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Selection and Identification of a Newly Isolated Thermotolerant and Amylolytic *Saccharomyces cerevisiae* Strain for Ethanol Production

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Authors' contributions

This work was carried out in collaboration between all authors. Author BHJ designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors RMD and RVP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Thermotolerant and ethanogenic *Saccharomyces cerevisiae ETGS1* was isolated from the grape samples by an enrichment technique using yeast extract peptone dextrose (YEPD) broth. This was obtained after the extensive screening of 67 samples from diverge ecosystem. The strain was found to tolerate ethanol concentration up to 12%. Enzyme profiling of this selected strain also revealed its amylolytic potentials. This isolate of *S. cerevisiae* was found to ferment YPS medium at elevated temperature in a single step with fermentation efficiency of 70.47%. It was found to produce 31 g/l of ethanol with yield of 0.4 (p/s, g/g). Looking to the prospects of this newly isolate for the conversion of starch based waste into ethanol in a single step without any complex pretreatments, it was selected for further thorough characterization. Based on the morphological, cultural and biochemical characterization followed by molecular analysis of 18S rDNA, it was identified as the strain of *S. cerevisiae*.

Keywords: Thermotolerance; ethanol tolerance; ethanol yield; amylolytic; molecular characterization.

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1. INTRODUCTION

In recent years, concerns have been growing worldwide regarding the environmental consequences of heavy dependence on fossil fuels, particularly due to climate change. As concern about global warming and dependence on fossil fuels grows, the search for renewable energy sources that reduce CO_2 emissions becomes a matter of widespread attention. Bioethanol is an alternative energy source of particular interest whose production by microbial fermentation is increasing to replace gasoline [1,2,3]. Yeasts, particularly Saccharomyces spp., are the most common ethanol producers employed in industry [4].

Ethanol fermentation at elevated temperature is a key requirement for effective ethanol production in tropical countries where the average daytime temperatures are usually high throughout the year and also in many non-tropical countries during the summer [5]. The advantages of rapid fermentation at high temperatures are not only a reduction in the cooling costs but also a decreased risk of contamination. Besides these, the use of thermotolerant yeast for bioethanol production has several process advantages. These includes broad substrate utilization range, higher saccharification and fermentation rates, minimized contamination risk, lower costs of pumping and stirring, no cooling problems, less energy requirement for mixing and product recovery [6,7,8,9]. To achieve ethanol production at elevated temperature it is necessary to use an effective yeast strain that can tolerate high temperatures.

Ethanol fermentation efficiency depends on many factors including the yeast strain employed in the fermentation. Different yeast species may display different ethanol fermentation performances when different feedstocks are used. Starch is one of the most abundant polysaccharides occurring as reserve energy in many plants and also occurs extensively in waste materials produced from food processing industries. Starch can be effectively used for the production of ethanol. Glucoamylase [α-1, 4-glucan glucohydrolase E.C. (3.2.1.3)] is one of the most important enzymes used for direct conversion of starch to glucose which can be further fermented to ethanol. It breaks successive bonds from the non-reducing end of the straight chains producing glucose [10]. The production process of ethanol from starch involves two steps that is enzymatic hydrolysis followed by fermentation, which are performed by two different organisms. But in direct conversion of starch to ethanol also known as consolidated bioprocessing (CBP), amylolytic yeast is also capable of fermenting the end product of hydrolysis that is glucose to ethanol. Selection of Thermotolerant and amylolytic yeast is vital component to bio-convert starch based waste into ethanol using CBP as cost effective process [11,12,13].

Looking to the present short supply of ethanol and further growth of ethanol demand at the rate of 10% annually needs to strengthen the existing technology to improve ethanol productivity per unit time. Utilization of alternative cheap substrates, besides molasses for its bioconversion into ethanol is one of the key challenges for biofuel production. Therefore, present work was attempted to screen out an efficient thermotolerant and amylolytic strain for ethanol production using starch as a substrate at elevated temperature.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of Yeast

Total of 67 samples from the diverse ecosystem such as dairy effluent, grapes, muskmelon, banana, idli batter, sugar cane juice, cane bagasse, soybean sauce, honey, jaggery and distillery effluent were collected and used for isolation. Samples were collected in the sterile plastic container and their details recorded. All the samples were stored in the refrigerator at 4 ± 0.5°C until further isolation process. For direct isolation, 1 g (or 1 ml if the sample was a liquid) of a sample was mashed and added to 100 ml Erlenmeyer flasks containing 10 ml of YEPD medium; 1% yeast extract (Difco), 2% peptone (Difco) and 2% glucose (Sigma-Aldrich). While for enrichment, samples were added in the YEPD each supplemented with cellulose, starch and lactose separately. Then it was incubated at 35°C for 3 days with occasional shaking. From this isolation on YEPD plates was carried out and individual distinct colony was selected and purified using YEPD medium. From this individual colonies, 1% (v/v) inoculum was prepared and inoculated into YEPD broth containing 6% (v/v) ethanol and incubated at 35°C for 48 h, which were employed as selective pressures for obtaining thermotolerant and ethanol tolerant yeasts [2]. Isolates able to grown

were again sub-cultured using YEPD agar containing 6% (v/v) ethanol at 35°C incubation temperature for 24 h to obtained pure culture. The isolated strains were carefully studied and distinct colonies selected based on the morphology, size and color. A single colony of each isolate was streaked on YEPD agar containing an ethanol concentration of 6% (v/v) and incubated at 35°C for 24 h. Isolates thus obtained were coded and stored at 4°C.

2.2 Screening for Thermotolerant and Ethanol Tolerant Ethanogenic Yeast

First of all the isolates obtained were studied for the ethanogenic potential. The YEPD fermentation broth was inoculated with the isolates at 35°C for 48 h. After fermentation was over, the fermented medium was centrifuged at 5000 rpm for 25 min at 4°C and supernatant of which was used for estimation of alcohol. The ethanol content was estimated by potassium dichromate method [14]. The 100 ml of potassium dichromate reagent solution was prepared. On the other hand, saturated s-Diphenylcarbazide solution was prepared by dissolving 1 g of s-Diphenylcarbazide to 1 ml of 95% ethanol. The mixture was then added with 1 ml of a 40% potassium sodium tartrate solution to stabilize the color. The ethanol absorbance values were measured at 575 nm. The ethanol % was calculated as v/v. Based on the ethanol productivity, isolates were screened out for further evaluation of the thermotolerance. Each isolates were grown at four different temperatures in YEPD broth on incubating shaker for 30 h. The temperature selected was 30°C, 35°C, 40°C and 45°C. After the course of incubation evidence of turbidity was measured spectrophotometrically at 600 nm. Viability of yeast cells was further reconfirmed by staining it with crystal violet followed by its microscopic observation [15]. Selected ethanogenic yeast strains showing temperature tolerance in primary screening were further evaluated for their ethanol tolerance. YEPD broth containing different ethanol concentrations (6%, 8%, 10% and 12% v/v) was inoculated with each of the selected isolate of yeast, and incubated at 35°C for 48 h. The growth was estimated using spectrophotometer at 600 nm and compared with control where yeast strain was cultured in YEPD broth without ethanol [16]. Further the ethanol tolerance threshold of yeast under study was increased up to 12% by adaption. For the same the isolates was allowed to grow in higher concentration of ethanol at an increment of 0.5 v/v of ethanol in YEPD broth for prolong period of time at 35°C. Viability and purity of these adapted yeast cells were confirmed by crystal violet staining during the study. The isolates grown at highest ethanol concentration was considered as its ethanol tolerance threshold. From this it was streaked on YEPD agar plates and incubated up to 4 days at 40°C.

2.3 Screening for Amylase Activity

Selected thermotolerant and ethanol tolerant yeast were studied for its ability to produce amylase. Distinct colony of each of the selected isolate was activated using YEPD broth for 36 h at 35°C. This activated culture was now spotted on starch agar plates. Then it was incubated at 35°C for 36 h. The starch present in the bacteria induction medium was considered the major carbon source. However, it was not the sole carbon source, since peptone, which is considered a complex additive, was added to the medium. After the incubation period, the plates were exposed to 1% iodine solution for 5 min to reveal the starch hydrolysis zone. Microorganisms were considered amylolytic if they will able to grow in the presence of starch as the major carbon source and formed a hydrolysis zone around the colony [16,17]. This was followed by quantitative estimation of amylase from this selected yeast strain. Activated log grown culture of yeast was inoculated in to YPS broth containing yeast extract, 10 g/l; peptone, 20 g/l; starch 20 g/l. This was then incubated at 35°C for 48 h. Then, the aliquots drawn for the enzymatic assays were centrifuged to remove the cells followed by filter sterilization. From this, 1 ml was added to test tube containing 1 ml; 0.5% (w/v) soluble starch in 50 mM sodium phosphate buffer (pH 6.5). Then it was incubated at 35°C for 10 min and the amount of reducing sugars released was measured using 2,5-dinitrosalicylic acid (Analytical grade) reagent [18]. One unit of enzyme activity is defined as the amount of enzyme that liberates 1 µmol of reducing sugar as glucose equivalents in 1 min under the assay condition.

2.4 Evaluation of Potential of *S. cerevisiae* ETGS1 for CPB Using Starch as Substrate

After screening and selection of thermotolerant and amylolytic yeast isolates, it was further evaluated for its ethanol production potential using starch as a sole source of carbon. Each

isolated colony from YPS agar plates was inoculated in 100 ml YPS broth as inoculum medium in (250 ml) flask. Then it was incubated at 35°C on orbital shaker at 120 rpm for 30 h to initiate fermentation [3]. 12% (v/v) of this inoculum was added in 400 ml production medium prepared in 500 ml fermentation glass bottle. These bottles were incubated at 35°C for 48 h and samples withdrawn at an interval of 12 h for estimation of sugar consumption and ethanol production. Conversion rate of sugar into ethanol in terms of ethanol yield is calculated according to following formula:

$$\text{Yield} = \frac{\text{Ethanol produced (g)}}{\text{Sugar utilized (g)}}$$

The fermentation process efficiency (FPE) was calculated for selected yeast was studied in the shake flake experiment as follow:

$$\text{FPE} = \frac{\text{Practical yield x 100}}{\text{Theoretical yield}}$$

2.5 Analytical Methods Used for the Evaluation of Ethanol Productivity

Yeast cell count was monitored with a haemocytometer and the cell viability was assessed by staining the cells with 0.1% methylene blue solution [19]. Samples were quickly withdrawn from fermentation flasks, filtered on 0.45 µm GF/A filters (Millipore, Bedford, MA USA) and the filtrates was analyzed to determine residual sugar and ethanol concentrations. Reducing sugar was determined with the dinitrosalicylic acid (DNS) method [18]. Ethanol production was determined using ethanol assay kit (Megazyme, K-ETOH). Two millilitre of distilled water was dispensed in a glass test tube. Then 0.1 ml of ethanol sample was added to it, followed by addition of 0.2 ml of buffer; 0.2 ml of NAD^+ and 0.05 ml of aldehyde dehydrogenase. Mixed it properly and after 2 min of incubation the absorbance A1 was read at 340 nm using spectrophotometer. Then in same tube 0.02 ml of alcohol dehydrogenase was added. Mixed it properly and after incubation of 5 min the absorbance A2 was measured at 340 nm. Simultaneously one tube with all additions except sample (volume of sample was made up by distilled water) was run as blank. The ethanol concentration was calculated by difference of absorbance (A2-A1) for both blank and sample. ΔA ethanol was obtained by taking the absorbance differences of the blank from the absorbance of the sample. The concentration of bioethanol can be calculated as follows:

$$C = \frac{V \times MW}{\varepsilon \times d \times v \times 2} \times \Delta A \quad c[g/l]$$

Where:

V = Final volume of ethanol assay (ml)
MW = Molecular weight of ethanol (g mol¯1)
ε = Extinction coefficient of NADH at 340 nm
= 6300 (1×mol×cm¯1)
d = Light path (cm)
v = Sample volume of the fermentation broth (ml)
2 = 2 moles of NADH produced for each mole of ethanol.

2.6 Characterization of Selected Yeast Isolate

2.6.1 Morphological, cultural and biochemical characterization

Selected ETGS1 isolate was further studied for its morphological, cultural, biochemical and molecular characterization. Vegetative cell and colony morphologies of the isolated yeasts grown on YEPD agar were observed and compared with type strain as described in The Yeast: a taxonomic study, 4thed [20]. Sugar utilization was studied using various sugars such as D-glucose, D-raffinose, D-sucrose, D-galactose, D-maltose, D-mannose, D-lactose and starch. The nutrient sugar (g/l00 ml) contains: 10 ml (10%) respected test sugar solution; 90 ml 1% peptone water; 1 ml Andrade's indicator. Broth was inoculated with loopful ETGS1 as a test culture and incubated at 35°C for 30 h. The tubes for acid and gas production were observed.

2.6.2 Molecular characterization

18S rDNA sequencing and phylogenetic analysis of the ETGS1 isolate was carried out. Yeast DNA was extracted using method described by Maniatis et al. [21]. The rDNA was amplified using universal fungal forward primer, 1F-5'- TGGTGCCAGCAGCCGCGGYAA-3' and 4R-5'-CKRAGGGCATYACWGACCTGTTAT-3' as reverse primer [22]. Amplicon purified by Gel/PCR DNA Fragment Extraction Kit (Geneaid Biotech Ltd., Taipei, Taiwan) was directly sequenced by ABI Prism™ Big Dye™ terminator cycle sequence ready reaction kit (Applied

Biosystems, Stafford, USA) according to manufacturer's instruction. The resultant sequenced file were processed, trimmed and developed the contigs by using Chromas and BioEdit software. Developed contig were used for the identification of isolate at species level by using online tool BLAST of NCBI. Contigs were processed by offline tool sequin and resulted sequin processed files were submitted to genebank for accession number. Phylogenetic tree was constructed by using online BLAST tool.

3. RESULTS AND DISCUSSION

3.1 Isolation of Yeast

Total of 67 samples from the diverse ecosystem such as dairy effluent, grapes, muskmelon, banana, idli batter, sugar cane juice, cane bagasse, soybean sauce, honey, jaggery and distillery effluent were collected from the region surrounding Anand, Gujarat and used for isolation. Direct isolation using YEPD as well as enrichment using YEPD supplemented with starch, cellulose, lactose were carried out from each of the sample before isolation. The isolated strains were carefully studied for characteristics and growth pattern studies using microscope. Total of 165 yeast isolates were obtained from diverge ecosystem over the period of 2 yr. These were evaluated for preliminary ability to ethanol fermentation, thermotolerance and ethanol tolerance studies.

3.2 Screening for Ethanogenic and Thermotolerant Yeast

A wide variety of yeasts are present in the different ecosystems, which can be harnessed for the purpose of mankind. The isolation and screening of the efficient thermotolerant ethanol producing yeasts can be helpful to overcome the current challenges in bioethanol production. Therefore, the thermotolerant trait was taken into the consideration in this study. 165 isolates of yeast were primarily screened out based on their ethanol fermentation ability in the laboratory experiment. Laboratory fermentation results showed potential of 43 isolates ethanogenic which produced more than 25 g/l ethanol with fermentation process efficiency also more than 70% (Table 1). These selected 43 ethanogenic yeast isolates were further evaluated for their thermotolerance. The isolates growing above 40°C were screened out in shake flask experiment. Total 18 ethanogenic isolates were found to have temperature tolerance at 40°C and above were selected for further studies (Table 1 and Fig. 1).

Table 1. Primary screening of for ethanogenic and thermotolerant yeast

Source of isolation	Primary screening	
	Ethanogenic yeast isolates	Ethanogenic and thermotolerant yeast isolates
Dairy effluent	7	3
Grapes	6	3
Banana	5	2
Idli batter	10	3
Sugar cane Juice	4	1
Cane Bagasse	4	1
Distillery Effluent	2	0
Muskmelon	2	2
Honey	1	1
Soy bean sauce	1	1
Jaggery	1	1
Total isolates	**43**	**18**

3.3 Screening for Ethanol Tolerance of Selected Yeast

These eighteen selected yeast isolates were evaluated for its ethanol tolerance. Majority of isolates tolerated ethanol concentrations up to 6% (v/v). But, only eight isolates i.e. ETB1T, ETB2T, ETDLT1, ETSBT1, ETGS1, ETMT2, ETJT1 and ETHT1 were found to tolerate ethanol concentration more than 10% (Fig. 1). Interestingly isolates ETDL1, ETGS1 ETMT2, ETJT1 and ETHT1 were found to tolerate 12% ethanol with significant growth.

Despite *S. cerevisiae* showing high ethanol tolerance, there have been many efforts to enhance this trait and select strains that tolerant to higher concentrations [23]. The relatively high ethanol tolerance of *C. bescii* found to significant improvements in ethanol production [24]. In that sense, thermotolerant and ethanol tolerant yeast isolates obtained from different ecosystem are potential candidate for bioethanol production and thus were further investigated for their diverge biochemical potential which enable them to bio-convert various cheaper substrate into ethanol without pretreatments. The ability to produce

ethanol in a single consolidated step without pretreatment such as acid or enzyme from cellulosic, starchy and lactose waste which generally abundantly produces as a part of agricultural practices or processing and is otherwise difficult to manage. This potential of yeast is very much important so far as the economics of the bioethanol production is concerned. Therefore, all selected isolates were then evaluated further for their potentials.

3.4 Screening for Amylolytic Potential of Selected Yeast

Out of eight selected ethanogenic, thermotolerant, and ethanol tolerant isolates were studied for their amylolytic potential, ETGS1 isolates was found to shown significant amylolytic activity (Fig. 2). Quantification of amylase production by ETGS1 isolate was studied using YPS broth. The results of fermentation show that ETGS1 strain found to produce 12 IU/ml after 48 h of fermentation at 35°C. This confirms ETGS1 strain as the potential thermotolerant and amylolytic ethanogenic yeast, which can able to bio-convert the starch based waste in to ethanol.

Consolidated Bioprocessing (CBP) integrates enzyme production, saccharification and fermentation into a one-step process, and represents a promising strategy for the cost-effective production of ethanol from starchy biomass. To design commercial systems of ethanol production by CBP, it is necessary to develop customized microorganisms. Search for novel amylolytic and ethanogenic yeast for ethanol production in single step using various microorganisms is attempted by several research groups and is continuous process [24,25,26]. Present studies revealed amylolytic potential of ethanogenic strain ETGS1 which is also thermotolerant as well as ethanol tolerant. These features strongly suggests the possibility of designing a starch based ethanol production process through CPB using this selected strain ETGS1 and therefore, it was further studied for the detailed characterization for their identification and further evaluation of their ethanol production ability using starch based medium in shake flask studies.

3.5 Evaluation of Potential of *S. cerevisiae* ETGS1 for CPB Using Starch as Substrate

Preliminary studies for the evaluation of selected isolate for the ethanol production using starch as substrate was carried out in laboratory fermentation experiments. *S. cerevisiae* ETGS1 found suitable candidate for CPB of starch containing medium at elevated temperature. The fermentation kinetics of laboratory studies is depicted in Fig. 3. The *S. cerevisiae* ETGS1 strain was found to produce 31 g/l of ethanol with yield of 0.4 (p/s, g/g). Similarly, Yuangsaard, et al., isolated *Pichia kudriavzevii* DMKU 3-ET15 from the traditional fermented pork sausage, which produced ethanol concentration of 4% (w/v) with productivity of 1.27 g $l^{-1}h^{-1}$ and yield of

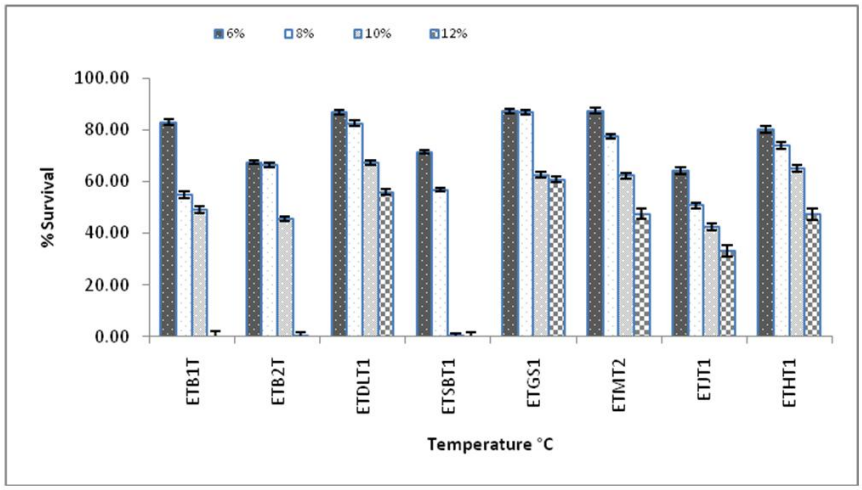

Fig. 1. Screening of selected yeast isolates based on the percentage of survivals of thermotolerant ethanogenic yeast isolates at different ethanol concentrations

42% of the theoretical yield in a cassava starch hydrolysate medium at pH 5 and 45°C [5]. It reported that isolation of *P. kudriavzevii* from sugarcane juice at 40°C, which produced 71.9 g/l of ethanol with a productivity of 4 g l^{-1} h^{-1}[3]. Two thermotolerant yeasts Kluyveromyces sp. GU133329 and Kluyveromyces sp. GU133331 isolated from plum fruit and cantaloupe, which produced 9.55 (w/v) and 11.72% (w/v) of ethanol, respectively at pH 5.5 and temperature of 35°C [27]. These results imply that these isolated strains could be able to produce ethanol at high temperature. Looking to these all, *S. cerevisiae* ETGS1 strain is quite comparable. Importantly, *S. cerevisiae* ETGS1 strain is capable of bioconversion of starch based waste from the agro-processing industries which is otherwise difficult to manage, into ethanol in a single step fermentation. This feasibility to develop CBP of starch waste for bioethanol production using *S. cerevisiae* ETGS1 strain and the ability to tolerate elevated temperature may have profound effect on economics of the process due to their thermotolerant nature. In ethanol production, cooling costs have great effect, which makes the process expensive. Hence, by using these thermotolerant yeasts, cooling and distillation costs can be reduced during process development. Besides, higher saccharification and fermentation rates, continuous ethanol removal and reduced contamination have stimulated a search for routes to thermotolerant yeasts. The overall fermentation process efficiency in preliminary evaluation was quite significant 70.47%. Overall studies suggest that this newly isolated strain has all potential for its further utilization for development of CPB of starch based food processing waste into ethanol. Therefore, it was further studied for their characterization for identification.

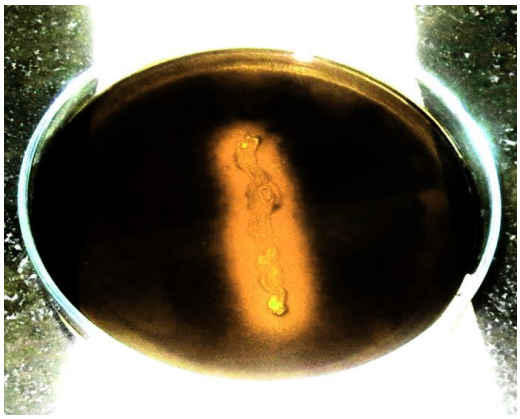

Fig. 2. Starch hydrolysis by amylolytic strain of *S. cerevisiae* ETGS1

3.6 Characterization of Selected ETGS1 Isolate

3.6.1 Morphological characterization

Morphological characteristics of isolate ETGS1 were studied. The morphological features are shown in Fig. 4 A and summarized in Table 2. The cell size of ETGS1 is 2 to 8 µm in length and 0.5 to 3 µm in breadth. The cells normally occurred mainly in small clusters or occasionally singly.

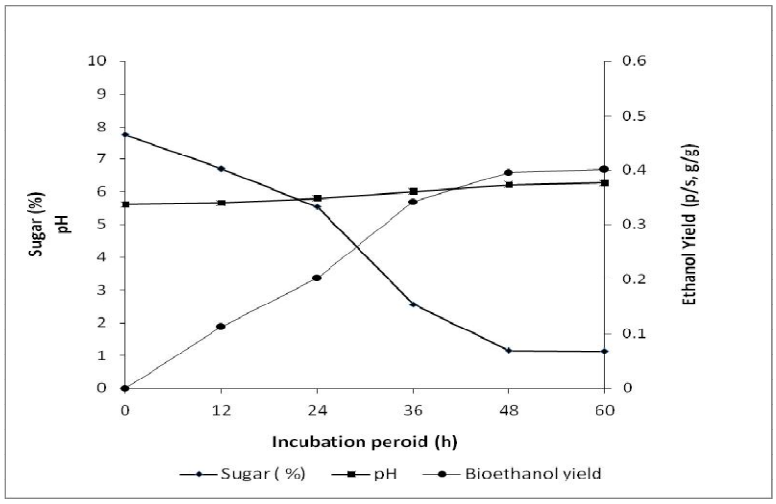

Fig. 3. Fermentation profile of selected strains of *S. cerevisiae* using starch as carbon source

Table 2. Characterization of selected ETGS1 isolate

Morphological characteristics	
Size	Small
Shape	Ovoid to elongate
Arrangement	Single or cluster
Cultural characteristics	
Size	Large
Shape	Round
Margin	Entire
Texture	Buttery
Elevation	Slightly raised
Opacity	Opaque
Colour	Milky white
Biochemical characteristics	
Assimilation of Carbon;	
Glucose	+
Galactose	+
Maltose	+
Mannose	+
Raffinose	+
Sucrose	+
Lactose	-
Starch	+
Cellulose	-
Cellobiose	-
Assimilation of Nitrogen;	
Peptone	+
Yeast Extract	+
Ammonium Sulphate	+
Asparagine	+
Nitrate	-
L-lysine	-
Cycloheximide resistance	-

3.6.2 Cultural characterization

The cultural characteristics of selected isolate ETGS1 were studied. The colony was Bright, milky white, large sized, opaque colony, butter like texture and slightly raised elevation, Oblong/Eclipse (Fig. 4 B). The cultural characteristics are depicted in Table 2. ETGS1 strain mediated the formation of larger cellular aggregates and seems to be flocculating.

3.6.3 Biochemical characterization

Biochemical properties were determined for selected ETGS1 isolate. The strain was found to utilise D-glucose, maltose, D-mannose, D-galactose, D-reffinose and D-sucrose. Negative assimilations and fermentations were observed for lactose, D-cellobiose, cellulose. Negative assimilations were observed for nitrate, and L-lysine which were used as nitrogen sources. Negative growth was observed in the presence of 100 ppm (µg/ml) of cycloheximide. Vigorous growth was observed at 35°C.

3.6.4 Molecular characterization of selected isolate

Data analysis using online BLAST tool of NCBI, ETGS1 has shown very close homology (99%) with the *S. cerevisiae YMC990* confers that the isolate belong to species level similarity with *S. cerevisiae*. This revels that our ETGS1 isolate is novel strain of *S. cerevisiae* yet been not reported in public domain. Further phylogenetic relation of *S. cerevisiae ETGS1* with the other available sequenced genotype is shown below in the form of tree dendogram Fig. 5 Sequence of selected strain of yeast was further processed by sequin stand-alone software and sequin generated file submitted to Genebank. The accession number was obtained for *S. cerevisiae* ETGS1 is KU173539.

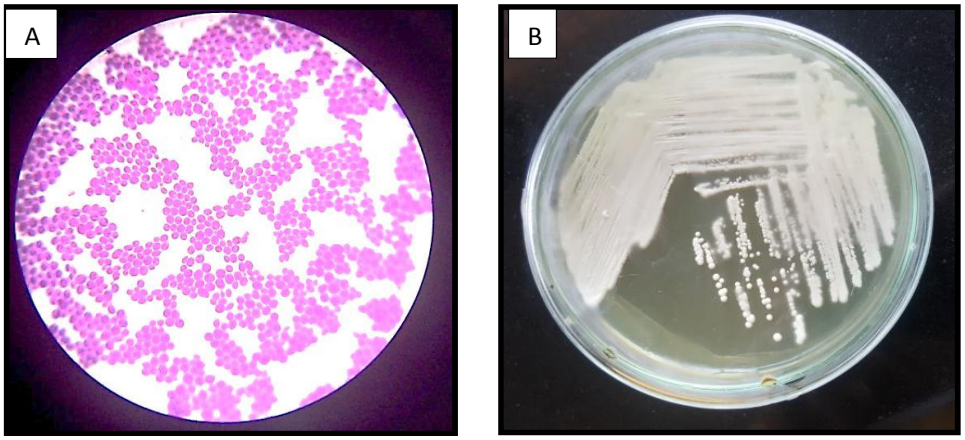

Fig. 4. A. Morphological and B. Cultural characteristics of selected ETGS1 isolate

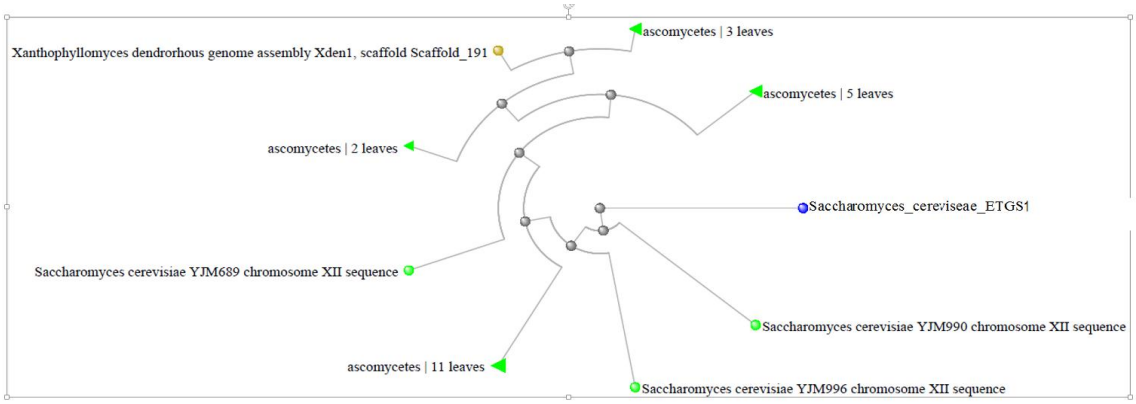

Fig. 5. Distance tree of query sequence for *S. cerevisiae* ETGS1 with available reference sequence in circular lay out form

4. CONCLUSION

Potential yeast isolates obtained from the diverge ecosystem were screened out based on their ethanogenic, thermotolerance and ethanol tolerance. These extensive screening resulted into selection of 18 efficient ethanogenic and thermotolerant isolates. These were further studied for their ability to produce the various enzymes. Results of enzyme profiling of selected isolates showed that ETGS1 strain is amylolytic. Looking to potentials of selected ETGS1 strain for bioconversion of cheaply available substrate i.e. starch waste into ethanol, it was further investigated thoroughly. Based on morphological, cultural and molecular characteristics, ETGS1 is identified as novel strain of *S. cerevisiae.* Preliminary fermentation studies confirm the *S. cerevisiae* ETGS1 as potential candidate for the process development for the bioconversion of starch processing wastes into ethanol at elevated temperature in single step without hydrolysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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