves. Electron. J. Biotechnol. 18: 29–34. doi.org/10.1016/j.ejbt.2014.11.005

Roongrojmongkhon, N., Rungjindamai, N., Vatanavicharn, T., Ochaikul, D. 2020. Isolation and identification of fungi with glucoamylase activity from loog-pang-khao-mak (a Thai traditional fermentation starter). J. Pure Appl. Microbiol. 14: 233–247. doi.org/10.22207/jpam.14.1.24

Schoch, C.L., Seifert, K.A., Huhndorf, S., et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc. Natl. Acad. Sci. U.S.A. 109: 6241–6246. doi.org/10.1073/pnas.1117018109

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G., Berghofer, E. 2011. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. Food Chem. 124: 132–140. doi.org/10.1016/j.foodchem.2010.05.115

Taechavasonyoo, A., Thammarut, J., Yompakdee, C. 2013. Identification of the molds and yeasts characteristic of a superior loogpang, starter of Thai rice-based alcoholic beverage sato. Asian J. Food Agro-Ind. 6: 24–38.

Triyono, A. andriansyah R.C.E., Luthfiyanti, R., Rahman, T. 2017. Development of modified starch technology (maltodextrin) from commercial tapioca on semi production scale using oil heater dextrinator. IOP Conf. Ser.: Earth Environ. Sci. 101: 012026. doi.org/10.1088/1755-1315/101/1/012026

González, G.V., Torres, E.F., Aguilar, C.N., Gomez, S.D.J.R., Godínez, G.D., Augur, C. 2003. Advantages of fungal enzyme production in solid state over liquid fermentation systems. Biochem. Eng. J. 13: 157–167. doi.org/10.1016/S1369-703X(02)00128-6

Wan, B., Tian, T., Xiong, Y., et al. 2025. Isolation and evaluation of *Rhizopus arrhizus* strains from traditional rice wine starters (jiuqu): enzyme activities, antioxidant capacity and flavour compounds. Foods 14: 312. doi.org/10.3390/foods14020312

Yan, X. 2017. Short-chain polysaccharide analysis in ethanol–water solutions. J. AOAC Int. 100: 1134-1136. doi.org/10.5740/jaoacint.16-0426

Zhang, Y.J., Zhang, S., Liu, X., Wen, H.A., Wang, M. 2010. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Lett. Appl. Microbiol. 51: 114–118. doi.org/10.1111/j.1472-765X.2010.02867.x