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# Prioritizing Wild Yeast Strains for Macroalgal Bioethanol Production

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## Abstract

Macroalgal biomass for bioethanol production has proved to be a viable alternative to feedstocks of first-generation (food crops rich in starch) and second-generation biofuel (agricultural residues and woody biomasses rich in lignocellulosic components). Production of bioethanol from biomass involves fermentation of mixed monosugars such as glucose, xylose, galactose, rhamnose, mannose, and fucose, and abundant monomer is found in algal biomass as well as lignocellulosic biomass. The inability of commonly used *Saccharomyces cerevisiae* to ferment xylose (pentose) sugar has led to the exploration of robust yeast strains that can utilize mixed sugars to produce ethanol. This study focuses on the isolation of yeast strains from various fruits and fermented products to determine efficacy in ethanol production using synthetic and macroalgal sugar. Two strains prioritized based on ethanol yield are *Meyerozyma caribbica* (isolated from cashew-fermented juice) and *Pichia kudriavzevii* (isolated from toddy). Strain *P. kudriavzevii* is thermotolerant (at 45 °C), whereas *M. caribbica* is tolerant to high salinity and produced ethanol of 2.6 g/L from 5.95 g/L of sugar, achieving 88.8% fermentation efficiency. *P. kudriavzevii* strain exhibits ethanol tolerance up to 4%. Fermentation of synthetic glucose produced 1.35 g/L and 1.44 g/L ethanol by *M. caribbica* and *P. kudriavzevii* strains with fermentation efficiencies of 83.6% and 94.8% respectively. *M. caribbica* strain fermented xylose and produced 1.4 g/L of ethanol achieving 14.9% fermentation efficiency, while simultaneous saccharification and fermentation process using *P. kudriavzevii* strain exhibited efficiency of 65.1% and 80.9% for *Enteromorpha intestinalis* and *Ulva lactuca* respectively. Cellulolytic activity of the prioritized strains was determined to carry out consolidated bioprocess.

**Keywords** Bioethanol · Macroalgae · Fermentation · Yeast · Thermotolerant · Ethanologenic

## Abbreviations

| | |
|---|---|
| CBP | Consolidated bioprocess |
| FAN | Free amino acid nitrogen |
| GHG | Greenhouse gas |
| GRAS | Generally recognized as safe |
| HMF | Hydroxymethyl furfurals |
| PCA | Principal component analysis |
| RS | Reducing sugar |
| SHF | Separate hydrolysis and fermentation |
| SSF | Simultaneous saccharification and fermentation |

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## Introduction

The global energy demand is projected to escalate beyond 37% by 2040 [1, 2], posing severe threats to fossil fuel reserve; for the foreseeable future, approximately 31% of crude oil remains the most significant energy source [3, 4]. The enhanced greenhouse gas (GHG) footprint [3, 5–7] with the concerns of climate changes has necessitated exploring alternative and renewable sources such as biofuels. Biofuels are produced from plant biomasses, and widely used biofuel comes from the feedstock that involves food crops like corn grains and molasses (first generation). This is followed by lignocellulose biomass such as rice straws, wheat straws, and sugarcane bagasse (second generation). Both these feedstocks faced the constraints of arable land, water, and higher production cost. In this context, algal biomass gained significance as a potential third-generation feedstock

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for bioethanol production [8–13]. Production of bioethanol from algae involves (i) degradation of feedstock to release fermentable sugars [14] and (ii) fermentation of variants of sugar using appropriate organisms to produce bioethanol.

The macroalgal genera *Kappaphycus*, *Gelidium*, *Gracilaria*, *Sargassum*, and *Ulva* are regarded as potential feedstock for bioethanol production [7]. Macroalgae are composed of structural and storage polysaccharides, which serve as a raw material for bioethanol production [7]. Polysaccharides of macroalgal feedstock constitute monosaccharide: glucose (26–30%), xylose (10–15%), rhamnose (3.3–12.7%), mannose (0.1–0.29%), galactose (1–6%), arabinose (0–0.08%), uronic acid (20–25%), and glucuronic acid (0–10%) [13, 15–18]. Xylose, glucose, and rhamnose are the three most abundant monomers found in green macroalgae. Maximum ethanol production is achieved by converting all the sugars present in the biomass [19]. Glucose (hexose sugar, C6) is ubiquitous in nature and is readily fermented by the yeast strain *S. cerevisiae*. Xylose (pentose sugar, C5) is the second most abundant sugar in nature and is not fermented by *S. cerevisiae*, limiting its usage in bioethanol production from lignocellulosic biomass [20]. Rhamnose is a deoxy sugar present in green macroalgae, mostly in the range of 3.3–12.7% [21–23]. Earlier studies indicate that the microorganisms cannot grow on rhamnose as a sole source of carbon as the uptake of rhamnose by these organisms is extremely slow [24, 25]. However, fermentation of these deoxy sugars like rhamnose and fucose is solely investigated to produce a high concentration of 1,2-propanediol [23, 26], which is used to synthesize polymer resins, non-ionic detergents, cosmetics, liquid detergents, biodegradable plastics, etc. The economic value of 1,2-propanediol is estimated over 1 billion pounds [26]. Therefore, the production of 1,2-propanediol by bacteria and yeast using deoxy sugars (rhamnose and fucose) is more economical than by-passing rhamnose sugar for bioethanol production. Therefore, this study highlights the utilization of glucose and xylose efficiently for bioethanol production by isolated wild yeast strains. Several studies have focused on investigating the potential of wild (non-domesticated) yeast strains for bioethanol production [27]. Co-fermentation for fermenting xylose and glucose using two different species of yeast also has been reported. *Candida shehatea*, *Scheffersomyces stipitis* (*Pichia stipitis*), and *Pacchysolen tannophilus* are the most commonly used yeast species for converting xylose [19]. *Scheffersomyces stipitis* strain (UFMG-IMH 43.2) proved to be the most efficient yeast strain, as it fermented glucose, xylose, and cellobiose with high ethanol yield and low quantities of co-products [19] with the ethanol yield of 0.91 g/g from 30 g/L of xylose [28].

Bioethanol production from macroalgal biomass is carried out either by separate hydrolysis (acid/enzyme) and fermentation (SHF) or simultaneous saccharification (enzyme) and fermentation (SSF) process. SHF involves two separate stages: (i) biomass is hydrolyzed by acid or enzyme to release sugars and (ii) fermentation of sugars to produce ethanol. In SSF process, acid pretreated biomass is subjected to enzyme hydrolysis and fermentation in a single reactor. However, both these processes are with relative merits and demerits. SHF is a faster process but encounters the formation of hydroxymethyl furfurals (HMF), an inhibitor during acid hydrolysis of biomass, which is detrimental to yeast organisms. SSF requires a more extended period as it involves enzyme hydrolysis and fermentation. Lower concentrations of inhibitors are formed in the SSF process. Bioethanol production from cellulosic feedstock involves four unit operations: pretreatment, enzyme production, enzyme hydrolysis, and microbial fermentation. Consolidated bioprocess (CBP) combines three-unit operations (enzyme production, enzyme hydrolysis, and microbial fermentation) into a single-unit operation. This brings down the cost of bioethanol production from macroalgae. However, wild yeast microorganisms with the capability of high cellulolytic activity and saccharification of lignocellulosic biomass and ethanol production are still unexplored.

The fermentation process is exothermic and causes an increase in temperature during industrial scales. However, higher temperatures are not tolerated by yeast organisms as it shortens the exponential phase of the yeast cell [20], affecting ethanol production. Other stresses such as sugar concentration and changes in pH inhibit cell growth. Microorganisms tolerant to these stresses naturally occur in nature [29]. Yeast strains are prioritized for the fermentation process based on the characteristics such as (i) rapid and relevant fermentation ability, (ii) genetic stability, (iii) osmo-tolerance, (iv) ethanol tolerance, (v) cell viability, and (vi) thermotolerance. The fermentation process is highly influenced by the type of yeast strain utilized [30, 31], due to which there is a perpetual quest for isolation of a novel, robust, and tolerant yeast strain with a potential of fermenting all the sugars available for higher bioethanol production and industrial application through bioprospecting. Catering to the challenges, the present study deals with bioprospecting of ethanologenic wild yeasts with a potential to produce bioethanol and screening of cellulolytic yeast strain and bioethanol production by CBP. The exploration of wild (non-domesticated) yeast strains with desirable characteristics would strengthen yeast strains' current repository for optimal bioethanol production.

## Materials and Methods

### Isolation and Screening of Fermentative Yeast

Yeast strains were isolated from various sources, namely fruits and fermented products (Supplementary Table S1). Fruit samples were cut into small pieces and incubated at

room temperature overnight and 1 ml of the fruit extract was serially diluted ($10^{-1}$ to $10^{-6}$ dilutions) and plated on Yeast Extract Peptone Dextrose Agar (YEPDA) which consists of 2% peptone, 2% yeast extract, 5% dextrose, and 1.5% agar supplemented with streptomycin 30 µg/mL, incubated for 24 h at 30 °C. After incubation, yeast colonies on agar were characterized based on size, shape, and pigmentation [32, 33]. Colonies were sub-cultured on YEPDA by streak plate technique and subsequent pure culture maintained on agar slants for further characterization. Screening of ethanologenic wild yeast strains for ethanol production was carried out in two steps: (i) First, ethanol fermentation was carried out in a Durham fermentation tube in six different sugars: 50 g/L of glucose, galactose, xylose, lactose, maltose, and sucrose; 10 g/L peptone; 5 g/L NaCl; and 0.5 g/L phenol red and inoculated for 24 h at 35 °C. The fermentation activity of yeast strains was confirmed by observing the volume of gas in the Durham tube filled with $CO_2$; based on this, positive yeast strains were selected for further studies [27]. Strains producing gas in glucose and xylose media were explicitly selected for the study. (ii) Next, biomass in glucose and xylose media was recorded by inoculating in 20 g/L yeast extract and 20 g/L peptone broth with 50 g/L of glucose and xylose separately and incubated at 35 °C on an orbital shaker at 100 rpm for 24 h, and biomass was recorded at 600 nm. Fresh YEPD broth was prepared, and yeast organism sample from the axenic culture was inoculated and incubated at 30 °C on an orbital shaker at 100 rpm. At every 1-h interval, 5 mL sample was drawn, and the absorbance was measured at 600 nm. This experiment was carried out until the attainment of the stationary phase with the recurring values.

## Characterization of Yeast Strain: Temperature, Ethanol, and Salt Tolerance

The selected yeast strains were inoculated in glucose media at various temperatures from 30 to 50 °C at an interval of 5 °C, ethanol concentration 0–10%, and salt concentration 0–14% with 2% interval and incubated for 24 h on an orbital shaker at 100 rpm and the absorbance was measured at 600 nm along with negative control (without yeast strain) and positive control using baker's yeast (*Saccharomyces cerevisiae*) [27, 34]. Experiments were carried out in triplicates.

## Identification of Yeast Strain Using rDNA Sequencing and Molecular Phylogenetic Analysis

Genomic DNA was isolated, the quantity was measured using a NanoDrop spectrophotometer, and the quality was determined using 2% agarose gel. A single band of high-molecular-weight DNA has been observed. 18S rRNA gene was amplified by 18SrRNAF and 18SrRNAR primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. A consensus sequence of 18S rRNA gene was generated from forward and reverse sequences. 18S rRNA gene sequence was compared to type strains in the National Center for Biotechnology Information (NCBI). Based on the maximum identity score, first ten sequences were selected and aligned using Clustal *W*, and a phylogenetic tree was constructed using the neighbor-joining method with MEGA version 7.0 with a bootstrap number 1000 [35].

## Fermentation

### Efficacy of Yeast Strain to Produce Ethanol Using Synthetic Sugars

Carbohydrates of *Enteromorpha intestinalis* and *Ulva lactuca* mainly composed of glucose and xylose are the source of carbon for fermentation. Fermentation efficiency of the selected yeast strains was evaluated in a 250-mL Erlenmeyer flask containing 100 mL of 2 g/L yeast extract and 2 g/L peptones with 5 g/L glucose, 5 g/L xylose, and 5% v/v yeast inoculum in 3 different flasks subjected to fermentation at 35 °C, pH 4, for 24 h using prioritized yeast strains in different combinations to determine its efficacy.

### Efficacy of Yeast Strain to Produce Ethanol Using Macroalgal Hydrolysate

Fermentation was carried out in a 250-mL Erlenmeyer flask containing 150 mL of clear hydrolysate. Macroalgal biomass (5 g) of *Enteromorpha intestinalis* and *Ulva lactuca* was acid pretreated using 0.7 N and 0.5 N $H_2SO_4$ at 121 °C for 45 min to determine the efficacy of isolated yeast strain to ferment seaweed sugars. The acid hydrolysate was obtained and neutralized using $Na_2CO_3$. It results in lower sugar removal [7] and the fermentation medium was adjusted to pH 4 and subjected to SHF using prioritized yeast (5% v/v) strains in different combinations at 35 °C, 100 rpm for 24 h. For SSF, acid pretreated biomass (2 g) was subjected to fermentation using an enzyme (5% v/v) extracted from S9 (*V. parahaemolyticus*) [14] and prioritized yeast (5% v/v) strains in different combinations at 55 °C, 100 rpm for 24 h. Macroalgal biomass contains abundant carbon sources and essential minerals for yeast growth; fermentation was carried out without exogenous nutrients.

### Analytical Method and Data Analysis

Reducing sugar obtained in both the process was estimated before and after fermentation by the DNS method [36]. Theoretically, 1 kg of glucose produces 510 g of ethanol, i.e., 51%. Theoretical yield is 51% of the fermented sugar by each of the yeast strain (Eq. (2)), and fermentation efficiency is the percentage ratio of ethanol yield obtained from the experiment to theoretical yield as indicated in Eq. (1)

$$\% \text{ Fermentation efficiency} = \frac{\text{Ethanol yield}}{\text{Theoretical yield}} \quad (1)$$

$$\text{Theoretical yield} = 0.51 * \text{Fermented sugar by each yeast strain} \quad (2)$$

The ethanol obtained was estimated using GCMS with an FID as a detector. The sample was injected using an Agilent gold standard syringe with an accuracy of ± 1%. The analysis was performed under the following conditions: injector volume 1 µL, inlet temperature 180 °C, mode was split-less, flowrate of 1.2 mL/min, runtime of 24.6 min by ramping method with a temperature of FID at 280 °C. The gasses used were hydrogen with a flow of 30 mL/min, zero airflow of 300 mL/min, and helium flow of 10 mL/min. The identification of ethanol was done by MS at temperature 230 °C and quadrupole temperature 150 °C. MS filament was switched on and off at 1.82 min and 2.82 min, respectively, to identify the ethanol ions in the sample, and the ethanol was identified through the NIST database.

Agglomerative hierarchical clustering of screened yeast strains was carried out for the ethanol produced from the study and the literature-integrated data using R studio version 3.4.4. Multivariate analysis through principal component analysis (PCA) was carried out using R studio software version 3.4.4, to determine strain responses to temperature, ethanol, and salt tolerance and provide an overview of similarities and differences among the yeast strains.

### Screening for Cellulolytic Yeast and Ethanol Production by Consolidated Bioprocess

Prioritized yeast strains were screened for cellulolytic activity by inoculating on a 1% CMC plate supplemented with agar and incubated at 35 °C for 72 h. Plates were flooded with Gram's iodine. Colonies producing zone of clearance were considered positive for cellulolytic activity. The hydrolytic activity of each strain was determined [14]. Macroalgal biomass was subjected to saccharification and fermentation using cellulolytic yeast strain in the consolidated bioprocess.

## Results and Discussion

### Isolation and Screening of Ethanologenic Wild Yeast Strains

Fruits serve as microhabitats for a variety of yeast species. Therefore, bioprospecting of yeasts from fruits is advantageous [32]. Yeasts isolated from 100 samples (98 fruit sources and two fermented products) were screened in the current study. Colonies were observed in 89 samples, and colony morphology was recorded by incubating the strains at 35 °C for 24 h. Colony morphology of isolated yeast presented elongated (24.72%), oval (14.61%), and rounded (60.67%) shape; large (6.7%), medium (23.6%), or small (69.66%) size; cream (39.33%) or white (57.3%) or yellow (3.37%); irregular (48.31%) or regular (51.69%) borders; and bright (49.44%) or opaque (22.47%) or smooth (28.09%) texture.

Yeasts exhibit variation in the ability to ferment and assimilate various sugars, which also aids in the identification of yeast than morphological and physiological characteristics. Certain genera of yeast such as *Saccharomyces*, *Torulaspora*, and *Zygosaccharomyces* ferment glucose readily, whereas *Lipomyces* and *Sterigmatomyces* are strictly non-fermentative genera of the yeast. In this study, it was seen that about 74.16% and 71.91% of yeast strains readily fermented sucrose and glucose, respectively. Glucose as a carbon source allows faster growth within 24 h, which is due to the presence of 20 different glucose transporters in their plasma membrane [32]. Sucrose, a disaccharide, is assimilated extracellularly by secreting enzyme invertase. Lactose was fermented by 57.3% yeast strains, galactose by 55.06% yeast strains, and lowest being maltose by 41.57% yeast strains. Production of biofuels from second- and third-generation biomass encourages isolation of yeast strains capable of fermenting xylose. About 69.66% of yeast strains fermented xylose in the current study. In a similar study [32], 45 yeast strains were isolated from fruits and chicken litter and it was observed that yeast strains readily fermented glucose (22%), sucrose (12.5%), lactose (2.1%), xylose (40%), galactose (8.3%), and maltose (2.1%). Yeast strains were tested in glucose and xylose medium as these sugars are major constituents across the taxonomical groups of green macroalgae. Strains with the highest biomass in glucose media (> 0.5 $OD_{600}$) and xylose media (> 0.1 $OD_{600}$) were considered for further investigations (Supplementary, S1). The screening was done using the phenotypic microarray method by eliminating strains with lower redox signal intensity (RSI) in glucose

medium [37]. About 40.45% of yeast strains exhibited good growth in glucose media with the biomass > 0.5 OD, and 47.19% of yeast strains obtained biomass > 0.1 OD in xylose medium. Yeast strains with full gas production in Durham's fermentation tube were selected. About 19 yeast strains (CY, TY, CHY, MY, MFY, GVY, TNY, PLY FBY2, BAY, FBJY, RJY, GWY, CKY, PWY, WTY, YKY, POY, CUIY) were screened down for further characterization and fermentation capabilities. A growth curve study was carried out for the strains, incubated at 35 °C for 24 h with samples drawn at every 1-h interval and biomass growth observed at 600 nm. Strains exhibiting a more prolonged exponential phase (Supplementary, S1) were selected, as it is a proxy for higher ethanol production as most of the primary metabolites are formed during this phase [38, 39].

## Screening of Multi-Stress Tolerant Ethanologenic Yeast Strain

Ethanol endurance is an important property that decides the fermentation efficiency of the yeast strain. Ethanol tolerance of yeast has been determined as the accumulation of ethanol during the fermentation process, and ethanol is toxic to yeast organisms as it inhibits the activity of crucial glycolytic enzymes involved in ethanol production and hinders amino acid and glucose transport leading to the loss of cell viability and inhibition of cell growth [34, 35]. Isolated yeast growth was recorded in a spectrophotometer (600 nm) in terms of turbidity at different ethanol concentrations. CHY had the highest ethanol tolerance, up to 10%, followed by CY, TY, and MY, after which the growth decreased (Supplementary, S2). In a similar study, yeast strains CHY1011 and CHFY0901 belonging to the *Saccharomyces* genus exhibited ethanol tolerance of up to 5% [40]; comparable tolerance level was recorded for baker's yeast (positive control) in the current study, whereas an isolated yeast (Y-1) from wine (Jiuqu) had ethanol tolerance of up to 14% [41]. *S. cerevisiae* isolated from Nuruk [42, 43], cashew [44], and soil [45] exhibited tolerance in a medium containing 15% alcohol.

Yeast growth at different temperatures was monitored. Thermotolerance of yeast is evident up to 37 °C, and growth is inhibited at higher temperatures. PLY strain exhibited consistent high growth of up to 40 °C, whereas TY strain exhibited higher thermotolerance of up to 45 °C. In a similar study earlier [46], *Pichia kudriavzevii* DMKU 3-ET15 isolated from fermented pork sausage displayed thermotolerance of up to 45 °C, which is comparable to TY strain (*Pichia kudriavzevii*) in the current study (Supplementary, S3). *Candida tropicalis*, *Pichia kudriavzevii*, *Candida orthopsilosis*, *Candida glabrata*, and *Kodamaea ohmeri* were reported as thermotolerant and high ethanol-producing yeast strains [47]. *Pichia caribbica* isolated from ripe banana was subjected to different temperatures, which displayed good growth at temperatures 28, 30, and 40 °C, which declined at 45 °C [48]; contrary to which, CY strain (identified as *P. carribbica*) exhibited good growth at 35, 40, and 45 °C, which declined after 45 °C.

Bioethanol process from seaweeds encounters a high concentration of NaCl due to its habitat [49]. Therefore, isolated yeast strains were subjected to different salt concentrations, which show a decline of cell growth with an increase in salt concentration, similar to *S. cerevisiae* KCTC 1126. But, *S. cerevisiae* KCTC 1126 adapted to NaCl and yielded an ethanol concentration of 0.48 [50]. *Issatchenkia orientalis* MF-121 produced 2.9% (w/v) ethanol in a medium containing $Na_2SO_4$ (50 g/L), while tolerant to multi-stress factors such as temperature, ethanol, and salt [51]. Halotolerance of up to 14% NaCl concentration was exhibited by WTY strain; however, the strain achieved a lower ethanol conversion efficiency of glucose and xylose fermentation (Supplementary, S4).

Multivariate analyses through principal component analysis (PCA), given in Fig. 1a, illustrate strains clustering into three groups with the clustering of temperature-tolerant strains and overlapping of ethanol- and salt-tolerant strains. Principal components are accounting for 56.5% of the total variance, with PC1 contributing 38.8% and PC2 contributing 17.7%. Salt tolerant strains POY, YKY, CUIY, and PWY were located at the positive side along PC1; on the other hand, thermotolerant species CY, TY MY, MFY, and CHY were closely loaded at the negative side along PC1. Ethanol-tolerant strains were loaded at the positive side of PC2.

## Ethanol Production by Yeast Strains Using a Synthetic Medium

Fermentation of glucose is an established technology; however, fermentation of xylose has been posing challenges. During the fermentation process, 70% of the sugar is converted to ethanol, whereas 20% assimilated by the yeast cells yields glycerol, organic acids, etc. [52, 53]. Production of glycerol at a higher concentration inside the yeast cell is stimulated by factors such as higher pH, a lower flux of pyruvate (due to the utilization of glycolytic intermediates), and increase in osmotic pressure, with the formation of by-products (higher alcohols and organic acids at lower level), affecting the ethanol yield as the growth of yeast cells invariably directs the glycolytic intermediates to corresponding pathways. Ethanologenic yeast strains are evaluated based on the ability of strains to utilize all sugars (glucose, xylose, galactose, mannose, rhamnose, and arabinose) and convert to ethanol with minimal by-product formation [54]. Conventionally, ethanol yield at an industrial scale is calculated based on the total sugar fed into the fermentation system,

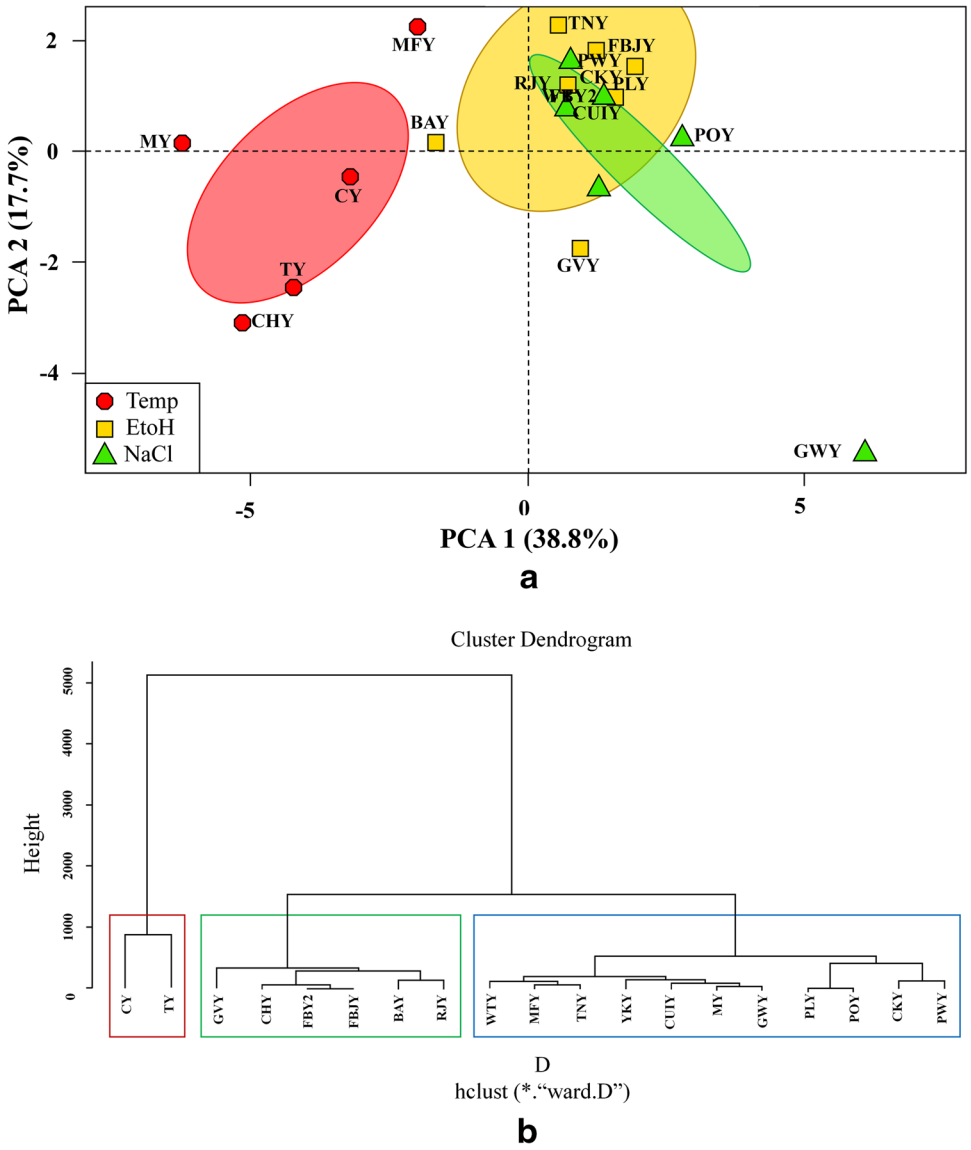


**Fig. 1** **a** PCA score plot of yeast strains tolerant to stress factors; temperature, ethanol, and salt concentration. **b** Multivariate cluster analysis of yeast strains

and 90–93% ethanol bioconversion is considered for an efficient ethanologenic strain.

Glucose is an abundantly found sugar in the feedstock and is readily fermented by yeast microorganisms. Yeast prefers glucose over xylose, and xylose uptake is regulated by glucose concentration [55, 56]. Glucose is metabolized in a series of enzyme catalyzed reaction process called glycolysis; to yield two molecules of three carbon compound pyruvate, under hypoxia or anaerobic condition, pyruvate is decarboxylated and acetaldehyde is reduced to ethanol through alcohol dehydrogenase [57]. Xylose is converted to xylulose and phosphorylated to xylulose-5-phosphate and further metabolized to glyceraldehyde-3-phosphate and fructose-6-phosphate, which then enters the glycolysis pathway for subsequent pyruvate and ethanol production [58]. It was seen that about 60–80% of glucose was assimilated and fermented by yeast strains within 24 h except for CY and TY strains, which consumed less glucose but achieved a higher conversion efficiency of 83% and 94%, respectively, compared to other yeast strains. The highest ethanol concentration of 5.04 g/L was recorded for MY strain with 65.3% conversion efficiency. The least ethanol concentration was recorded for PLY, CKY, and FBJY strains.

Xylose is the main component (1/3) of lignocellulosic biomass, and xylose is not fermented by *Saccharomyces cerevisiae* due to lack of transport system. Yeast species capable of fermenting xylose belong to the genera *Brettanomyces*, *Candida*, *Clavispora*, *Kluyveromyces*, *Pachysolen*, *Pichia*, and *Schizosaccharomyces*. Among which, *Candida shehatea*, *Pachysolen tannophilus*, and *Pichia stipitis* ferment xylose at high concentrations [58], and studies are being carried out to isolate xylose-fermenting yeast strains [59–61].

In the current study (Table 1), a higher ethanol concentration of 28.2% was recorded for GVY followed by CY strain with 15.3% conversion efficiency, indicating the ability to ferment xylose sugar. GVY strain produced lower conversion efficiency in glucose medium. Co-fermentation using two different yeast species has been carried out to efficiently utilize both xylose and glucose [19, 62]. Co-fermentation of sugars present in lignocellulose biomass was carried out using *P. stipitis* and *K. marxianus*, and *P. stipitis* and *S. cerevisiae* yielded 31.87 g/L and 29.45 g/L of ethanol [15]. In this study, co-fermentation of glucose and xylose using yeast strain was carried out. Acid hydrolysis of biomass releases mixed sugars into the medium, and therefore, mixed sugar fermentation was carried out, and CY strain produced 1.83 g/L of ethanol, achieving 27% efficiency, whereas GVY strain produced 1.8 g/L of ethanol achieving 20.6% conversion efficiency. Ethanol production was affected in other yeast strains due to xylose, hence the lower ethanol yield despite the presence of glucose. Studies report that, in order to improve xylose fermentation, isolated yeast strain should be grown on xylose-rich media, and efficacy of yeast strain in ethanol production was evaluated by supplementing xylose in concentration of 10 g/L or 25 g/L [63].

## Ethanol Production by Prioritized Strain Using Macroalgal Sugars

Fermentation of macroalgal hydrolysate was carried out to validate the potential of isolated wild yeast strains for selection of ethanol production. Hydrolysates of *E. intestinalis* and *U. lactuca* obtained from acid hydrolysis were neutralized and subjected to fermentation using each of the screened yeast individually at 35 °C for 24 h on an orbital shaker with 100 rpm. Reducing sugar profile of *Ulva* and *Enteromorpha* is illustrated in Table 1.

Fermentation progress was determined by measuring the reducing sugar (after the fermentation process) and comparing it with the theoretical yield (51% of fermented sugar) by estimating the sugar conversion efficiency of each strain, which is detailed in Table 2. A higher conversion efficiency of 49.4% was obtained for CY strain for *E. intestinalis* followed by TY strain 42.9% (Table 3). A similar study was carried out using wild yeast strains *S. cerevisiae* Y12 and YPS128, derived from clean lineages with no alternations to their genome due to human interventions or domestication [37, 64], and this strain fermented the hydrolysate of *U. lactuca* producing 7 g/L of ethanol (Table 3). A multi-tolerant strain of six *Saccharomyces* strains was selected and utilized for fermentation of lignocellulose hydrolysate. This study indicates that the natural strains outcompeted other strains for specific traits. Yeast strains isolated from the natural environment have the potential for bioethanol production and superior to industrial strains obtained by tweaking the strain through breeding, experimental evolution, or genetic engineering [34].

Free amino acid nitrogen (FAN) content in green seaweeds is > 0.15 g/L, and this avoids the need to supply additional nitrogen sources during fermentation [37]. The FAN required for yeast growth during fermentation and metabolism is 0.15 g/L. Hence, fermentation of seaweed hydrolysate was carried out without the addition of nitrogen sources. Multivariate cluster analysis was performed by selecting biochemical compositions of fruit sources (carbohydrate, protein, fat, dietary fibers, vitamins, moisture content, and minerals) as independent variables and ethanol production from synthetic sugar as a dependent variable. Figure 1 b illustrates the clustering of strains CY and TY from the rest of the isolated strains indicating its unique properties with the higher performance capabilities. These two strains achieved higher biomass, longer exponential growth, and maximum conversion efficiency concerning glucose fermentation, and exhibited temperature tolerance. Based on these criteria, strains CY and TY were prioritized for fermentation of macroalgal sugar.

## Yeast Identification

The identity of the prioritized yeast strains CY was confirmed as *Meyerozyma* (*Pichia*) *caribbica* and TY as *Pichia kudriavzevii* based on 16S rRNA nucleotide sequences homology match within the NCBI GenBank (Supplementary, S5). The yeast cells were stained with methylene blue and observed under an Olympus BX-51 bright field, phase contrast microscope; live cells reduce the dye (methylene blue) [65] and remain colorless, whereas dead cells retain the color and are stained blue (Fig. 2a, b). *Pichia kudriavzevii* cells are oval or ellipsoidal to elongate in the study. *P. kudriavzevii* is a thermotolerant yeast strain isolated from

**Table 1** Reducing sugar profile of macroalgal feedstock

| Macroalgae | Glucose | Xylose | Mannose | Galactose | Arabinose | References |
|---|---|---|---|---|---|---|
| *Ulva* sp. | 8.2 | 4.5 | 0.29 | 1 | 0.08 | Wal et al., 2013; Yaich et al., 2011 |
| *Enteromorpha* sp. | 26.3 | 3.5 | | 6 | | Cho et al., 2010 |

**Table 2** Fermentation capacities of yeast strains for synthetic sugar: glucose, xylose, and glucose + xylose

| Glucose | | | | | | Xylose | | | | Glucose + xylose | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yeast strains | Initial sugar g | Fermented sugar g | EtOH g | Theoretical yield | % conversion | Fermented sugar g | EtOH g | Theoretical yield | % conversion | Fermented sugar g | EtOH g | Theoretical yield | % conversion |
| POY | 20 | 17.01 ± 0.06 | 1.80 ± 0.04 | 8.68 | 20.8 | 14.63 ± 0.01 | 0.07 ± 0.01 | 7.47 | 0.1 | 18.08 ± 0.01 | 0.33 ± 0.05 | 7.47 | 3.6 |
| BAY | | 16.36 ± 0.14 | 3.71 ± 0.10 | 8.34 | 44.6 | 15.63 ± 0.01 | 0.01 ± 0.00 | 7.98 | 0.1 | 18.27 ± 0.01 | 1.28 ± 0.17 | 7.98 | 13.8 |
| GVY | | 16.78 ± 0.06 | 0.98 ± 0.20 | 8.56 | 11.5 | 15.69 ± 0.01 | 2.25 ± 0.04 | 8.00 | 28.2 | 17.50 ± 0.01 | 1.83 ± 0.05 | 8.00 | 20.6 |
| F3 | | 15.26 ± 0.22 | 0.48 ± 0.01 | 7.78 | 6.2 | 13.43 ± 0.03 | 0.05 ± 0.02 | 6.85 | 0.8 | 17.05 ± 0.06 | 0.08 ± 0.02 | 6.85 | 1.0 |
| TY | | 2.97 ± 0.18 | 1.42 ± 0.02 | 1.52 | 93.7 | 16.01 ± 0.1 | 0.04 ± 0.01 | 8.17 | 0.6 | 16.63 ± 0.03 | 0.02 ± 0.01 | 8.17 | 0.3 |
| CY | | 3.21 ± 0.24 | 1.35 ± 0.04 | 1.83 | 82.5 | 18.40 ± 0.04 | 1.43 ± 0.01 | 9.39 | 15.3 | 13.35 ± 0.03 | 1.83 ± 0.05 | 9.39 | 27.0 |
| CHY | | 14.20 ± 0.01 | 4.16 ± 0.22 | 7.24 | 57.5 | 10.36 ± 0.01 | 0.02 ± 0.01 | 5.29 | 0.5 | 13.96 ± 0.02 | 0.04 ± 0.01 | 5.29 | 0.6 |
| MY | | 15.12 ± 0.04 | 5.04 ± 0.03 | 7.72 | 65.3 | 9.54 ± 0.06 | 0.03 ± 0.01 | 4.87 | 0.7 | 11.57 ± 0.03 | 0.05 ± 0.02 | 4.87 | 0.9 |
| MFY | | 15.25 ± 0.02 | 4.64 ± 0.03 | 7.78 | 59.7 | 10.21 ± 0.12 | 0.02 ± 0.01 | 5.21 | 0.5 | 11.57 ± 0.11 | 0.04 ± 0.02 | 5.21 | 0.8 |

**Table 3** Fermentation capacities of yeast strains for acid-treated hydrolysate of *E. intestinalis* and *U. lactuca*

| Yeast strains | *Enteromorpha intestinalis* | | | | | *Ulva lactuca* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Initial sugar g | Fermented sugar g | Ethanol g | Theoretical yield | % conversion | Initial sugar g | Fermented sugar g | Ethanol g | Theoretical yield | % conversion |
| CY | 3.74 ± 0.15 | 3.23 ± 0.01 | 0.81 ± 0.01 | 1.6 | 49.4 | 3.74 ± 0.24 | 1.97 ± 0.01 | 0.42 ± 0.02 | 1.0 | 41.8 |
| TY | | 3.20 ± 0.02 | 0.70 ± 0.02 | 1.6 | 42.9 | | 2.02 ± 0.05 | 0.59 ± 0.01 | 1.0 | 57.1 |
| CHY | | 3.39 ± 0.01 | 0.57 ± 0.02 | 1.7 | 33.2 | | 2.75 ± 0.02 | 0.46 ± 0.02 | 1.4 | 32.8 |
| MY | | 3.32 ± 0.01 | 0.42 ± 0.03 | 1.7 | 24.8 | | 2.85 ± 0.01 | 0.36 ± 0.04 | 1.5 | 25.2 |
| MFY | | 3.32 ± 0.01 | 0.50 ± 0.02 | 1.7 | 29.5 | | 2.61 ± 0.02 | 0.42 ± 0.03 | 1.3 | 31.8 |
| GVY | | 3.12 ± 0.03 | 0.38 ± 0.01 | 1.6 | 24.3 | | 2.82 ± 0.01 | 0.23 ± 0.02 | 1.4 | 16.4 |
| BAY | | 3.08 ± 0.01 | 0.46 ± 0.04 | 1.6 | 29.6 | | 2.73 ± 0.03 | 0.43 ± 0.01 | 1.4 | 31.5 |
| YKY | | 3.14 ± 0.03 | 0.51 ± 0.01 | 1.6 | 32.3 | | 2.51 ± 0.01 | 0.18 ± 0.01 | 1.3 | 14.7 |
| POY | | 3.01 ± 0.04 | 0.24 ± 0.02 | 1.5 | 16.1 | | 2.62 ± 0.01 | 0.44 ± 0.03 | 1.3 | 33.6 |

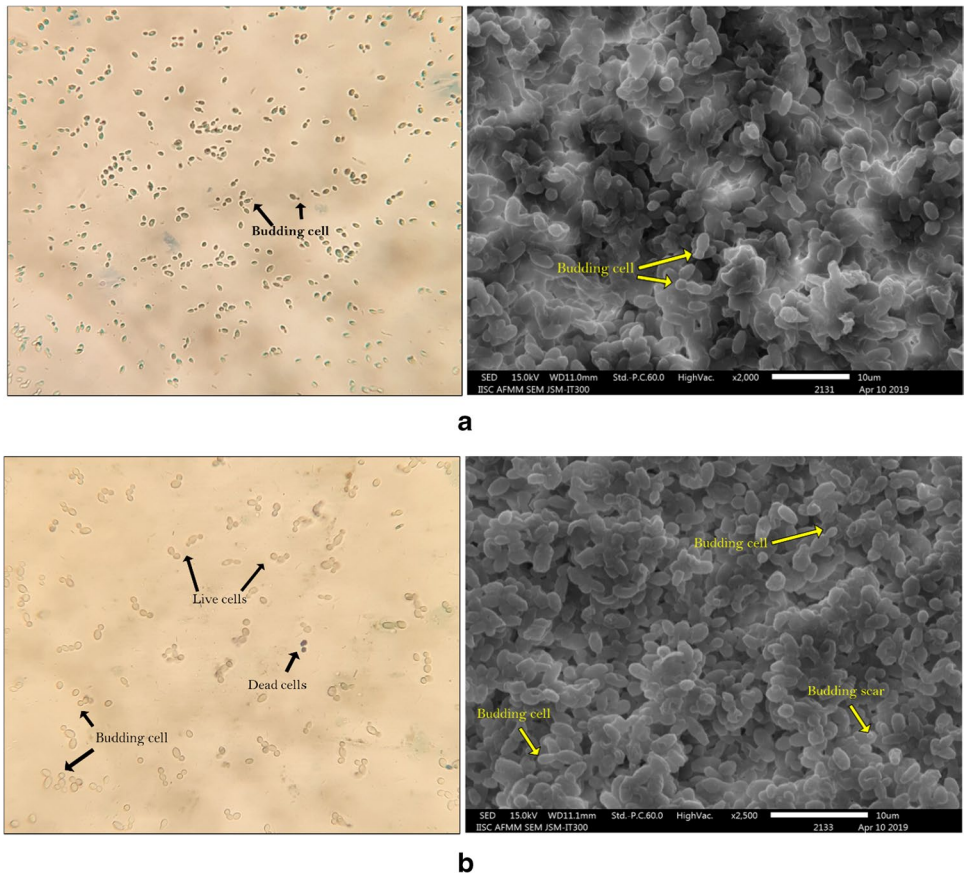


**Fig. 2** **a** Microscopic image and scanning electron micrographs of (CY) *Meyerozyma* (*Pichia*) *caribbica.* **b** Microscopic image and scanning electron micrographs of (TY) *Pichia kudriavzevii*

fruits and food sources. In contrast, *Meyerozyma caribbica* are isolated from fermented beverages having the capabilities to ferment xylose with high efficiency [66], which was observed in this study as well.

## Fermentation of Macroalgal Sugar Using Prioritized Yeast Strains

### Separate Hydrolysis (Acid) and Fermentation (SHF)

Reducing sugar from *E. intestinalis* and *U. lactuca* was obtained by using 0.7 N and 0.5 N $H_2SO_4$ concentration. About 22.4% and 19.2% sugar conversion with respect to biomass was achieved for *E. intestinalis* and *U. lactuca*, respectively (Table 4). The acid hydrolysate obtained was subjected to fermentation using prioritized yeast strains CY and TY in different combinations at 35 °C, 100 rpm for 24 h. Fermentation of *E. intestinalis* hydrolysate using CY and TY produced 0.14 g/L and 0.16 g/L of ethanol with fermentation efficiencies of 46.9% and 51.8%, respectively. Co-fermentation of *E. intestinalis* hydrolysate using CY and TY yielded lower fermentation efficiency of 33%. *Candida* sp. was isolated from marine fermented red algae, *Kappaphycus alvarezii*, acid hydrolysate achieving 50% fermentation efficiency [27]. *E. intestinalis* subjected to SHF produced 8.6 g/L of ethanol with 30% conversion efficiency within 48 h (Cho et al., 2013). SHF of *K. alvarezii* [67] and *Gelidium amansii* [68] yielded 0.25 g and 3.33 g of ethanol achieving 55.9% and 74.7% efficiency, respectively. Acid hydrolysis (1% v/v, $H_2SO_4$ for 90 min) of sugarcane bagasse pith was subjected to fermentation obtaining 2.58 g/L of ethanol in 30 h. Fermentation time for lignocellulose biomass is longer than of macroalgal biomass due to the presence of complex polysaccharide lignin. Fermentation of *U. lactuca* hydrolysate yielded lower ethanol of 0.04 g/L and 0.05 g/L for both CY and TY strains with fermentation efficiencies of 24% and 48.7%, respectively. Bioethanol has been obtained from all the three types of algae; however, appropriate microorganism is yet to be isolated which consumes pentose sugar and mixed sugar [69]. Lower ethanol yield in this study can be attributed to inhibitor formation during acid hydrolysis.

### Simultaneous Saccharification and Fermentation (SSF)

Acid pretreated macroalgal biomass was subjected to enzyme hydrolysis using enzyme extracted from *V. parahaemolyticus* [14] and subjected to the subsequent fermentation. SSF of *E. intestinalis* and *U. lactuca* using CY

**Table 4** Separate hydrolysis and fermentation of dilute acid hydrolysis of macroalgal biomass

| Seaweed hydrolysate | Biomass (g) | Acid pretreatment | Yeast strain and fermentation process condition | Initial sugar (g) | Fermented sugar (g) | Ethanol (g) | Theoretical yield | % conversion efficiency |
|---|---|---|---|---|---|---|---|---|
| *E. intestinalis* | 5 | 0.7 N $H_2SO_4$, 121 °C for 45 min | CY (35 °C, 100 rpm for 24 h) | 1.12 ± 0.03 | 0.58 ± 0.03 | 0.14 ± 0.02 | 0.30 | 46.9 |
| | | | TY (35 °C, 100 rpm for 24 h) | | 0.61 ± 0.01 | 0.16 ± 0.02 | 0.31 | 51.8 |
| | | | CY and TY (35 °C, 100 rpm for 24 h) | | 0.48 ± 0.08 | 0.08 ± 0.01 | 0.25 | 33.0 |
| *U. lactuca* | | 0.5 N $H_2SO_4$, 121 °C for 45 min | CY (35 °C, 100 rpm for 24 h) | 0.96 ± 0.07 | 0.30 ± 0.07 | 0.03 ± 0.01 | 0.15 | 24.0 |
| | | | TY (35 °C, 100 rpm for 24 h) | | 0.20 ± 0.04 | 0.05 ± 0.01 | 0.11 | 48.7 |
| | | | CY and TY (35 °C, 100 rpm for 24 h) | | 0.30 ± 0.01 | 0.06 ± .01 | 0.16 | 40.4 |

strain produced ethanol of 0.12 g/L and 0.08 g/L achieving conversion efficiencies of 45.5% and 48.7% (Table 5). Higher conversion efficiency of 80.9% was achieved for *U. lactuca* followed by 65.2% for *E. intestinalis* biomass using TY yeast strain indicating its thermotolerance capabilities. Similarly, *Kluyveromyces marxianus* was recognized as a safe (GRAS) thermotolerant yeast strain with tolerance range of 38–45 °C and producing high ethanol concentration SSF process [66].

Higher sugar conversion efficiency by the non-domesticated ("wild") strains *Pichia kudriavzevii* and *Meyerozyma caribbica* indicates their potential to be used at industrial level, with strain improvement through experimental evolution, hybridization, or genetic engineering.

## Effect of Salt on Ethanol Production

Marine yeast is utilized in several applications as they thrive in harsh conditions, hence tolerate higher process conditions (salinity and temperature) [70]. In the bioethanol process, pretreatment using dilute acid hydrolysis of marine macroalgal biomass results in salty hydrolysate, which requires a desalination process when employing terrestrial yeast strains, but in the case of halotolerant yeast strains, the hydrolysate is directly fermented to bioethanol.

**Table 5** Simultaneous saccharification and fermentation of acid pretreated macroalgal biomass

| Seaweed hydrolysate | Biomass (g) | Acid pretreatment | Enzyme and yeast strain fermentation process condition | Initial sugar (g) | Fermented sugar (g) | Ethanol (g) | Theoretical yield | % conversion efficiency |
|---|---|---|---|---|---|---|---|---|
| *E. intestinalis* | 2 | 0.7 N $H_2SO_4$, 121 °C for 45 min | S9 and CY (55 °C 100 rpm for 24 h) | 0.87 ± 0.08 | 0.53 ± 0.02 | 0.12 ± 0.02 | 0.27 | 45.48 |
| | | | S9 and TY (55 °C 100 rpm for 24 h) | | 0.31 ± 0.02 | 0.10 ± 0..01 | 0.16 | 65.19 |
| | | | S9, CY, and TY (55 °C 100 rpm for 24 h) | | 0.33 ± 0.01 | 0.06 ± 0.01 | 0.17 | 39.04 |
| *U. lactuca* | | 0.5 N $H_2SO_4$, 121 °C for 45 min | S9 and CY (55 °C, 100 rpm for 24 h) | 0.86 ± 0.01 | 0.33 ± 0.04 | 0.08 ± 0.01 | 0.17 | 48.74 |
| | | | S9 and TY (55 °C 100 rpm for 24 h) | | 0.34 ± 0.01 | 0.14 ± 0.02 | 0.18 | 80.94 |
| | | | S9, CY, and TY (55 °C 100 rpm for 24 h) | | 0.21 ± 0.02 | 0.04 ± 0.01 | 0.11 | 44.67 |

*CY*, cashew yeast (*P. caribbica*); *TY*, toddy yeast (*P. kudriavzevii*); *S9*, *V. parahaemolyticus*

Several industrial applications have utilized salt-tolerant yeast strains such as *Debaryomyces hansenii* and *Zygosaccharomyces rouxii*, but not all the yeasts have the ability to tolerate high salt conditions. However, in this study, it was seen that CY strain has consistent ethanol production until 4% salt concentration and produced the highest ethanol of 2.6 g/L from 5.95 g/L of reducing sugar achieving 88.8% fermentation efficiency and reduced at 5% and 6% salt concentration along with biomass. Similar results of luxuriant growth were observed for *Candida* sp. isolated from a marine source in the presence of 2–13% salt, which subsequently decreased at 14 and 15% salt [27]. *Dabaryomyces*, *Rhodotorula*, *Candida*, and *Saccharomyces* exhibit tolerance to NaCl ranging from 0 to 16% [71]. The TY strain (Fig. 3b) biomass gradually decreased with the increase in salt concentration and intermittent ethanol production. Highest ethanol of 2.5 g/L from 11.94 g/L fermented sugar was obtained at 3% salt concentration achieving 41.45% fermentation efficiency. At 5 and 6%, sugars were left unutilized by TY strain.

## Screening of Cellulolytic Yeast and Ethanol Production by CBP

A single strain of microorganisms that expresses cellulolytic activity and fermentation capabilities is of potential interest in bioethanol production as it brings down the economic burden of enzyme production and the overall bioethanol production and is regarded as the low-cost biomass processing [72, 73]. In this study, prioritized strains were isolated on plates comprising 1% CMC as the sole source of carbon (Fig. 4). Hydrolytic activity was recorded for both strains CY: 2.06 and TY: 2.69. Enzyme activity of 1.15 U/mL and 1.19 U/mL was recorded for *M. caribbica* and *P. kudriavzevii* at 24 h respectively (Table 6).

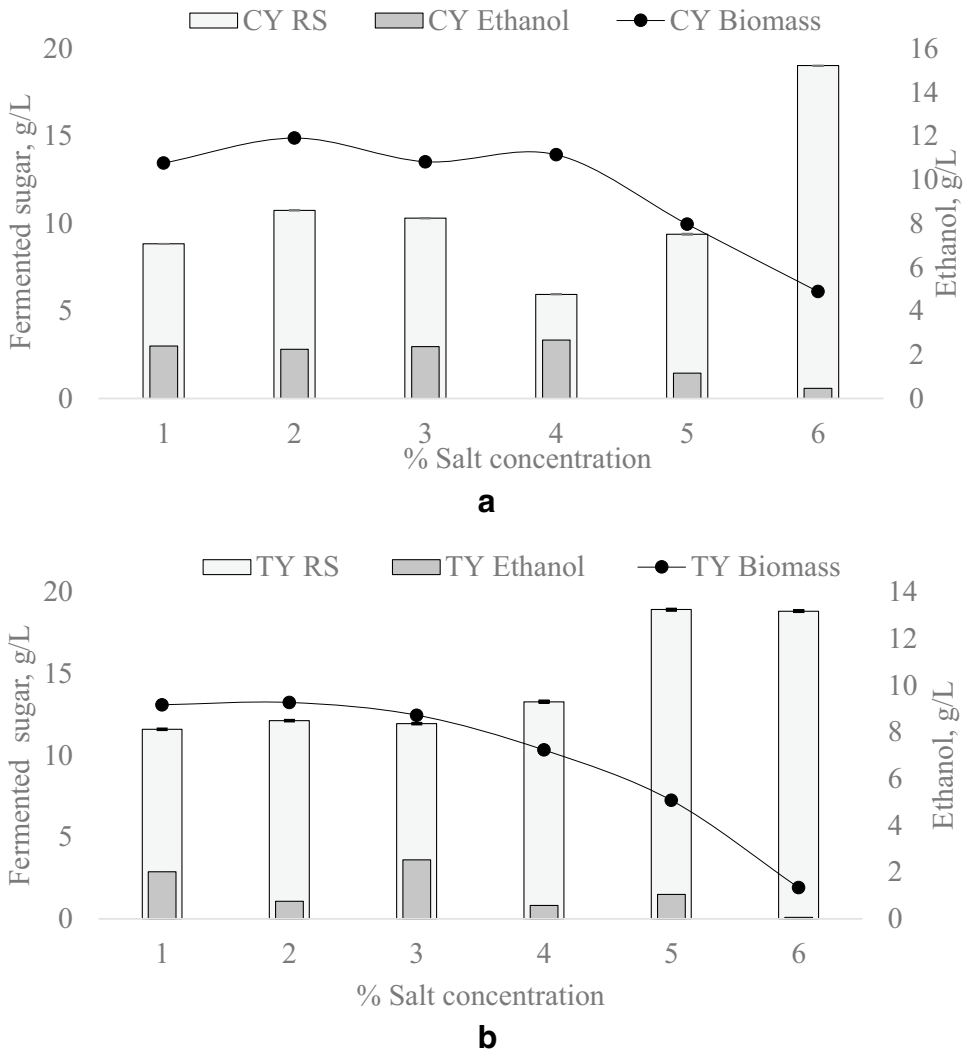


**Fig. 3** **a** Effect of different concentrations of salt on CY strain growth and ethanol production (RS, reducing sugar). **b** Effect of different concentrations of salt on TY strain growth and ethanol production (RS, reducing sugar)

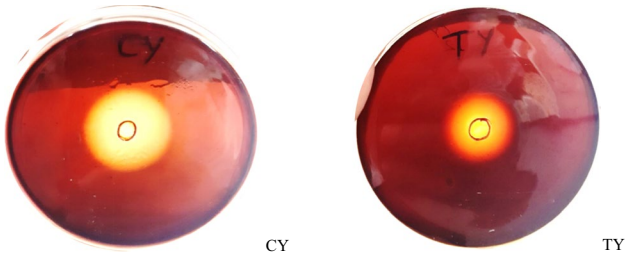


**Fig. 4** Hydrolytic activity observed for prioritized yeast strain CY: *Meyerozyma caribbica* and TY: *Pichia kudriavzevii* on CMC medium

In CBP, cellulase production, cellulose hydrolysis, and fermentation of subsequent sugar occur in a single reactor by a single microbial community [74] compared to SSF. The advantage of CBP is lower or zero capital costs for enzyme production and compatibility of enzymatic and fermentation processes. Anaerobic bacteria have been tested for ethanol production via CBP. However, lower ethanol tolerance (< 2%) of bacteria is a limitation for its application at an industrial scale. In this study, wild ethanologenic yeast strains *M. caribbica* and *P. kudriavzevii* were used to ferment the pretreated macroalgal biomass *E. intestinalis* and *U. lactuca* (Table 7). Higher ethanol conversion efficiency was recorded for *P. kudriavzevii* fermenting *E. intestinalis* and *U. lactuca* compared to the conversion efficiency achieved through SSF process. *K. marxianus* PT-1 isolated from grape fermented Jerusalem artichoke tuber flour consisting of inulin at 40 °C for 48 h and achieved 90% conversion efficiency through CBP [39]. CBP was carried out for brown algae *Saccharina japonica* using engineered *E. coli* (BAL1611) bacteria for 150 h and obtained 4.7% ethanol [75].

SEM analysis was carried out for macroalgal biomass after CBP process; the initial dilute-acid pretreatment provided surface area for the yeast cells to attach and secret enzymes to degrade the biomass. Biomass was disintegrated after CBP process indicating the cellulolytic yeast activity (Fig. 5).

CBP-compatible microorganism extensively studied is *S. cerevisiae*; however, it is not suitable while employing second-generation feedstock as it only yields higher ethanol from hexose and not from pentose sugar [72]. Therefore, there is a need to explore wilder ethanologenic yeast strains that exhibit higher cellulolytic activity and be employed for

**Table 6** Enzyme activity of yeast strains at 24 h

| Yeast Strain | Protein (mg) | Total activity (U/mL) | Specific activity (U/mg) |
|---|---|---|---|
| *Meyerozyma caribbica*, CY | 5.23 | 1.15 | 0.22 |
| *Pichia kudriavzevii*, TY | 5.73 | 1.19 | 0.21 |

**Table 7** Fermentation of pretreated macroalgal biomass using cellulolytic yeast strain through CBP

| Seaweed | Acid pretreatment | Fermentation | | Initial sugar, g | Fermented sugar, g | Ethanol, g | Theoretical yield | % conversion efficiency |
|---|---|---|---|---|---|---|---|---|
| *E. intestinalis* | 0.7 N $H_2SO_4$, 121 °C for 45 min | CY (35 °C, 100 rpm for 72 h) | 1.07 ± 0.09 | | 0.56 ± 0.02 | 0.12 | 0.29 | 43.06 |
| | | TY (35 °C, 100 rpm for 72 h) | | | 0.19 ± 0.01 | 0.07 | 0.10 | 74.14 |
| *U. lactuca* | 0.5 N $H_2SO_4$, 121 °C for 45 min | CY (35 °C, 100 rpm for 72 h) | 1.51 ± 0.09 | | 0.95 ± 0.02 | 0.32 | 0.49 | 64.22 |
| | | TY (35 °C, 100 rpm for 72 h) | | | 0.94 ± 0.01 | 0.46 | 0.49 | 94.84 |

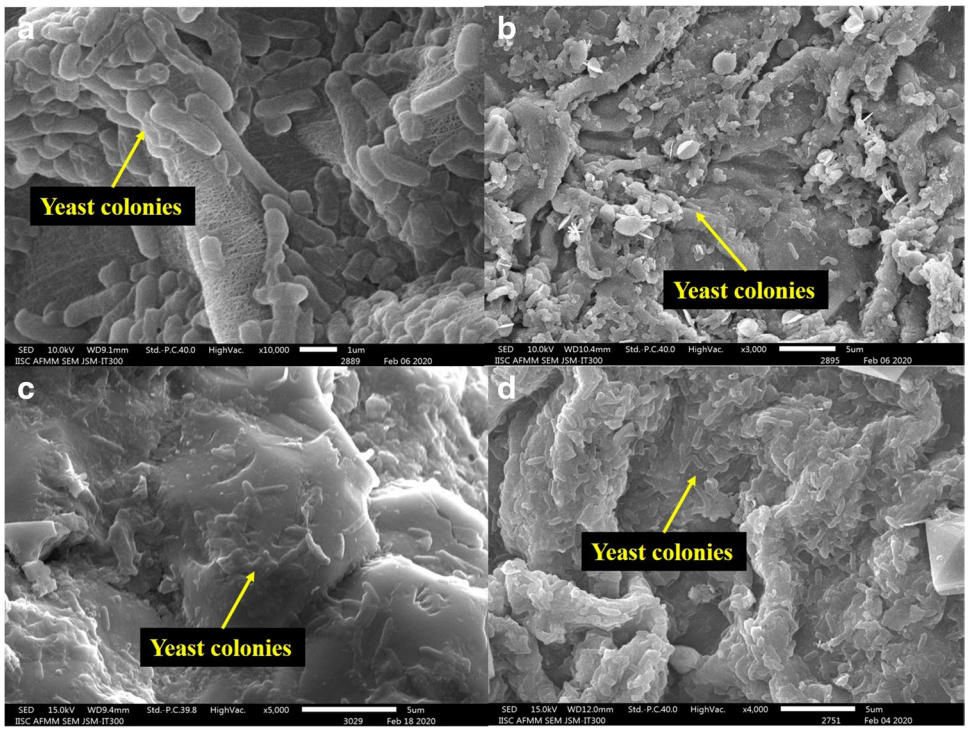


**Fig. 5** SEM micrographs of the interaction between cellulolytic yeast strains *Meyerozyma caribbica* on **a** *E. intestinalis* and **b** *U. lactuca*, and *Pichia kudriavzevii* on **c** *E.intestinalis* and **d** *U. lactuca*

CBP of third-generation macroalgal feedstock and second-generation feedstock.

## Conclusion

The study isolated yeast strain from 100 samples (98 fruit sources and 2 fermented products), and 19 strains were prioritized based on the growth in glucose and xylose media, with the carbohydrate fermentation capabilities. Yeast strains CY, TY, CHY, and MY exhibited longer exponential phase during growth kinetics. Around 21% of yeast strains exhibited good ethanol tolerance of up to 10% NaCl concentration. Good growth was observed at 35 °C for all the yeast strains. Synthetic sugar fermentation produced higher ethanol conversion efficiency for the two strains CY (83.5%) and TY (94.7%). Xylose fermentation by GVY produced 28.56% conversion efficiency. Yeast strains CY and TY were prioritized based on higher ethanol conversion and ethanol and temperature tolerance and identified as *Meyerozyma caribbica* (cashew yeast: CY) and *Pichia kudriavzevii* (toddy yeast: TY). Thermotolerant yeast *Pichia kudriavzevii* (TY) could withstand temperature of up to 45 °C and *Meyerozyma caribbica* (CY) could tolerate salt concentration up to 4% and produce highest ethanol of 2.6 g/L achieving 88.8% fermentation efficiency, providing basis for new insights on tolerance levels for different stressors by these wild yeast strains. Glucose fermentation by CY and TY strains produced 3.45 g/L and 13.6 g/L ethanol with fermentation efficiencies of 24.69% and 60.62%, respectively. Xylose fermentation was achieved by CY strain producing 1.43 g/L of ethanol with 10.45% fermentation efficiency exhibiting xylose-fermenting capabilities, whereas TY strain was seen efficient in fermenting the macroalgal sugars. The cellulolytic nature of the prioritized yeast strain revealed its activity through consolidated bioprocess.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12155-021-10283-3.

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