Original research paper

Biolog Identification of Fermenting Yeasts from Fermented Teff (Eragrostis teff (Zucc.)) Dough

ABSTRACT

Background: Injera is one of baked product, which is commonly prepared from teff (Eragrostis teff (Zucc.)) flour. It is a staple food and believed to be consumed on daily basis by two-thirds of Ethiopians, a population of nearly 100 million. As it is a product of naturally fermented dough, the course of fermentation is done by consortia of microorganisms. Therefore, this study was aimed to isolate and identify yeasts from fermenting Teff (Eragrostis teff) dough.
Place and Duration of Study: Samples were collected from different sources in Addis Ababa, Ethiopia. Laboratory isolation and identification of isolates were carried out at Holeta Biotechnology Institute, Microbial Biotechnology laboratory and Ethiopian Institute of Biodiversity, Microbial laboratory from December to April, 2015 to June, 2016 G. C.
Methodology: A total of 200 dough samples were collected from households with different fermentation stage. Twenty (20) yeast isolates with different cultural characteristics were selected and further identified by ominilog identification systems.
Results: The seven yeast isolates obtained from teff dough were identified as *Pichia fermentans*, *Pichia* spp., *Rhodotorula aurantiaca* B, *Pichia fluxuum*, *Candida humilis*, *Trichosporon beigelii* B and, *Cryptococcus albidus Var aerus*
Conclusion: This study has confirmed the presence of different yeast in the fermenting teff dough and also supports the involvement of consortia of various groups of microorganisms in the course of the fermentation.

Keywords: Teff dough, *Injera*, Microbial Identification, ominilog

11.INTRODUCTION

Yeasts are unicellular, eukaryotic and polyphyletic organisms classified in the kingdom fungi. They are ubiquitous, and commonly found on fruits, vegetables, insect and other plant materials. Some yeast is found in association with soil and water. Approximately 100 genera comprising more than 1500 species of yeast have been described (Kurtzman and Fell, 2006). The significance of yeasts in food technology in a world of low agricultural production and rapidly increasing population makes the production of food grade yeasts extremely important (Bekatorou *et al*., 2006). In Ethiopia there are several fermented foods such as, kocho, bulla, tella, tej, milk product and injera, etc. A wide variety of fermented foods and beverages are consumed in Ethiopia being prepared from a wide range of raw materials using traditional techniques. These include: Injera, kocho, tella, awaze, borde and tejj (Askal and Kebede, 2013). Injera is one of the fermented foods that is made from different cereals, including sorghum, teff, corn, wheat, barley, or a combination of some of these cereals (Mogesse, 2006). Injera from teff (Eragrostis teff) is much more relished by most Ethiopians than that from any other source. It is a thin soft fermented baked food usually obtained after the flour of cereals has been subjected to 24 to 96h of traditional fermentation depending on the ambient temperature (Askal and Kebede, 2013; Bultosa *et al*., 2008)

The fermentation process uses natural inoculants from different sources in a mixed form

(Stewart and Getachew, 1962). Teff injera is getting popularity in the developed world because of its gluten free nature and being a whole grain product (Hiwot *et al*., 2013). Teff is a cereal crop which is mainly cultivated in Ethiopia for the purpose of making injera (Bultosa *et al*., 2008; Mogesse, 2006). For injera making, teff grain is considered by many as superior when compared to other cereal grains used in the country (Yetneberk *et al*., 2005).

A lot of research was undertaken on microbial profile of these commodities through conventional methods. In most cases, strain of Saccharomyces cervisiae, Rhodotorula spp, pichia, were found to dominate fermenter in tella, injera, milk product and other fermented foods. However, the yeast species involved in injera fermentation not studied well using standard Biolog MicroStation identification technology for shortening fermentation time and selecting the potential fermenter yeast in future. The aim of this study was isolation, identification and characterization of yeast species involved in injera fermentation by using biolog identification system.

2. MATERIALS AND METHODS

2.1. Sample collection and Description of the Study sites

A total of 200 teff dough samples (two hundred gram each) were collected from each of 14 sampling sites of fermenting dough samples with different times of fermentation (Brhanu, 1985) from around Addis Ababa, the capital of Ethiopia. The samples were transported aseptically to Holeta Biotechnology Institute, Microbial Biotechnology laboratory, for processing and microbial isolation. Sample processing and laboratory isolation of yeast were carried out in Holeta Biotechnology Institute, Microbial Biotechnology laboratory. Ominilog identification was carried out at microbial laboratory at Ethiopian Biodiversity Institute.

2.2. Isolation and selection of yeasts

After the samples were transported to the laboratory, 10 g dough sample was transferred aseptically into separate flask with 90 mL sterile 0.1% peptone water and homogenized. Thereafter, a tenfold serial dilution was made. From appropriate dilution factor, 1ml of the suspension was streaked onto pre-solidified yeast extract glucose Chloramphenicol Bromophenol blue agar medium. The plates were incubated at 25°C for 24h. Then after 24h incubation, the selected colonies (10-20) were sub-cultured on yeast extract glucose Chloramphenicol Bromophenol blue agar medium three times to purify the isolates. Yeast isolates were selected according to their cultural characteristics (colony size, colony color, colony texture) on yeast extract glucose Chloramphenicol Bromophenol blue agar based on Bergey"s Manual (Whitman, 2009). `

2.3. Biolog Omnilog identification and characterization of isolates

Yeasts were sub cultured to Biolog Universal Yeast (BUY) agar and Biolog Universal and were incubated at 26°C for 24 to 48 h. Yeast suspension were prepared in 9ml sterile distilled water and adjusted to 47% T using Biolog YT turbidity standard. One hundred micro liters of inoculums were added to each well of the YT Micro Plate (Biolog Inc) and incubated at 26°C (Kurtzman and Fell, 2006). A YT Micro Plate was read by the Biolog Micro Station Reader (BiologInc) at 24 h, 48 h, and 72 h at a single wavelength of 590 nm. The Biolog software micro log3 ver. 4.20.05 compared the results obtained with the test strain to the database and provided identification based on distance value of match and separation score

82 produces similarity index value and probability. An acceptable species identification must

83 have similarity index value>0.5 or probability >75% were Chosen only for species
84 identification and characterization (Biolog, 1993). Since, some yeast species are inhibited by
85 the tetrazolium violet redox dye used in Biolog Micro Plates, the YT Micro Plate was
86 configured with both oxidation tests and assimilation tests. The first 1-36 column panel
87 contained carbon source for oxidation tests using tetrazolium violet as a colorimetric
88 indicator of oxidation. Because, the last column panel of 49-60 wells contained 2 carbon
89 sources; these wells test was used for co-utilization of various carbon sources with D-xylose.
90 The read is going to be positive on the Micro Plate, if <X>, and when the database result for
91 that well was negative the printout showed <X- to indicate a mismatch where the database
92 reaction was negative. If there were a negative read, X with no brackets and database value
93 for that well were positive the well reads X+ indicating a positive reaction in the database. At
94 the time of a read the data were compared to the database in order to determine the ID
95 (Kurtzman and Fell, 2006).
96
97 2.4. Data Analysis
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99 Omnilog clustering of yeasts based on the carbon utilization was done using past software.
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1023. RESULTS AND DISCUSSION
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1043.1. Isolation of yeast and gas production from glucose
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106 The yeast isolates recovered were observed to have different features of colonial
107 morphology. The yeast isolates showed diverse cultural characteristics with regard to colour
108 (white, gray pigmentation or blue), shape (circular or irregular), edge (irregular or smooth)
109 and elevation (flat or raised). Furthermore, the sizes of the colonies ranged from medium to
110 large.
111 And a total of 20 yeast isolates were selected on the basis their potential in gas production
112 (Bakheit, 2008). Yeast isolates with good potential of gas production were believed to be
113 good fermenter and for the formation of many eyes on ready- to-eat *injera,* and these were
114 selected for molecular identification. The percentage occurrence on culture media recorded
115 as, 5% *Pichia fermentans*, 15% *Pichia spp., Rhodotorula aurantiaca* B, 15% *Pichia fluxuum*,
116 10% *Candida humilis,* and, 10% *Cryptococcus albidus Var aerus* other 35% were not
117 identified. Six yeast species were identified which is involved in dough fermentation by
Omni
118 Log identification system.
119
120 3.2. Biolog Omnilog identification and characterization of isolates
121
122 Selected yeasts isolates were aseptically transferred on BUY agar were used for Biolog
123 identification. Seven yeast species were identified which may involve in dough fermentation
124 by Omni Log identification system. Based on the result, Table 1 shows those species of
125 yeasts: *Pichia fermentans*, *Pichia spp., Rhodotorula aurantiaca* B, *Pichia fluxuum*, *Candida*
126 *humilis, Trichosporon beigelii* B and, *Cryptococcus albidus Var aerus* with their identification
127 statuses in the YT Biolog Micro Plate.
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134 Table 1. Biolog Micro station identification result.
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Species	Probability	Similarity	Distance	Remark
Cryptococcus albidus var. aerus	100%	0.73	5.98	Hid
Pichia fermentans	100%	0.653	5.33	Hid
Rhodotorula acheniorum	100%	0.623	5.78	Hid
Trichosporon beigelii B	100%	0.615	5.91	Hid
Pichia spp	99%	0.693	4.62	Hid
Pichia fluxuum	98%	0.668	4.87	Hid
Candida humilis	86%	0.553	5.47	Hid

136 Based on the result, Table 2 shows those species of yeasts: *Pichia fermentans*, *Pichia spp.,*
137 *Rhodotorula aurantiaca* B, *Pichia fluxuum*, *Candida humilis, Trichosporon beigelii* B and,
138 *Cryptococcus albidus Var aerus* which exhibited positive result for oxidation test and
139 assimilation potential of different carbon coated in the YT Biolog Micro Plate. Yeast Micro
140 Plate has two different reactions: assimilation of carbohydrates and oxidation. Six yeast
141 species which were identified by BioLog (*Pichia fermentans*, *Pichia spp., Rhodotorula*
142 *aurantiaca* B, *Pichia fluxuum*, *Candida humilis, Trichosporon beigelii* B and, *Cryptococcus*
143 *albidus Var aerus*) have positive oxidative test for Dextrin, stachyose, D-galactose, glucose,
144 L-aspartic acid, D-gluconic acid, D-melibiose, sucrose, turanose, D-mannitol, tween 80, D-
145 arabitol, D-meleitose, L-sorbose, Xylitol, Glycerol, Gentiobiose, Sucrose, Salicin, Maltotriose,
146 L-sorbose carbon sources.
147
148 **Table 2. Oxidation and assimilation result of yeast using YT Biolog MicroPlate**

Isolate code	Oxidation	Assimilations
AAUYT30B (*P. fermentans*)	Dextrin, stachyose, D galactose, glucose,	Fumaric acid, L-malic, methyl succinate, bromo succinic acid, L-glutamic acid, a-keto- glutaric acid, D- gluconic acid, Dextrin, Cellobiose, Gentiobiose, Maltose, Maltotriose, Palatinose, D- raffinose, Stachyose, Sucrose, D-trehalose, Turanose, glucose, D-galactose, L-rhamnose, D-glucoside, Amygdalin, Arbutin, Salicin, D-mannitol, D-sorbitol, adonitol, xylitol, glycerol, tween 80, D- ribose, 1,2-propanediol+ D-xylose a
AAUYT26C AAUYT32A AAUYT34A (*Pichia* spp.)	Dextrin, stachyose, D galactose, glucose,	Fumaric acid, L-malic, methyl succinate, bromo succinic acid, L-glutamic acid, a-keto- glutaric acid, D- gluconic acid, Dextrin, Inulin, Cellobiose, Gentiobiose, Maltose,

		Maltotriose, D-melibiose, Palatinose, D- raffinose, Stachyose, Sucrose, D trehalose, Turanose, D-glucosamine, a-D-glucose, D-galactose, L-rhamnose, L-sorbose, Amygdalin, Arbutin, Salicin, D-maltitol, D-mannitol, D-sorbitol, adonitol, xylitol, glycerol, tween 80, D- ribose, D xylose, D-galactose+ D-xylose, ,2-propanediol+ D xylose
AAUYT21B AAUYT27A AAUYT25A (*P. fluxuum*)	L-aspartic acid, D-gluconic acid, dextrin, D-melibiose, sucrose, turanose, D mannitol and tween 80	Gentiobiose, Maltotriose, Arbutin and D-mannitol, L-rhamnose, L-sorbose, Amygdalin, Salicin, D sorbitol, adonitol, xylitol, glycerol, tween 80, D ribose, D-xylose N-acetyl-L-glutamic acid, D-xylose, D-glucuronic acid+ D-xylose, dextrin, D-xylose, D melibiose+ D-xylose, D-galactose+ D-xylose, 1,2-propanediol+ D-xylose

Species	**Probability**	**Similarity**	**Distance**
Cryptococcus albidus var. aerus	100%	0.73	5.98
Pichia fermentans	100%	0.653	5.33
Rhodotorula acheniorum	100%	0.623	5.78
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Pichia fluxuum	98%	0.668	4.87
Candida humilis	86%	0.553	5.47

UNDER PEER REVIEW

AAUYT31A (C. albidus Var aerus)	Dextrin, D- arabitol	Fumaric acid, L-malic, methyl succinate, bromo succinic acid, Gentiobiose, Amygdalin, D-mannitol, D-sorbitol, Adonitol and D-arabitol
AAUYT23B (*Candida humilis*)	L-aspartic acid, D-gluconic acid, Dextrin, D-melibiose, L-sorbose, Xylitol, Glycerol and tween 80	D- gluconic acid, Cellobiose, Palatinose, D raffinose, Stachyose, D-trehalose, N-acetyl-D glucosamine, Arbutin, Salicin, D-xylose and a-D lactose+ D-xylose
AAUYT22A (*R. aurantiaca B*)	Gentiobiose, Sucrose, Salicin and tween 80	Fumaric acid, methyl succinate, 2-keto-D- gluconic acid, D- gluconic acid, Dextrin,

		Cellobiose, Gentiobiose, Maltose, Maltotriose, D-melibiose, D raffinose, Stachyose, Sucrose, D-trehalose, Turanose, N-acetyl-D-glucosamine, Arbutin and D xylose
AAUYT28A AAUYT24C AAUYT35A (Unidentified)	L-aspartic acid, D-gluconic acid, Dextrin, Maltotriose, L-sorbose, D- sorbitol, Glycerol and tween 80	L-rhamnose, L-sorbose, Amygdalin, Arbutin, Salicin, D-maltitol, D-mannitol, D-sorbitol, adonitol, xylitol, l-erythritol, glycerol, tween 80, D- ribose, D galactose+ D-xylose, 1,2-propanediol+ D-xylose

Based on Biolog reading result different yeast isolates were utilizing different carbon sources. But all isolates utilized dextrin in common and L-aspartic acid, D-gluconic acid and tween 80 were utilized more than seven isolates and the extant of utilizing were presented in Figure 1.

Figure 1. Number of yeast isolates utilizing the same carbon in the microplate.

Based on the carbon utilization and assimilation the yeast was clustered using past analysis software as indicated in Figure 2. The cluster shows six clusters which resembles similar carbon utilization and assimilation characteristics.

Figure 2. Clustering of yeast isolates by carbon utilization using past

The percentage occurrence on culture media recorded as, 5% Pichia fermentans, 15% Pichia spp., Rhodotorula aurantiaca B, 15% Pichia fluxuum, 10% Candida humilis, and, 10% Cryptococcus albidus Var aerus other 35% were not identified. Six yeast species were identified which is involved in dough fermentation by Omni Log identification system. Those selected isolates were identified using Biolog OmniLog identification system using Biolog OmniLog Micro plates. Six yeast species were identified by OmniLog identification system. From those identified yeast species by OmniLog 54% were similar in genus, 15% were similar at species level and 8% were different with yeast species which identified by molecular identification system and 23% were unidentified. Yeast Micro Plate has two different reactions: assimilation of carbohydrates and oxidation. Six yeast species which were identified by BioLog (*Pichia fermentans, Pichia spp., Rhodotorula aurantiaca B, Pichia fluxuum, Candida humilis, Trichosporon beigelii B* and*, Cryptococcus albidus Var aerus* have positive oxidative test for Dextrin, stachyose, D-galactose, glucose, L-aspartic acid, D-gluconic acid, D-melibiose, sucrose, turanose, D-mannitol, tween 80, D- arabitol, D-meleitose, L-sorbose, Xylitol, Glycerol, Gentiobiose, Sucrose, Salicin, Maltotriose, L-sorbose carbon sources.

4. CONCLUSION

The study indicated that the fermenting teff dough is composed of different yeast groups. The identification of these isolates could possibly contribute pieces of information needed for understanding and verification of microbial consortia involved in the course of injera dough fermentation. And those microorganisms were appeared from 48h to 72h but some were

appearing in early stage and others were processed up to the last stage (i.e. 72h).

COMPETING INTERESTS

I declare that this research and the information presented in are my own and has been generated by me as the result of my own original experimental research.

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