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Potential for single cell protein production by yeast isolated from fermented foods

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Tiềm năng sản xuất protein đơn bào bởi nấm men phân lập từ thực phẩm lên men

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Tóm tắt

Trong nghiên cứu này, 36 chủng nấm men được phân lập từ các nguồn thực phẩm lên men thu thập được ở các khu vực khác nhau của miền Bắc Việt Nam. Quá trình sàng lọc đã thu được 15/36 chủng có khả năng sinh trưởng tốt với khối lượng khô tế bào (CDW) dao động từ 10-14 g/L, và 10/36 chủng có tiềm năng sản xuất protein đơn bào với tổng hàm lượng protein (TPC) vượt quá 57% (g/100 g khối lượng khô tế bào) sau 24 giờ nuôi cấy trong môi trường lỏng YPD. Chủng nấm men YPI-Y2 (với CDW đạt 14,03 g/L; TPC đạt 62,3%) được xác định là có quan hệ gần gũi với loài *Pichia kudriavzevii* (99,77%) dựa trên phân tích và so sánh trình tự ITS rDNA. Các đặc điểm dinh dưỡng và nuôi cấy của chủng *Pichia kudriavzevii* YPI-Y2, hướng tới sản xuất sinh khối tế bào và tích lũy protein, đã được xác định. Nhiệt độ nuôi cấy tối ưu và điều kiện pH ban đầu của môi trường để sinh trưởng và tích lũy protein của chủng nấm men YPI-Y2 lần lượt là 30°C và 6,5, trong khi nguồn carbon phù hợp được xác định là D-fructose. Đồng thời, chủng nấm men này vẫn thể hiện khả năng sinh trưởng và tích lũy protein tốt trên các nguồn nitơ vô cơ (ngoại trừ urê với 35,62% TPC). Dưới điều kiện nuôi cấy và dinh dưỡng thích hợp, giá trị CDW và TPC đạt lần lượt 16,12 g/L và 66,62% (g/100g CDW).

Từ khóa: *Protein đơn bào, Pichia kudriavzevii, nấm men, thực phẩm lên men, YPI-Y2*

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Potential for single cell protein production by yeast isolated from fermented foods

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Abstract

In this study, 36 yeast strains were isolated from fermented food sources collected in different areas of Northern Vietnam. The screening process yielded 15/36 strains capable of good growth with Cell Dry Weight (CDW) ranging from 10-14 g/L, and 10/36 strains having the potential for single cell protein production with Total Protein Content (TPC) exceeding 57% (g/100 g cell dry weight) after 24 hours of cultivation in YPD liquid medium. The yeast strain YPI-Y2 (with CDW reaching 14.03 g/L; TPC reaching 62.3%) was identified as closely related to the species Pichia kudriavzevii (99.77%) based on ITS rDNA sequence analysis and comparison. The nutritional and cultivation characteristics of the Pichia kudriavzevii YPI-Y2 strain, oriented towards cell biomass production and protein accumulation, were determined. The optimal cultivation temperature and initial medium pH condition for the growth and protein accumulation of the YPI-Y2 yeast strain were 30°C and 6.5, respectively, while the suitable carbon source was identified as D-fructose. Simultaneously, this yeast strain still demonstrated good growth and protein accumulation capability on inorganic nitrogen sources (except urea with 35.62% of TPC). Under suitable cultivation and nutritional conditions, the CDW and TPC values reached 16.12 g/L and 66.62 % (g/100g CDW), respectively.

Keywords: Single cell protein (SCP), Pichia kudriavzevii, yeast, fermented food, YPI-Y2

1. INTRODUCTION

Climate change, coupled with population growth, constitutes a primary determinant of the stability and sustainability of the global food security system. The global population is projected to reach a threshold of approximately 9.3-10 billion by 2050. Consequently, addressing the escalating demand for food to sustain human life has become an urgent imperative. Concurrently, nutritional instability poses severe risks to human health, leading to adverse consequences such as malnutrition and muscle atrophy. Collectively, achieving equilibrium between food production and consumption poses a formidable challenge for traditional food production systems that are heavily reliant on agriculture [1].

According to the Food and Agriculture Organization of the United Nations (FAO), human nutritional requirements are categorized into 3 primary macronutrients: fats, carbohydrates and proteins. Among these, protein is considered the most critical and essential nutrient, given its ubiquitous involvement in virtually all cellular functions and physiological processes, distinguishing it from fats and carbohydrates. Consequently, the exploration of alternative protein sources that are non-animal and non-plant in origin, specifically those that are cost-effective and independent of arable land and freshwater resources, represents a vital strategy in the current global context. In this regard, single-cell protein (SCP) derived from microorganisms emerges as a potent solution. SCP satisfies the rigorous criteria established to address food security challenges, being nutrient-dense with minimal reliance on land and freshwater, while simultaneously mitigating environmental impacts [1].

SCP refers to protein-rich microbial biomass produced from various microorganisms, including yeast, filamentous fungi, algae, and bacteria [1]. Among these, yeast-derived single-cell protein (SCP-Y) represents a particular promising avenue for both research and industrial application [1, 2]. This is primarily because SCP-Y boasts a crude protein content of up to 60% of its dry cell weight, significantly surpassing conventional protein sources such as meat (45%), milk (25%), and soybean (35%) [2]. The issue is worth discussing, and the primary rationale for our selection of yeast as the subject for SCP production is its comprehensive amino acid profile. SCP-Y contains all 8 essential amino acids that the human cannot synthesize *de novo,* including isoleucine (Ile), leucine (Leu), lysine (Lys), phenylalanine (Phe), methionine (Met), threonine (Thr), tryptophan (Trp) and valine (Val). A notable example is lysine. A study by Jach et al. (2022) reported that yeast can synthesize lysine at concentrations up to 70 mg/g protein, which is 2.5 times higher than the 28 mg/g typically found in wheat [2].

These findings underscore the potential of yeast SCP as a viable solution to global nutritional challenges. It offers a low-cost, scalable production method that is independent of arable land and freshwater resources, thereby supporting sustainable food security and minimizing environmental footprints. Consequently, microbial SCP in general, and SCP-

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Y in particularly, is currently garnering significant attention and being intensively exploited worldwide, including in Vietnam [1, 2].

In Vietnam, yeast has been present in many food products for a long time, and mostly focused in alcoholic beverages. Early research on SCP was carried out by Pham Thanh Ho using oyster mushroom (*Pleutorus*) as producer, but not yeast [3]. By the advantages such as popularity and safety, some reseachers have been chosen Saccharomyces cerevisiae as SCP potential producer. Trang et al. (2028) optimized biomass production on cost-effective substrates such as molasses, establishing a promising basis for protein-enriched biomass applicable to food and animal feed industries [4]. Hai et al. (2024) utilized resultant seaweed hydrolysate as nutrient for *S. cerevisiae* biomass production resulted 1.2x10^6 cfu/ml after 72 hrs of cultivation [5]. By-product of pulp industry was hydrolysated for SCP production by *Candida utilis* at pilot scale of 1000 liters for animal feed applications [6].

Pichia kudriavzevii is a particularly non-conventional yeast. Although the safety of *Pichia kudriavzevii* remains controversial, it is found in many fermented foods. Therefore, *Pichia kudriavzevii* is attracting attention for food and biotechnology applications [7].

In this study, we isolated and screened yeast strains, that capability of rapid growth and concomitant high protein accumulation. Subsequently, 01 superior strain was selected to investigate its biological characteristics for SCP production applications. The results of this research contribute to the effective exploration and exploitation of non-*Saccharomyces* yeast diversity for SCP production, proposing an effective alternative to animal and plant-based protein in the current context.

2. MATERIALS AND METHODS

2.1. Materials

5 distinct fermented food samples were collected directly from local household producers (table 1). The samples were subsequently transported to Bioresource Research Center, Phenikaa University, and stored at 4°C before analysis.

Table 1. Sample informations for yeast isolation

Sample name (code)		Sample information (location, collector, …)
Vegetable pickle (YPI)	:	Location: Bui Xuong Trach, Hanoi, Vietnam Collector: Le Thi Hoang Dung
Tré (fermented meat) (YFPS)	:	Location: Bac Ninh, Vietnam Collector: Hoang Quoc Viet
Kimchi (YKCS)	:	Location: Ha Loc, Phu Tho, Vietnam Collector: Hoang Quoc Viet
Fermented grape (NHC-V)	:	Location: Hoai Duc, Hanoi, Vietnam Collector: Tran Huu Phong
Grape pomace (BN-V)	:	Location: Ha Thach, Phu Tho, Vietnam Collector: Hoang Quoc Viet

2.2. Chemicals, standards

Standard chemicals: Methyl red (CAS: 493-52-7), and bromocresol green (CAS: 76-60-8, Xilong). Other reagents and solvents consisted of: yeast extract, peptone-r, D-glucose, D-fructose, 98% sulfuric acid, sodium hydroxide, boric acid, potassium sulfate, copper sulfate pentahydrate and double-distilled water (ddH_2O).

2.3. Equipment, apparatus

General laboratory equipment included: Class II-Biological Safety Cabinet, laminar flow cabinet, 4°C refrigerator, microbial stock storage unit, autoclave, fume-hood, optical microscope, shaking incubator, and cooled incubator. Specialized instrumentation included: Biochrom WPA CO8000 Cell Density, UV-Vis spectrophotometer D2800, biological sample digestion system, Nexus Gradient PCR thermal cycler, Kjeldahl system KDN-04.

2.4. Methods

2.4.1. Enrichment, isolation and screening of potential yeast strain

Five grams of each sample were homogenized in 45 mL of Yeast – Peptone – Dextrose broth (YPD broth). The suspension was incubated at 30°C with shaking at 200 rpm for 1h to facilitate pre-activation. Subsequently, 10% (v/v) inoculum of the suspension was transferred into 45 mL of fresh YPD medium. This enrichment culture was maintained at 30°C, 200 rpm for 48h to establish the 1st generation (T_1). Subculturing was performed sequentially 3 times at 48h intervals to obtain the 3rd generation (T_3). The T_3 enriched community was observed at 1000x magnification by crystal violet staining and subjected to isolation via the serial dilution method. 1 mL of the T_3 culture underwent ten-fold serial dilutions (up to 10^{-6}) in 9 mL of 0,9% NaCl solution. Then, 100 µL of the culture was spread on YPD agar plates and incubated at 30°C for 48h. Visible colonies were selected and purified by repeated streaking on YPD agar until pure cultures were obtained. The isolates were preserved in 30% glycerol at -20°C.

The suitability of yeast strains for SCP production is critically determined by their capacity for rapid biomass proliferation and high intracellular protein accumulation. Initially, 36 yeast strains were reactivated from -20°C storage on YPD agar plates at 30°C for 24h. A loopful of activated cells was then inoculated into erlenmeyer flasks containing 20 mL of YPD broth. Cultivation was performed at 30°C, 200 rpm for 24h. To evaluate biomass production, the entire culture volume was centrifuged at 6000 rpm for 10 minutes. The cell pellet was collected and dried at 80°C until constant weight. The cell mass was used for total protein analysis by Kjeldahl method.

2.4.2. DNA extraction and phylogenetic analysis

Genomic DNA was recovered through an integrated physicochemical cell lysis protocol [8]. Cell pellets were resuspended in lysis buffer fortified with 20%, followed by thermal incubation at 65°C for 120 minutes. Subsequently, 400 µL of Chloroform – Isoamyl alcohol was added, and the mixture was inverted thoroughly before centrifugation at 12000 rpm for 10 minutes. The supernatant was collected and mixed with Isopropanol at a 1:1 ratio to precipitate the DNA, followed by incubation at -20°C for 1h. The DNA pellet was recovered by centrifugation at 12000 rpm for 10 minutes and subsequently resuspended in 50 µL of Milli-Q water. The ITS rDNA region was amplified via PCR using the primer pair ITS_1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS_4 (5' TCCTCCGCTTATTGATATGC 3'), as described by White et al. (1989) [9]. PRC programe was detailed in figure 1. The PCR samples were sequenced by Phusa Biochem Co., Ltd and then were analyzed for homology against the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) using the BLAST algorithm. A phylogenetic tree was constructed using MEGA 11 software based on the Neighbor–Joining method with 1000 bootstrap replications [10].

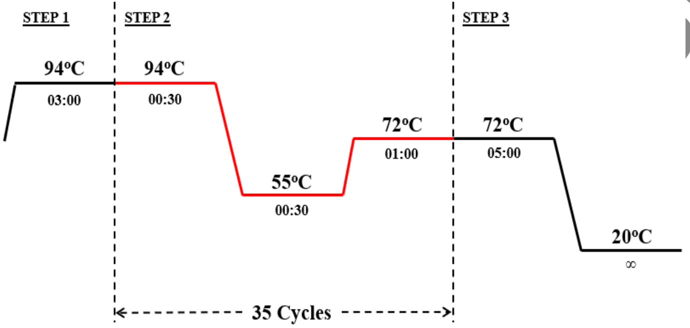

Figure 1. *PCR program for ITS-rDNA gene amplification.*

2.4.3. Effect of culture volume on YPI-Y2 biomass production

Effect of volume culture ratio was carried out with a range of 5 to 45 ml of YPD broth in 100 ml conical flask. The seed culture of YPI-Y2 strain was activated in YPD medium for 24h. After that seed culture was transferred to YPD broth with different volume ratio to reach 1.0 value of OD$_{600}$. Then the flasks were incubated at 30°C and shaken at 200rpm. After 24h, 5 mL of culture from each flask was centrifuged at 6000 rpm for 10 minutes. The resulting biomass was harvested and dried 80°C until a constant weight was achieved. Biomass weight and cell density data were recorded. The experiments were performed in three independent replicates.

2.4.4. Optimization of nutritional components and culture conditions for growth and protein accumulation

Yeast cells YPI-Y2 transferred into 45 mL YPD broth to establish the primary seed culture. Incubation was performed at 30°C with shaking at 200 rpm for 24h. An appropriate volume of the seed culture was inoculated into 10 mL of the experimental media to achieve an OD$_{600}$=1,0. This setup was designed to evaluate the effects on growth and concomitant protein accumulation across various parameters, including: 8 carbon sources (20g/L of D-fructose, D-glucose, Xylose, Sucrose, Maltose, Maltose dextrin, Molasses and Starch soluble), 6 nitrogen sources (YP, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, NH_4NO_3, NH_4Cl and $(NH_2)_2CO$ with total nitrogen of 3.8 g/L), 4 temperature points (25-40°C, increments 5°C), and 13 pH levels (3.0-9.0, increments 0.5). Key parameters, including: biomass, OD$_{600}$, and total protein content, were quantified. The experiments were repeated three times.

2.4.5. Analytical methods for biomass and total protein content

Cell dry weight (CDW) was determined according to the protocol described by Thuoc et al. (2014) [11]. Total protein content (TPC) was quantified using the Kjeldahl method [12, 13].

2.4.6. Methodology for data analysis

6

3. RESULTS AND DISCUSSIONS

3.1. Enrichment, isolation, screening and identification of yeast strains

The implementation of 3 consecutive enrichment cycles established an effective selective pressure, significantly suppressing non-adapted bacteria while fostering the dominance of yeast populations within the culture medium. The accumulation of ethanol, coupled with mild acidic conditions, acted as a natural competitive filter, thereby stabilizing the target microbial community. By the 3rd generation (T_3), the microbial composition was notably refined, consisting predominantly of yeast species exhibiting robust growth kinetics and efficient substrate conversion capabilities (Fig. 3). A total of 36 yeast strains were successfully isolated from 5 enriched communities.

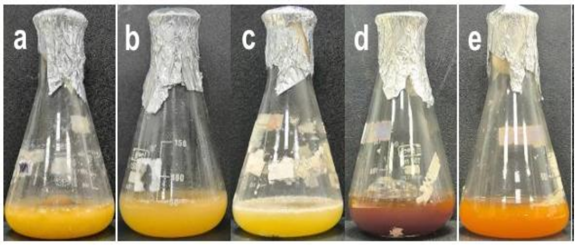

Figure 2. Enrichment results of the 5 collected samples: (a) vegetable pickle, (b) Tré (fermented meat), (c) kimchi, (d) fermented grape, and (e) grape pomance

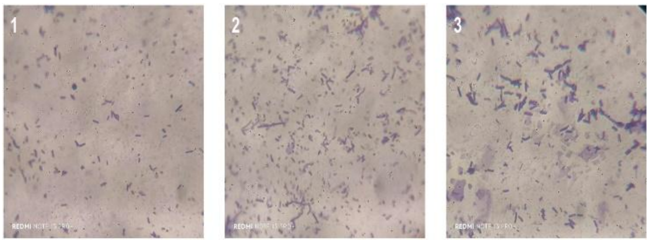

Figure 3. Microbial communities through successive enrichment processes of vegetable picle sample: (1) 1st generation, (2) 2nd generation, and (3) 3rd generation.

After 24h of cultivation, 15/36 strains (41,7%) yielded a CDW exceeding 10 g/L. The CDW values for this high-yield group ranged from 11-16 g/L. The highest CDW was observed in strains YFPs-M6 (15,6±0,42 g/L) and YFPs-M10 (15,2±0,21 g/L) (Fig.4). Of there, 3 strains were isolated from Kimchi exhibited CDW values of approximately 11 g/L. Among the remaining isolates, 15 strains displayed CDW values between 6-10 g/L, while 8 strains produced less than 6 g/L. In a study by Trang et al. (2017), *S. cerevisiae* SC2.75 strain achieved a CDW of 10.71 g/L after 18h of cultivation [4]. These comparisons indicate that the 15 effectively regulate metabolism to maximize biomass production, even under non-optimized conditions.

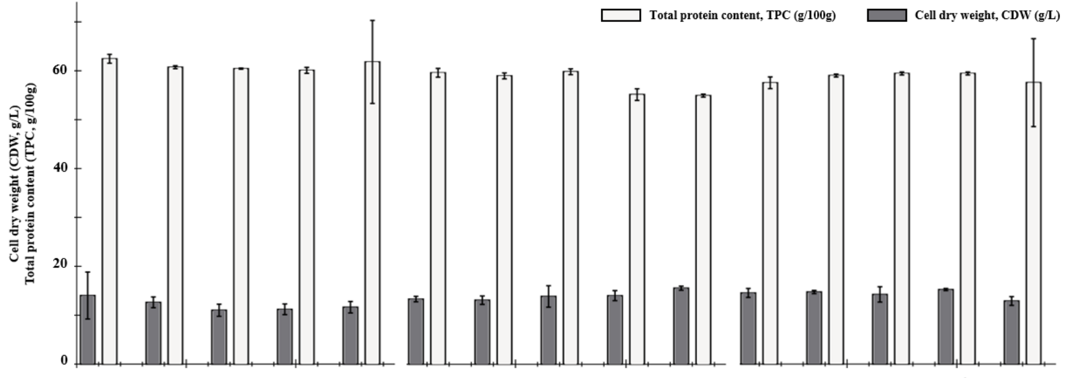

Figure 4. *CDW and TPC values of promised yeast strains.*

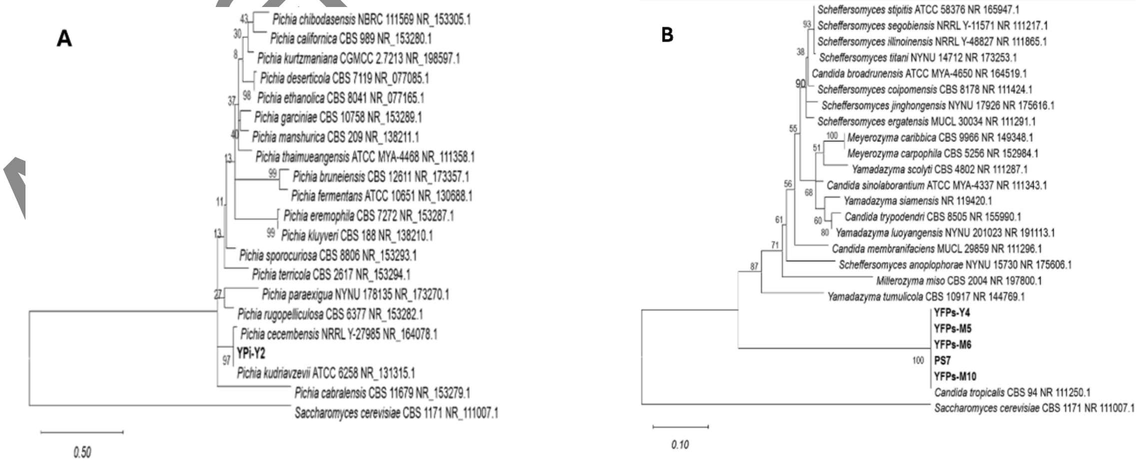

Figure 5. Phylogenetic tree of yeast strains based on ITS rDNA sequences BLASTed on NCBI. A – YPI-Y2 relationship with Pichia genus; B – Other strain relationship with Candida genus

Total protein content (TPC) analysis of the ten yeast strains with highest biomass revealed significant variation in SCP production capacity, with values ranging from 57 to 62.3% (g/100g of CDW). Strains named YFPS-M5, YFPS-M6, YFPS-M7, YFPS-M8 and YFPS-M10 could produce relatively high cell mass ranging from 13 to 15 g/L, but

TPC values achieved not superior. Conversely, strains YPI-Y2 and YKCS-Y3 exhibited outstanding protein accumulation capacity with TPC value at 62.3% and 61.5%, respectively (Fig. 4). A study by Rachamontree et al. (2015), reported that the strain *P. kudriavzevii* MSY-2 achieved a TPC of 66.8% (g/100g of biomass) only under optimized culture conditions [14]. Remarkably, several strains in this study achieved TPC values comparable to this benchmark without any prior optimization. This finding underscores the robust physiological potential of these isolates for efficient SCP production.

Predominant yeast strains were successfully identified to genus level based on ITS sequence analysis. The constructed phylogenetic tree showed that the yeast strain designated YPI-Y2 belongs to the genus *Pichia* with the similarity level of 99.77% to the species *Pichia kudriavzevii* (Fig. 5A). The other strains were all identified as belonging to the genus *Candida* with a similarity level of over 99% to the species *Candida tropicalis* (Fig. 5B). Strain YPI-Y2 was selected as the best candidate for further investigation.

3.2. Culture characteristics of YPI-Y2 strain for biomass production and protein accumulation

3.2.1. Effect of culture volume ratio

The investigation across 8 different culture volumes revealed that the V5 and V10 conditions supported the most favorable growth. Specifically, the maximum optical OD_{600} of 19,7 was recorded at V10. Correspondingly, the CDW at V10 (13,7 g/L) surpassed that of V5 (12,4 g/L). Notably, a progressive decline in growth was observed as the volume increased from V15 to V40. The lowest performance was recorded at V40, with an OD_{600} of 12,95 and a CDW of only 7,2 g/L (Fig. 6). These findings demonstrate a critical correlation between the medium volume and the liquid-air interface area, which directly influences the O_2 transfer rate and consequently, the biomass productivity of the yeast strain. This observation aligns with the study by Nayana et al. (2023), which corroborated that culture volume and surface area availability significantly impact the yield and physiological activities of aerobic microorganisms due to O_2 limitation [15]. Based on these results, a medium to flask volume ratio of 1/10 was selected as the ideal condition for further characterization of the strain.

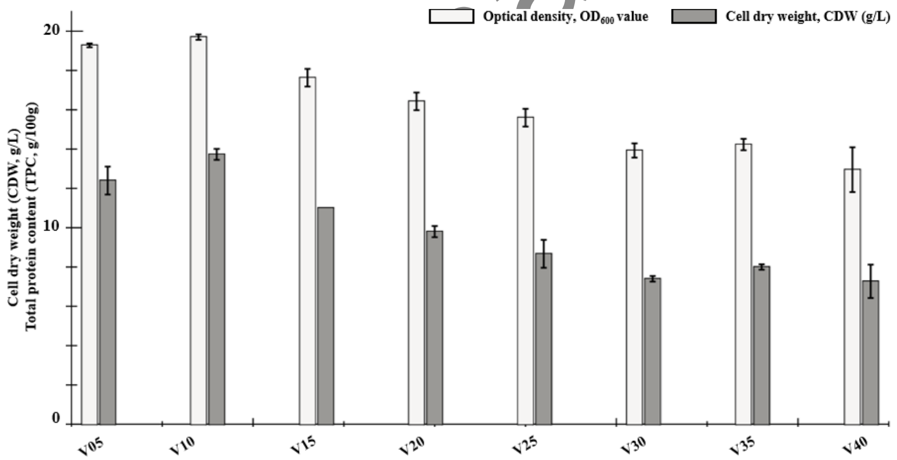

Figure 6. *Effect of culture volume ratio on CDW and OD_{600} values of yeast strain YPI-Y2*

3.2.2. Effect of pH on growth and protein accumulation

The TPC of the yeast strain exhibited significant fluctuations in response to variations in the initial pH of the culture medium. As the pH increased from 3.0 to 4.0, the TPC remained relatively stable within the range of 51% to 52% (Fig. 7). This observation indicates that the strain retains its capacity for protein biosynthesis even under mildly acidic conditions. The obtained results Within the pH range of 5.0 to 6.5, a progressive increase in protein content was observed, reaching a peak at pH 6.5 (64.75%). This peak coincided with the maximal values recorded for OD_{600} of 19.65 and CDW of 13.63 g/L. Under relatively acidic pH conditions (pH 3.0), the CDW value of strain YPI-Y2 reached 88.6% compared to at the optimal pH point (pH 6.5). The results is quite similar to Thu et al. (2023) and Xu et al. (2024) for *P. kudriavzevii* [16, 17].

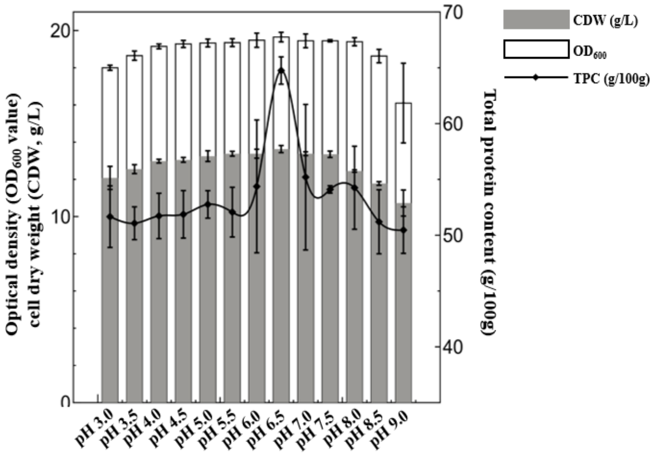

Figure 7. *Effect of initial pH on growth and protein accumulation*

3.3.3. Effect of temperature

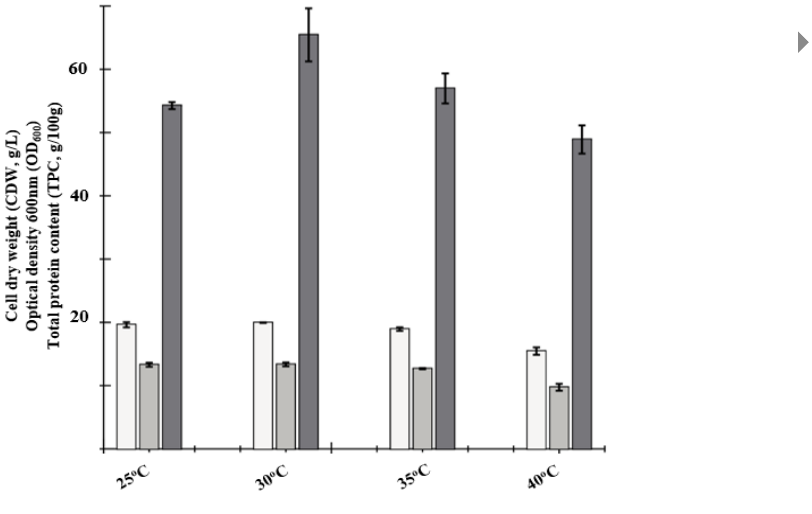

Figure 8. *Effect of culture temperature on growth and protein accumulation*

The results indicate that incubation temperature exerts a significant influence on both the growth capacity and protein accumulation of the yeast strain. A substantial increase in protein content was observed as the temperature rose from 25 to 30°C. The value peaked at 65.44% at 30°C, coinciding with maximal OD_{600} of 19.95 and CDW of 13.45 (Fig. 8). This temperature was identified as the optimum, enabling cells to maintain a high metabolic rate and robust protein synthesis.

Publications on the yeast *Pichia kudriavzevii* show similar results to the impact of temperature on growth as in this study. Nieto-Sarabia et al. (2022) showed wide temperature range for growth of strain *P. kudriavzevii* 4A and best exhibition at 30-40°C [18]. In the study of Chamnipa et al. (2018), the strain *P. kudriavzevii* RZ8-1 could grow well in the temperature range of 30-40°C and slightly decreased at 42°C but sharply at 45°C [19].

3.3.4. Effect of carbon sources

Carbon serves as a fundamental macronutrient for all living organisms. Furthermore, it functions as the primary energy source driving microbial growth and the concomitant accumulation of associated secondary products. In this study, various carbon sources were investigated at a concentration of 2% (w/v) with absence of carbon source as negative control to evaluate the metabolic performance of yeast strain YPI-Y2 using minimal salt medium (MSM) [20]. The results indicated that strain YPI-Y2 was capable of assimilating 7/8 tested carbon sources for growth, while maintaining a state of survival on the remaining source (xylose).

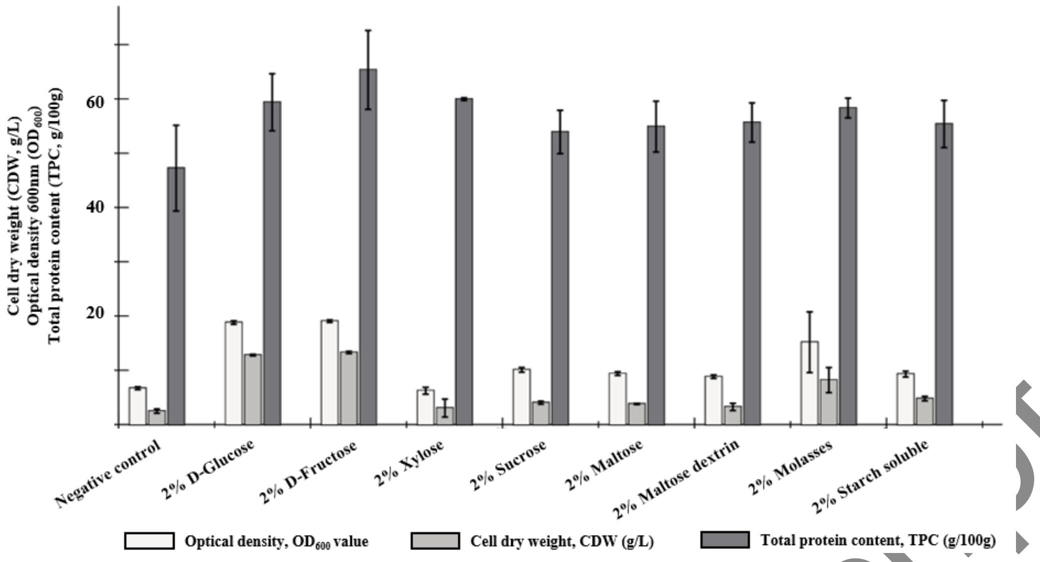

Figure 9. *Effect of carbon sources on growth and protein accumulation of yeast strain YPI-Y2*

Among the assimilable sources, YPI-Y2 exhibited superior growth and protein accumulation when cultivated on D-fructose and D-glucose. Specifically, maximal values for CDW, OD$_{600}$, and TPC were recorded with D-fructose (13.3 g/L, 19.05, and 65.35%, respectively). In comparison, D-glucose yielded slightly lower values of 12.8 g/L, 18.8, and 59.39% (Fig. 9). These findings suggest that D-fructose is metabolized more efficiently than D-glucose by this specific strain. Compared to monosaccharides, complex carbon sources (sucrose, maltose, maltodextrin, and starch) resulted in significantly reduced metabolic efficiency. This reduction is attributed to their complex chemical structures (polymers, oligosaccharides), which necessitate additional energy expenditure for hydrolysis before assimilation. This energy diversion limits the resources available for cellular proliferation and protein synthesis. Consequently, the average OD$_{600}$ for this group ranged from 8,82-10,08, approximately 1.4-fold higher than the control but 2.03-fold lower than that of D-fructose. Similarly, CDW values ranged from 3.2 to 4.7 g/L (4.1-fold lower than D-fructose), and TPC values were moderate, ranging from 54.9% to 58.3%. Molasses, a complex substrate containing various sugars, minerals, and potential inhibitors that may induce cellular stress or inhibit hydrolytic enzymes, supported moderate growth. Strain YPI-Y2 demonstrated a metabolic capability on molasses second only to the monosaccharides, achieving an OD$_{600}$ of 15.17, CDW of 8.2 g/L, and TPC of 58.34%. High total protein content and biomass due to molasses utilization is an advantage of YPI-Y2 strain by solving an inexpensive by-product and thus increasing the competitiveness at industrial production scale. Despite its ability to utilize complex sugars, YPI-Y2 failed to assimilate xylose effectively. This limitation is likely due to the absence of key enzymes, such as xylose reductase and xylitol dehydrogenase, which are required to convert xylose into xylulose for entry into the Pentose Phosphate Pathway [21, 22]. Our results align with those of Nava et al. (2017) demonstrating that *P. kudriavzevii* ITV-S42 preferentially metabolized glucose and fructose as primary carbon sources, with negligible xylose catabolism and limited sucrose utilization due to deficient metabolic enzymes [23]. Notably, Koutinas et al. (2016) reported that *P. kudriavzevii* KVMP10 possessed efficient xylose-fermenting capacity at elevated temperatures [24], indicating genetic diversity within this species. This finding highlights genetic diversity among different *P. kudriavzevii* strains. The OD$_{600}$ of 6.23 and CDW of 3.05 g/L on xylose showed minimal deviation from the negative control (OD$_{600}$ of 6.68; CDW of 2.48 g/L). However, the TPC remained relatively high at 59.96%. This suggests that while xylose does not support proliferation, a fraction may be partially metabolized to provide maintenance energy for organelle function and cellular survival.

3.3.5. Effect of nitrogen sources

Nitrogen is an indispensable element for biological functions, serving as a fundamental constituent of vital cellular macromolecules, including protein, nucleic acids, and ATP... Consequently, it plays a critical governing role in microbial growth. To evaluate the growth and concomitant protein accumulation of yeast strain YPI-Y2, 6 distinct nitrogen sources were investigated: combined Yeast-Peptone (YP), $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, NH_4NO_3, NH_4Cl, and $(NH_2)_2CO$ with absence of nitrogen source as negative control.

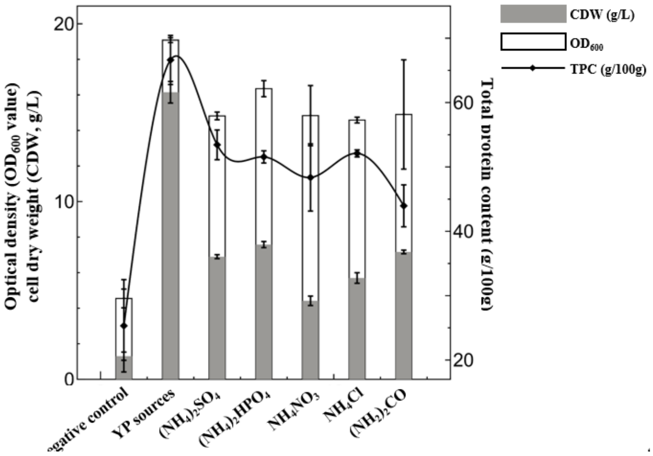

Figure 10. *Effect of nitrogen sources on growth and protein accumulation of yeast strain YPI-Y2*

The results indicated that strain YPI-Y2 exhibited optimal performance when cultivated on yeast – Peptone combination (YP), the organic nitrogen source. Specifically, the TPC reached 66.62%, while the maximal OD_{600} and CDW peaked at 19.08 and 16.15 g/L, respectively (Fig. 10). Compared to other groups, cultivation on YP resulted in a 4-fold increase in cell density (OD_{600}) and a 2.6-fold increase in protein accumulation. This superiority is likely attributable to the presence of readily absorbable amino acids and peptides in peptone and yeast extract. These components not only provide a rapid nitrogen supply but also act as precursors that stimulate the synthesis of intracellular structural proteins and metabolic enzymes. Xu et al. (2022) reported that *P. kudriavzevii* XTY1 strain demonstrated efficient utilization of organic nitrogen during heterotrophic nitrification, which is consistent with our findings showing that organic nitrogen uitilization capacity by *P. kudriavzevii* YPI-Y2 exhibited superior performance relative to inorganic nitrogen sources [21]. Among the inorganic sources, $(NH_4)_2SO_4$ (53.44%), $(NH_4)_2HPO_4$ (51.56%), and NH_4Cl (52.11%) yielded superior efficiency compared to ammonium nitrate and urea. These salts provide readily metabolizable ammonium ions NH_4^+, and facilitate pH stability, thereby supporting moderate biomass production (CDW ranging from 5.7 to 7.6 g/L) and enhancing protein accumulation. NH_4NO_3 in a lower TPC of 48.36%. This reduction is likely because the nitrate ion NO_3^- requires energy-intensive reduction before assimilation, effectively slowing down the rate of nitrogen incorporation. Although urea supported relatively high OD_{600} and CDW values, the total protein content reached only 35.62%. This disparity suggests that the rate of urea hydrolysis into utilizable NH_4^+ is slower compared to the direct uptake available from ammonium salts. By low-cost and availability, ammonium could be potentially important nitrogen when using YPI-Y2 strain in single cell protein production at industrial scale.

4. CONCLUSION

The potential yeast strains (36 strains) were isolated from fermented food that exhibited good growth and high protein accumulation ranging from 57% to 62.3% (g/100g cell biomass). Among them, the most promising strain *Pichia kudriavzevii* YPI-Y2 was selected for further investigation. The optimal conditions for the growth and concomitant protein accumulation of YPI-Y2 were determined as follows: an ideal culture volume ratio of 10%, D-fructose (20 g/L) and combine of Yeast – Peptone (YP, 3.8 g/L) as the carbon and nitrogen sources, pH of 6.5, and temperature of 30°C. Under these optimal conditions, the average CDW and TPC reached 16.12 g/L and 66.62% (g/100g biomass), respectively, within 24h of cultivation. These results substantiate YPI-Y2's superior protein accumulation capacity and short fermentation duration, demonstrating its promising potential for industrial single-cell protein production. Further studies on safety and fermentation technology should be conducted to provide robust data for the application of YPI-Y2 strain for single cell protein production in industrial scale.

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