Screening of Yeasts from Thai Traditional Fermentation Starter (Loog-pang) for Alcoholic Fermentation Products in Community Enterprise

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Abstract:

Thai traditional fermentation starter or Loog-pang is a starter culture in dry including bacteria, yeast and fungi used for traditional Sato processing in Thailand. Alcoholic fermentation products in community enterprise are not good quality enough because of alcohol content are not stable. Thus, this study aimed to isolation of yeasts from Loog-pang. Microbes in Loog-pang Sato from 3 sources including Roi-Et Province, Maha Sarakham Province and Phangnga Province were isolated. Yeasts were isolated using yeast extract-peptone-dextrose (YPD) agar and incubated at 30 °C for 3 days. Yeast culture in YPD broth was incubated in a temperature controlled agitator at 37 °C at 200 rpm for 48 hours. The yeast concentration was adjusted to 10^8 cells /ml to be used as a starter. The isolates were determined for total dissolved solid (TDS), alcohol production and alcohol tolerance. The result found that isolate LPR5 showed the highest total dissolved solid at 19.07 °Brix, the highest alcohol production capacity at 1.54% and gave the highest alcohol tolerance. Consequently, the yeast isolate LPR5 was identified by DNA sequencing and morphology. The results indicated that LPR5 as *Wickerhamomyces anomalus*.

Keywords: Yeast, Thai traditional fermentation starter, Loog-pang, Alcoholic fermentation, Sato

Introduction

Loog-pang is a Thai traditional fermentation starter culture crafted in a form of ball shape with an important role in providing quality and flavors of Sato fermentation. Many herbs such as *Allium sativa*, *Zingiber officinale* and *Alpinia siamensis* were mixed with bacteria, yeasts and fungi for dried starter preparation. Loog-pang is the source of beneficial microorganisms for ethanol fermentation process [1]. Microorganisms that frequently found in Loog- pang are yeasts such as *Saccharomyces*, *Pichia*, *Issatchenkia*, *Candida* and *Saccharomycopsis* [2-4].

Sato or Thai rice wine is made using glutinous rice as raw material and produced under non-sterile conditions at home scale using Loog-pang [5]. The fungi are mainly responsible for the hydrolysis of starch in the glutinous rice into sugar and yeasts play basic roles in converting sugars into ethanol, carbon dioxide and hundreds of other secondary products [1, 6]. If Loog-pang was kept for too long, it may result in less effective fermentation.

The problem of home scale production of Sato is inconsistency in Sato quality produced from each batch. This problem can be related to the variability of the microbial communities between different Loog-pangs. The use of good pure culture of desirable microorganisms should lead to better consistency in Sato quality from each bacth of fermentation [1]. The aim of this research was to isolate yeasts in Loog-pang with a potential to produce high alcohol in Sato.

Materials and methods

Yeast isolation

Loog-pang balls were collected from 3 provinces including Roi Et, Mahasarakham and Phangnga, Thailand. Each Loog-pang was milled using sterile mortar before used. Fifty grams of steamed sticky rice were mixed with each milled Loog-pang (250 mg) in a sterile-glass bottle and incubated at room temperature for 3 days. The produced water from fermentation was used as source of yeasts. Yeast was isolated by spreading the produced water on yeast extract peptone dextrose agar (YPD agar) and incubated at 30 °C for 3 days. Single colony was picked and cross streaked on YPD agar for further experiment.

Determination the efficiency of sugar fermentation and ethanol production

One milliliter of each isolated yeast was cultured in 10 ml of sterile 20 °Brix sugar solution mixed with 0.05% (W/V) diammonium phosphate (DAP) and contained glass Durham tube. The tubes were static incubated at 37 °C for 5 days. The remaining sugar was determined using hand refractometer. Alcohol content was measured by Gas chromatography (GC). Yeast that can produce the highest amount of alcohol was selected.

Determination of alcohol tolerance

One milliliter of all isolate were tested for alcohol tolerance so how did the represent active isolate was select according to this parameter was cultured in 10 ml of sterile 20 °Brix sugar solution mixed with 0.05% (W/V) diammonium phosphate (DAP), 10% and 15% ethanol and contained glass Durham tube. The tubes were static incubated at 37 °C for 5 days. The alcohol tolerance was indirectly observed by gas production in Durham tube.

Yeast identification

Isolated yeast with high efficiency in ethanol production was streaked onto YPD agar and cultured using YBD broth for 2 days. Yeast colony and cell morphology were observed under the microscope. For yeast identification, isolated yeast was sent to Thailand institute of scientific and technology research (TISTR) for automated DNA sequencing. The resulting sequences were compared with nucleotide database from PubMed using the BLAST program.

Results and discussion

Determination the efficiency of sugar fermentation and ethanol production

The results found that 9 yeast isolates were isolated from Loog-pang collected from Roi Et (2 isolates), Mahasarakham (2 isolates) and Phangnga (5 isolates). All isolated yeasts were determined for the efficiency of sugar consumption and ethanol production. The results found that LPR5 isolates showed the lowest remaining sugar and the highest ethanol production at 19.07 °Brix and 1.54%, respectively (Table 1.)

Table 1 The efficiency of sugar fermentation and ethanol production from isolated yeasts.

Isolate number	Gas level at Day 1	Gas level at Day 5	Remaining sugar (°Brix)	Ethanol content (%)
LPR4	-	++	19.47±0.06	1.15±0.04
LPR5	++	++++	19.07±0.06	1.54±0.05
LPS5	-	+	19.37±0.12	0.67±0.03
LPS6	-	+++	19.20±0.10	1.20±0.03
LPP5	-	-	19.80±0.10	0.07±0.03
LPP6	-	-	19.77±0.06	0.05±0.04
LPP7	-	-	20.03±0.06	0.01±0.01
LPP8	-	-	19.87±0.06	0.03±0.02

LPP9	-	-	19.87±0.06	0.03±0.01

+; gas level at ¼ Durham tube, ++; gas level at ½ Durham tube, +++; gas level at ¾ Durham tube, ++++; gas level at full Durham tube

Determination of alcohol tolerance

The result found that LPR5 isolate was the most tolerant against culture broth containing 10% and 15% ethanol followed by LPR6 and LPR4 at 5 day incubation (Table 2).

Table 2 Alcohol tolerance of isolated yeasts

Isolate	10% Ethanol		15% Ethanol	
	Gas level at Day 1	Gas level at Day 5	Gas level at Day 1	Gas level at Day 5
LPR4	-	++	-	+
LPR5	+	++++	-	+++
LPS5	-	+	-	-
LPS6	-	+++	-	+
LPP5	-	-	-	-
LPP6	-	-	-	-
LPP7	-	-	-	-
LPP8	-	-	-	-
LPP9	-	-	-	-

+; gas level at ¼ Durham tube, ++; gas level at ½ Durham tube, +++; gas level at ¾ Durham tube, ++++; gas level at full Durham tube

Yeast characterization and identification

Colony of LPR5 has a morphology of white to cream-colored, smooth and pherical to ellipsoidal budding blastoconidia form. DNA sequences were compared with nucleotide database from PubMed using the BLAST program. The result indicated that LPR5 was identified as *Wickerhamomyces anomalus* at 100% identity (1-589 bases). The LPR5 sequences were subjected to phylogenetic tree using MAGA X program. Many yeast strains were isolated from Loog-pang including *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera*, *Pichia anomala*, *Issatchenkia orientalis*, *Tolulaspora delbrueckii* and *Candida glabrata* were isolated from 114 Loog-pangs collected from small-scale factories and villages in central, northern and northeastern Thailand [1]. In addition, another study [6] presented that *Hanseniaspora uvarum*, *Hanseniaspora occidentalis*, *Metschnikowia pulcherrima*, *Candida zemplinina*, *Hanseniaspera vineae*, *Issatchenkia orientalis*, *Zygosaccharomyces bailii*, *Pichia kluyveri* and *Saccharomyces cerevisiae* were isolated from Cabernet Sauvignon musts in three vineyardsat the beginning, middle and final stages of spontaneous fermentations. One finding [7] reported that *S. cerevisiae*, *Pichia anomala*, *Trichosporon sp*, *Candida tropicalis*, *Pichia guilliermondi*, *Candida parapsilosis*, *Torulaspora delbrueckii*, *Pichia fabianii* and *Candida montana* were found in 54 'Hamei' samples collected from household rice wine preparations in tribal villages of Manipur. It was also reported [8] that 3 isolates, *Saccharomyces cerevisiae*, *Pichia kudriavzevii* and *Candida glabrata* were isolated from 35 from rice wine starters (Loogpang) in Chiang Mai

province, Thailand. They found 3 isolated yeast, *Candida tropicalis*, *Saccharomyces cerevisiae* and *Saccharomycopsis* spp. from Medombae [9].

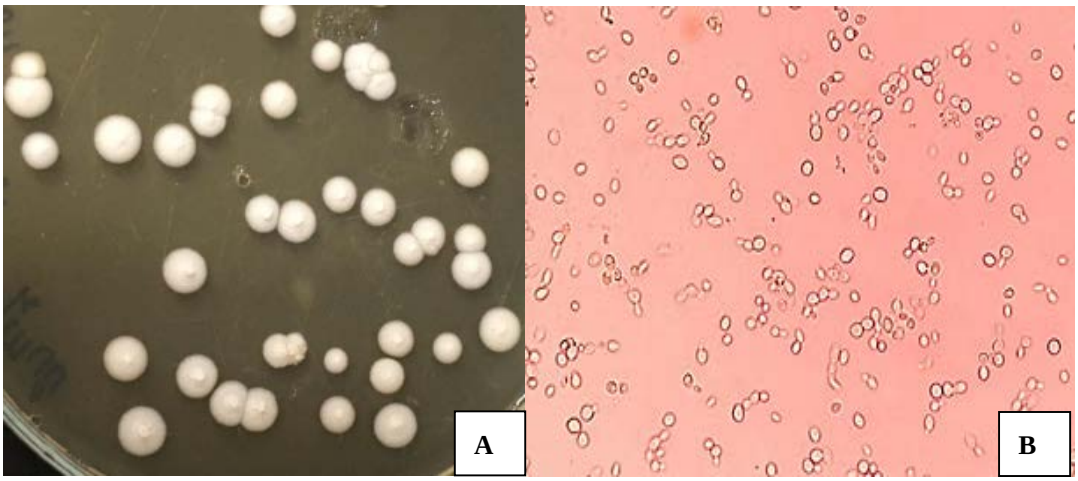

Figure 1 Cell morphology of LPR5 isolates
A: Colony of LPR5, B: Cell structure of LPR5

Conclusions

LPR 5 was isolated from Loog-pang collected from Roi Et province, Thailand. This isolate showed the highest sugar consumption, alcohol production (1.54%) and alcohol tolerance and was identified as *Wickerhamomyces anomalus*. This isolated yeast will combine with potential fungi and herbs for Loogpang preparation. The produced Loogpang will be sent to the community enterprise for improving sato production.

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