Ethiop. J. Biol. Sci. **14(2): 171–184, 2015**
 ISSN: 1819-8678

ISOLATION AND CHARACTERIZATION OF SOME DOMINANT YEAST STRAINS FOR ETHANOL PRODUCTION FROM COFFEE (*COFFEA ARABICA* L.) WASTES

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ABSTRACT: The current study was initiated to isolate and characterize yeasts from wet Arabica coffee processing wastes for bioethanol production. Yeast isolates were collected from wet Arabica coffee processing effluent 1, effluent 2, effluent 3, pulp 1 and pulp 2. They were screened and characterized for ethanol production following their carbohydrate fermentation using standard methods. The selected ethanol producing isolates from pulp (ACP12) and effluent (ACE12) showed significantly high counts of $2.16 \pm 1.00 \times 10^8$ and $1.21 \pm 1.00 \times 10^8$ CFU/ml, respectively at 20% glucose concentration. The isolate ACP12 showed even higher population number ($9.7 \pm 1.00 \times 10^7$ CFU/ml) than the standard culture (*Saccharomyces cerevisiae*) with colony count of $8.7 \pm 1.00 \times 10^7$ CFU/ml at 30°C and at pH 5. Based on morphological, physiological and biochemical characteristics, the two isolates (ACE12 and ACP12) were tentatively identified to the genus *Saccharomyces*. Isolate ACP12 showed the maximum ethanol production (6.2 g/l) from pulp 1 with sugar concentrationon (90%) compared to the standard isolate (5.49 g/l) and the other test yeasts. From this study, it can be concluded that isolate ACP12 has the potential for ethanol production from coffee pulps compared to the other test yeast isolates and needs further supplementary activities to qualify it for industrial application.

Key words/phrases: Arabica coffee, Coffee wastewater, Ethanol production, Fermentation.

INTRODUCTION

In the 20th century, the world economy has been dominated by technologies that depend on energy obtained from fossil fuels (Sun and Cheng, 2002). The use of fossil fuels contributes to 73% of the CO_2 emission in the atmosphere that poses global warming (Demirbas *et al*., 2004; Wildenborg and Lokhorst, 2005). This necessitates the search for alternative and advanced technologies for energy supply from renewable sources that increase energy efficiency and reduce CO_2 emission (Oliveria *et al*., 2005; Demirbas, 2006).

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Ethanol production is an appropriate technology for the dual purpose of management of agro-industrial residues and generation of energy (Demirbas, 2006). It can be produced by fermentation of sugars from agricultural products or waste plant materials in the presence of saccharophilic yeast strains. The most commonly used ethanol producers are strains of yeasts such as *Saccharomyces cerevisiae*. Efficient ethanol production requires a rapid fermentation leading to high ethanol concentrations.

Bioethanol production from these wastes is one of the promising strategies to minimize energy problems besides reduction of environmental pollution. According to Sree *et al*. (2000), many countries produce ethanol from various agricultural residues and mix it with petroleum to reduce the cost and amount of petrol consumption. In Ethiopia, about 5.6 million litres of ethanol is annually produced, but there is an urgent need to maximize this yield in the years to come using different cheap and locally available agricultural wastes (i.e., coffee wastes).

These days there is a lot of interest in using wastewater from wet coffee processing firms for the production of useful commodities such as fertilizers and biofuels (Deepa *et al*., 2002). Therefore, this study was initiated to isolate, characterize some potential yeast strains for the production of ethanol from wet Arabica coffee processing effluents and pulps in order to utilize these agro-wastes for the production of bioethanol to meet the rising energy demand and to reduce environmental pollution.

MATERIALS AND METHODS

Sample collection

Wet Arabica coffee wastewater (three effluents) and two pulp samples were collected from Goma and Jimma weredas where wet coffee processing is common practice. The samples were collected using sterile plastic containers (10 litres capacity) placed in icebox taken to Addis Ababa University, Mycology Laboratory for analysis (Urbaneja *et al*., 1996).

Isolation of yeasts from Arabica coffee wastes

Yeasts were isolated from the samples on pre-solidified plates of yeast extract peptone glucose (YEPD) agar medium with 50 µg chloramphenicol/ml). Ten (10 ml) of Arabica coffee effluent 1, 2, and effluent 3 and 10 g of pulp 1 and pulp 2 were separately mixed with 90 ml sterile distilled water to prepare a ten-fold serial dilution. From appropriate dilution, a 0.1 ml aliquot was spread-plated on pre-solidified YEPD agar

medium. All the inoculated plates were incubated at 25–28°C for 2 to 3 days. The yeast isolates were purified and preserved on YEPDA slants at 4°C for further study.

Characterization of yeast isolates

Testing of isolates for carbohydrate fermentation

The isolates were tested for carbohydrate fermentation on the minimal medium containing (g/l) 4.5 yeast extract, 7.5 peptone, with respective carbohydrates in test tubes with Durham tubes. The carbohydrates used were: glucose (dextrose), galactose, maltose, sucrose, lactose, fructose, trehalose, raffinose, starch and cellulose. After preparation of the medium, one drop of 72 hrs old yeast culture grown in YEPD broth was added to each tube and incubated at 30°C for one week.

Tolerance of yeast isolates to some physicochemical factors

Two yeast isolates (ACP12 and ACE12) were selected for further characterization since they showed rapid fermentation on the tested carbohydrates.

Tolerance to glucose concentration

The ability of the two isolates to grow at different concentrations of glucose was undertaken according to Subashini *et al*. (2011). Glucose concentrations (10%, 20%, 30%, 40%, 50%, and 60%) were separately added to 100 ml YM broth containing (g/l) yeast extract, 3 g; peptone, 5 g; glucose, 10 g and 1000 ml distilled water. The broth was dispensed in test tubes and inoculated with 1 ml of 24 hrs old yeast culture. The test tubes were flasks incubated at 30°C for 7 days. After incubation, the yeast cells were counted by serial dilution and plating.

Tolerance to ethanol

A 1 ml of 24 hrs old culture grown in YEPD broth was inoculated to 100 ml yeast mannitol broth containing different concentrations of ethanol 4%, 8%, 12%, 16%, 20%, 24% (v/v) and incubated at 30°C for 7 days. Growth was estimated by plate counting according to Subashini *et al*. (2011).

Tolerance to temperature

One ml of 24 hrs old yeast culture grown in YEPD broth was inoculated aseptically into 100 ml yeast mannitol broth and incubated at 10, 20, 25, 30, 40, 50, and 60°C for 7 days. Growth was estimated by plate counting according to Subashini *et al*. (2011).

Tolerance to pH

In a 100 ml yeast mannitol broth, the pH was adjusted to 2.5, 3.5, 4.5, 5.5, and 6.5 using 1N HCl and NaOH. One ml of 24 hrs old yeast culture was inoculated into flasks and incubated at 30°C for 7 days. After incubation, the population was estimated by serial dilution and plating (Subashini *et al*., 2011).

Morphological characterization

Morph typing of the isolated yeast isolates was done following the methods of Barnett *et al*. (2000).

Total sugar determination in the coffee waste samples

The sugar content of the coffee effluents was calculated by Fehling method using the following formula (Periyasamy *et al*., 2009).

$$\text{Sugar content (\%)} = \frac{300\ \text{ml} * f * 100}{V}$$

Where: f = -Fehling factor (0.051); v = volume used in the titration (titrate value) (ml).

Fermentation of coffee wastes

The flasks containing the coffee effluent (750 ml) were diluted with 250 ml of distilled water (v/v). The flasks were covered, autoclaved for 15 minutes at 121°C and allowed to cool at room temperature. Fermentation was carried out in 1000 ml capacity Erlenmeyer flask with optimum inoculum 3 g/l of yeast isolates following standard method (Turhan *et al*., 2010). The flasks were incubated at 30°C and fermented for 72 hrs.

The powdered pulp (20 g) was hydrolyzed with 1000 ml of distilled water contained in a flask for 4 hrs. The flasks were covered, autoclaved and allowed to cool at room temperature. Fermentation was carried out in 1000 ml capacity Erlenmeyer flask with 3 g/l of yeast isolates and standard *S. cerevisiae* with incubation temperature of 30°C for 72 hrs (Franca *et al*., 2008; Thuesombat *et al*., 1990).

Determination of ethanol in the fermentation broth

After centrifugation at 10,000 rpm for 5 minutes, the supernatant was filtered. Ethanol concentration was measured using Ebulliometer at Balezaf Alcohol and Liquors Factory in Sabata town, Oromia Regional State, Ethiopia.

Cell biomass determination

After 72 hrs, the fermentation broth with coffee effluents and pulps in each flask was filtered and centrifuged at 10,000 rpm for 5 minutes. Each yeast biomass (pellet) was measured using Methler balance (Scaltec).

A standard yeast *S. cerevisiae* was obtained from Mycology Laboratory, Addis Ababa University and used as a positive control for some experiments.

Data analysis

Data were analyzed statistically on the basis of tolerance to different factors and/or concentration of substrates and ethanol yield using SPSS window version 22.0, SPSS Inc, Chicago, IL, USA. Analysis of variance (ANOVA) was used to indicate significant mean differences at 95% confidence limit.

RESULTS

Isolation of fermentative yeasts

A total of fifteen (15) yeast isolates were retrieved from five samples of Arabic coffee wastes and most of them showed smooth surfaces with circular margins, and creamy white texture. However, a few isolates showed slightly red and pinkish colonies (data not shown).

Screening for fermentative yeast isolates

The yeast isolates were capable of utilizing different carbon (Table 1). Almost all the isolates utilized glucose, galactose, fructose and maltose. The most versatile fermenters were ACE12 and ACP12 and were taken for further morphological and physiological characterization for ethanol production.

Table 1. Carbohydrate fermentation by yeast isolates.

Isolates	Fermentation											
	Glucose	Galactose	Fructose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	Starch	Cellulose	Xylose	Total carbohydrate fermented
ACE11	+++	++	++	+	+	+	+	+	−	−	−	8
ACE12	+++	++	+	++	+	+	++	+	−	+	+	10
ACE13	+	+	+	+	+	−	−	+	−	−	+	8
ACE21	++	++	+	−	+	+	+	+	−	−	−	7
ACE22	++	+	+	+	+	++	+	+	+	−	−	9
ACE23	+	+	+	+	+	++	−	+	+	−	−	8
ACE31	++	+	++	++	++	+	+	+	−	−	+	9
ACE32	++	++	+	−	+	+	−	+	+	−	+	8
ACE33	+++	++	++	++	++	+	−	+	−	−	−	7
ACP11	++	+	++	+	+	+	+	+	−	−	−	8
ACP12	+++	+	+	+	+	++	+	+	+	−	+	10
ACP13	+	+	+	+	+	−	+	−	−	+	−	7
ACP21	++	+	+	−	+	−	+	+	−	−	+	7
ACP22	+	+	++	−	+	+	+	−	−	−	−	6
ACP23	++	+	+	+	+	−	−	+	−	−	−	6
S.cerevisiae	++	+	+	++	+	−	+	+	−	−	−	7

+ = Fermentative, ++ = moderately fermentative, +++ = Highly fermentative (Durham tube empty), − = No carbohydrate utilization

Physiological characterization of the yeast isolates

Sugar tolerance

The growth of ACP12 and ACE12 gradually increased with concentrations of sugar with maximum population at 20% glucose concentration (Table 2). However, as the sugar concentration increased from 20% to 60%, the growth of both isolates and standard yeast *S. cerevisiae* decreased gradually. The yeasts isolated from pulps (ACP12) and effluents (ACE12) recorded maximum population count at 20% glucose concentration with the mean counts of $2.16 \pm 1.00 \times 10^8$ and $1.21 \pm 1.00 \times 10^8$ CFU/ml, respectively (Table 2). At glucose concentrations of 30–60%, isolate ACP12 showed higher counts compared to the other strain. There was significant difference ($p<0.05$) within the yeast isolates in terms of glucose concentrations tolerance.

Table 2. Growth of yeast isolates at different glucose concentrations.

Glucose (%)	Mean count (CFU x 10^8/ml) of yeast isolates		
	ACP12	**ACE12**	***S. cerevisiae***
10	1.00 ± 0.77^{g}	0.50 ± 0.48^{j}	1.00 ± 0.56^{i}
20	2.16 ± 1.00^{a}	1.21 ± 1.00^{c}	1.53 ± 1.00^{b}
30	1.12 ± 1.00^{d}	1.00 ± 0.91^{f}	1.06 ± 0.94^{e}
40	1.00 ± 0.65^{h}	1.00 ± 0.43^{k}	1.00 ± 0.48^{j}
50	1.52 ± 0.49^{j}	1.00 ± 0.23^{l}	1.00 ± 0.36^{l}
60	1.00 ± 0.37^{l}	1.00 ± 0.13^{m}	1.00 ± 0.21^{m}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p<0.05$)

Ethanol tolerance

Difference ($p<0.05$) in ethanol tolerance was observed among the yeast isolates and the standard *S. cerevisiae* (Table 3). The yeast isolate ACP12 showed the highest count ($9.6 \pm 1.00 \times 10^7$ CFU/ml) followed by isolate ACE12 with maximum mean count of $7.8 \pm 1.53 \times 10^7$ CFU/ml. ACP12 exhibited tolerance up to 16% ethanol with a mean count of $7.8 \pm 1.00 \times 10^7$ CFU/ml similar to that of the standard strain *S. cerevisiae* ($6.8 \pm 1.00 \times 10^7$ CFU/ml). As the concentration of ethanol increased from 4% to 24%, cell number drastically decreased.

Table 3. Tolerance of the yeast isolates to different ethanol concentrations.

Ethanol (%)	Mean count (CFU x 10^7/ml) of yeast isolates		
	ACP12	**ACE12**	***S. cerevisiae***
4	9.6 ± 1.00^{a}	7.7 ± 1.53^{de}	8.9 ± 1.00^{b}
8	8.7 ± 1.00^{bc}	6.4 ± 1.00^{gh}	7.7 ± 1.00^{de}
12	8.1 ± 1.00^{cd}	5.7 ± 1.53^{h}	7.2 ± 1.00^{ef}
16	7.8 ± 1.00^{de}	4.5 ± 2.00^{i}	6.8 ± 1.00^{fg}
20	2.4 ± 2.00^{j}	2.0 ± 1.9^{j}	4.0 ± 2.40^{j}
24	2.01 ± 0.20^{k}	1.0 ± 0.90^{k}	1.0 ± 1.00^{k}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p<0.05$)

Temperature tolerance

The yeast isolate ACP12 showed highest mean count ($9.7 \pm 1.00 \times 10^7$ CFU/ml) at 30°C followed by the standard culture *S. cerevisiae* and isolate ACE12 with maximum population of $8.7 \pm 1.00 \times 10^7$ CFU/ml and $6.8 \pm 1.54 \times 10^7$ CFU/ml), respectively (Table 4). Growth was declined as the temperature increased, except for isolate ACP12 that performed better at 40 and 50°C compared to the other isolates.

Table 4. Temperature tolerance by the yeast isolates.

Temperature (ºC)	Mean count (CFU x 10^7/ml) of yeast isolates		
	ACP12	**ACE12**	***S. cerevisiae***
15	2.2 ± 1.00^{k}	1.2 ± 1.0^{m}	1.7 ± 1.00^{l}
20	2.6 ± 1.00^{i}	1.5 ± 1.00^{m}	2.3 ± 1.00^{jk}
25	7.1 ± 0.58^{c}	5.6 ± 1.53^{fg}	5.9 ± 1.00^{f}
30	9.7 ± 1.00^{a}	6.7 ± 1.54^{d}	8.7 ± 1.00^{b}
40	6.3 ± 1.00^{e}	5.4 ± 1.00^{g}	5.5 ± 1.00^{g}
50	3.6 ± 1.53^{h}	2.1 ± 1.00^{k}	3.2 ± 1.53^{i}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p<0.05$)

pH tolerance

The isolates ACP12 and ACE12) gave the maximum mean counts of 9.8 ± 1.00 x 10^7 CFU/ml), 7.8 ± 1.00 x 10^7 CFU/ml) and the standard strain 8.7 ± 1.00 x 10^7 CFU/ml) at pH 5.0, respectively (Table 5). In all cases, there was a decline in yeast growth above pH 5.5.

Table 5. pH tolerance of the yeast isolates.

pH	Mean count (CFU x 10^7/ml) of yeast isolates		
	ACP12	**ACE12**	***S. cerevisiae***
2.5	1.4 ± 1.00^{j}	1.2 ± 1.00^{j}	1.2 ± 1.00^{j}
3.5	2.6 ± 1.00^{i}	1.5 ± 1.00^{j}	2.3 ± 1.00^{i}
4.5	4.8 ± 0.58^{f}	3.5 ± 1.00^{h}	4.3 ± 1.00^{g}
5	9.8 ± 1.00^{a}	7.8 ± 1.00^{c}	8.7 ± 1.00^{b}
5.5	9.5 ± 1.00^{a}	7.6 ± 2.65^{c}	8.7 ± 1.53^{b}
6.5	5.8 ± 0.58^{d}	4.5 ± 1.00^{fg}	5.3 ± 1.00^{e}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p<0.05$)

Identification of the yeast isolates

The growth of the selected yeast isolates was smooth and white cream colour on YPD agar (Table 6). Ascospores were formed in ascospore forming medium after incubating at 30°C for 3 weeks. All the dominant yeasts isolated from the Arabica coffee effluents and pulps did have round or oval shape or spherical or ellipsoidal (Table 6). Both test isolates and the standard strain (*S. cerevisiae*) reproduced asexually by budding (Fig. 1) and sexually by forming round ascospores in which their asci contained four ascospores (Table 6). The isolated yeasts also showed a filamentous growth when they were inoculated into corn meal agar, a nitrogen-deficient medium (data not shown).

Table 6. Morphological characteristics of the two selected yeast isolates.

Character	ACE12	ACP12	Standard *S. cerevisiae*
Surface	Smooth	Smooth	Smooth
Margin	Circular	Circular	Circular
Colour	Creamy, white	Creamy, white	Creamy, white
Cells	Ellipsoidal/oval	Ellipsoidal/oval	Spheroidal, ellipsoidal
	Single/Multilaterial budding	Single/Multilaterial budding	Single/Multilaterial budding
Ascospores	+	+	+
Filamentous	+	+	+

+ = Formation of ascospores and filamentous growth

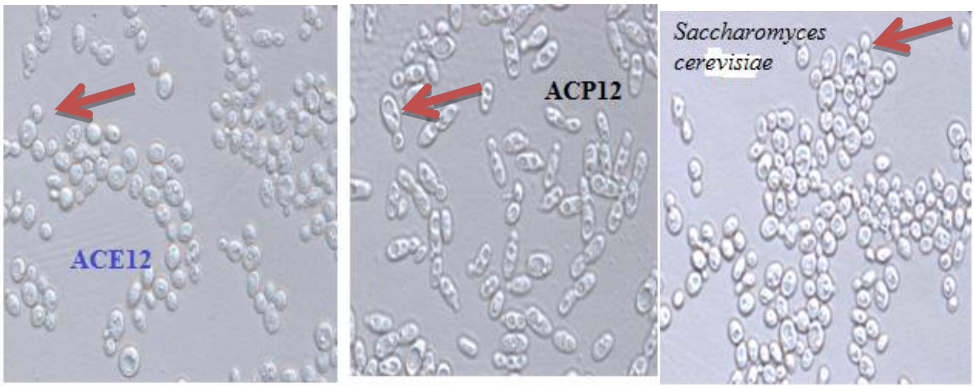

Fig. 1. Asexual reproduction (arrows) of yeast isolates and standard *S. cerevisiae*.

Determination of sugar content

The maximum reducing sugar concentration of 90% (Fig. 2) was produced from distilled water hydrolysate of coffee pulp 1 followed by pulp 2 (85%), effluent 1 (51%), effluent 2 (43.71%) and effluent 3 (40.26%).

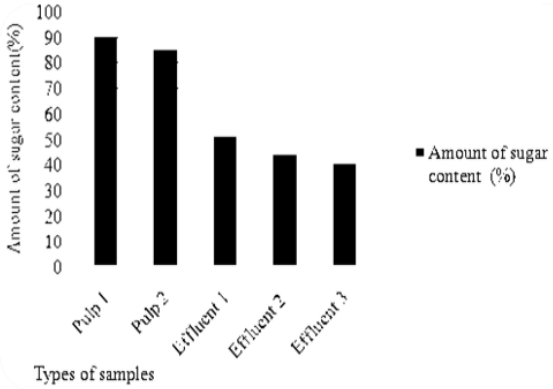

Fig. 2. Total sugar content of Arabica coffee wet processing wastes.

Fermentation and bioethanol concentration

The alcohol yields from different substrates by the yeast isolates (Table 7) ranged from 4–6.2% (g/l). The isolates showed different pattern of ethanol production of 4.5% (g/l) for standard *S. cerevisiae*, 6.20% (g/l) for isolate ACP12 and 5.01% (g/l) for isolate ACE12 from pulp 1 (Table 7).

Table 7. Comparison of ethanol production from Arabica coffee effluents, pulps and standard sucrose.

Types of substrates	Yeast isolates	Alcohol contents(g/l)
Standard sucrose	ACE12	4.0^{fg}
	ACP12	5.8^{ab}
	Saccharomyces cerevisiae	4.83^{c}
Pulp 1	ACE12	5.01^{bc}
	ACP12	6.20^{a}
	S. cerevisiae	5.49^{b}
Pulp 2	ACE12	4.14^{ef}
	ACP12	5^{cd}
	S. cerevisiae	4.34^{de}
	ACE12	2.1^{i}
Effluent 1	ACP12	2.5^{hg}
	S. cerevisiae	2.3^{i}
Effluent 2	ACE12	1.86^{j}
	ACP12	2.01^{i}
	S. cerevisiae	1.98^{j}
Effluent 3	ACE12	0.96^{j}
	ACP12	1.23^{j}
	S. cerevisiae	1.01^{j}

Means ± SD from two replications and mean values followed by different letters in the same column indicate significant differences ($p<0.05$)

Biomass yield at the end of fermentation

The maximum cell density was recorded for ACP12 and ACE12 compared to standard *S. cerevisiae* at initial sugar concentration of 20% standard sucrose, pulp 1 and pulp 2 as well as coffee effluents (Table 8). ACP12 showed the higher cell densities in all the substrates with the highest biomass from pulp 1.

Table 8. Biomass of isolates after fermentation.

Types of substrate	Isolates and standard isolates		
	Net weight (g/l)		
	ACP12	ACE12	Standard *S. cerevisiae*
Standard sucrose	2.4 ± 0.30^{a}	1.2 ± 1.00^{k}	2.3 ± 0.00^{c}
Pulp 1	2.4 ± 1.52^{a}	1.2 ± 0.60^{k}	2.3 ± 0.20^{b}
Pulp 2	2.2 ± 0.30^{d}	1.1 ± 0.80^{l}	2.2 ± 1.00^{ef}
Effluent 1	2.2 ± 0.40^{e}	1.0 ± 0.10^{m}	2.1 ± 0.51^{g}
Effluent 2	1.9 ± 0.80^{h}	1.0 ± 0.11^{n}	1.7 ± 0.60^{j}
Effluent 3	1.8 ± 0.01^{i}	0.5 ± 0.00^{mn}	1.6 ± 0.50^{j}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p<0.05$)

DISCUSSION

Some yeast isolates with notable fermentative potential were retrieved from samples of coffee wet processing wastes. All the test isolates were capable of utilizing 6–10 sugars indicating their potential in utilization of diverse sugars to produce more ethanol. Among the test isolates, ACP12 and ACE12 were capable of fermenting ten (10) sugars out of the eleven (11) sugars compared to the standard *S. cerevisiae*. However, the rate of fermentation varied among isolates because of their inherent difference in carbohydrate utilization that could be attributed to difference in strain types. Among the isolates, two of them (ACP12 and ACE12) were highly fermentative and were selected for ethanol production.

The test isolates showed remarkable tolerance to high (20%) glucose concentrations compared to standard yeast (*S. cerevisiae*) but their growth gradually decreased when the glucose concentrations increased. Similarly, Osho (2005) reported that wine yeast (*S. cerevisiae*) strains could tolerate a maximum (20%) sugar concentration. Very recently, Ali and Khan (2014) reported maximum ethanol production from 20% of glucose concentration within 72 hrs of incubation, but the yield abruptly decreased when the concentration of sugar increased. Bekatorou *et al*. (2006) showed that high substrate concentration would lead to catabolic repression by glucose and sucrose and may lead to several problems such as incomplete fermentation, development of off flavours and undesirable by products as well as decreased biomass and yeast vitality.

From this study, the isolate from coffee pulp (ACP12) recorded more tolerance (up to 16%; v/v) ethanol than isolate from coffee effluent (ACE12) and the standard strain *S. cerevisiae*. The data also showed as the concentration of ethanol increased from 4% (v/v) to 24% (v/v), the growth of isolates slightly, but not significantly decreased at higher concentrations. This is similar with the report of Subashini *et al*. (2011) that showed that *S. cerevisiae* was tolerant to ethanol concentration as high as (15%) concentration with cell count of 6.2 x 10^7 CFU/ml, but slightly lower than the reports of Casey and Ingledew (1986) and Teramoto *et al*. (2005), showing yeasts tolerating up to 16%–16.5% (v/v) ethanol.

Regarding the effect of temperature on the growth of the selected yeast strains, maximum yeasts count recorded at 30°C, but beyond this temperature the population number sharply decreased. Similar study was done by Subashini *et al*. (2011) who demonstrated yeast growth inhibition at higher temperatures. The environmental isolates and the standard strain

showed maximum population at pH 5 and pH 5.5, respectively. Similarly, Linden *et al*. (1992), have carried out fermentations, with *S. cerevisiae* at pH 4.5, 5.0, 5.5, 6, 7 and 8 and found that the optimal pH for ethanol production and maximum population of yeast cells were around 5 and 5.5.

Morphological observations and other studied parameters of the yeast isolates shared similarities with the descriptions given by Lodder (1971) and Boekhout and Kurtzman (1996). Accordingly, the coffee waste (pulps and effluents) isolates (ACP12 and ACE12) are tentatively assigned to a genus *Saccharomyces* type unicellular ascomycete. Furthermore, the features depicted by the isolates are consistent with the previous findings (Berhanu Abegaz Gashe *et al*., 1982; Samuel Sahle and Berhanu Abegaz Gashe, 1991; Tamene Milkessa, 2009) reported for yeast isolates recovered from cereals based products.

The maximum reducing sugar concentration of 90% was produced from distilled water hydrolysate of coffee pulp 1 compared to the other coffee wastes. The result showed that the amount of sugar obtained decreases along the sampling points due in part to the formation of organic acids by fermentation process (pulp 2) during storage and further dilution of sugars by water (coffee effluents).

The isolates showed different patterns of ethanol production from coffee wet processing wastes. Maximum amount of ethanol was produced by ACP12 isolate from pulp 1 substrate compared to the two isolates from the other samples. Similarly, Ayele Kefale (2011) confirmed the maximum bioethanol concentration of 7.4 g/l from Arabica coffee pulp, but the author noted that as hydrolysis time extended beyond 4 hrs, ethanol yield drastically decreased. Generally, the current findings indicate the potential of coffee wet processing wastes for ethanol production which has an added advantage in reduction of environmental pollution where Arabica coffee wet processing is taking place. Maximum ethanol concentration was also obtained by batch fermentation of acid hydrolysate of other substrates such as coffee husk (13.6 g/l) (Franca *et al*., 2008) and from wheat stillage hydrolysate (11 g/l; Davis *et al*., 2005) using *S. cerevisiae*.

The biomass accumulation of ACP12 was the highest compared to the other yeast isolates. This shows that biomass accumulation was directly proportional to the ethanol yield. Optimal conversion of carbohydrates to ethanol requires maximum number of cells that are tolerant to high concentration of both substrate and product to efficiently produce good amount of ethanol yield (Walker *et al*., 2006).

CONCLUSION

The two screened and further tested yeast isolates showed closest morphological similarities to genus *Saccharomyces*. All the isolates were capable of utilizing 6–10 carbohydrates. Yeast isolate from pulp (ACP12) showed remarkable tolerances to different levels of sugar, ethanol concentration, temperature and pH with production of high amount of ethanol yield from coffee wastes. This study demonstrates that Arabica coffee wet processing wastes have untapped potential and are a promising alternative feedstock for bioethanol production in Ethiopia which in turn contributes much to the proper management of environmental pollution.

ACKNOWLEDGEMENTS

The authors would like to thank Addis Ababa University for providing all kinds of support and laboratory facilities to carry out this research work.

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