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Characterization of film yeast isolated from fermented bamboo shoots

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Abstract

The characterization of film yeasts (*Saccharomyces cerevisiae* J1, *Candida krusei* J2 and *C. krusei* J3) isolated from fermented bamboo shoots were studied. The morphological change of *C. krusei* J2 and *C. krusei* J3 showed the cell extension about 6% on the nitrogen limitation in liquid synthetic low-ammonia dextrose medium (SLAD), while *S. cerevisiae* J1 exposed the highest of the cell extension about 21% within 24 h on solid yeast extract peptone dextrose medium (YPD) containing 0.5 %(v/v) isoamyl alcohol. The viable cells of film yeasts were investigated at various temperatures. These film yeasts can grow at 0, 40 and 50 °C but they were not survived at high temperature than 60 °C. *S. cerevisiae* J1 can tolerate to 18% (w/v) glucose and the gas production was shown higher than 12 ml in liquid YPD. But *C. krusei* J2 and *C. krusei* J3 produced low a gas production. *S. cerevisiae* J1 was 0.10 g/ml of the glycerol production in liquid YPD medium containing 2.5% (w/v) NaCl, whereas *C. krusei* J3 and *C. krusei* J2 were only 0.077 and 0.058 g/ml, respectively. Killer activity of these film yeasts to five sensitive reference yeast strains (*C. tropicalis* TISTR 5045, *Hansenula anomala* TISTR 5113, *S. cerevisiae* TISTR 5020, *S. cerevisiae* TISTR 5055 and *Torulopsis glabrata* TISTR 5241) was examined on medium with 0.003%(w/v) methylene blue. All film yeasts were killer yeasts to five sensitive reference yeast strains.

Keywords: film yeast, fermented bamboo shoots, characterization, glycerol, killer activity

1. Introduction

Fermented bamboo shoot is one of the fermented food in the south of Thailand. This product can be stored for a year or more. The problem of the product was film yeast on the surface of fermented solution that the consumers dislike after opening the container. The yeast occurred in samples of liquid at the surface of fermented bamboo shoot product from villagers in Pattani Province. Many species of yeasts were identified as *Saccharomyces cerevisiae* J1, *Candida krusei* J2 and *C. krusei* J3. All species tolerated 2.5% NaCl addition (1). Garcia *et al.* (2) reported that in the presence of 1 M NaCl showed a reduction in growth rate and adaptation of yeasts. Because yeast has an adaptative mechanism to increase sodium efflux or restrict sodium uptake during

continuous growth in media with NaCl. Yeast can defend against salt stress by the osmolytes such as Glycerol. Also know as 'budding yeast', is able to undergo a morphology to produce filaments of elongated cells in diploids and invasive filaments in haploids (3). But the switch from budding to filamentous has been found in the pathogen (e.g., *Candida albicans*). Dickinson (4) discovered 'fusel' alcohols induce hyphal-like extensions and pseudohyphal formation in yeasts. Fusel alcohols are the products of amino acid catabolism which accumulate under nutrients limitation (4,5). While much of work carried out to date in relation to food preservation was using chemical treatments such as benzoic acid and sorbic acid, or adding salt solution to inhibit the growth of film yeasts. A tolerance for the preservatives were a one problems for the food industry. It need to increasing the levels to prevent yeast spoilage (6). In addition, *C. krusei* spoilage is often characterization to tolerate the conditions in foods, the fermentation of available sugars can produce CO_2 and leading to bloating (7,8). Other, there is considerable for possibility to relation. Therefore, the focus of our research was to study the morphology and physiology of film yeast.

2. Materials and Methods

2.1 Cultures

S. cerevisiae J1, *C. krusei* J2 and *C. krusei* J3 were isolated from liquid at the surface of fermented bamboo shoot product from villagers in Pattani Province. (1). Pure cultures were grown on yeast extract peptone dextrose medium (YPD; 2% glucose, 2% peptone, 1% yeast extract and agar) at 30 °C for 24 h and stored at 4 °C.

2.2 Morphology

All film yeasts were cultured in liquid and solid of YPD or synthetic low-ammonia dextrose (SLAD; 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 2% dextrose, and 6.6 (mg/l) ammonium sulfate) for nitrogen limitation. YPD and SLAD containing 0.5 % (v/v) isoamyl alcohol or 10% (v/v) serum were determined for induction of the morphological change. An elongated cell was defined as one 3 times as an ordinary cell or longer; pseudohyphal cells were not counted as elongated cells. At least 300 cells were counted, and results were given as percentage.

2.3 Growth at various temperature

All film yeasts were tested to grow at 0, 40, 50, 60, 70 and 80 °C in YPD media. A loopful of inoculum was taken from a pure culture of film yeast and inoculated into 5 ml of YPD broth. Then, it was incubated at 30 °C for 24 h. The growth so obtained was adjusted to 10^6 cfu/ml and kept at different temperature for 0, 15, 30, 45 and 60 min. The samples was counted the viable cells by spread plate on YPD agar medium and incubated at 30 °C for 24 h.

2.4 Gas production

Gas produced by all film yeast strains was measured in 5 ml YPD media containing 2 and 15% (w/v) glucose. Incubation was carried out at 30 °C for 24 h, in bottle capped with syringe. Gas volume (ml) was measured in 12 ml gas syringes through to the tops of the bottles.

2.5 Glycerol determination

Film yeast strains were cultured in YPD and YPD medium containing 2.5% (w/v) NaCl for 24 hr. Samples (1ml) were centrifuged and washed twice with a solution containing 1.5 M sorbitol and 20 mM $MgCl_2$. Cells were resuspended in 1 ml 0.5 M Tris/HCl, pH 7.6, and heated at 85 °C for 15 min. After centrifugation at 4000g for 10 min, the supernatant was used to measure the glycerol content. Glycerol content was determined by an enzymic assay kit (Boehringer Mannheim, cat. no. 148270)

2.6 Killer activity

The killer activity was determined by killer-zone assay in petri dish using YPD containing 0.003% methylene blue (YPD-MB) (9,10,11,12). The single loop of killer-sensitive *C. tropicalis* TISTR 5045, *Hansenula anomala* TISTR 5113, *S. cerevisiae* TISTR 5020, *S. cerevisiae* TISTR 5055 and *Torulopsis glabrata* TISTR 5241 were cultivated in YPD broth medium for 24 h at 30 °C. The suspensions were swabbed in YPD-MB plates and incubated for 24-48 h until colonies were formed. The YPD-MB plates were streak inoculated with the strain and incubated at 30 °C for 24-48 h to be tested for its killer character. Killer activity was determined by the occurrence of methylene blue strained dead cells and in YPD-MB plates by the clear zones of growth inhibition. The diameter of the inhibition zone was used as a measure of the yeast killer activity.

3. Results and Discussion

3.1 Morphological characteristics

Film yeasts grown in liquid and solid of YPD or SLAD medium (Table 1). The percentage of cell extension in SLAD medium was higher than YPD medium. *C. krusei* J2 and *C. krusei* J3 showed the cell extension about 6% in liquid SLAD medium, while *S. cerevisiae* J1 had a few cell extension. This result was different to the mutant J19 which had pseudohyphal growth in both liquid YPD and SLAD media (13). *S. cerevisiae* J1 exposed the highest of the cell extension about 21% within 24 h on YPD solid medium containing 0.5 % (v/v) isoamyl alcohol (Table 2). This result suggests that *S. cerevisiae* J1 may interact in the isoamyl alcohol-induced production of this phenotype. Dickinson (4) reported that on a solid medium and low concentration of isoamyl alcohol causes pseudohyphal growth and a high concentration causes hyphal-like growth. Cells that were grown in media and addition 10% (v/v) fetal bovine serum showed a little morphological change (data not shown). Therefore, *S. cerevisiae* J1 showed the characterization of morphological change. It is useful for investigating the morphological transition. Further research should probably focus on some environmental stimuli cause the morphological transition.

3.2 Physiological characteristics

3.2.1 Growth at various temperature

In all film yeast strains were tested the ability to grow at 0, 40, 50, 60, 70 and 80 °C. These film yeasts can grow at low temperature but they were not survived at higher than 60 °C within 15 min (Table 3). It was quite sensitive to heat. These results are also found in some yeasts spoilage which isolated from syrups and candied fruit nougats are a capability displayed at 37 °C. All strains tested were killed at 43 and 52.5 °C (14). This observation indicates that the ability of temperature at 60 °C or more had potential for control spoilage yeasts in food preservation.

Table 1. Effects of solid and liquid YPD or SLAD medium on cell morphology of film yeasts

Medium		Strain	Cell extension (%)		
			0 h	24 h	48 h
Solid	YPD	*S. cerevisiae* J1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		C. krusei J2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		C. krusei J3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	SLAD	*S. cerevisiae* J1	0.0 ± 0.0	1.0 ± 0.5	0.0 ± 0.0
		C. krusei J2	0.0 ± 0.0	4.0 ± 1.9	3.0 ± 0.6
		C. krusei J3	0.0 ± 0.0	5.0 ± 1.9	5.0 ± 0.7
Liquid	YPD	*S. cerevisiae* J1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		C. krusei J2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0
		C. krusei J3	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0
	SLAD	*S. cerevisiae* J1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		C. krusei J2	0.0 ± 0.0	6.0 ± 0.5	5.0 ± 0.6
		C. krusei J3	0.0 ± 0.0	6.0 ± 0.2	5.0 ± 0.2

Table 2. Effects of solid and liquid YPD or SLAD medium containing isoamyl alcohol on cell morphology of film yeasts

Medium		Strain	Cell extension (%)		
			0 h	24 h	48 h
Solid	YPD	*S. cerevisiae* J1	0.0 ± 0.0	21.0 ± 2.2	14.0 ± 3.5
		C. krusei J2	0.0 ± 0.0	7.0 ± 1.3	1.0 ± 0.1
		C. krusei J3	0.0 ± 0.0	3.0 ± 0.7	1.0 ± 0.1
	SLAD	*S. cerevisiae* J1	0.0 ± 0.0	7.0 ± 0.7	4.0 ± 2.8
		C. krusei J2	0.0 ± 0.0	5.0 ± 1.1	2.0 ± 0.2
		C. krusei J3	0.0 ± 0.0	5.0 ± 1.0	3.0 ± 0.7
Liquid	YPD	*S. cerevisiae* J1	0.0 ± 0.0	11.0 ± 2.5	12.0 ± 1.2
		C. krusei J2	0.0 ± 0.0	10.0 ± 1.7	7.0 ± 1.3
		C. krusei J3	0.0 ± 0.0	4.0 ± 0.2	3.0 ± 1.5
	SLAD	*S. cerevisiae* J1	0.0 ± 0.0	4.0 ± 0.8	2.0 ± 0.5
		C. krusei J2	0.0 ± 0.0	3.0 ± 0.3	4.0 ± 0.1
		C. krusei J3	0.0 ± 0.0	4.0 ± 0.6	5.0 ± 2.7

Table 3. Growth of film yeasts at various temperature for 15 min in liquid YPD medium

Temperature (°C)	Viable cells (cfu/ml)[a]		
	***S. cerevisiae* J1**	***C. krusei* J2**	***C. krusei* J2**
0	8.0×10^6	6.1×10^6	1.3×10^7
40	1.4×10^7	1.5×10^7	1.4×10^7
50	8.4×10^6	1.2×10^7	1.0×10^7
60	0	0	0
70	0	0	0
80	0	0	0

[a] Initial viable cells of *S. cerevisiae* J1 about 8.3×10^6cfu/ml, *C. krusei* J2 about 8.5×10^6 cfu/ml and *C. krusei* J3 about 1.1×10^7 cfu/ml

3.2.2 Gas production

This present study was tested for film yeasts to form gas. All strains were quite similar in YPD media containing 2 and 15% glucose. *S. cerevisiae* J1 was grow and showed a high gas production more than 12 ml in YPD containing 18% glucose and 3.2 ml in 2% glucose. *C. krusei* J2 and *C. krusei* J3 produced a volume of gas in YPD containing 2% glucose about 5.4 ml and decreased the gas production in high glucose media. These results demonstrate that *S. cerevisiae* J1 was a high gas producer and spoilage capability. Anothor feature which contribute to the spoilage capacity of yeast, *Zygosaccharomyces* genus is ability to ferment hexose sugars, such as glucose and fructose (8,15).

3.2.3 Glycerol determination

Glycerol is the main by product of yeast metabolism in high-osmolarity environments. Film yeast strains were enhanced by adding NaCl to the medium. Cells of film yeast strains were cultivated for 24 h and glycerol syntheses are compared in Table 4. The glycerol production of film yeast strains were enhanced by adding NaCl to the medium. *S. cerevisiae* J1 was able to accumulate glycerol to 0.1 g/ml, following to *C. krusei* J3 and *C. krusei* J2 about 0.07 and 0.05 g/ml, respectively. In the case of *C. tropicalis* glycerol synthesis is lower than in *S. cerevisiae* (2). Therefore, *S. cerevisiae* J1 showed a well adaptation to osmotic stress and exhibited glycerol production.

Table 4. Glycerol production of film yeasts in liquid YPD medium and adding 2.5% NaCl for 24 h.

Strain	Glycerol content(g/ml)	
	YPD	**YPD+2.5%NaCl**
S. cerevisiae J1	0.02 ± 0.003^b	0.10 ± 0.001^a
C. krusei J2	0.03 ± 0.007^b	0.05 ± 0.006^a
C. krusei J3	0.04 ± 0.003^b	0.07 ± 0.007^a

3.2.4 Killer activity

Film yeast strains were tested for killer acitivity in YPD-MB medium and a bluish coloured zone of dead sensitivity yeast strains was apparent surrounding killer yeast (Fig. 1). All of film yeasts showed killer activity to 5 sensitive yeast strains (*C. tropicalis* TISTR 5045, *Hansenula anomala* TISTR 5113, *S. cerevisiae* TISTR 5020, *S. cerevisiae* TISTR 5055 and *Torulopsis glabrata* TISTR 5241). The results in this study indicated that killer toxin produced by film yeasts. It may have some advantages to controlling growth of pathogenic yeast or bacteria in the future.

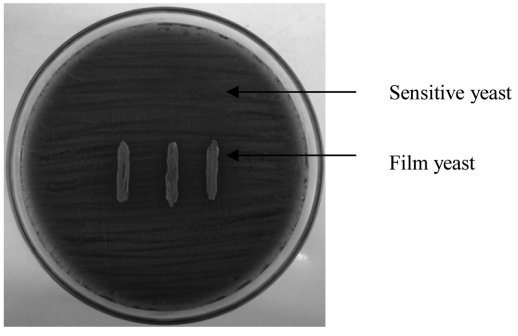

Figure 1. Inhibition zones of sensitive yeast strains surrounding the growth of film yeast strains in YPD-MB medium.

4. Conclusion

S. cerevisiae J1, *C. krusei* J2 and *C. krusei* J3 isolated from fermented bamboo shoot displayed particular morphological and physiological, including to growth at low temperature, ability to ferment glucose, adapt to NaCl concentrations and killer activity. Therefore, the characterization of these strains can be contribute to understanding of food preservation methods and to the strategies for food preservation.

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6. References

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