

Indigenous *Saccharomyces cerevisiae* Strains from Coconut Inflorescence Sap: Characterization and Use in Coconut Wine Fermentation

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ABSTRACT

This study isolated Saccharomyces cerevisiae strains from coconut inflorescence sap and evaluated their suitability for coconut wine fermentation. Twenty-six S. cerevisiae strains were isolated from coconut inflorescence sap in Thailand. They were identified based on their morphological, physiological, and biochemical characteristics, as well as by sequence analysis of the D1/D2 region of the large-subunit ribosomal RNA gene. All 26 isolates grew at 37°C and 11 strains (42%) produced ethanol at more than 50 g/L from 180 g/L glucose containing medium at 30°C. Sensory evaluation using a nine-point hedonic scale of the coconut wines fermented by five selected yeast strains from 23°Brix coconut juice showed no significant differences ($p > 0.05$) in the color and clarity except for the wine fermented by strain NL010, whose flavor profile and overall acceptance were rated exceptionally high. Thus, coconut inflorescence sap is a potential source of autochthonous S. cerevisiae strains for coconut wine fermentation.

Keywords: *Saccharomyces cerevisiae*, Coconut inflorescence sap, Wine fermenting, Ethanol

INTRODUCTION

Coconut inflorescence sap is the exudate obtained from the unopened inflorescence of coconut palm (*Cocos nucifera*). It is a yellowish brown, clear liquid of pH 7.0 that contains 12-15% (w/w) sucrose; approximately 0.23% (w/w) protein; 0.02% (w/w) fat; trace amounts of glucose, fructose, maltose, and raffinose; and is rich in Na and K ions (Kalaiyarasi et al.,

2013). Coconut inflorescence sap is rapidly fermented by a heterogeneous population of microorganisms that originate from the environment of the coconut inflorescence. More than 50% of the microorganisms isolated from fermenting coconut inflorescence sap can produce $\geq 9\%$ (v/v) ethanol in batch cultivation using coconut inflorescence sap containing 15–18% (w/v) sucrose (Wijesinghe and Samarajeewa, 1988). Wellala et al. (2004) isolated 12 yeasts from naturally fermenting coconut inflorescence sap in Sri Lanka and reported that all isolates were identified as members of the *Saccharomyces* genus (species not determined). These *Saccharomyces* isolates could be categorized into three groups based on the shape and size of the single cells.

Saccharomyces cerevisiae has long been used to produce alcoholic beverages and as baker's yeast (Bekatorou et al., 2006). Today, *S. cerevisiae* strains are also used to produce single cell proteins (SCP), ethanol, acetic acid, biocontrol agents, and recombinant vaccines, among others (Ghosh, 2011). The use of indigenous wine yeasts selected from each wine production region is widespread (Lopes et al., 2007). These indigenous yeasts are presumed to be more competitive than commercial yeasts, because they are better adapted to the ecological and technological conditions of their own region. Therefore, they have the potential to dominate fermentation and become the most important biological agent in making wine (Senses-Ergul and Ozbas, 2016). The use of autochthonous yeast strains can contribute to promoting or retaining the natural *S. cerevisiae* biodiversity (Capece et al., 2016).

The objective of this research was to explore coconut inflorescence sap for indigenous *S. cerevisiae* strains that have the desired characteristics for coconut wine fermentation.

MATERIALS AND METHODS

Coconut inflorescence sap and yeast isolation

Fifty-six samples of coconut inflorescence sap, freshly harvested from coconut trees, were collected from Samut-Songkram province, Thailand and kept at 4 °C for no longer than 12 h before use. One mL of the coconut inflorescence sap was inoculated into 5 mL of medium containing 2% (w/v) glucose, 0.3% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (w/v) chloramphenicol, and 3% (v/v) ethanol at pH 5.6 per 16 × 150 mm test tube and incubated in an anaerobic pouch (Kenki, Mitubishi Gas Chemicals., Inc. Tokyo, Japan) at 30 °C for 5 d. To screen for strong fermenting *S. cerevisiae*, the obtained culture was purified by the streak plate method on 10% glucose agar medium (10% (w/v) glucose, 0.3% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (w/v) chloramphenicol, 2% (w/v) agar, pH 5.6) and incubated under anaerobic conditions, as above. The resultant single colony was transferred onto YM agar (1% (w/v) glucose, 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 0.3% (w/v) malt extract, 2% (w/v) agar, pH 5.6) and incubated at 30 °C under aerobic conditions for 2 d. The obtained culture was kept at 4 °C for further studies.

Identification of yeast isolates

Conventional identification. Isolated yeasts were identified based on their morphological, physiological, and biochemical characteristics according to the methods described in Kurtzman et al. (2011). Cultures grown on YM agar at 30 °C for seven days

were examined for ascospore production. Formation of pseudohyphae was determined by the Dalmau plate culture method at 25 °C for 7-14 days using cornmeal agar. Vegetative cell, ascospore, and colony morphologies of YM agar grown cultures were observed and compared to those of the *Saccharomyces cerevisiae* type strain. Carbon assimilation was determined using the API kit ID 32 C (Biomerieux, France). Two-day-old cells suspended in ultra-pure water at a final MacFarland concentration of 2 (250 µL) were inoculated into C-medium, transferred (135 µL) into well (included in the API kit), and incubated at 30 °C. The cell turbidity was monitored at 24, 48, and 72 h after incubation.

Carbon fermentation was performed using basal yeast fermentation broth (0.45% (w/v) yeast extract, 0.75% (w/v) peptone, 1% (w/v) bromothymol blue) containing 2% (w/v) of the specific carbon source (glucose, galactose, sucrose, maltose, lactose, raffinose, or trehalose). The ability to ferment the carbon source was examined anaerobically from gas formation in a Durham tube and color change of bromothymol blue (Wickerham, 1951).

Molecular identification. One loopful of yeast cells suspended in 200 µL lysis buffer (100 mM Tris-Cl (pH 8.0), 30 mM EDTA (pH 8.0), 0.5% (w/v) SDS) was boiled for 15 min in a boiling water bath, mixed with 200 µL of 2.5 M potassium acetate (pH 7.5), incubated for 1 h in ice-water, and then centrifuged (18,000xg, 4 °C, 5 min). The supernatant was extracted twice with 100 µL of chloroform; isoamyl alcohol (24:1) and DNA was precipitated by ethanol precipitation method. The DNA precipitate was dried at 37 °C, dissolved in sterile water (30 µL). The resultant DNA solution (10-20 µg /µL) was used as a DNA template for PCR amplification using the F63 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and LR3 (5'-GGTCCGTGTTTCAAGACG-3') primers and thermal cycling at 94 °C for 3 min followed by 35 cycles of 94 °C for 20 s, 52 °C for 30 s, and 72 °C for 30 s, and then a final 72 °C for 5 min. The PCR product (600 bp) was purified using a Gel/PCR DNA fragment extraction kit (PCR clean up: Genaid Biotech Co. Ltd., Taipei, Taiwan), and then direct DNA sequenced commercially at Macrogen Inc., Korea. Each obtained DNA sequence was compared pairwise by BLASTn search and aligned with sequences retrieved from GenBank using the CLUSTALX ver 1.8 multiple alignment program (Thompson et al., 1997). A phylogenetic tree of representative strains of the *S. cerevisiae* isolates was constructed by Kimura's model using the neighbor-joining (NJ) method (Saitou and Nei, 1987) with bootstrap random re-sampling from 1,000 replicates. Sequences determined in this study were deposited at DDBJ (DNA Data Bank of Japan), with their accession numbers shown in Figure 1.

Determination of ethanol production by *S. cerevisiae* isolates

A single colony was inoculated into 50 mL of fermentation medium (18% (w/v) glucose, 0.45% (w/v) yeast extract, 0.75% (w/v) peptone, pH 5.6) in a 250-mL Erlenmeyer flask and incubated at 30 °C and 200 rpm for 24 h. The obtained culture was then transferred at 1% (v/v) into 50 mL of the same medium, incubated under the same condition for 24 h, and used as the inoculum. The inoculum was inoculated at 10% (v/v) into the fermentation medium (42.5 mL) in a 50-mL Erlenmeyer flask and incubated at 30 °C under an oxygen-limited condition for 48 h. The oxygen-limited condition was performed by cultivating 47.5 mL of the inoculated medium in a 50-mL Erlenmeyer flask without shaking; the cotton plug of the culture flask was tightly wrapped with parafilm. After 48 h the culture was centrifuged at 4 °C and 16700xg for 5 min; the supernatant was analyzed for ethanol by gas chromatography (Hewlett-Packard,

HP5890 series, USA) using a Porapak QS (cabowax 20 M) column (2 m × 0.32 m) at an oven temperature of 175 °C and a flame ionization detector at 150 °C. Helium at a flow rate of 35 mL/min was used as the carrier gas (Jutakanoke et al., 2012). The *S. cerevisiae* TISTR 5596 strain was used as a reference ethanol producing (control) strain. Those *S. cerevisiae* strains that produced a high ethanol level relative to the control strain were selected for studying coconut wine production.

Coconut wine fermentation

High ethanol producing *S. cerevisiae* strains were grown on a YM agar slant at 30 °C for 30 h, eluted with 5 mL of coconut juice (8.0 °Brix, pH 5.6), and inoculated into 45 mL of coconut juice in a 250-mL Erlenmeyer flask and incubated at 30 °C and 200 rpm for 24 h. One mL of this culture was transferred into 50 mL of coconut fermentation medium (20% (w/v) coconut sugar in coconut juice, 23 °Brix, pH 5.6) and incubated at 30 °C under a static condition for 8 d; the *S. cerevisiae* cells were then removed by centrifugation (4 °C, 16,120x g, 5 min). The supernatant was filtered through 0.45 µm membrane, determined for total soluble solid (TSS) level (°Brix) by hand refractometer, total acidity (as tartaric acid) by the AOAC (2000) method, and ethanol concentration by ebulliometer (Per Vinum J. Salleron Dujardin, Paris). Coconut wine that contained more than 9% (v/v) alcohol was selected for sensory testing.

Sensory test

Coconut wine with an alcohol content of more than 9% (v/v) was presented to a trained panel of 15 randomly selected 20-25-year-old men and women who regularly drank wine. The panelists were asked to evaluate the coconut wine for color, clarity, odor, flavor, and overall acceptance using a nine-point hedonic scale, where 9 = extremely like, 8 = very much like, 7 = moderately like, 6 = slightly like, 5 = neither like nor dislike, 4 = slightly dislike, 3 = moderately dislike, 2 = very much dislike, and 1 = extremely dislike. The data were statistically analyzed by ANOVA and the significance of means using Duncan's multiple range test (DMRT) accepting significance at the $P < 0.05$ level.

RESULTS

Yeast isolation and identification

Among the yeasts isolated, 26 isolates showed circular colonies with a smooth margin and cream color of 1-3 mm diameter. Cells were spherical to ovoid in shape and reproduced by multilateral budding. The vegetative cell transformed into asci containing 1-4 spherical and smooth ascospores that did not liberate. All of them grew at 37 °C, but only one isolate, NL009, could grow at 40 °C. Isolate NL010 produced rudimentary pseudohyphae after incubation at 25 °C for 7 d (Figure 2). Analysis of their 26S rRNA (D1/D2) sequence revealed a 99.8-100% match to the nucleotide of *S. cerevisiae* NRRL Y-12632^{NT} (AY048154) (Table 1). Their morphological, physiological, and biochemical characteristics (Table 2) were consistent with *S. cerevisiae*; the NJ phylogenetic tree also placed all strains within this species (Figure 1).

Table 1. Isolate number, nearest relative, 26S rRNA gene (D1/D2 domain) sequence identity and GenBank/DDBJ accession number of the *S. cerevisiae* strains.

Isolate no.	Nearest relative	Identity (%)	^a Accession number
NT001	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC021478
NT016	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051017
NT017	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC016751
NT018	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051018
NT019	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC021480
NT021	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051019
NT024	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051020
NT025	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051021
NC003	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC021481
NC004	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC050997
NC007	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC050999
NC008	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC021469
NC010	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051001
NC012	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051002
NC013	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051003
NC016	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051006
NC020	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC021474
NC023	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC002242
NC025	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051008
NC026	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC051009
NL009	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC016755
NL010	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC016750
NL015	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC021475
NL018	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC021476
NL019	<i>S. cerevisiae</i> NRRL Y-12632 ^T	99.8	LC021471
NL033	<i>S. cerevisiae</i> NRRL Y-12632 ^T	100	LC016752

Note: ^aAccession number: LSU rRNA (D1/D2) sequences determined in this study and deposited at the DDBJ (DNA Data Bank of Japan) gene databank in Japan.

Table 2. Morphological, physiological, and biochemical characteristics of the four *S. cerevisiae* groups.

Characteristic	<i>S. cerevisiae</i> group			
	A (9 strains)	B (4 strains)	C (12 strains)	D (1 strain)
No. of strains	A (9 strains)	B (4 strains)	C (12 strains)	D (1 strain)
Cell shape	Oval	Oval	Oval	Oval
Budding	Multilateral	Multilateral	Multilateral	Multilateral
Ascospore	SS	SS	SS	SS
Pseudohyphae	-	-	-	Rudimentary
Growth at 37 °C	+	+	+	+
Growth at 40 °C	-(+1)	-	-	-

Table 2. Continued.

Characteristic	<i>S. cerevisiae</i> group			
Assimilation of:				
N-acetyl-glucosamine	-	-	-	-
L-Arabinose	-	-	-	-
D-Cellobiose	-	-	-	-
Cyclohexamide (actidizone)	-	-	-	-
Esculin	+	-	-	-
Erythritol	-	-	-	-
D-Galactose	+	+	+	+
Glucosamine	-	-	-	-
D-Glucose	+	+	+	+
Glycerol	-	-	-	+
Inositol	-	-	-	-
Lactic acid	-	-	-	-
D-Lactose (bovine origin)	-	-	-	-
levulinic acid (LevulinaTe)	-	-	-	-
D-Maltose	+	+	+	+
D-Mannitol	-	-	-	-
D-Melezitose	-	-	-	-
D-Melibiose	-	-	-	-
Methyl- α D glucopyranoside	-	+	-	+
No substrate	-	-	-	-
PalatinosE	-	-	-	-
Potassium gluconate	-	-	-	-
Potassium 2 ketogluconate	-	-	-	-
D-Raffinose	+	+	+	+
L-Rhamnose	-	-	-	-
D-Ribose	-	-	-	-
D-Saccharose (sucrose)	+	+	+	+
Sodium glucuronate	-	-	-	-
L-Sorbose	-	-	-	+
D-Trehalose	+	+	+	+
D-Sorbitol	-	-	-	-
D-Xylose	-	-	-	-
Fermentation of:				
Galactose	-	-	-(+1)	-
Glucose	+	+	+	+
Lactose	-	-	-	-
Maltose	+	+	+	+
Raffinose	+	+	+	+
Sucrose	+	+	+	+
Trehalose	-	-	-	-

Note: A, strains NL009, NT001, NT016, NT019, NC010, NC012, NC023, NC025 and NC026; B, strains NL033, NT017, NC013 and NC020; C, strains NL015, NL018, NL019, NC003, NC004, NC007, NC008, NT018, NT021, NT024, NT025 and NC016; D, strain NL010. SS, spherical and smooth. Numbers in parentheses indicate the number of strains showing the reaction. Data represent the results from 3 independent assays.

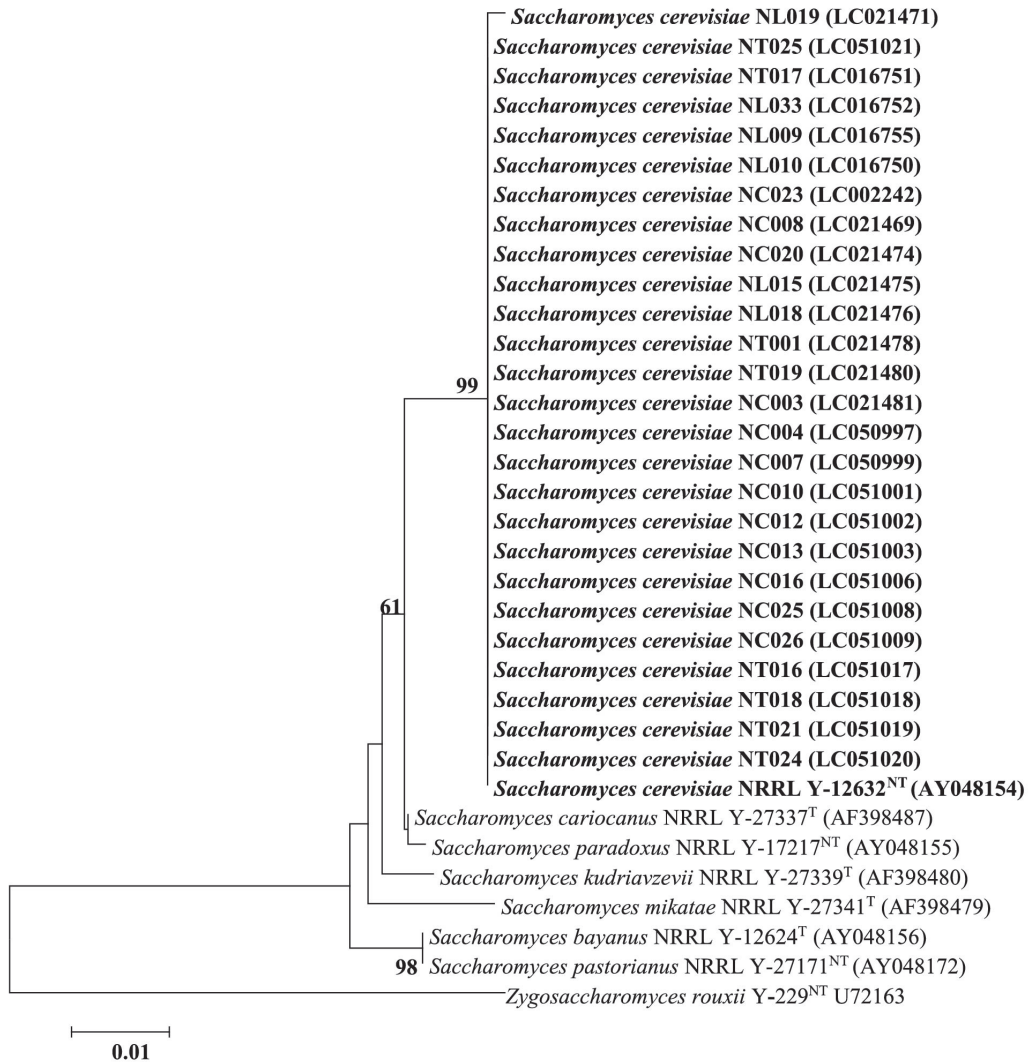


Figure 1. Phylogenetic tree constructed by the NJ method based on 600 bp of the 26S rRNA gene (D1/D2 domain) sequences.

Note: Bold type indicates the position of the reference *S. cerevisiae* strain (NRRL Y-12632^{NT}) and those strains of this study. GenBank/DDBJ accession codes are given in parenthesis after the species/strain designation. Numbers at nodes represent the percentages from 1,000 replicate bootstrap resamplings when over 50%. Scale bar represents the number of substitutions.

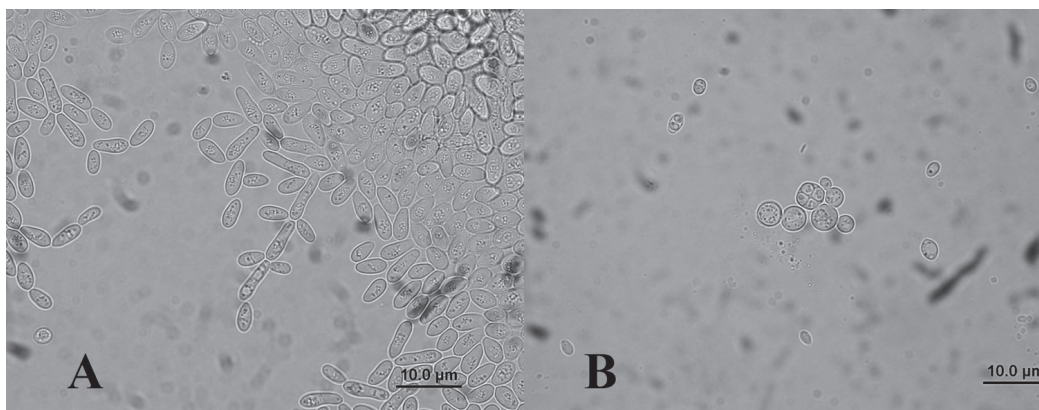


Figure 2. (A) Pseudohyphae cells and (B) ascospore of *S. cerevisiae* strain NL010 (100X magnification). Images shown are representative of those seen from at least 6-7 such fields of view.

Based on these results, they were identified as *S. cerevisiae* (Pretorius et al., 1999). The 26 strains of *S. cerevisiae* were divided into four groups (A–D) based upon their morphology, carbon assimilation, and fermentation differences (Table 2).

Group A contained nine strains (NL009, NT001, NT016, NT019, NC010, NC012, NC023, NC025, and NC026) that all assimilated glucose, galactose, sucrose, raffinose, maltose, trehalose, and esculin, and fermented glucose, sucrose, maltose, and raffinose. Only strain NL009 could grow at 40 °C (Table 2).

Group B contained four strains (NL033, NT017, NC013, and NC020) that were different from Group A in their ability to assimilate methyl- α D glucopyranoside, but not esculin (Table 2).

Group C contained 12 strains (NL015, NL018, NL019, NC003, NC004, NC007, NC008, NC016, NT018, NT021, NT024, and NT025) that differed from Groups A and B in their ability to assimilate esculin and methyl- α D glucopyranoside, respectively. However, one strain (NL019) could also ferment galactose (Table 2).

Group D contained one strain (NL010) that differed from Groups A-C in the presence of rudimentary pseudohyphae and its ability to assimilate glycerol and L-sorbose, and from Groups A and C by its ability to assimilate methyl- α D glucopyranoside (Table 2).

Ethanol production

From the four groups of *S. cerevisiae*, 11 representative strains – NL009, NL010, NL015, NL018, NL019, NL033, NT001, NT017, NT019, NC008, and NC023 – were selected and examined for their ethanol production in 18% (w/v) glucose containing medium at 30 °C for 48 h. All of the representative strains selected produced ethanol at more than 50 g/L with an ethanol productivity of more than 1.04 g/L/h (Figure 3). Strain NC008 produced the highest maximum ethanol yield at 67.22 g/L, which was higher than that produced by the reference *S. cerevisiae* TISTR 5596 strain (66.52 g/L).

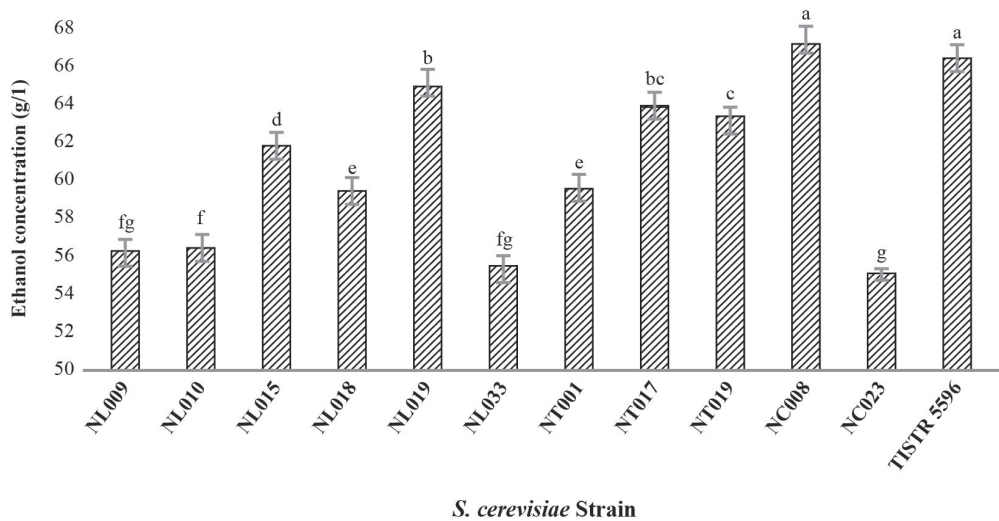


Figure 3. Ethanol production by *S. cerevisiae* strains at 30 °C.

Coconut wine fermentation and sensory test

After eight days of fermentation, the coconut wine fermented by strains NL009, NL010, NL033, NC023, and NT017 all contained more than 9% (v/v) alcohol with a pH in the range of 2.9-3.8. Strain NL010 gave the highest alcohol content wine at 10.3% (v/v), with the other three strains being slightly lower at 9.3-9.4% (v/v), and the lowest TSS content except for that produced by strain NL009 (Table 3).

Table 3. The total soluble solid, pH, total acidity, and ethanol concentration of coconut wine fermented by the selected five *S. cerevisiae* strains.

Strain	Characters			
	Total soluble solid	pH	Total acidity	Ethanol % (v/v)
NL009	12.17 ^b ± 0.76	3.70 ^a ± 0.20	0.46 ± 0.01	9.73 ^b ± 0.25
NL010	13.6 ^{ab} ± 0.46	3.83 ^a ± 0.06	0.45 ± 0.01	10.3 ^a ± 0.2
NL033	15.17 ^a ± 0.93	2.97 ^b ± 0.06	0.44 ± 0.02	9.37 ^b ± 0.15
NC023	14.0 ^a ± 1.00	3.70 ^a ± 0.10	0.43 ± 0.01	9.30 ^b ± 0.35
NT017	15.33 ^a ± 1.17	2.93 ^b ± 0.06	0.45 ± 0.01	9.33 ^b ± 0.34

Note: Data are shown as the mean ± SD, derived from three independent replicates. Means within the same column followed by a different letter are significantly different (*P* < 0.05; DMRT).

Fifteen panelists evaluated these coconut wines fermented by the five selected *S. cerevisiae* strains (NL009, NL010, NL033, NC023, and NT017) for their sensory qualities. The result indicated that the wines did not markedly differ, although the wine fermented by strain NL010 had the highest overall acceptance and best flavor, while that fermented by strain NL009 had the best odor (Table 4).

Table 4. Sensory test of coconut wine fermented by the five selected *S. cerevisiae* strains.

Strains	Criteria				
	Color	Clarity	Odor	Flavor	Acceptance
NL009	7.38 ± 1.03	7.71 ± 1.01	7.82 ^a ± 0.86	7.33 ^b ± 1.09	7.17 ^{bc} ± 1.09
NL010	7.71 ± 0.84	7.53 ± 0.69	7.68 ^{ab} ± 0.97	7.93 ^a ± 1.07	7.97 ^a ± 1.25
NL033	7.69 ± 0.73	7.56 ± 0.76	7.40 ^{bc} ± 0.72	7.26 ^b ± 0.84	7.00 ^c ± 0.77
NC023	7.78 ± 0.93	7.69 ± 0.67	7.22 ^c ± 0.90	7.40 ^b ± 0.78	7.44 ^b ± 0.94
NT017	7.76 ± 0.91	7.78 ± 0.67	7.71 ^{ab} ± 0.87	7.04 ^b ± 0.71	6.77 ^c ± 0.76

Note: Data are shown as the mean ± SD, derived from three independent replicates. Means within the same column followed by a different letter are significantly different ($P < 0.05$; DMRT).

DISCUSSION

From the 26 strains of *S. cerevisiae* isolated from coconut inflorescence sap, only one strain (NL009) could grow at 40 °C. This result agreed well with Cassey and Ingledew (1996) in that yeast cell viability decreased with increasing temperature, and D'Amore et al. (1989) that growth and budding of yeast were fast at 30-37 °C, but growth decreased at higher temperatures. Fermenting yeast tolerant to high temperature has several advantages – it minimizes contamination risk, decreases the cost of cooling during fermentation, and reduces the energy required for proper agitation rate due to the decrease in fermentation broth viscosity at high temperature.

Carbon assimilation is an important criterion in the identification of yeasts that utilize organic carbon compounds as an energy source for growth. All the isolated *S. cerevisiae* strains were able to ferment glucose, sucrose, maltose, and raffinose; could not ferment lactose and trehalose; and had a variable response to galactose. This result agreed with Dash et al. (2015) and Jimoh et al. (2011, 2012) that most yeasts that fermented glucose, galactose, maltose, sucrose, and raffinose were *S. cerevisiae*.

The maximum alcohol concentration of the coconut wine fermented by the five strains of *S. cerevisiae* selected was 10.3% (v/v). This accorded with Nzabuhahaheza and Nyiramugwera (2014) who fermented must containing 18° Brix sugar that yielded a wine with 10% (v/v). The wine fermented by strain NL010 had the highest overall acceptance and best flavor according to the trained sensory panel.

Studies have shown that changes in the composition and sensory quality of coconut wine can indicate the variety of the *S. cerevisiae* strains. In particular, distinct strains of *S. cerevisiae* induced considerably different flavor characteristics when the same must is fermented (Mauriello et al., 2009). Currently, commercially available strains of *S. cerevisiae* are used to produce the majority of wine. However, many researchers and wine producers opt for the autochthonous strains of *S. cerevisiae* as the starter culture (Capece et al., 2014) because indigenous *S. cerevisiae* strains adapt better to the micro-area conditions in certain wine producing regions. Moreover, locally chosen yeast strains and strain-specific metabolic characteristics can be used together, beneficially affecting the quality of the wine produced (Capece et al., 2012).

CONCLUSION

An indigenous coconut inflorescence sap *S. cerevisiae*, strain NL010, was isolated that could produce a coconut wine of 10.3% (v/v) alcohol with a satisfactory overall acceptance and flavor profile, as determined by a sensory panel based on a nine-point hedonic scale.

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