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Original Paper

Isolation and Comparative Characterization of *Saccharomyces Cerevisiae* From Indigenous Fermented Tea "Miang" Under Fermentation Stress Conditions

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***Abstract*- This study investigated the isolation and comparative characterization of *Saccharomyces cerevisiae* strains from indigenous fermented tea "Miang" and their comparison with the commercial strain *Saccharomyces cerevisiae* EC1118 under various fermentation stress conditions. Yeast strains were isolated from Miang, and their performance was evaluated in terms of sugar utilization, pH tolerance, ethanol concentration, potassium metabisulfite (KMS), sodium chloride (NaCl), and osmotic pressure tolerance. Optical density (OD) at 600 nm was measured using a spectrophotometer over a 24-h incubation period to assess yeast growth. The results showed that the newly isolated strain, *S. cerevisiae* MXH-1, exhibited superior growth in ethanol concentrations of up to 15%, with an OD increase of 1.22 compared to 0.12 for EC1118. Additionally, MXH-1 demonstrated enhanced tolerance to KMS at 200 ppm and NaCl at 5%, with significantly higher OD changes than EC1118. These findings underscore the potential of *S. cerevisiae* MXH-1 for industrial applications, particularly in high-stress fermentation processes. This study provides valuable insights into the fermentation capabilities of indigenous yeast strains under challenging environmental conditions positioning MXH-1 as a promising candidate for food and beverage industries. Further research is recommended to explore the genetic mechanisms underlying its resilience and tolerance to fermentation stressors.**

Keywords-*Miang, Saccharomyces cerevisiae, ethanol fermentation, Stress tolerance*

I. INTRODUCTION

The *S. cerevisiae*, commonly known as yeast for baker and brewer is a widely studied eukaryotic microorganism due to its essential role in various fermentation processes including bread, beer, and wine production [1;2]. This yeast is renowned for its ability to metabolize sugars into alcohol and carbon dioxide making it indispensable in the food and beverage industries [3;4]. *S. cerevisiae* has proven multipurpose in various applications, including the bioconversion of fruit wastes into single-cell proteins offering a sustainable approach to addressing protein shortages while reducing pollution and waste [5]. Its resilience under various environmental stresses, such as fluctuations in pH, ethanol concentration, salt content, and osmotic pressure, has led to extensive research into its industrial applications [6]. These features make *S. cerevisiae* a model organism for understanding stress tolerance mechanisms in yeast [7;8].

The traditional fermented tea "Miang" provides a unique environment for microbial fermentation [9;10]. The fermentation of Miang relies on various microorganisms including yeasts to break down the natural sugars in tea leaves [11;12]. The presence of yeast in Miang fermentation is particularly interesting because this indigenous strain may exhibit fermentation characteristics distinct from commercial yeast strains [13]. Miang fermentation is occurred over long periods and fluctuating environmental conditions offers an ideal natural setting for isolating robust yeast strains capable of adapting to stress conditions [14;15].

In industrial fermentation, *S. cerevisiae* must withstand several stress conditions that significantly impact its growth and metabolic activity including pH fluctuations, ethanol toxicity, and osmotic pressure due to high sugar concentrations [16;17]. High ethanol tolerance is particularly crucial in alcoholic fermentation as ethanol accumulation inhibits yeast growth and reduces fermentation efficiency [18]. Similarly, tolerance to osmotic pressure and salt concentrations is essential in high-sugar and high-salt environments such as in the production of fermented foods and beverages [19]. Potassium metabisulfite also creates a significant stress condition, and yeast tolerance to this preservative is critical in certain industrial processes [20]. These stress responses are often used as benchmarks for selecting yeast strains suitable for industrial fermentation [21;22]. This study aims to isolate and compare *S. cerevisiae* strains from Miang with the commercial strain (*S. cerevisiae* EC1118). The strains were evaluated under various stress

conditions, including ethanol concentration, pH levels, KMS, sodium chloride, and osmotic pressure.

II. MATERIALS AND METHODS

A. Raw material and chemicals

Miang samples were collected from main production areas in the northwestern region of Laos, specifically from 3 districts within Xayaboury Province. The chloramphenicol was sourced from Bio Basic Inc. (Ontario, Canada), while 95% food-grade ethyl alcohol was obtained through Union Chemical and Equipment CO. LTD (Bangkok, Thailand). The reference strain *S. cerevisiae* Lalvin EC-1118 was purchased from Lallemand Inc. (Montreal, Canada).

B. Methods

1) Sample collection

A total of 30 Miang samples were collected from 3 districts in Xayaboury Province, Laos PDR: Saysthan (19.405888°N, 101.387887°E), Xienghorn (19.587956°N, 100.807902°E), and Khop (19.41032°N, 100.30071°E). These areas are well-known for their long-standing tradition of Miang cultivation and fermentation, which is deeply embedded in the local culture and customs of the villagers in northwestern Laos. The samples were aseptically collected in sterile plastic bags, transported on ice, and stored at 4°C until further analysis.

2) Yeast isolation and characterization

Each Miang sample (10 g) was mixed with 50 mL of sterile water, and 1 mL of the mixture was inoculated into YPD broth (yeast extract, peptone, glucose ratio 10:20:20 g/L) supplemented with 100 ppm of chloramphenicol as an antibacterial agent. A Durham tube was used to detect gas production, then the cultures were incubated at 30°C for 2-5 days. Positive cultures were streaked on YPD agar to isolate yeast colonies, which were then examined microscopically for typical *Saccharomyces* morphology. The selected colonies were preserved in YPD broth with 15% glycerol at -20°C.

3) Molecular-based identification method

The obtained isolates with ethanol-producing activity were identified through sequence analysis of the D1/D2 domain of the LSU rRNA gene. All yeast strains were cultured in YPD broth at 30°C for 24 h, followed by centrifugation (14,000 × g, 1 min, 4°C) and resuspension in 480 µL of 50 mM ethylenediaminetetraacetic acid. Cells were treated with 40 µL of 50 mg/mL lysozyme (Bio Basic Inc., Canada), and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's protocol. DNA amplification was carried out using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) on an MG-96 MyGeneTM Thermal Cycler (LongGene Scientific Inc., China). The PCR products were purified and sequenced by 1st BASE Pte Ltd. (Singapore). The sequence products were aligned against the GenBank database, and a phylogenetic tree was constructed using the neighbor-joining method with MEGA software version 4.0.

4) Fermentation efficiency for ethanol production

Ethanol production was assessed at 24-h intervals using an ebulliometer to measure ethanol concentration. The ethanol content was calculated as a percentage of the ethanol concentration. Fermentation efficiency was determined by comparing the practical yield of ethanol to the theoretical yield, expressed as a percentage. These calculations provide a quantitative evaluation of the yeast strains to produce ethanol.

5) Assessment of strees condition

The performance of yeast isolates from Miang and the commercial strain EC-1118 was evaluated under various stress conditions to assess their resilience and adaptability. The stress factors included ethanol concentrations (5%, 10%, 15%, and 20%), pH levels (2, 3, 4, 5, and 6), osmotic pressure created by sugar concentrations (10%, 15%, 20%, and 25%), potassium metabisulfite (KMS) concentrations (50, 100, 150, and 200 ppm), and salt (NaCl) levels (5%, 10%, and 15%). These conditions were prepared in YPD broth.

The growth rate of the yeast strains was monitored by measuring the optical density at 600 nm (OD_{600}) at the initial time point and after 24 h of incubation to evaluate their ability to grow under each condition. Additionally, the capacity of the yeast strains to metabolize different sugars (glucose, fructose, xylose, and lactose) was examined. This comprehensive evaluation aimed to identify yeast strains with enhanced stress tolerance, which is essential for consistent and efficient ethanol production under challenging fermentation conditions.

C. Data analysis

Differences in the observed data were analyzed using a T-test and analysis of variance (ANOVA) to determine statistical significance ($p<0.05$). Data analysis was performed using SPSS software, version 17.

III. RESULT AND DISCUSSION

A. Isolation and molecular identification

Among the 30 Miang samples collected from three districts in Xayaboury Province, only two samples exhibited positive gas production. From these samples, a total of eight yeast isolates were obtained. Each isolate was subsequently tested for gas production, and only isolates MXH-1 from Xienghorn and MMK-4 from Khop were confirmed to produce gas in the confirmation step (Table 1). The colony morphology and microscopic observations are shown in Fig. 1. On YPD agar, MXH-1 exhibited ivory-colored, smooth, circular colonies with a slightly creamy texture and well-defined edges. Under the microscope, MXH-1 cells displayed an oval shape with prominent budding, with some cells appearing spherical (Fig. A1 and A2). In contrast, MMK-4 formed round, white colonies with a non-smooth, raised surface on YPD agar. These colonies had distinct edges and a unique spider-tail-like feature, suggesting potential hyphal growth. Microscopically, MMK-4 cells appeared oval to elongated, showing clear evidence of budding. The cell morphology indicated a potential ability to transition between yeast and hyphal forms under specific conditions (Fig. B1 and B2)

The identification of the 2 ethanol-producing isolates (MXH-1 and MMK-4) were conducted through sequence

analysis of the D1/D2 domain of the LSU rRNA gene (Table 2). The analysis revealed that isolate MXH-1 had 100% similarity to *Saccharomyces cerevisiae* (NG_042623.1), while isolate MMK-4 was found to be closely related to *Candida tropicalis* (KU729147.1) with 100% similarity. The phylogenetic relationships of the isolates were illustrated using the neighbor-joining method, as shown in Fig. 2. Since *Candida tropicalis* (MMK-4) is a known pathogenic yeast [23], further investigations focused solely on MXH-1 comparing its characteristics with the commercial strain *S. cerevisiae* EC1118

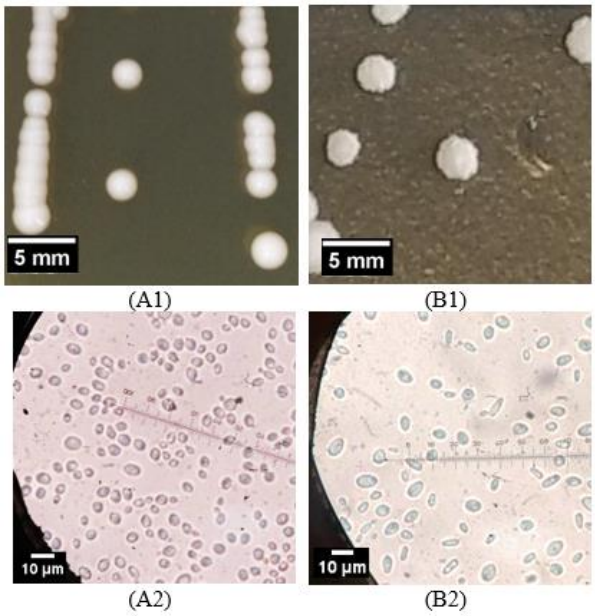

Fig. 1. Morphology of yeast isolated form Miang sample.

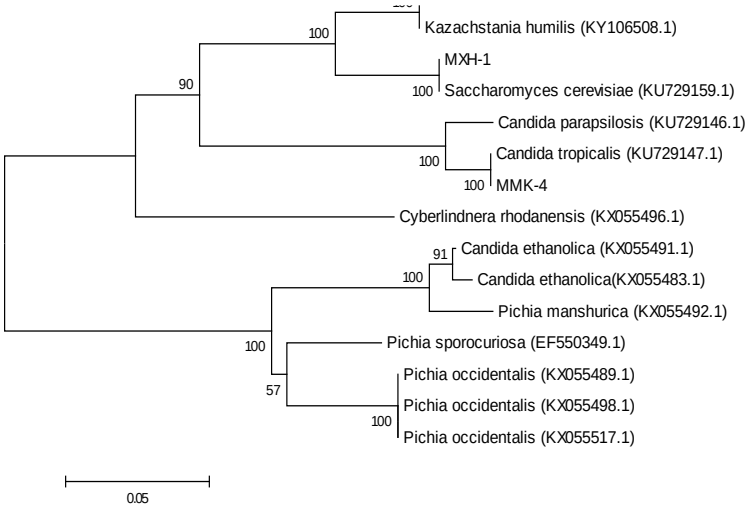

Fig. 2. Phylogenetic tree constructed using the neighbor-joining method based on the complete D1/D2 sequence.

B. The efficacy of ethanol production

The ethanol production of the two yeast strains, MXH-1 and EC1118, was compared after 24 h of fermentation in YPD broth containing 5% glucose. As shown in Table 3, there was no statistically significant difference in ethanol content between the strains. MXH-1 produced 2.35% ethanol, while EC1118 produced 2.40%. Similarly, the theoretical yield of ethanol fermentation was comparable between the 2 strains, with MXH-1 achieving 92.21% and EC1118 reaching 94.11%. This finding aligns with previous studies highlighting the robust ethanol production performance of *S. cerevisiae* strains. Wild-type *Saccharomyces cerevisiae* strains isolated from local environments have demonstrated high ethanol production rates comparable to those of commercial strains [24;25].

TABLE I. YEAST ISOLATES AND GAS PRODUCTION LEVELS

Sampling site		**Gas level**	**Isolate number**	**Gas confirmation**
District	Positive sample			
Xienhorn	1/10	+++	MXH-1	+++
			MXH-2	-
			MXH-3	-
			MXH-4	-
Khop	1/10	+++	MMK-1	-
			MMK-2	-
			MMK-3	-
			MMK-4	+++
Saysathan	0/10	-	-	-

Remark: Gas production levels were classified as follows: (-) negative, (+) low, (++) moderate, (+++) high.

TABLE II. IDENTIFICATION OF THE D1/D2 DOMAIN OF THE LSU RRNA GENE

No	**S. Name**	**Per.Ident %**	**Query Length**	**Accession number**
MXH-1	*Saccharomyces cerevisiae*	100	603	NG_042623.1
MMK-4	*Candida tropicalis*	100	594	KU729147.1

TABLE III. COMPARISON OF THE ETHANOL PRODUCTION AT 24 H

Parameter	**MXH-1**	**EC1118**
Ethanol content (%) ^{ns}	2.35 ± 0.19	2.40 ± 0.16
Theoretical yield of ethanol fermentation (%) ^{ns}	92.21 ± 5.36	94.11 ± 6.70

Remark: ns: non-significant difference ($p > 0.05$)

C. The comparison of fermentation under stress conditions

1) Acidic condition

The growth performance of *S. cerevisiae* strains MXH-1 and EC1118 were compared under varying pH conditions (Table 4). At neutral pH (6) and mildly acidic pH (5), both strains exhibited robust and nearly identical growth, with MXH-1 showing OD changes of 1.47 and 1.41, and EC1118 showing changes of 1.46 and 1.41, respectively. At pH 4, both strains maintained similar growth levels with MXH-1 showing a slightly higher OD change (1.34) compared to EC1118 (1.32). However, at pH 3, both strains experienced a significant reduction in growth, with MXH-1 showing an OD change of 0.10 and EC1118 at 0.13. At pH 2, MXH-1 exhibited slightly better tolerance (0.13) compared to EC1118 (0.06.). Low pH environments have been shown to increase cell mortality and extend the lag phase in yeast growth, as indicated by decreased OD and reduced cell counts [26;27]. Acidification of the cell interior disrupts essential cellular processes, leading to impaired growth and increased mortality, with viability further reduced due to membrane damage at pH levels below 3 [28;29]

TABLE IV. COMPARISON OF GROWTH PERFORMANCE IN ACIDIC CONDITION

No	pH	Initial OD	After 24 h OD	Change in OD
MXH-1	6	0.18	1.65	1.47
	5	0.22	1.63	1.41
	4	0.22	1.57	1.34
	3	0.25	0.35	0.10
	2	0.25	0.38	0.13
EC1118	6	0.25	1.72	1.46
	5	0.24	1.65	1.41
	4	0.24	1.56	1.32
	3	0.24	0.38	0.13
	2	0.34	0.41	0.06

2) Potassium metabilsulfite concentration

The effect of KMS on growth performance was presented in Table 5. At a KMS concentration of 50 ppm, MXH-1 exhibited an OD change of 1.33, slightly higher than the 1.25 observed for EC1118. As the KMS concentration increased to 100 ppm, MXH-1 continued to demonstrate higher tolerance, with an OD change of 1.35 compared to 1.30 for EC1118. At 150 ppm, MXH-1 maintained stable growth with an OD change of 1.35, while the growth of EC1118 decreased further showing an OD change of 1.23. At the highest KMS concentration of 200 ppm, MXH-1 exhibited the greatest tolerance, with an OD change of 1.37 compared to 1.25 for EC1118.

The enhanced tolerance of MXH-1 to KMS likely stems from genetic and physiological adaptations, specifically in stress response and detoxification pathways. Resistant strains of MXH-1 exhibited upregulated pathways that maintained redox balance and facilitated efficient KMS export, supporting growth at high KMS concentrations [30]. Additionally, modifications in membrane structure and transport proteins preserved potassium homeostasis and cell wall integrity, preventing cellular acidification and damage under sulfite stress [31;32]. These defense mechanisms allowed MXH-1 to maintain stable growth under conditions that inhibited EC1118, highlighting its robustness for fermentation processes involving sulfites.

TABLE IV. COMPARISON OF GROWTH PERFORMANCE IN KMS CONCENTRATION

No	KMS (PPM)	Initial OD	After 24 h OD	Change in OD
MXH-1	50	0.31	1.64	1.33
	100	0.31	1.66	1.35
	150	0.30	1.65	1.35
	200	0.29	1.66	1.37
EC1118	50	0.24	1.49	1.25
	100	0.21	1.51	1.30
	150	0.26	1.49	1.23
	200	0.21	1.46	1.25

3) Ethanol concentation

The effect of ethanol concentrations on growth performance was presented in Table 6. At 5% ethanol, MXH-1 demonstrated a higher OD change of 1.46 compared to EC1118, which exhibited a change of 1.04. As the ethanol concentration increased to 10%, MXH-1 maintained strong growth with an OD change of 1.42, while EC1118 showed a significant reduction in growth, with an OD change of 0.33.

At 15% ethanol, MXH-1 exhibited moderate growth with an OD change of 1.22, whereas EC1118 showed minimal growth, with an OD change of 0.12. These findings suggest that MXH-1 is more tolerant to higher ethanol concentrations compared to EC1118, demonstrating superior growth performance, particularly at 10% and 15%. This indicates that MXH-1 has potential as a more robust strain for ethanol fermentation under stress conditions.

TABLE VI. COMPARISON OF GROWTH PERFORMANCE IN ETHANOL CONCENTRATION

No	Ethanol (%)	Initial OD	After 24 h OD	Change in OD
MXH-1	5	0.28	1.74	1.46
	10	0.21	1.63	1.42
	15	0.18	1.40	1.22
	20	0.12	0.15	0.04
EC1118	5	0.31	1.35	1.04
	10	0.13	0.46	0.33
	15	0.13	0.25	0.12
	20	0.11	0.17	0.05

4) Salinity concentration

The growth performance under different NaCl concentrations was summarized in Table 7. At 5% NaCl, MXH-1 exhibited a significant OD change of 0.34 indicating good tolerance to salt stress, while EC1118 showed a lower OD change of 0.23. However, as the NaCl concentration increased to 10%, MXH-1 showed no growth with a slight decrease in OD (-0.02), while EC1118 maintained a minimal OD change of 0.02. At the highest NaCl concentration of 15%, MXH-1 showed no change in OD (0.00), whereas EC1118 exhibited a small decline in growth, with a negative OD change (-0.04). These results suggested that MXH-1 had higher tolerance to moderate salt stress (5% NaCl) compared to EC1118, which displayed lower growth under the same condition.

High NaCl concentrations were observed to trigger osmotic stress forcing cells to produce protective osmolytes like glycerol, which reduced energy availability for growth. Elevated sodium levels disrupted intracellular ionic balances, compromising enzyme function and protein stability [33]. Furthermore, NaCl interfered with nutrient uptake and metabolic processes as cellular resources were diverted from growth to maintaining osmotic balance [34].

TABLE VII. COMPARISON OF GROWTH PERFORMANCE IN NACL CONCENTRATION

No	NaCl (%)	Initial OD	After 24 h OD	Change in OD
MXH-1	5	0.18	0.51	0.34
	10	0.16	0.14	- 0.02
	15	0.16	0.16	0.00
EC1118	5	0.18	0.41	0.23
	10	0.19	0.21	0.02
	15	0.20	0.15	-0.04

5) Osmotic pressure condition

The growth performance under different osmotic pressure conditions was shown in Table 8. At 10% sucrose, MXH-1 exhibited stronger growth, with an OD change of 1.52 compared to 1.43 for EC1118. As the sucrose concentration increased to 15%, MXH-1 maintained strong growth with an OD change of 1.49, while the growth of EC1118 declined significantly to 1.14. At 20% sucrose, MXH-1 continued to show relatively high growth, with an OD change of 1.47, while the growth of EC1118 further decreased to 0.95. At 25% sucrose, MXH-1 displayed an OD change of 1.32, which was significantly higher than the 0.92 observed for EC1118.

The initial growth differences between MXH-1 and EC1118 at 10% sucrose were consistent with strain-specific variations in osmotic stress responses [35]. As sucrose concentrations increased, the divergent growth patterns between the strains reflected observations from previous studies [36], where yeast strains demonstrated varying abilities to maintain cellular homeostasis under osmotic pressure. Notably, the sustained growth of MXH-1 at high sucrose concentrations (20%) suggested enhanced osmoadaptation mechanisms, potentially attributed to increased glycerol production and accumulation as key osmolytes, a mechanism previously explained by Tamás and Hohmann [37].

TABLE VIII. COMPARISON OF GROWTH PERFORMANCE IN OSMOTIC PRESSURE CONDITION

No	Sucrose (%)	Initial OD	After 24 h OD	Change in OD
MXH-1	10	0.33	1.85	1.52
	15	0.33	1.82	1.49
	20	0.27	1.73	1.47
	25	0.3	1.62	1.32
EC1118	10	0.35	1.79	1.43
	15	0.34	1.49	1.14
	20	0.36	1.31	0.95
	25	0.36	1.28	0.92

6) Sugar type consumption

The growth performance in utilizing different sugar types is presented in Table 9. On glucose, MXH-1 showed a change in OD of 1.38, slightly higher than the 1.34 observed for EC1118. On fructose, MXH-1 exhibited similar growth, with an OD change of 1.34, comparable to the 1.33 recorded for EC1118. When xylose was used as the carbon source, MXH-1 demonstrated better growth with an OD change of 0.85, while EC1118 showed significantly lower growth, with an OD change of 0.45. For lactose, MXH-1 achieved an OD change of 0.90, slightly higher than the 0.86 observed for EC1118. The findings suggest that MXH-1 is more efficient in utilizing xylose and performs comparably to EC1118 in the utilization of glucose, fructose, and lactose. This indicates the potential of MXH-1 for fermentation processes involving a variety of sugars. The sugar consumption differences between MXH-1 and EC1118 align with established glucose transport mechanisms [38]. Notably, MXH-1 showed superior growth on xylose suggesting enhanced pentose phosphate pathway activity and xylose transport [39]. Both strains exhibited similar growth on lactose, but MXH-1 demonstrated broader sugar utilization capabilities, particularly with non-conventional sugars, indicating its potential in diverse fermentation processes.

TABLE IX. COMPARISON OF GROWTH PERFORMANCE IN SUGAR TYPE CONSUMPTION

No	Sugar	Initial OD	After 24 h OD	Change in OD
MXH-1	Glucose	0.21	1.6	1.38
	Fructose	0.28	1.62	1.34
	Xylose	0.43	1.28	0.85
	Lactose	0.18	1.08	0.90
EC1118	Glucose	0.41	1.75	1.34
	Fructose	0.28	1.66	1.38
	Xylose	0.53	0.99	0.45
	Lactose	0.33	1.18	0.86

IV. CONCLUSION

In summary, *S. cerevisiae* MXH-1 demonstrated superior performance compared to EC1118 in several key areas, including higher tolerance to ethanol, KMS, and osmotic pressure as well as more efficient utilization of xylose. These attributes make MXH-1 a strong candidate for various industrial fermentation applications, particularly in high-stress environments or processes involving diverse sugar sources. Its strong performance under stress conditions highlights its potential as a valuable alternative to the commercial strain EC1118, especially for fermentations requiring resilience to harsh conditions. For future research, it is recommended to investigate the genetic and molecular mechanisms underlying the resilience of the strain. Additionally, pilot-scale trials and applications in real food or beverage fermentation systems would help validate its practical utility and optimize its performance for commercial use.

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