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Isolation of Multi Stress Tolerant Yeast for Ethanol Production at Elevated Temperature

Upma Pal[1,2], Rizwan Saifi[2], Sumit Pal[3] & Shilpa Vij[2]*

[1]School of Biotechnology, Department of Biotechnology, Gautam Buddha University, Greater Noida 201 312, Uttar Pradesh, India
[2]Dairy Microbiology Division, ICAR- National Dairy Research Institute, Karnal 132 001, Haryana, India
[3]Centre for Rural Development and Technology, Indian Institute of Technology 110 016, New Delhi, India

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In recent decades, bioethanol and biofuels have emerged as alternatives to fossil fuels globally. Utilizing dairy waste (whey) as substrate is lucrative option for utilizing it and producing ethanol. Therefore, in deliberating the cost-effectiveness of ethanol fermentation there is a requirement of a microorganism strain that can convert substrate (lactose) into ethanol at higher temperatures. The study aims to isolate multi-stress tolerant lactose fermenting yeast strain for economical production of ethanol. Out of 215 samples of lactose fermenting yeast 9 strains were selected for ethanol production. Taxonomical identification and multi-stress tolerance (thermal, sugar, ethanol) of the 9 strains were conducted. After optimizing ethanol production at different temperatures (37°C, 40°C, 42°C, 45°C), significant ethanol production (8% v/v) could be observed at 37°C in 40 h from 15% w/v lactose (YPL medium) while at 40°C and 42°C the productivity of ethanol slightly decreased, i.e., 7.5% v/v after 30 h with 0.5% residual lactose and at 45°C, only 3.5% v/v ethanol has been produced with approximately 7.5% w/v residual lactose. Thus, the isolated *Kluyveromyces* sp. 6C17 strain showed notable stress tolerance, making it promising for economical bioethanol production using lactose containing substrates at high temperature.

Keywords: Bioethanol, Ethanol tolerance, High-temperature fermentation, *Kluyveromyces marxianus*, Lactose

Introduction

The rising world population has led to a spurt in demand for fuels, as these are responsible for propelling the engines of the economy in this rapidly developing phase of the anthropogenic era. However, the rampant usage of fossil fuels has had a devastating impact on the environment as these have invariably contributed to global warming by contributing a lion's share to the generation of greenhouse gases, which are notorious for causing climate change. These have set alarm bells ringing across continents, as efforts are being carried out on a war footing to find alternatives that are sustainable, renewable, and have cleaner emissions. The paradigm shifts in the strategies to combat climate change and global warming have started focusing on eco-friendly solutions to ensure the safety of environmental health.[1]

Bioethanol is simply ethanol that is produced by the microbial fermentation of substrates. It is colourless, has cleaner emissions, and has received widespread attention across scientists and policymakers. It is widely used in developed and developing nations as a blended fuel consisting of 10% ethanol and 90% gasoline, popularly known as E10.[1] Bioethanol is mainly produced by fermentation using conventional yeast, *Saccharomyces cerevisiae*. However, *S. cerevisiae* requires glucose to convert it to ethanol and a low temperature for incubation, which are the major bottlenecks. As a result, extensive research has been conducted to develop yeasts capable of producing high-yielding bioethanol from various agricultural waste sugars such as lactose and xylose. Also, Fermentation at elevated temperature is advantageous for temperate countries such as India because it minimises cooling costs and minimizes the risk of contamination.[2] During fermentation, yeast faces many stresses that affect its productivity. These stresses may be biological (i.e., competition of microbes and ageing of cells), chemical (i.e., product toxicity or metabolites and change in pH), or physical (i.e., change in temperature, osmotic flux, and membrane perturbing).[3] The ability of a yeast strain to adapt to shifting surroundings and undesirable growth conditions, known as stress resistance, has a direct relationship with fermentation performance.[4] Therefore, isolation and screening of strains are usually performed for effective utilisation of substrate and higher production.

Meyerozyma caribbica MJTm3 isolated from bio-waste showed thermotolerance of 45°C and osmo-

*Author for Correspondence
E-mail: shilpa.vij@icar.gov.in

tolerance of 50% with 12.7% (v/v) of ethanol production utilizing sucrose as substrate.[5] In another study for isolating lactose-degrading *K. marxianus* from kefir grain, and out of 15 isolates, K9 was found to be the superior strain due to its higher growth rate and ethanol titer.[6] The *K. marxianus* ETP87 ethanologenic yeast isolated from Ethiopian fermented dairy beverages and were able to produce 7.97 g/L ethanol compared to 210 isolates.[7] Thus, very few studies have been conducted in non-conventional strains that can yield a maximum ethanol titer from utilising lactose as a substrate.[8–11] Therefore, the aim of the present research was to isolate a yeast strain with multiple stressors that was capable of ethanol fermentation at an elevated temperature. The isolates were characterised based on their physical and physiological traits, in addition to phylogenetic analysis of the D1/D2 domains of the (26S) rDNA large subunit coding gene.

Materials and Methods

Isolation of Lactose Fermenting Yeast

Isolation of lactose fermenting yeast was performed with method used by Golubev *et al.*[12] Yeast strains were isolated from different milk cream samples from the National Dairy Research Institute, Karnal, India. The samples were collected in a sterilised 50-mL polypropylene bottle (Himedia, India) and suspended in Yeast Extract Dextrose (YPD) (yeast extract, 1 g/100 mL; peptone, 2 g/100 mL; dextrose, 2 g/100 mL; and agar, 1.5 g/100 mL) medium containing chloram-phenicol (100 µg/mL). The samples were incubated for 48 h at 37°C. Aliquots (100 µL) of the culture supernatants were used for plating onto YPD agar and kept in incubator at 37°C for 48 h. Yeast strains were purified by picking single colonies.

Taxonomic Identification

Taxonomical identification of yeast was performed using method given by Latorre-García *et al.*[13] The chosen yeast strain was taxonomically characterised by 26S rDNA sequencing. All the strains partial 26S rDNA was synthesized by using universal primers in PCR and immediately sequenced. The BLAST system of the GenBank NCBI was used for homology of sequence. The neighbour-joining approach with the MEGA 4 program and bootstrap analysis based on 1000 replicates were used to accomplish the phylogenetic analysis.

Multi Stress Determination

Ethanol Tolerance Determination

For determining ethanol tolerance, method used with some modification given by Thammasittirong *et al.*[14] The yeast cultures were grown in YPL medium (Yeast extract; 1 g/100 mL; peptone; 2 g/100 mL; and lactose; 2 g/100 mL) containing 5–16% (v/v) ethanol and kept in shaker incubator at 37°C with 100 rpm. The OD_{600nm} of samples was measured after 48 h of incubation.

Sugar Tolerance Determination

For sugar tolerance determination method used given by Sree *et al.*[15] The yeast isolates were tested for their osmo-tolerance by inoculating YPL broth media containing 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, and 35 g/L of lactose. The OD_{600nm} of samples was measured after 48 h of incubation.

Thermal Tolerance Determination

For thermal tolerance determination method used given by Osman *et al.*[16] All the isolates were cultured, inoculated in 100 mL YPL broth medium, and incubated at 30°C, 37°C, 42°C, 45°C, and 50°C for 48 h. The OD_{600nm} of samples was measured after 48 h of incubation.

Screening for Ethanol Production

Screening for ethanol production was performed by using method given by Thammasittirong *et al*[14]. Fermentation for ethanol production was performed at 37°C with a 2% v/v pre-culture (cells initially adjusted to 1×10^6 cells/mL) in 100 mL of YPL broth containing of 10% (w/v) lactose. Fermentation samples were taken after 48 h of incubation for determining ethanol concentration, residual lactose sugar in the medium, other by-products, and the biomass of yeast.

Ethanol Production in a Bioreactor

Ethanol production using bioreactor was conducted using method given by Saini *et al.*[17] 15% YPL medium was used as a substrate. A 3-liter jar fermenter was used for the batch fermentations. Fermenter (BIOFLO/CELLIGEN 115, New Brunswick, New Jersey, USA) contain 1 litre of YPL 15% medium. 50 mL inoculum was prepared in a 100-mL flask containing YPL medium. Throughout the fermentation process, the temperature was kept at 37°C and the agitation speed at 150 rpm. Initially, the aerobic phase was maintained for the first 12 h by supplying sterile air at 0.5 L/min rate. The samples were taken during 48 h fermentation to monitor the ethanol, residual lactose concentration, cell dry weight, and by-products.

Sugar and Ethanol Determination

Sugar, ethanol, and glycerol concentrations were determined using a HPLC with a TSK-gel SCX (H^+) column (TOSOH, Stuttgart, Germany) and RID 10A

(refractive index detector) (Shimadzu) method given by Saini *et al.*[17] The column temperature was 35°C. The mobile phase was a (5 mM) H_2SO_4 (sulfuric acid) at a flow rate of 0.8 mL/min. With a run time of 20 min 20 µL sample was injected into a manual injector (Shimadzu). The cell biomass was determined in 10 mL sample by centrifuging for 10 min at 10000 rpm (Thermo Scientific, Germany). The cell pellets were weighed after being rinsed with distilled water, centrifuged once more, and dried in an oven set to 75°C for 24 hours.

Statistical Analysis

The experiments were conducted in triplicates. Graph data shows average of triplicates and the error bar indicates the standard deviation.

Results and Discussion

Isolation of Lactose Fermenting Yeast

Screening of lactose-fermenting yeast strains has been conducted among 215 samples from the National Dairy Research Institute, Karnal, and local markets. We obtained nine yeast isolates from milk cream samples that ferment lactose efficiently. All the isolates were found positive for the -galactosidase enzyme. Fermented dairy products have been reported as an indigenous source for isolating lactose-fermenting microorganisms. In similar study, Lactose-fermenting *Candida kefyr* has been isolated and identified from different cheese samples.[18] In other studies, different strains of lactose-fermenting yeast have been reported in literature, taxonomically identified, and explored in various applications.[19]

Taxonomic Identification of Stress Tolerant Yeasts

For the phylogenetic analysis, the chromosomal DNA of all the isolates was successfully extracted, and the 18S rRNA sequence was amplified efficiently. All the isolates of yeast were found to be members of the genus *Kluyveromyces*. The D1/D2 region of the 18S rDNA gene was sequenced. The homology of all the sequences was determined by the BLAST algorithm. The sequence of 18S rDNA of all the isolates showed 99% identity to *Kluyveromyces* sp. In taxonomic studies on nucleotide sequences of rDNA, the sequences of the strain *Kluyveromyces* sp. 6C17 and the *K. marxianus* strain CCT 7735 (UFV-3) were identical. *K. marxianus* CCT 7735 (UFV-3) is a Highly Lactose-Fermenting Yeast Isolated from the Brazilian Dairy Industry. Therefore, the strain 6C17 was identified as *K. marxianus*. The 18S rDNA sequence of strain *Kluyveromyces* sp. 6C17 has been submitted to GenBank under the accession number KF815067. In other study author reported the isolating lactose-degrading yeast from a local dairy sample; out of 4 isolates, 1 showed maximum -galactosidase enzyme activity (27.88 nmol/min/mL); also, this strain has wide range temperature (0–50°C) activity in the degradation of lactose.[20] The *K. marxianus* strain has been isolated from Jerusalem artichoke by combined bioprocessing and the yeast was able to produce 73.6 g/L ethanol which was 90% theoretical yield.[21] The thermotolerant *K. marxianus* DBKKU Y-102 isolated from plant leaves and soil; the strain was capable of fermenting inulin from Jerusalem artichoke and produced 104.83 g/L and 97.46 g/L of ethanol at 37°C and 40°C, respectively.[22]

Screening for Ethanol Production

To investigate ethanol production from all the isolates, the fermentation was carried out in YPL medium (10% lactose) (Fig. 1). To examine the fermentation ability of yeast strains, the ethanol production and utilization of lactose from YPL

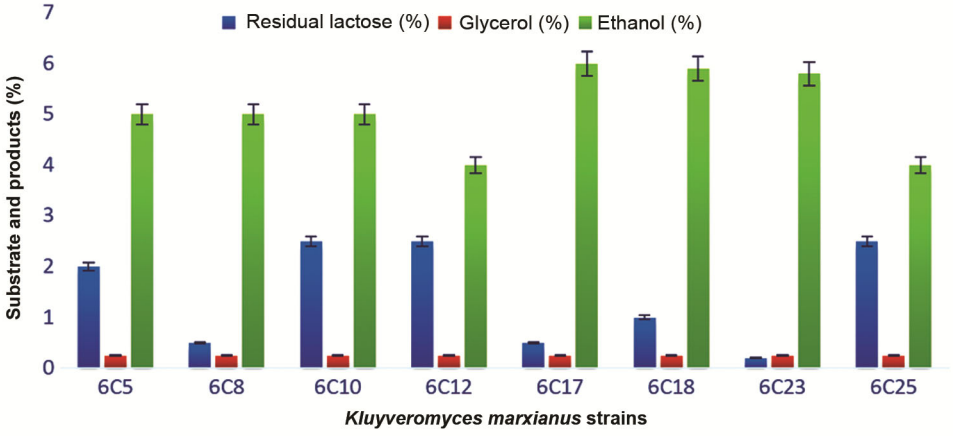

Fig. 1 — Screening of different yeast isolates for ethanol production at shake flask conditions (Values are mean ± SD)

medium were examined by HPLC equipped with RID detectors. All the isolated yeasts were taxonomically identified by 26S rRNA sequencing. In our study, we found that out of 8 isolated yeast strains, *Klyuveromyces* sp. 6C17 (isolated from a cream sample) exhibited high lactose fermentation ability, higher conversion of lactose to ethanol, and the lowest residual lactose these results was correlate to study conducted by Tikka *et al.*[23] *Klyuveromyces* sp. 6C17 was selected for further determination of multi-stress tolerance and ethanol production in a 3-L bioreactor at an elevated temperature.

Determination of Ethanol Tolerance

The viability of yeast cells of different strains in the presence of exogenous ethanol was examined. In the presence of 5%, 6%, or 7% (v/v) ethanol, the viability (OD_{600nm}) of this strain was identical and did not decrease even after 24 h of incubation in YPD medium. The viability of *Kluyveromyces* sp. 6C17 in the presence of 8% ethanol had decreased after 24 h, shown in Fig. 2. The high cell counts at 8% ethanol amended in YPD medium indicated that *Kluyveromyces* sp. 6C17 possessed efficient tolerance against ethanol. After 9% ethanol, however, the viability of *Kluyveromyces* sp. 6C17 cells had fallen below the optical density of zero h of the treatment, i.e., 0.200. The current study's range of ethanol tolerance was 6–8%, which is consistent with earlier findings.[2,14] The enhanced ethanol tolerance of *K. marxianus* MTCC1389 due to adaptation, which increased ethanol production by 42.9% from its parent strain.[24] The isolation of a highly ethanol-tolerant yeast strain from dairy products will allow further exploration into the practical uses of the isolated strains in using dairy whey for ethanol generation, which is a lucrative solution for cheese whey bioremediation and utilization. There is a constant need for multi-stress-tolerant yeast strains for further application in sustainable ethanol production. Yeast strains with hyper-ethanol tolerance might be a potential candidate for bioethanol production with concentrated or condensed cheese.

Determination of Sugar Tolerance

Results obtained with strain 6C17 of *Kluyveromyces* sp. showed the rise in the initial concentration of lactose yields an enhance in ethanol production, however cell biomass/viability was increased as the concentration of lactose increased from 15% w/v. Moreover, the amount of residual lactose present at the end of fermentation in Erlenmeyer flasks also increased. Initial concentration of 10% w/v lactose yields 6.5% v/v ethanol after 48 h. Ethanol production was significantly increased with an increase in osmotic pressure (lactose) up to 15% w/v, i.e., 7.5% v/v ethanol produced. However, increases in lactose concentration up to 20%, 25%, 30%, and 35% w/v were found to produce less ethanol as compared with the 10% and 15% w/v initial lactose (Fig. 3). Also, the residual lactose concentration was found to be increasing with an increase in osmotic stress. The possible reason behind the decrease in cell viability, ethanol production, and residual lactose is attributed to multiple explanations, viz., a high content of saccharides resulting in high osmotic pressure and low water activity. Fermentation of high-gravity substrates was attempted by various researchers. Isolated strains of yeast that were capable of fermenting 35% of their initial saccharide's concentration though, the possibility of fermenting a high sugar concentration in the media is of great economic interest to the fuel ethanol industry.[15] However, growth inhibition and low ethanol titers with an increase in sugar concentration could be regulated with stepwise addition or fed batch strategies.[1]

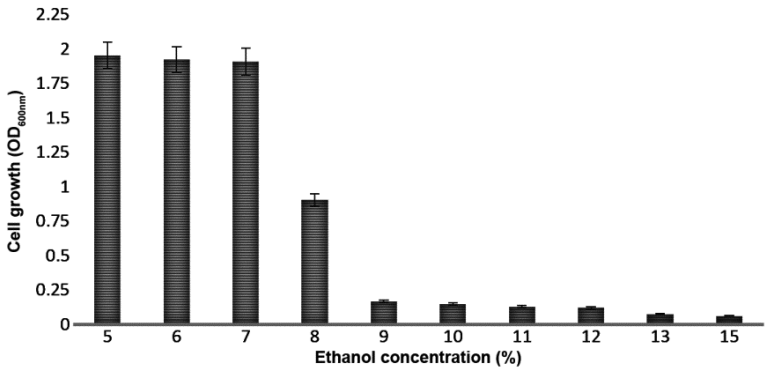

Fig. 2 — Ethanol tolerance determination of *Kluyveromyces* sp. strain 6C17 at different ethanol concentrations (Values are mean ± SD)

Thermal Tolerance Determination

In the present study, the thermo-tolerance of strain *K. marxianus* 6C17 sp. was evaluated by incubating it at different temperatures (viz., 37°C, 40°C, 42°C, and 45°C) up-to 24 h in YPL medium. The O.D. (optical density) of each treatment was measured at 600 nm, along with the ethanol produced and residual lactose after each shake flask experiment. Strain *K. marxianus* 6C17 sp. was found to be thermotolerant, and the viability of cells was more than 80% after incubation at 45°C as compared to the 37°C (Fig. 4). On the other hand, ethanol production was found to be constant, i.e., 7.5% v/v up to 42°C and residual lactose was 2 g/L. However, when the experiment was carried out at the 45°C -ethanol production dropped drastically to 3.5% v/v, and even the residual lactose concentration was 7.5% v/v. Probably the reason behind the drop in ethanol production and lactose utilisation was the performance and stability of the β-galactosidase enzyme responsible for the lactose hydrolysis. The isolated strains from Greater Mekong Subregion countries, out of 234 yeast isolates, 5 strains were found to be thermotolerant, such as *S. cerevisiae* KKU-VN8, VN20, VN27, and *Pichia kudriavzevii* TH33 and TH43. Strains were capable to thriving at 37°C, and maximum ethanol (7–7.5% v/v) was produced by the VN8 strain.[25] Another study isolated thermotolerant *K. marxianus* DBTIOC-35 for lignocellulose biomass fermentation to produce ethanol. And by using the strain, there was elimination of the pre-saccharification step, which was able to cut the overall process time of fermentation.[26] This means, high-temperature fermentation can be converted to the conventional process using thermotolerant yeast.[27]

Ethanol Production in a Bioreactor

Ethanol production was performed at 37°C, 40°C, 42°C, and 45°Cin 1 Liter substrate (YPL 15% w/v) in 3 Liter capacity jar fermenter and having an initial pH of 5.0 (uncontrolled) with other fermentation conditions including: 200 rpm of agitation,0.5 LPM of aeration for the initial 12 h, and followed by an anaerobic growth phase. The maximum ethanol titer was 8 % (v/v) in 40 h at 37°C (Fig. 5(a)). However, when batch fermentation was conducted at 40°C and 42°C the productivity of ethanol slightly decreased, i.e., 7.5% v/v after 30 h with 0.5 % residual lactose (Fig. 5(b) and (c)). It was evident that the increase in incubation temperature up to 42°C did not significantly reduce the ethanol productivity. Even the amount of residual lactose was similar to 37°C. On the contrary,

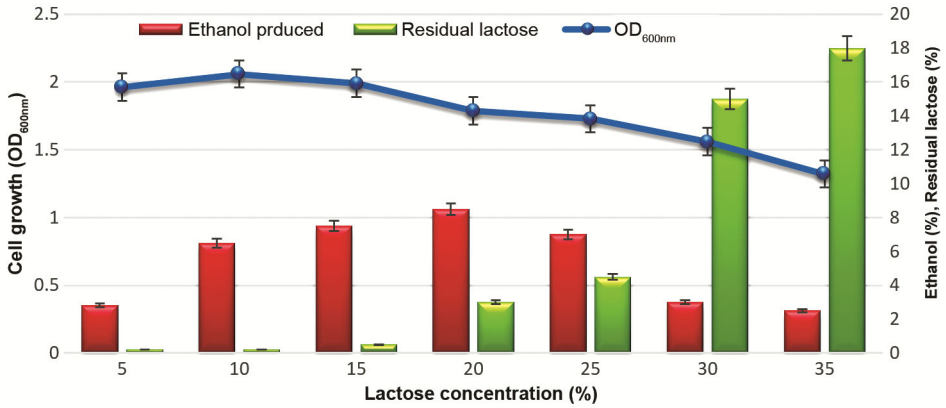

Fig. 3 — Sugar tolerance and ethanol production by *Kluyveromyces* sp. strain 6C17 at different lactose concentrations (Values are mean ± SD)

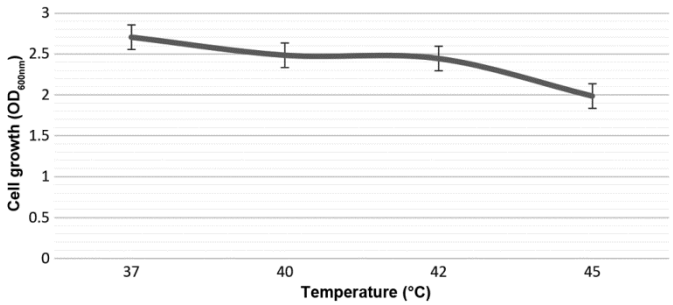

Fig. 4 — Growth (OD600) of *Klyuveromyces* sp. 6C17 at different temperatures (Values are mean ± SD)

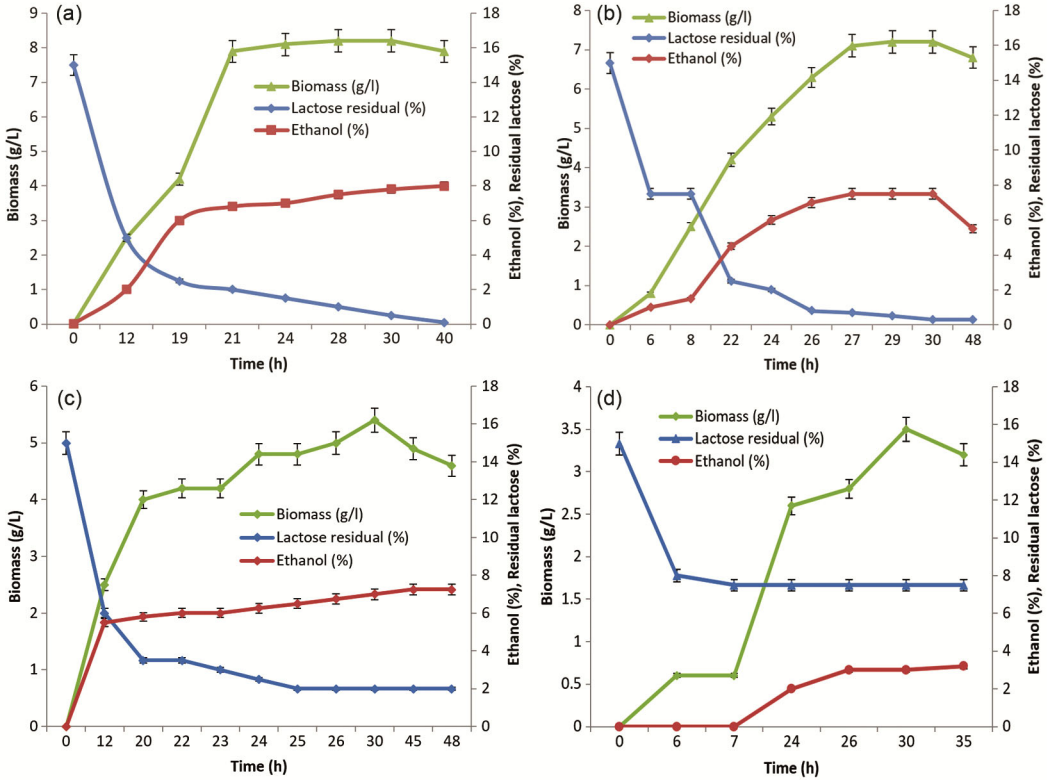

Fig. 5 — Batch fermentation by *Kluyveromyces* sp. 6C17 in YPL 15 % w/v medium at: (a) 37°C, (b) 40°C, (c) 42°C and (d) 45°C (Values are mean ± SD)

the biomass slightly declined from 8 g/L to 6.5 g/L, possibly due to the low cell density at the elevated temperature. Although, ethanol titer was significantly reduced at 45°C *i.e.,* only 3.5 % v/v ethanol has been produced with approximately 7.5 % w/v residual lactose (Fig. 5(d)). Biomass at 45°C was also declined to 3.5 g/L after 30 h of fermentation and further declined drastically. It shows that under high temperature yeast cell loses viability that led to low ethanol titer. Another study found that *K. marxianus* MTCC 1389 was a thermally tolerant yeast that could grow at temperatures as high as 52°C, however efficient yeast growth was optimized at 37°C, and the ethanol titer was 78 g/L utilizing whey containing 20% lactose as a substrate.[17]

Conclusions

The newly isolated thermotolerant yeast strain *Kluyveromyce s*sp. 6C17 has shown the potential to be used for significant ethanol production at high temperatures. The study revealed that the characteristics of the yeast strain allow it to grow efficiently at high temperatures (45°C) with high lactose concentrations (15%) and significant ethanol tolerance (8%). Such characteristics have the potential to lead to the development of a bioprocess in which lactose containing substrates can be used to produce ethanol. Though, further research is required to increase the strain's ability to produce bioethanol using high lactose-content substrates. Further studies with *Kluyveromyces* sp. 6C17 should be focused on enhancement of stress tolerance (sugar or ethanol) and gene level study to identify the genes involved in increasing stability of strain for further modification to create economic cell factory for utilising whey to produce bioethanol.

Declaration

The authors confirm no possible conflict of interest regarding the authorship, investigation, and publishing of the current article.

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References

1 de Souza J P, do Prado C D, Eleutherio E C, Bonatto D, Malavazi I & da Cunha A F, Improvement of Brazilian bioethanol production–challenges and perspectives on the identification and genetic modification of new strains of

Saccharomyces cerevisiae yeasts isolated during ethanol process, *Fungal Biol*, **122(6)** (2018) 583–591, https://doi.org/10.1016/j.funbio.2017.12.006.

2 Mo W, Wang M, Zhan R, Yu Y, He Y& Lu H, *Kluyveromyces marxianus* developing ethanol tolerance during adaptive evolution with significant improvements of multiple pathways, *Biotechnol Biofuels*, **12(1)** (2019) 1–15, https://doi.org/10.1186/s13068-019-1393-z.

3 Walker G M & Basso T O, Mitigating stress in industrial yeasts, *Fungal Biol*, **124** (2020) 387–397, https://doi.org/10.1016/j.funbio.2019.10.010.

4 Lahtvee P J, Kumar R, Hallström B M & Nielsen J, Adaptation to different types of stress converge on mitochondrial metabolism, *Mol Biol Cell*, **27(15)** (2016) 2505–2514,https://doi.org/10.1091/mbc.E16-03-0187.

5 Hawaz E, Tafesse M, Tesfaye A, Beyene D, Kiros S, Kebede G & Muleta D, Isolation and characterization of bioethanol producing wild yeasts from bio-wastes and co-products of sugar factories, *Ann Microbiol*, **72(1)** (2022) 39, https://doi.org/10.1186/s13213-022-01695-3.

6 Kurniawati M, Nurliyani N, Budhijanto W & Widodo W, Isolation and identification of lactose-degrading yeasts and characterisation of their fermentation-related ability to produce ethanol, *Ferment*, **8(4)** (2022) 183, https://doi.org/10.3390/fermentation8040183.

7 Tesfaw A, Oner E T & Assefa F, Optimization of ethanol production using newly isolated ethanologenic yeasts, *Biochem Biophys Rep*, **25 (2021) 100886**, https://doi.org/10.1016/j.bbrep.2020.100886.

8 Valderrama M J, De Silóniz M I, Gonzalo P & Peinado J M, A differential medium for the isolation of *Kluyveromyces marxianus* and *Kluyveromyces lactis* from dairy products, *J Food Prot*, **62(2)** (1999) 189–193, https://doi.org/10.4315/0362-028X-62.2.189.

9 Limtong S, Sringiew C & Yongmanitchai W, Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*, *Bioresour Technol*, **98(17)** (2007) 3367–3374, https://doi.org/10.1016/j.biortech.2006.10.044.

10 Anderson P J, McNeil K & Watson K, High-efficiency carbohydrate fermentation to ethanol at temperatures above 40°Cby *Kluyveromycesmarxianus var. marxianus* isolated from sugar mills, *Appl Environ Microbiol*, **51(6)** (1986) 1314–1320, https://doi.org/10.1128/aem.51.6.1314-1320.1986.

11 Koukoumaki D I, Papanikolaou S, Ioannou Z, Mourtzinos I & Sarris D, Single-cell protein and ethanol production of a newly isolated *Kluyveromyces marxianus* strain through cheese whey valorization, *Foods*, **13(12)** (2024) 1892, https://doi.org/10.3390/foods13121892.

12 Golubev W I & Golubev N W, Selection and study of potent lactose-fermenting yeasts, *Appl Biochem Microbiol*, **40** (2004) 280–284, https://doi.org/10.1023/B:ABIM.0000025952.55462.7a.

13 Latorre-García L, del Castillo-Agudo L & Polaina J, Taxonomical classification of yeasts isolated from kefir based on the sequence of their ribosomal RNA genes, *World J Microbiol Biotechnol*, **23** (2007) 785–791, https://doi.org/10.1007/s11274-006-9298-y.

14 Thammasittirong S N R, Thirasaktana T, Thammasittirong A & Srisodsuk M, Improvement of ethanol production by ethanol-tolerant *Saccharomyces cerevisiae* UVNR56, *Springer Plus*, **2(1)** (2013) 1–5, https://doi.org/10.1186/2193-1801-2-583.

15 Sree N K, Sridhar M, Suresh K, Banat I M & Rao L V, Isolation of thermotolerant, osmotolerant, flocculating *Saccharomyces cerevisiae* for ethanol production, *Bioresour Technol*, **72(1)** (2000) 43–46, https://doi.org/10.1016/S0960-8524(99)90097-4.

16 Osman M E, Khattab O H, Hammad I A & El-Hussieny N I, Optimization of bio-fuel production by *Saccharomyces cerevisiae* isolated from sugar cane bagasse, *Am J Sci*, **7(5)** (2011) 485–492.

17 Saini P, Beniwal A, Kokkiligadda A & Vij S, Evolutionary adaptation of *Kluyveromyces marxianus* strain for efficient conversion of whey lactose to bioethanol, *Process Biochem*, **62** (2017) 69–79, https://doi.org/10.1016/j.procbio.2017.07.013.

18 Ianieva O D, Voronina G O & Pidgorskyi V S, Isolation and characteristics of the lactose-fermenting yeasts Candida kefyr, *Cytol Genet*, **47** (2013) 359–365, https://doi.org/10.3103/S0095452713060066.

19 Afolabi F T, Adewale O P & Adeyemo S M, Isolation, production and optimization of beta-galactosidase by utilizing yeasts isolated from selected dairy product, *J Food Saf Hyg*, **8(3)** (2022) 170–182, https://doi.org/10.18502/jfsh.v8i3.11019.

20 Dahal B, Karki S, Adhikari N & Shrestha U T, Characterization of β-Galactosidase from Lactose Utilizing Yeast Isolated from the Dairy Sample, *Tribhuvan Univ J Microbiol*, **7** (2020) 133–141, https://doi.org/10.3126/tujm.v7i0.33874

21 Hu N, Yuan B, Sun J, Wang S A & Li F L, Thermotolerant *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* strains representing potentials for bioethanol production from Jerusalem artichoke by consolidated bioprocessing, *Appl Microbial Biotechnol*, **95** (2012) 1359–1368, https://doi.org/10.1007/s00253-012-4240-8.

22 Charoensopharat K, Thanonkeo P, Thanonkeo S & Yamada M, Ethanol production from Jerusalem artichoke tubers at high temperature by newly isolated thermotolerant inulin-utilizing yeast *Kluyveromyces marxianus* using consolidated bioprocessing, *Antonie Van Leeuwenhoek*, **108** (2015) 173–190, https://doi.org/10.1007/s10482-015-0476-5.

23 Chiranjeevi T, Osuru H P, Atluri N, Chakravarthi P, Raghavulu V, Mannur I S, Prasad U V, Aluru S & Bhaskar M, Isolation and characterization of ethanol tolerant yeast strains, *Bioinform*, **8** (2013) 421, https://doi.org/10.6026/97320630009421.

24 Pal U & Vij S, Adaptive evolution of *Kluyveromyces marxianus* MTCC1389 for high ethanol tolerance, *Biocatal Agric Biotechnol*, **45** (2022) 102533, https://doi.org/10.1016/j.bcab.2022.102533.

25 Techaparin A, Thanonkeo P & Klanrit P, High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion, *Braz J Microbiol*, **48** (2017) 461–475, https://doi.org/10.1016/j.bjm.2017.01.006.

26 Saini J K, Agrawal R, Satlewal A, Saini R, Gupta R, Mathur A & Tuli D, Second generation bioethanol production at high gravity of pilot-scale pretreated wheat straw employing newly isolated thermotolerant yeast *Kluyveromyces marxianus* DBTIOC-35, *RSC Adv*, **5(47)** (2015) 37485–37494, DOI https://doi.org/10.1039/C5RA05792B.

27 Abdel-Banat B M, Hoshida H, Ano A, Nonklang S & Akada R, High-temperature fermentation: how can processes for ethanol production at high temperatures become superior to the traditional process using mesophilic yeast, *Appl Microbiol Biotechnol*, **85** (2010) 861–867, https://doi.org/10.1007/s00253-009-2248-5.