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Isolation, Identification and Characterization of Candida utilis from Some of the Sudanese traditionally Fermented Food Products

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ABSTRACT

The aims of this study were to isolate, identify and characterize the yeast Candida utilis (as a source of single cell protein SCP) from various local Sudanese fermented foods (Kissra, Hulu Mur and Marisa). Hulu Mur samples were found to contain the highest counts of yeast (6.89 cfu/g 6.78 cfu/g) while the low counts were found in Kissra samples (5.95 cfu/g - 5.84 cfu/g). Most of the C. utilis isolates had the same biochemical profiles with some slight variations. The study showed that C. utilis can utilize aerobically and anaerobically dextrose, sucrose, and raffinose and could assimilate maltose under aerobic conditions only. The isolates could not utilize lactose, glactose. cellubiose and arabinose under both aerobic and anaerobic conditions. It had an ability to assimilate nitrate and grew at high concentration of ethanol. The study showed that the biomass yield of C utilis was 2.5 g\l using batch fermentation. On the other hand the protein and moisture content of the product were 42% and 61%, therefore. It can be used in the production of single cell protein (SCP).

Key words: Yeast, Hulu Mur, Kissra, Marisa.

INTRODUCTION

Yeasts are unicellular fungi that can be classified into two phylogenetic groups i.e. teleomorphic and anamorphic ascomycetous or teleomorphic and anamorphic basidiomycetous yeasts that reproduce by budding or fission and that form their sexual states (i.e. asci), which are not enclosed in a fruiting body (Boekhout and Kurtzman, 1996; Kurtzman and Fell, 1998; Querol and Belloch, 2003).

Many workers reported on the presence of yeasts in the Sudan (Abdelgadir ei ale, 1976; Agab, 1983; Ahmed, 1994). The presence of yeasts in carbonated beverages in the Sudan was studied by Abdelgadir and Mustafa (1978), who identified five strains of Saccharomyces cerevisiae and grouped the rest of the isolates in the genera Kleveromyces and Candida.

Hamad (1986), isolated 200 pure yeast cultures from different food processing factories and surroundings. Ali (1997), isolated 74 pure yeast cultures, and identified five isolates to the level of genus and species. Mirghani (1999), isolated 19 yeasts from Sudanese sources, and examined these isolates as baker's yeast.

The possibility of growing Candida utilis as foodstuff on commercial scale was first recognized by German workers in Berlin in the Institute Fur Garungsgewerbe during World War I. Because C. ulilis is capable of utilizing pentoses, pulping-waste liquors from the paper industry have been used for about three decades as a commercial substrate for culturing this yeast (Kurtzman et ale, 1979).

The general acceptance of C. utilis by the food and the feed industries as a safe and nutritious form of single cell protein (SCP) had made it an important species for cultivation on other types of biological waste. Despite the importance of C. utilis, its taxonomic affinities have remained uncertain because of its failure to undergo sexual reproduction (Kurtzman ei ale, 1979).

The objectives of the present work include: isolation, identification and characterization of C. utilis from some Sudanese fermented foods namely, Hulu Mur, Kissra and Marisa.

MATERIALS AND METHODS

Collection of Samples

Samples of Hulu Mur, Kissra, and Marisa (Sudanese indigenous traditionally cereal-based fermented foods) were collected from local households at Wad Madani and Sinnar local markets (Central Sudan) in September 200T All samples were kept in sterile bottles and transported on the same day to the Department of Food Science and Technology, Faculty of Engineering and Technology, University of Gezira.

Methods

Total microbial viable counts

Nine milliliters of distilled water were piptted into tubes and labeled. Then one gram of the sample was diluted to 10 ml of distilled water to make a cell suspension. One ml of the suspension was removed and transferred to one of the tubes. The procedure was repeated serially so that each tube contained l/ 10th of the number of the cells.

One ml of each tube was transferred into petridishes containing nutrient agar medium and spreaded over the surface of the agar. Then incubated at 25 ^{0}c for 48 hours.

Yeast and mould total count

Serial dilutions from the samples were done, and one ml of each tube was transferred into a petri dish containing potato dextrose agar medium. The pH of the medium was adjusted to 4- 6 using 0. IN HCI. Then

the suspension was spread over the surface of the agar. Then incubated at 25 ^{o}C for 48 hours (Stivan, 1984).

Recovery of the Yeast from Samples

The yeasts were isolated by the direct-yeast extract-malt extract agar (YM agar). To suppress bacterial growth, 100mg Rose Bengal were added to one liter medium. The plates were incubated at 2800 for three days. Morphologically different colonies were examined microscopically Separate colonies were streaked on plates of the same medium (Stivan, 1984).

Purification of the yeast isolates

Different yeast colonies were purified by inoculating them onto solidified plates• of YM agar medium in a quadratic streaks manner, then they were incubated at room temperature for three days. Then the slants were stored in a refrigerator at 400 for further studies and were subcultured every three months on the same medium.

Identification of yeasts

Selected yeast isolates were identified according to the methods described by Kreger van-Rij (1984), Barnett et al., (1983) and Lodder (1970). These methods included: Microscopic appearance of non-filamentous vegetative cell, microscopic examination for pseudomycelium formation, utilization of carbohydrates aerobically and anaerobically, utilization of nitrogen compounds for aerobic growth, growth at high concentration of ethanol and growth in 10% NaCl plus 5% Glucose medium. Propagation of culture.

The yeast isolates were grown in wort broth. The medium was sterilized for 30 min at 121 OC. The pH adjusted at 4.5. Then then appropriate amount of inoculum was added and the fermentation began at 30^{0}C. After propagation, samples were transferred into dry centrifugation tubes and centrifuged for 10 min at 2000 rpm. The samples were then washed twice, each time suspended in deionized water and again centrifuged.

Determination of moisture and protein contents

The moisture and protein contents of the yeast isolate were determined according to AOAC (1990) method.

RESULT AND DESCUSSION

Screening for C. utilis

As indicated in Table (1), 60 pure cultures of yeast were isolated from various samples of Hulu Mur, Kissra and Marisa, the number of the cultures isolated from the sources were 26, 16 and 18, respectively.

Four isolates were identified as C. utilis according to conventional methods. Three of them from Hulu Mur and one from Marisa. This indicates the possibility of the presence of C. utilis in the Sudanese traditionally fermented foods. Paskevicius (2005) isolated C. utilis from cereal grains and fodder.

It is clear from Table (2) that the total viable counts of Kissra from Wad Madani and Sinnar were 7.77 cfu/g and 7.62 cfu/g, respectively. While the samples of Hulu Mur from Wad Madani and Sinnar were found to contain 8.01 cfu/g and 8 06 cfu/g, respectively. For the sample of Marisa from Wad Madani and Sinnar the total viable counts were 7.7 cfu/g and 7.73 cfu/g, respectively.

Table (2) also indicates that higher yeast count were recorded in Hulu Mur samples which ranged between 6.89 cfu/g and 6.78 cfu/g, followed by Marisa samples which contained 6.3 cfu/g and 6.04 in samples from Wad-Medani and Sinnar, respectively. On the other hand, the Kissra samples contained the lowest value of total yeast, and the yeast count of Medani and Sinnar samples were 5.95 cfu/g and 5.84 cfu/g, respectively.

Table (l): Number and the sources of culture isolates

Source of samples	No. of isolated cultures	No. of isolates as C. utilis
Hulu Mur	26	3
Kissra	16	-
Marisa	18	1
Total	60	4

Table (2): Total viable counts and the total yeast counts in Kissra, Hulu-mur and Marisa samples

sample	Madani		Sinnar	
	Nutrient agar	PDA	Nutrient agar	PDA
Kissra	7.77	5.95	7.62	5.84
Hulu Mur	8.01	6.89	8.06	6.78
Marisa	7.7	6.3	7.73	6.04

Morphological characteristics

Table (3) shows the morphological characteristics of yeast colonies and cells. All culture colonies were smooth and cream. The table also illustrates the vegetative method of multiplication, and the shape of all cells was oval. Also all the isolates showed pseudohyphae formation.

Table (3): Morphological characteristics of yeast colonies and cells

Yeast isolate	Colonies shape	Cells		
		Shape	Vegetative method of multiplication	Pseudohyphae
Hulu Mur 1	Smooth/cream	Oval to round	Budding	+
Hulu Mur 2	Smooth/cream	Oval	Budding	+
Hulu Mur 3	Smooth/cream	Oval	Budding	+
Marisa	Smooth/cream	Rod	Budding	+

Biochemical characteristics

Table (4) presents the biochemical characteristic of the yeast isolates. The table shows that the isolates assimilated aerobically and anaerobically glucose, sucrose, and raffinose and could assimilate maltose under aerobic conditions only. The isoltaes could not utilize lactose. glactose, cellubiose and arabinose under both aerobic and anaerobic conditions. This result is consistent with the findings of Stivan (1984) who investigated the yeast C. utilis and found that it can grow aerobically and anaerobically on many sugars as carbon and energy source.

From the result it was found that C. utilis grows on sucrose under aerobic and anaerobic conditions, so that molasses as substrate can therefore be used for SCP and ethanol production.

Assimilation of nitrate

The isolates showed growth on media contained nitrogen compound (Table 5). Two isolates showed weak growth.

The presence or absence of the ability to utilize nitrate is mainly used as a taxonomic criterion of the species level (Yarrow, 1998) so that the ability to utilize nitrate as sole source of nitrogen was an important criterion in defining C. ulilis, this can be confirmed by the same characteristics described by Stivan (1984).

Table (4): Biochemical characteristics of yeast isolates

Sugars	Code number of the isolates							
	Hulu Mur		Hulu Mur		Hulu Mur		Marisa	
	Fermentation	Growth	Fermentation	Growth	Fermentation	Growth	Fermentation	Growth
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-
Maltose	-	+	-	-	+	+	-	+
Galactose	-	-	-	-	-	-	-	-
Raffinose	+	+	weak	weak	weak	+	+	weak
Cellobiose	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-

Growth at high concentration of ethanol

The growth of the isolates tested in media containing absolute ethanol shown in Table (5). Two isolates grew well while two isolates showed weak growth. The result agreed with Stivan (1984) who investigated the ability of C. utilis to grow in media containing high concentration of ethanol and found that C. utilis grew well or weak. Also Sestakova (1976) tested the growth of C. utilis in presence of 21 different organic compounds, and he found that the highest yield of dry weight yeast was obtained with 72% ethanol.

This result give advantage for using ethanol as carbon source, and this was in agreement with Sestakova (1976) who investigated the growth of C. utilis on different carbon sources and found that the addition of ethanol resulted in an increased of the production and yield of the yeast dry weight but the cultivation time was prolonged.

Sodium chloride tolerance

The yeast isolates were tested for growth in liquid media containing 5% glucose and 10% sodium chloride. All isolates grew well in this media, this indicates that the yeast can be regarded as osmotolerant. Such yeasts are interesting for biotechnology and food microbiologist (Table 5).

Many Sudanese researchers isolated more than one type of yeast. this indicates that the osmotolerant yeasts are found in abundance in the Sudanese environment (Agab, 1983; Ahmed, 1994).

Table (5): Assimilation of nitrate, Growth at high conc. of ethanol and growth at 10% NaCl+ 5% glucose

Source	Assimilation of nitrate	Growth at absolute ethanol	Growth at 10% NaCl+ 5% glucose
Hulu Mur 1	+	W	+
Hulu Mur 2	W	+	+
Hulu Mur 3	W	W	+
Marisa	+	+	+

W= Weak reaction

Propagation of culture

C. utilis isolate was separated from the media by centrifugation (centrifuge model M13 IOJ, OSK). As presented in Table (6), C. utilis produced low yield of biomass (2.5 g/liter wet weight) this was due to the fermentation conditions which was applied under anaerobic conditions. When yeast are grown in shake flasks, they always produce ethanol, because the dissolved oxygen in the shake flasks was not enough to support aerobic growth. This result corresponds with that of Reed and Papler (1973) who found that, under anaerobic conditions, the yields of baker's yeast was low while in aerobic system a yield was high.

Paredes et al., (1976) studied the growth of C utilis in batch and continuous culture and found that the best yields occurred at the pH range of 3.5 to 4.5 and temperature of 30 ^{o}C in batch system.

Also Sestakova (1976) cultivated C. utilis under batch conditions with different carbon sources and found that the yield increased due to monosaccharides but the yeild with respect to total carbon sources was lower.

In this study the result showed high moisture content of 6100 and a protein content of 4200 on dry bases (Table 6). The protein content is the most important factor beside nucleic acid content influencing the nutrition

value of SCP. Therefore this result is better compared with the SCP of C. utilis cultivated at 30^0C and at dilution rate of 0.12 contained 39.8% protein (Hamad, 1986).

Kurbanoulu, (2000) investigated the growth of the yeast Candida utilis on horn hydrolysate for single-cell protein production and found that the biomass yield of C. utilis and its protein content were found to be 6.8 g /l and 49.8% respectively. Consequently, both the biomass yield of the microorganism and the protein ratio of the biomass (Table 6) were found to be near to the results of investigations in which some other yeasts such as C. pseudotropicalis, C. utilis, C krusei and C. Iropicalis (Michel et al., 1987) were grown on sweet whey and vinasse medium and C. utilis (Kurbanoulu, 2000) was grown on Ram horn hydrolysate, C. utilis (Nigam, 1998) grown on pineapple cannery effluent in batch culture, and we can suggest the growing of yeasts in continuous and semicontinuous processes obtain higher yields.

Table (6): Biomass yield of C. utilis isolate and its protein and moisture contents.

Sample	Biomass Yield g/l	Protein %	Moisture %
Hulumur	2.3	42	63
Marisa	2.5	42	61
Kissra	2.2	41	66

CONCLUSION

The objective of this study was to isolate and identify C utilis from various local Sudanese fermented foods (Kissra, Hulu Mur and Marisa), and characterization of the isolates.

The investigation revealed the presence of C. utilis in low percentage (9%) with respect to the total culture isolates. Also it revealed the presence of C. utilis in Hulu Mur and Marisa samples.

The study showed that C. ulilis can utilize some sugars aerobically and anaerobically and it had an ability to assimilate nitrate and grow in high concentration of ethanol. C. utilis had grown better in medium contained 10% sodium chloride plus 50 0 glucose.

From the results we can conclude that C. utilis is interesting for food microbiologists due to its characteristics and, it has a good potential to be used for single cell protein production.

It is highly recommended to use advanced techniques to identify C. utilis to the level of strain or even sub-strain, these technique include: Polymerase Chain Reaction (PCR), DNA analyser etc.. Protein quality assessment of C. utilis in any further studies must include its biological value and amino acids analysis.

REFERENCES

Abdelgadir, A.M.; khalid, A.S.; Mudathir, A. and Agab, M. (1976). Microbiological examinations of dehydrated foods. Sudan J. Fd. sci. Technol. 8: 50-54.

Abdelgadir, A.M. and Mustafa, M.M. (1978). Yeast causing spoilage in carbonated beverages in the Sudam Sudan J. Fd. Sci. Technol. 10: 55-64.

Agab, M.A. (1983). Isolation of Yeast Strains Suitable for Ethanol and Microbial Production from Sugar Cane Molasses. MSc. Thesis, University of Khartoum, Faculty of Agriculture, Sudan.

Ahmed, A.M (1994). Single Cell Protein from Yeasts Using Molasses. MSc. Thesis, University of Khartoum, Faculty of Agriculture, Sudan.

Ali, I.M.A. (1997). Screening for Thermotolerant Yeasts in The Sudan. MSc. Thesis, University of Khartoum, Faculty of Agriculture, Sudan

AOAC (1990). Official Methods of Analysis. Helrichk, ed 15th Association of Official Analytical Chemists, Inc. USA. 777 pp.

Barnett, J. A. , Payne, R. W. and Yarrow, D. (1983). Yeasts: Characteristics and Identification. Cambridge University Press, Cambridge.

Boekhout, T. and Kurtzman, C. P. (1996). Principles and Methods used in Yeast Classification: an overview of currently accepted genera. In: Wolf K. (ed), Non conventional yeasts in Biotechnology: A Handbook, Springer-Verlag, Berlin, Heidelberg, pp. I -80.

Hamad, S.H (1986). Screening of Yeasts Assosiated with Food from the Sudan and their Possible Application for Single Cell Protein and Ethanol Production. PhD. Thesis, Technischen Universitat, Berlin, Germany.

Kreger-van Rij, N. J. W (1984). General Classification. In: Kreger-van Rij N. J. W. (ed), The Yeasts, a Taxonomic Study, 3rd edn. Elsevier Science B V, Amsterdam, The Netherlands, pp. 15-16.

Kurbanoulu E B. (2000). Production of Single-Cell Protein from Ram Horn Hydrolysate. Atatork University, Science and Letters Faculty, Department of Biology, Turkey.

Kurtzman, C. P. and Fell, J. W. (1998). Definition, classification and nomenclature of the yeasts. In: Kurtzman C. P. & Fell J. W. (eds), The Yeasts, A Taxonomic Study, 4th edn. Elsevier Science B V, Amsterdam, The Netherlands, pp. 3-5.

Kurtzman, C. P. , Johnson, C. J. and Smiley, M. J. (1979). Determination of conspencifity of C. utilis and Hansenula jadinii through DNA Reassociation. Northern Regional Research Center, Peoria, Illinois 61604.

Lodder, J. (1970). General classification of the yeasts. In: Lodder J. (ed), The Yeasts, A Taxonomic Study, 2nd edn. North-Holland, Amsterdam, pp. 1-33.

Michel A, Simone P, Fran0 Ois J. and Joseph P, (1987). Biomass composition of a Candida pseudotropicalis new strain grown on ctude sweet whey. J. Sci. Food Agric., 39: 277-287.

Mirghani, S.S. (1999). Isolation and Stusy of Baker's Yeast from Sudanese Sources. MSc. Thesis, University of Khartoum, Faculty of Agriculture, Sudan

Nigam, LN. (1998). Single cell protein from pineapple cannery effluent. World J. of Microbiology & Biotechnology 14 (5).693-696.

Paredes, L. O, Camargo, R. E, and Orales, V. (1976). Influence of specific grwoth rate on biomass yeild production and composition of of C. utilis in batch and continuous culture. PMID 5055 [Pub Med index-ed for MEDLINE].

Paskevicius, A. (2005). Yeast distribution on various plant substrates and their biochemical peculiarties. Botanica, lithuanica. 11• 119-123.

Querol, A. and Belloch, C. (2003). Molecular evolution in yeast of biotechnological interest. Int. Microbiol. 6, 201-205.

Reed, G. and Papier, J,H. (1993). "Yeast Technology" AVI Pub co. West Port, Conn.

Seståková, M. (1976). Assimilation spectrum of the yeast Candida utilis used for producing fodder yeast from synthetic ethanol. Journal Folia Microbiol (Praha).

Stivan, K. (1984). Testing methods in food microbiology. Development in Food science 6 ed. Central Food Research Institute, Budapest, Hungary.

Yarrow, D. (1998), Methods for the isolation, maintenance, classification and identification of yeasts. In: Kurtzman C. P. and Fell J. W. (eds), The Yeasts, A Taxonomic Study, 4th ed. Elsevier.