Assiut Univ. J. of Botany
46(2), p-p23:43(2017)

GLYCEROL PRODUCTION BY OSMOPHILIC/OSMOTOLERANT MYCOBIOTA ASSOCIATED WITH MOLASSES

Abdel-Naser A. Zohri[*], Nemmat A. Hussein[*],

Mohamed Abdel-azim[**] and Shymaa F. Attia[**]

[*]*Botany and Microbiology Department, Faculty of Science, Assiut University, Egypt*
[**]*Sugar Technology Research Institute, Assiut University, Egypt*
nemmgoda@yahoo.com

Received: 8/5/2017 **Accepted:** 24/7/2017

Sugarcane molasses is a by-product of sugar production from sugarcane plants. The constituents of molasses may favor the presence of osmotolerants fungi. Twenty samples of cane molasses were collected from four sugar factories from different localities in Upper Egypt during August 2015 to February 2016. Twenty-two species of osmophilic and/or osmotolerant filamentous fungi belong to 8 genera were isolated on 30% sucrose Czapek's agar medium. The most common species were *Aspergillus fumigatus, A. flavus, Monascus ruber*. A total of 55 isolates belong to 22 species of the collected isolates were screened for their ability to produce extracellular glycerol using Czapek's medium supplemented with 10% NaCl and 3% sodium bisulfite (Cz10NaCl). All isolates could produce extracellular glycerol with variable degrees (0.03-0.55 g/L). The highest producers were *A. clavatus* STRI 1, *A. fumigatus* STRI 12, *A. terreus* STRI 21, *A. versicolor* STRI 23, STR1 25 and *Penicillium duclauxii* STRI 45. They produced extracellular glycerol ranging between 0.51- 0.55 g/L (= 5.54 – 5.98 ml moles). On the other hand, the mycelium of both *A. versicolor* STRI 25 and *A. clavatus* STRI 1, the highest extracellular glycerol producers (5.98 and 5.87 ml moles), released respectively 63.84 and 28.90 ml moles of intracellular glycerol, when transferred into sterilized distilled water for 24 hours.

***Keywords*:** Molasses, Fungi, 30% sucrose Czapek's agar medium, Glycerol.

INTRODUCTION

The most important crop from which sugar can be produced in commer cial quantity is sugarcane. Sugarcane molasses is a by-product generated from sugar industry. It has a viscous and dark liquids and it is a final effluent obtained during the preparation of sugar by repeated crystallization [1,2]. Chemical composition of sugarcane molasses is very varied; where sucrose (35-63%), reducing sugars (3-9%) and trace minerals are the principal components [3-6]. Other constituents such as

vitamins, non-nitrogeneous acids, pigments, waxes, sterols, lipids, few amino acids, and inorganic compounds are also present [5,7,8]. Sugar factory effluent produces obnoxious odour and unpleasant color when released into the environment. A large number of microbial diversity could be associated with sugarcane industrial molasses. Some potential fungal strains such as *Penicillium pinophilum, Alternaria gaisen, Aspergillus flavus, A. niger, Fusarium moniliforme* were isolated from sugarcane industrial effluent [9].

Molasses has been used in livestock and poultry feeds. Several studies were carried out using microorganisms in transformation and fermentation of molasses into ethanolic and organic acid products [10-15]. The availability and cost of sugarcane molasses make it an attractive feedstock for use in many countries [16].

Glycerol, is a simple alcohol, 1,2,3-propanetriol, has many uses. It is used in the cosmetics, paints, automotive, food, feeds, tobacco, pharmaceutic, pulp and paper, cellophane leather and textile industries [17,18]. Also, glycerol is used for chemical synthesis of poly trimethylene terephthalate, new polyester with novel fiber and textile applications. Glycerol can be produced either by microbial fermentation or by chemical synthesis from petrochemical feed stocks or can be recovered as a by-product of soap manufacture from fats [19]. In normal alcoholic fermentation, about 3% of the weight of sugar is converted into glycerin as a by-product. Alcoholic fermentation normally splits sugar into approximately equal parts of carbon dioxide and alcohol, with slight amounts of succinic acid and glycerin. Raw glycerol constitutes are a versatile carbon source with many possible applications in industrial fermentation [20]. Moreover, raw glycerol has been employed as a substrate for the production of other microbial metabolites, such as organic acids, polyols, microbial lipid and mass [21-25]. Commercial glycerol synthesis was primarily performed for propylene chlorine hydrolyzation in caustic environments [26]. Nowadays, the chemical synthesis of glycerol only account for about 10% of the current market because of the increasing cost of petrochemical precursors and decreasing price of pure glycerol [27]. Therefore, microbial species could convert glycerol to value-added products.

Glycerol formation rate can be increased in response to decreasing extracellular water activity. Under conditions of hyperosmotic stress, the channel is closed thereby conserving the glycerol within the cell in order to maintain an osmotic equilibrium with the external environment.

In contrast, in the absence of hyperosmotic stress, the channel remains open and glycerol freely permeates from the cell [28,29]. Increasing in sugar concentration generally results in greater synthesis of glycerol [30].

The current work was conducted to investigate filamentous fungi in molasses, as well as to estimate glycerol production by the isolated fungi.

MATERIALS AND METHODS

Collection of Samples

A total of 20 samples (500 g each) of sugarcane molasses were collected from four sugar factories in Upper Egypt (Nag Hammady, Dishna, Qus and Abo-Qurqas) during August 2015 to February 2016. Samples were brought to laboratory of sugar Technology Research Institute (STRI), and kept in a refrigerator (3-5^{o}C) till fungal analysis.

Isolation of osmophilic/osmotolerant fungi

Osmophilic and/or osmotolerant fungi were isolated from sugarcane molasses using dilution plate method as described by Johnson and Curl [31]. Ten ml of each sample were put in 100 ml conical flask containing 10 ml sterilized distilled water. The flask was shaken for 4 hours to get a homogenous suspension. One ml of the suspension was transferred to each agar plate using Menzies dipper [32]. About 15-20 ml of melted sterilized 30% sucrose Czapek′s (Cz30S) agar medium which contained sucrose, 300 g; $NaNO_3$, 3 g; KH_2PO_4, 1 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; agar, 15 g and 1000 ml distilled water, rose-bengal (1\15000) and chloramphenicol (250 mg/l) were added as bacteriostatic agents, were poured in each plate. Four replicates were prepared for each sample and the plates were stirred clockwise/anti-clockwise to dispense suspension in the medium. Plates were incubated at 28±2ºC for ten days. Pure fungal colonies were identified and transferred to Czapek′s agar slants, and kept for further studies.

Identification of fungi

The growing fungi were identified based on macro- and microscopic features following the keys and descriptions of Pitt [33], Raper and Fennell [34], Moubasher [35], Domsch *et al.*, [36] and Pitt and Hocking [37].

Screening of extracellular glycerol production by fungi

Preparation of inocula

Fifty-five isolates collected in the present investigation were screened for their glycerol production capability. Each inoculum was prepared by inoculating the fungal isolate in agar plate containing Czapek′s medium and incubated for seven days at 28±2^{o}C.

Cultivation

Extracellular glycerol production was assessed on fermentation Czapek′s medium which contained glucose, 10.0g; $NaNO_3$, 3.0g; KH_2PO_4, 1.0g; KCl, 0.5g; $MgSO_4.7H_2O$, 0.5g; $FeSO_4.7H_2O$, 0.1g and 1000 ml distilled water, (pH 6) [38]. This medium was supplemented with 15% NaCl (the optimum concentration for the best glycerol production). Inoculum from seven days- old fungal colony was inoculated in conical flask containing 50 ml of the medium. The flasks were incubated at 28±2ºC for 12 days under static condition in dark, then, the dry weight and extracellular glycerol were measured.

Effect of hypo-osmotic shock on extracellular glycerol accumulation

The aim of this experiment was to determine the effect of desalinization on extracellular glycerol accumulation by fungi. *A. versicolor* STRI 25 and *A. clavatus* TRSI 1 as the superior glycerol producers were grown for 12 days, on Czapek′s medium supplemented with 10% NaCl and 3% sodium sulfite (Cz10NaCl). At the end of incubation period, the fungal mycelia were transferred immediately into sterilized distilled water for 24 hours. Extracellular glycerol released in saline media as well as distilled water was measured.

Determination of mycelial dry weight

At the end of incubation time, fungal mycelia were filtered through pre-weighted whatman No.1 filter paper. The filter papers with the fungal mycelia were washed to remove medium residues and excess of NaCl residues, then dried in an electric oven at 60ºC up to constant weight. The biomass was then determined and the fungal growth was expressed as g dry weight/ 50 ml medium.

Estimation of glycerol

The glycerol produced in the filtrate was determined by the method of Chitlaru and Pick [39].

One ml of periodate reagent (65 mg $NaIO_4$ in 90 ml of water, 10 ml acetic acid, 7.7g ammonium acetate) was added to 200 µl of the fungal filtrate, then 2.5 ml of acetylacetone reagent (2.5 ml acetyl acetone and 247.5 ml isopropanol) were added and mixed. The mixture was incubated in water bath at 45ºC for 20 min. Optical density was measured at 410 nm by spectrophotometer and compared to calibration standard, which was prepared using different concentrations of glycerol (10-100 ppm).

RESULTS AND DISCUSSION

Isolation of osmophilic/osmotolerant fungi

Twenty-two species representing 8 genera were collected during the current study. These fungi were isolated from molasses samples collected from Nag Hammady (16 species and 7 genera), Dishna (11 and 3), Qus (6 and 4) and Abo-Qurqas (7 and 3) sugar factories (Table 1). *Aspergillus* (recorded in 95% of total samples) was found with high frequency, encountering 51.5% of total fungi. *Penicillium* was the runner-up, it was isolated from 80% of total samples, representing 39.8% of total fungi. *Monascus* (*M. ruber*) was detected moderately from 45% of total samples and 1% of total fungi. The remaining five genera namely *Byssochlamys* (*B. nivea*), *Eurotium*, *Paecilomyces* (*P. variotii*), *Rhizopus* (*R. stolonifer*) and *Scopulariopsis* were isolated in low or rare frequencies ranging between 5% - 15% of total samples and 0.053% - 6.78% of total fungi.

From 22 species, *A. fumigatus* (recorded in 50% of total samples and represented 7.06% of total fungi), *A.flavus* (45% and 42.1%), *P. corylophilum* (40% and 3.61%) and *P. restrictum* (35% and 1.44%) were the most common species. Molasses samples from Dishna sugar factory were highly contaminated by fungi (4660 CFUs/10 ml), although the maximum species diversity (16 species) were found in molasses of Nag Hammady sugar factory. *A. flavus* showed high frequency in samples of Qus and Abo-Qurqas factories (60% of total samples), but moderate (40%) and low (20%) frequencies in Dishna and Nag Hammady, respectively. In respect to *A. fumigatus*, it was highly isolated (60%) from all factories except Nag Hammady (low frequency). *A. niger* was detected only from samples of Nag Hammady and Dishna factories (40% of total samples). *A. terreus* was found in high frequency (80%) from Abo-Qurqas but not found in other factories.

Molasses may be suitable substrate for microorganisms due to high sugar and nitrogen contents [6].

In previous study, Seyis and Subasioglu [40] could cultivated *A. niger*, *Penicillium* and *Rhizopus* species on molasses. In the current study, *Byssochlamys nivea* was detected in high frequency (15% of total sample and 0.1% of total fungi) on molasses samples of Qus factory. This species was previously isolated from pineapple juice from Brasil [41]. The results obtained in the present study are consistent with Awasthi *et al.* [42], who reported that *Aspergillus* species were the most dominant species in sugar industry effluent and molasses in India. Also, El-Said [43] isolated *Aspergillus*, *Mucor* and *Penicillium* as the most common genera from molasses. Our results were in harmony with the findings of Abdel-Sater and Ismail [44], Abdel-Hafez *et al.,* [45] and Barakat *et al.,* [46].

In the current study, two species of *Eurotium* were recorded from Nag Hammady *(E. amstelodami*) or from Nag Hammady and Dishna sugar factories (*E. repens*) in low frequency of occurrence (20% of total samples each). In accordance, El-Said [43] recovered 3 species of *Eurotium* (*E. chevalieri, E. amstelodami* and *E. repens*) from molasses with *E. chevalieri* was the most common species. In this respect, *Eurotium* was also isolated with variable frequencies and populations from different substrates with high osmotic potential on sucrose Czapek′s agar medium [47-49]. During the study on microbiota of molasses in sugarcane industry of Madhya Pradesh, Pillai *et al.* [50] reported that *Aspergillus* was the most dominant genus representing with *A. niger, A. flavus* and *A. terreus*. In a study on fungi associated with 25 molasses samples collected from Aboqurqas, Nag Hammady, Dishna, Qus, and Kom Ombo sugar factories, Abdel- Sater *et al*. [51] isolated 12 species and 8 fungal genera on 25% sucrose Czapek′s agar medium. The present results are in agreement with the findings of Abdel-Sater *et al*. [51] in that *Aspergillus* was the most common fungus isolated from 64% of total molasses samples tested from which *A. flavus* and *A. niger* were the predominant species. In the present investigation, *A. fumigatus* was isolated from molasses of all sugar factories, while *A. parasiticus* was detected from molasses of Nag Hammady and Qus sugar factories. In this respect, Abdel-Sater *et al.,* [51] recorded the two mentioned species from Abo Qurqas molasses only. In contrast to our results *Alternaria, Fusarium,* and *Emericella* were collected from molasses samples [43,51].

Table 1. Total counts (TC calculated per 10 ml molasses) of fungal genera and species isolated from molasses from 4 factories (5 samples each) on Capek's 30% sucrose agar medium at 28±2°C.

Factories / Fungal Taxa	Nag Hammady factory			Dishna factory			Qus factory			Abo-Qurqas factory			**TC**	**TC %**	**F %**
	TC	TC%	F%	TC	TC%	F%	TC	TC%	F%	TC	TC %	F %			
Aspergillus	1135	81.4	100	1340	28.76	100	164	69.1	80	750	73.8	10	486	51.5	95
A. clavatus Desmazieres				20	0.43	20							20	0.21	5
A. flavus Link	1000	71.7	20	1245	26.72	40	109	45.94	60	641	63.1	60	397	42.1	45
A. fumigatus Fresenius	5	0.36	20	55	1.18	60	541	22.78	60	66	6.5	60	667	7.06	50
A. niger van Tieghem	10	0.72	40	10	0.21	40							20	0.21	20
A. ochraceus Wilhelm	5	0.36	20										5	0.05	5
A. parasiticus Speare	5	0.36	20				9	0.38	60				14	0.15	20
A. terreus Thom										34	3.35	80	34	0.36	20
A. versicolor (Vuillemin Tiraboschi	110	7.89	20	10	0.21	40				9	0.89	60	129	1.37	30
Byssochlamys nivea Westling							9	0.38	60				9	0.10	15
Eurotium	10	0.72	20	630	13.52	20							640	6.78	10
E. amestelodami Mangin	5	0.36	20										5	0.05	5
E. repens de Bary	5	0.36	20	630	13.52	20							635	6.72	10
Monascus ruber Van Tieghem	10	0.72	20				75	3.14	100	9	0.89	60	94	1.00	45
Paecilomyces variotii Bainier	10	0.72	20										10	0.11	5

Table1. Continued

Factories / Fungal Taxa	Nag Hammady factory			Dishna factory			Qus factory			Abo-Qurqas factory			**TC**	**TC%**	**F%**
	TC	TC%	F%	TC	TC%	F%	TC	TC%	F%	TC	TC%	F%			
Penicillium	165	11.83	60	2690	57.73	100	650	27.37	60	257	25.3	100	3762	39.8	80
P. chermesinum Biourge				1160	24.9	40							1160	12.3	10
P. citrinum Thom	30	2.15	40										30	0.32	10
P. corylophilum Dierckx	125	8.96	20	25	0.54	60				191	18.8	80	341	3.61	40
P. duclauxii Delacroix				5	0.12	20	650	27.37	60				655	6.93	20
P. funiculosum Thom				1435	30.8	20							1435	15.2	5
P. restrictum Gilman &Abbott	5	0.36	20	65	1.4	60				66	6.5	60	136	1.44	35
P. waksmanii Zaleski	5	0.36											5	0.053	5
Rhizopus stolonifer (Ehrenberg) Vuillemin	60	4.3	20										60	0.64	5
Scopulariopsis sp	5	0.36	20										5	0.053	5
Total count (TC)	1395			4660			2375			1016			9446	100	
No. of genera (8)	7			3			4			3					
No. of species (22)	16			11			6			7					

Glycerol production

Glycerol is a well-known metabolite formed by many microorganisms including filamentous fungi. Screening of 55 isolates of osmophilic and/or osmotolerant fungi related to 22 species and 8 genera collected in this study for extracellular glycerol production using Cz15NaCl medium was examined. Results in table (2) revealed that all isolates tested were able to release extracellular glycerol with various degrees. The highest producer isolates were *A. clavatus* STRI 1, *A. fumigatus* STRI 12, *A. terreus* STRI 21 *A. versicolor* STRI 23, STRI 25 and *P. duclauxii* STRI 45 produced extracellular glycerol levels ranging between 0.51-0.55 g/L (= 5.54 – 5.98 m moles). Glycerol has been previously reported to be accumulated in response to osmotic stress in filamentous fungi [52-57].

The mycelium of *A. versicolor* STRI 25 and *A. clavatus* STRI 1, which formed 0.55 g/L (5.98 m moles) and 0.54 (5.87 m moles) as extracellular glycerol in saline medium (Cz15NaCl), released 5.873 g/L (63.84 m moles) and 2.659 g/L (28.90 m moles) when transferred into sterilized distilled water for 24 hours from their intracellular glycerol, respectively (Table 3). These results are in harmony with those recorded by Zidan and Abdel-Mallek [54] and Saada [57]. Zidan and Abdel-Mallek [54] examined the accumulation of glycerol by three species of *Aspergillus* (*A. niger*, *A. ochraceus* and *A. tamarii*) in media with different concentration of NaCl (0.0 – 16%).They reported that both intracellular and extracellular glycerol increased with increasing NaCl levels and the major portion of glycerol was retained within the mycelium. Also, they found that the extracellular glycerol decreased at high levels of NaCl. Saada [57] examined the glycerol production by some species of *Eurotium* and reported that the growth of *E. amstelodami* and *E. chevalieri* in saline media showed an enhanced production of glycerol with the major portion of the glycerol produced retained within the mycelium. Also, she recorded that more than two- thirds of intracellular glycerol within the mycelia of the two tested *Eurotium* species were released to external water when the mycelia were transferred into distilled water for 10 hours. This increase in intracellular glycerol could be due to the metabolic conversion of osmotically inactive storage carbohydrate into osmotically active glycerol.

Table 2. Screening of glycerol production (measured by g/L) by different filamentous fungi isolated from molasses.

Fungal Taxa	No. of isolates	Source of isolates	Dry weight	Glycerol concentration	
				(g/L)	m mol
Aspergillus (26)					
A. clavatus	STRI 1	Dishna	0.816	0.54	5.86
A. flavus	STRI 2	Dishna	0.754	0.13	1.41
A. flavus	STRI 3	Nag Hammady	0.557	0.37	4.02
A. flavus	STRI 4	Qus	0.716	0.12	1.30
A. flavus	STRI 5	Abo-Qurqas	0.719	0.13	1.41
A. flavus	STRI 6	Dishna	0.704	0.11	1.19
A. flavus	STRI 7	Dishna	0.873	0.13	1.41
A. fumigatus	STRI 8	Nag Hammady	0.739	0.39	4.23
A. fumigatus	STRI 9	Dishna	0.639	0.14	1.52
A. fumigatus	STRI 10	Dishna	0.766	0.15	1.63
A. fumigatus	STRI 11	Qus	0.71	0.16	1.74
A. fumigatus	STRI 12	Abo-Qurqas	0.638	0.52	5.65
A. fumigatus	STRI 13	Abo-Qurqas	0.88	0.31	3.37
A. niger	STRI 14	Nag Hammady	0.7295	0.17	1.85
A. niger	STRI 15	Nag Hammady	0.688	0.14	1.52
A. niger	STRI 16	Dishna	0.84	0.10	1.09
A. niger	STRI 17	Dishna	0.754	0.03	0.33
A. ochraceus	STRI 18	Dishna	0.811	0.09	0.98
A. parasiticus	STRI 19	Nag Hammady	0.633	0.46	4.99
A. terreus	STRI 20	Abo-Qurqas	0.781	0.44	4.78

Table 2. Continued

Fungal Taxa	No. of isolates	Source of isolates	Dry weight	Glycerol concentration	
				(g/L)	m mol
A. terreus	STRI 21	Abo-Qurqas	0.6384	0.54	5.86
A. versicolor	STRI 22	Dishna	0.634	0.30	3.26
A. versicolor	STRI 23	Dishna	0.789	0.51	5.54
A. versicolor	STRI 24	Dishna	1.034	0.02	0.22
A. versicolor	STRI 25	Abo-Qurqas	0.835	0.55	5.97
A. versicolor	STRI 26	Nag Hammady	0.726	0.43	4.67
Byssochlamys nivea	STRI 27	Qus	0.509	0.04	0.43
Byssochlamys nivea	STRI 28	Qus	0.52	0.13	1.41
Eurotium (2)					
E. repens	STRI 29	Nag Hammady	1.075	0.13	1.41
E. repens	STRI 30	Dishna	0.493	0.08	0.87
Monascus (5)					
M. ruber	STRI 31	Abo-Qurqas	0.95	0.09	0.98
M. ruber	STRI 32	Qus	0.428	0.04	0.43
M. ruber	STRI 33	Abo-Qurqas	0.74	0.24	2.61
M. ruber	STRI 34	Nag Hammady	0.525	0.15	1.63
M. ruber	STRI 35	Qus	0.8	0.23	2.50
Penicillium (18)					
P. chermesinum	STRI 36	Dishna	0.855	0.43	4.67
P. chermesinum	STRI 37	Dishna	0.957	0.13	1.41
P. citrinum	STRI 38	Nag Hammady	0.956	0.27	2.93
P. corylophillum	STRI 39	Nag Hammady	0.911	0.35	3.80
P. corylophillum	STRI 40	Dishna	1.038	0.46	4.99

Table 2. Continued

Fungal Taxa	No. of isolates	Source of isolates	Dry weight	Glycerol concentration	
				(g/L)	m mol
P. corylophillum	STRI 41	Abo-Qurqas	0.944	0.09	0.98
P. corylophillum	STRI 42	Abo-Qurqas	0.8	0.11	1.19
P. corylophilum	STRI 43	Dishna	1.022	0.28	3.04
P. duclauxii	STRI 44	Qus	0.7272	0.44	4.78
P. duclauxii	STRI 45	Qus	0.678	0.53	5.75
P. funiculosum	STRI 46	Dishna	0.696	0.08	0.87
P. restrictum	STRI 47	Nag Hammady	0.888	0.32	3.47
P. restrictum	STRI 48	Dishna	0.658	0.15	1.63
P. restrictum	STRI 49	Dishna	0.65	0.22	2.39
P. restrictum	STRI 50	Dishna	0.834	0.12	1.30
P. restrictum	STRI 51	Abo-Qurqas	0.684	0.14	1.52
P. waksmanii	STRI 52	Nag Hammady	1.096	0.30	3.26
Paecilomyces	STRI 53	Nag Hammady	0.737	0.08	0.87
Rhizopus stolonifer	STRI 54	Nag Hammady	0.693	0.45	4.89
Scopulariopsis sp	STRI 55	Nag Hammady	0.525	0.09	0.98

Table 3. Effect of desalination by transferring the mycelia of *A. versicolor* STRI 25 and *A. clavatus* STRI 1 from saline growth medium into sterilized distilled water for 24 hours on glycerol outflow.

Fungal Isolate	Dry weight (g/L)	External glycerol in saline medium (m moles)	Glycerol outflow from mycelia into sterilized distilled water (m moles)	Total glycerol (m moles)
***A. versicolor* STRI25**	11.51	5.98	63.84	69.82
***A. clavatus* STRI1**	13.25	5.54	28.90	34.44

Glycerol and other polyols are poor inhibitors of enzyme function [58,59]. At lower water activity, glycerol concentration increased rapidly at first but on continuous incubation, the glycerol content was declined [60]. The four isolates tested of *A. niger* produced small concentration of glycerol ranging between 0.03-0.17 g/L. Adler *et al.* [52], in similar study, attributed the decrease in the polyols content of *A. niger* and *P. chrysogenum* to polyol consumption or conversion into storage saccharides. However, Hocking [61] attributed glycerol depletion to the formation of spores and their maturation and noted that production of mature conidia occurred at or near the peak of glycerol accumulation and sporulation was followed by a dramatic fall in the intracellular glycerol concentration. In the current study, two *E. repens* isolates showed low ability to produce glycerol (0.08 and 0.13 g/L). In accordance, it was stated that, the quantity of glycerol produced by *A. wentii* was twice higher than that obtained by *E. amstelodami* on whey treated with 8% NaCl [60].

Since glycerol often serves the function of an osmolyte, balancing external osmotic pressure [62], osmophilic fungi are of particular interest for glycerol production. In filamentous fungi, glycerol is repeatedly found as the major osmolyte [63]. *A. niger*, *Botrytis* sp., *P. italicum*, *R. javanicus*, and *R. nigricans*, as well as yeast are potential candidates for glycerol production [64-68]. Glycerol is the primary small storage molecule produced from sugar or glycolytic intermediates in *A. niger* [69]. On 2% sucrose, glycerol accumulates early [70]. Accumulation of glycerol in 10% glucose medium means that glycerolytic control is displaced from fructose-6-phosphate dehydrogenase to glyceraldehyde-3-phosphate dehydrogenase under citric acid production conditions.

Thus, Legisa and Grdadolnik [69] proposed that glyceraldehyde-3-phosphate dehydrogenase would be a promising target for metabolic engineering.

CONCLUSION

A large number of fungal diversity associated with sugarcane industrial effluent and molasses created novel record of the fungal diversity associated with sugarcane industrial waste, and provided a base in solving the problems associated with pollution of sugarcane industry. Furthermore, it may become a basis for the management of sugarcane industrial wastes. In addition, many fungal species which have the capability for glycerol production can be used for this purpose.

REFERENCES

1. Leeson, S. and Summers, J. D. V. (2000): Broiler and breeder production. University Books, Guelph, Ontario, Canada.

2. Olbrich, H. (2006): "Molasses"Fermentation of molasses. *Biotechnologie-Kempe GmbH*, 58-62.

3. Yepez, Y. (1995): Selección de unacepa de *Saccharomyces cerevisiae* con alta productividad de etanol y quetoleremayoresniveles de azúcarque los usados en la Planta Alcoquímica Sucromiles S.A. Tesis de maestria. Pontificia Universidad Javeriana. Facultad de ciencias. Bogotá, Colombia:34-48.

4. Tellez, D. (2004): Caracterización de lasmelazasempleadas en el procesofermentativo de la destilería San Martín-Industria de Licoresdel Valle. Santiago de Cali, Colombia.

5. El-Samman, A. A. (2010): Organic and inorganic constituents analysis of cane molasses and its effect on microbial fermentation industries, Ph.D Thesis, Sugar Technology Research Institute (Chemistry), Assiut University, Assiut, Egypt 147 pp.

6. Venna, V. R., Xu, Y., Doran, S. J., Patrizz, A. and McCullough, L. D. (2014): Social interaction plays a critical role in neurogenesis and recovery after stroke. *Translational Psychiatry* **4**:e351

7. Binkley, W. W. and Wolfrom, M. L. (1953): Principles of sugar technology, *Advanced Carbohydrate Chernistry* **8**:291.

8. Olbrich, H. and Honig, P. (1963). Molasses In Honig P. [ed.], Principles of sugar technology, Volume III, Elsevier Publishing Co, New York, 511-697.

9. Pant, D. and Adholeya, A. (2007): Biological approaches for treatment of distillery wastewater: A review. *Bioresource Technology* **98**:2321-2334. doi:10.1016/j.biortech.2006.09.027

10. Galazzo, J. L. and Bailey, J. E. (1990): Growing *Saccharomyces cerevisiae* in calcium-alginate beads induces cell alterations which accelerate glucose conversion to ethanol. *Biotechnology and Bioengineering* **36**:417-426. doi:10.1002/bit.260360413

11. Nigam, P., Banat, I. M., Singh, D., McHale, A. P. and Marchant, R. (1997): Continuous ethanol production by thermotolerant *Kluyveromyces marxianus* IMB3 immobilized on mineral kissiris at 45°C. W. Journal of Microbiolial Biotechnology **13**:283-288. doi:10.1023/A:1018578806605

12. Sheoran, A., Yadav, B. S., Nigam, P. and Singh, D. (1998): Continuous ethanol production from sugarcane molasses using a column reactor of immobilized *Saccharomyces cerevisiae* HAU-1. *Journal of Basic Microbiology* **38**:123-128.

13. Zohri, A. A., Ragab, W. S. M. and Ali, M. M. A. (2014): Ethanol production from Egyptian sugar cane molasses by six yeast strains using batch fermentation. *Journal of Basic and Applied Mycolology* **5**:43-49

14. Bakhiet, S. E. A. and Al-Mokhtar, E. A. I. (2015): Production of citric acid by *Aspergillus niger* using sugarcane molasses as substrate. *Jordan Journal of Biological Sciences* **8**(3):211-215. doi: 10.12816/0026960

15. Fadel, M., Zohri, A. A., Kamal El-Dean, A. M., Auob, A. H. and Deiab, B. (2016): Enhancing ethanol yield from sugar cane molasses fermentation by addition of depolymerising enzymes. *Indian Journal of Applied Research* **6**(8):291-294.

16. Bagy, M. M. K., Abd-Alla, M. H., Morsy, F. M. and Hassan, E. A. (2014): Two stage biodiesel and hydrogen production from molasses by oleaginous fungi and *Clostridium acetobutylicum* ATCC 824. *International Journal of Hydrogen Energy* **39**:3185-3197. doi:10.1016/j.ijhydene.2013.12.106

17. Biebl, H., Zeng, A. P., Menzel, K. and Deckwer, W. D. (1998): Fermentation of glycerol to 1, 3-propanediol and 2, 3-butanediol by *Klebsiella pneumonia*. *Applied Microbiology and Biotechnology* **50**:453-457. doi: 10.1007/s002530051251

18. Biebl, H., Zeng, A. P., Menzel, K. and Deckwer, W. D. (1999): Microbial production of 1,3-Propanediol. *Applied Microbiology and Biotechnology* **52**:289-296.

19. Wang, Z. X., Zhuge, J., Fang, H. and Prior, B. A. (2001): Glycerol production by microbial fermentation. *Biotechnology Advances* **19**:201-223. doi: 10.1016/S0734-9750(01)00060-x

20. Chatzifragkou, A., Makri, A., Belka, A., Bellou, S., Mavrou, M., Mastoridou, M., Mystrioti, P. and Onjaro, G. (2011): Biotechnological conversions of biodiesel derived waste glycerol by yeast and fungal species. *Energy* **36**:1097–1108. doi:10.1016/j.energy.2010.11.040

21. Imandi, S. B., Bandaru, V. V. R., Somalanka, S. R., Bandaru, S. R. and Garapati, H. R. (2007): Optimization of medium constituents for the production of citric acid from byproduct glycerol using Doehlert experimental design. *Microbial Enzyme* **40**(5):1367-1372. doi: 10.1016/j.enzmictec.2006.10.012

22. Wen, Z., Zeng, R. S., Niu, G., Berenbaum, M. R. and Schuler, M. A. (2009): Ecological significance of induction of broad-substrate cytochrome P450s by natural and synthetic inducers in *Helicoverpazea*. *Journal of Chemical Ecology* **35**:183–189. doi:10.1007/s10886-009-9598-4

23. Andre, A., Diamantopoulou, P., Philippoussisb, A., Sarris, D., Komaitis, M. and Apanikolaou, S. (2010): Biotechnological conversion of bio-diesel derived waste glycerol into added-value compounds by higher fungi: production of biomass, single cell oil and oxalic acid. *Industrial Crops and Products* **31**:407-416. doi:10.1016/j.indcrop.2009.12.011

24. Makri, M., Hitt, M. A. and Lane, P. J. (2010): Complementary technologies, knowledge relatedness, and invention outcomes in high technology mergers and acquisitions. *Strategic* Management Journal **31**(6):602-628. doi:10.1002/smj.829

25. Ethier, S., Woisard, K. and Vaughan, D. (2011): Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid. Bioresource Technology **102**(1):88–93. doi:10.1016/j.biortech.2010.05.021

26. Christoph, R., Schmidt, B., Steinberner, U., Dilla, W. and Karinen, R (2006): Glycerol. Ed. Morrison LR, Ullmann's Encyclopedia of Industrial Chemistry. Wiley, New York, USA.

27. Yazdani, S. S. and Gonzalez, R. (2007): Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. *Current Opinion in Biotechnology* **18**:213–219. doi: 10.1016/j.copbio.2007.05.002

28. Tamas, M. J., Luyten, K., Sutherland, F. C. W., Hernandez, A., Albertyn, J., Valadi, H., Li, H., Prior, B. A., Kilian, S. G., Ramos, J., Gustafsson, L., Thevelein, J. M. and Hohmann, S. (1999): Fps 1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. *Molecular Microbiology* **31**:1087–1094. doi:10.1046/j.1365-2958.1999.01248.x

29. Hofmann, C., Féraud, G. and Courtillot, V. (2000): Dating of mineral separates and whole rocks from the Western Ghats lava pile: Further constraints on duration and age of the Deccan traps. Earth and Planetary Science Letters **180**:13-27.

30. Albertyn, J., Hohmann, S., Thevelein, J. M. and Prior B. A. (1994): GPD1, which encodes glycerol-3-Phosphate dehydrogenase, is essential for growth

under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high-osmolarity glycerol response pathway. *American Society for Microbiology* **14**(6):4135-4144. doi:10.1128/MCB.14.6.4135

31. Johnson, L .F. and Curl, E. A. (1972): Methods for research on ecology of soil-borne pathogens. Burgess Publishing Co., Minneapois, MN, USA.

32. Menzies, J. D. (1957): A dipper technique for serial dilution of soil for microbial analysis. Soil *Science Society America Proceedings* **21**:660.

33. Pitt, J. I. (1979): The Genus *Penicillium* and Its Teleomorphic States *Eupenicillium* and *Talaromyces*. London: Academic Press, 635 pp.

34. Raper, K. B. and Fennell, D. I. (1965): The Genus *Aspergillus*, pp 686 Baltimore: Williams & Wilkins Company, 686 pp.

35. Moubasher, A. H. (1993): Soil fungi in qatar and other arab countries. The Scientific and Applied Research Centre. University of Qatar, Doha, Qatar, 566 pp.

36. Domsch, K. H., Gams, W. and Anderson, T. (2007): Compendium of soil fungi. 2nd edition. IHW Verlag. Eching, Germany, 672 pp.

37. Pitt, J. I. and Hocking, A. D. (2009): Fungi and food spoilage. 3rd edition, Springer Science and Business Media, London, 519 pp.

38. El-Kady, I. A., Moubasher, M. H. and Mostafa, M. E. (1995): Accumulation of sugar alcohol by filamentous fungi. *Folia Microbiologia* **40**:481-486. doi:10.1007/BF02814727

39. Chitlaru, E. and Pick, U. (1989): Selection and characterization of *Dunaliella salina* mutants defective in haloadaptation. *Plant Physiology* **91**:788-794.

40. Seyis, I. and Subasioglu, T. (2009): Screening of different fungi for decolorization of molasses. *Journal of microbiology* **40**:61- 65. doi:10.1590/S1517-83822009000100009

41. Zimmermann, M., Miorelli, S., Massaguer, P. R. and Aragão, G. M. F. (2011): Growth of *Byssochlamys nivea* in pineapple juice under the effect of water activity and ascospore age. *Brazilian Journal of Microbiology* **42**:203-210. doi:10.1590/S1517-83822011000100025

42. Awasthi, G., Prasad, G. B. K. S. and Das, A. (2011): Population genetic analyses of Plasmodium falciparum chloroquine receptor transporter gene haplotypes reveal the evolutionary history of chloroquine-resistant malaria in India. *International Journal Parasitology* **41**:705-709. doi: 10.1016/j.ijpara.2011.03.002

43. El-Said, A. H. M. (2002): Mycotoxins and invertase enzyme of the mycoflora of molasses in Upper Egypt. *Mycobiology* **30**(3):170-174. doi:10.3923/pjbs.2001.1386.1389

44. Abdel-Sater, M. A. and Ismail, M. A. (1993): Enzymatic studies on fungi associated with biscuits in Egypt. *International Biodeterioration and Biodegradation* **31**:277-292. doi:10.1016/0964-8305(93)90023-u

45. Abdel-Hafez, S. I. I., El-Said, A. H. M. and Maghraby, T. A. (1995): Studies on fungi isolated from skin diseases and associated fungi of students in Qena and Red Sea Governorates, Egypt. *Assiut Bulletin Faculty of Science, Assiut University* **24**(2-D):181–209. doi:10.4489/MYCO.2002.30.3.154

46. Barakat, L. P., Kazak, A. E., Gallagher, P. R., Meeske, K. and Stuber, M. (2000): Posttraumatic stress symptoms and stressful life events predict the long-term adjustment of survivors of childhood cancer and their mothers. Journal of Clinical *Psychology in Medical Settings* **7**:189–196.

47. Abdel-Sater, M. A. and Saber, S. M. (1999): Mycoflora and Mycotoxins of some Egyptian dried fruits. *Assiut Bulletin Faculty of Science, Assiut University* **28**(1-D):91-107.

48. Abdel-Gawad, K. M. and Zohri, A. A. (1993): Fungal flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. *Mycopathologia* **124**(1):55-64. doi:10.1007/BF01103057

49. Kozakiewicz, Z. (1989): Ornamentation types of conidia and conidiogenous structures in fasciculate *Penicillium* species using scanning electron microscopy. *Journal of the Linnean Society Botany* **99**:273-293. doi:10.1111/j.1095-8339.1989.tb00404.x

50. Pillai, D., Gerbig, C., Marshall, J., Ahmadov, R., Kretschmer, R., Koch, T. and Karstens, U. (2010): High resolution modeling of CO_2 over Europe: implications for representation errors of satellite retrievals. *Atmospheric Chemistry and Physics* **10**:83–94. doi:10.5194/acp-10-83-2010.

51. Abdel-Sater, M. A., Zohri, A. A., Ragab, W. S. M. and Noor El-Din, S. M. (2015): Filamentous fungi and their enzymatic activities associated with sugar cane molasses. *Egypt Sugar Journal* **8**:45-60.

52. Adler, L., Pedersen, A. and Tunblad-Johansson I. (1982): Polyol accumulation by two filamentous fungi grown at different concentrations of NaCI. *Physiological Plant* **56**:139 - 142.

53. Gadd, G. M., Chudek, J. A., Foster, R. and Reed, R. H. (1984): The osmotic responses of *Penicillium ochro-chloron*: Changes in internal solute levels in response to copper and salt stress. *Journal of General Microbiology* **130**:1969-1975. doi:0022-1287/84/0oO1-1767

54. Zidan, M. I. and Abdel-mallek, A. Y. (1987): Effect of NaCl on the accumulation of glycerol by three *Aspergillus* species. *Journal of Basic Microbiology* **27**:293-297. DOI: 10.1002/jobm.3620270713.

55. Seidl, V., Seiboth, B., Karaffa, L. and Kubicek, C. P. (2004): The fungal STRE-element-binding protein Seb1 is involved but not essential for glycerol dehydrogenase (gld1) gene expression and glycerol accumulation in *Trichoderma atroviride* during osmotic stress. *Fungal* Genetics *and Biology* **41**:1132–1140. doi:10.1016/j.fgb.2004.09.002

56. Khaleil, M. M. M. (2009): Physiological studies on some osmophilic fungi. Master thesis, Faculty of science, Zagazig University. Egypt.

57. Saada, H. G. H. (2014): Production of glycerol by some species of genus *Eurotium*. Ph D thesis, Botany and microbiology Dept, Fac Sci, Zagazig Univ 152 pp.

58. Adler, L. (1978): Properties of alkaline phosphatase of the halotolerant yeast *Debaryomyces hansenii.* Biochimica *et* Biophysica. Acta (BBA) Enzymology **522**(1):113-121.

59. Brown, A. D. (1978): compatible solutes and extreme water stress in eukaryotic microorganisms. *Advanced Microbial Physiology* **17**:181-242.

60. El-Kady, I., El-Maraghy, S. and Zihri, A. N. (1994): Mycotoxin producing potential of some isolates of *Aspergillus flavus* and *Eurotium* groups from meat products. *Microbiological Research* **149**:297-307. doi:10.1016/S0944-5013(11)80073-X

61. Hocking, A. D. (1986): Effect of water activity and culture age on the glycerol accumulation patterns of five fungi. *Z General Microbiology* **132**:269-275. doi:10.1099/00221287-132-2-269

62. Sunder, S., Singh, A. J., Gill, S. and Singh, B. (1996): Regulation of intracellular level of Na+, K+ and glycerol in *Saccharomyces cerevisiae* under osmotic stress. Molecular *and Cellular Biochemistry* **158**(2):4-121.

63. Adler, L. and Blomberg, A. (1992): Physiology of osmotolerance in fungi. *Advanced Microbiological Physiology* **33**:145-212. doi:10.1016/S0065-2911(08)60217-9

64. Ravji, R. G., Rodiguez, S. B. and Thornton, R. J. (1988): Glycerol production by four grape molds. *American Journal of Enology and Viticulture* **39**:77-82.

65.Lu, Q., Wallrath, L. L. and Elgin, S. C. R. (1995): The role of a positioned nucleosome at the *Drosophila melanogaster* hsp26 promoter. *European Molecular Biology Organization* **14**(19):4738-4746.

66. Ciani, M. and Ferraro L. (1996): Enhanced glycerol content in wines made with immobilized *Candida stellata* cells. *Applied and Environmental Microbiology* **62**:128–132. doi:0099-2240/96/$04.0010

67. Remize, F., Roustan, J. L., Sablayrolles, J. M., Barre, P. and Dequin, S. (1999): Glycerol overproduction by engineered *Saccharomyces cerevisiae*

wine yeast strain leads to substantial changes in byproduct formation and to a stimulation of fermentation rate in stationary phase. *Applied and environmental microbiology* **65**:143–149.

68. Mohammed, M. J., Marston, C. K., Popovic, T., Weyant, R. S. and Tenover, F. C. (2002): Antimicrobial susceptibility testing of *Bacillus anthracis*: Comparison of results obtained by using the National Committee for Clinical Laboratory Standard broth microdilution reference and test agar gradient diffusion method. *Journal of Clinical Microbiology* **40**:1902–1907. doi: 10.1128/JCM.40.6.1902-1907.

69. Legisa, M. and Grdadolnik, S. G. (2002): Influence of dissolved oxygen concentration on intracellular pH and consequently on growth rate of *Aspergillus niger*. *Food Technology Biotechnology* **40**: 27–32.

70. Witteveen, C. F. B. and Visser, J. (1995): Polyol pools in *Aspergillus niger*. *Fems Microbiology Letters* **134**:57-62. doi:10.1016/0378-1097(95)00381-e

الملخص العربى

إنتاج الجليسرول بواسطة الفطريات المقاومة أو المحبة للسكر والمصاحبة للمولاس

عبد الناصر احمد زهري* ، نعمات عبد الجواد حسين* ، محمد عبد العظي** ، شيماء فتحي عطية**.

* قسم النبات والميكروبيولوجي ، كلية العلوم ، جامعة اسيوط ، جمهورية مصر العربية

** معهد ابحاث تكنولوجيا السكر ، جامعة اسيوط ، جمهورية مصر العربية

nemmgoda@yahoo.com

يعتبر المولاس من النواتج الاساسية لصناعة السكر من قصب السكر وهو غنى بالمواد المفضلة من قبل الكائنات الدقيقة وخاصة الفطريات المحبة للسكر. بناءاً على ذلك تم عزل عدد ٢٢ نوعاً فطرياً تنتمى إلى ٨ أجناس من ٢٠ عينة مولاس جمعت من ٤ مصانع للسكر فى صعيد مصر على الوسط الغذائى شابكس ٣٠% سكروز اجار. اسبرجيلس فيوميجاتس ، فلافس، بالاضافة إلى مونأسكس روبر كانت أكثر الانواع شيوعاً. ونظراً للإستخدامات العديدة للجليسرول ولقلة موارده، تم بعد ذلك إختبار مقدرة عدد ٥٥ معزولة فطرية على إنتاج الجليسرول على الوسط الغذائى شابكس ١٠% كلوريد صوديوم بالاضافة إلى ٣% ثنائى كبرتيت الصوديوم. أظهرت النتائج مقدرة جميع العزلات المختبرة على إنتاج الجليسرول فى الوسط الغذائى (خارج الخلايا) بدرجات متفاوتة (٠.٠٣-٠.٥٥ جم/لتر) وتفوقت العزلات التابعة لأسبرجيلس كلافاتس (STRI 1)، فيوميجاتس (STRI 12)، تيريس (STRI ٢١)، فيرسكلر (STRI 23, 25)، وبينيسيليوم دكلوكسى (STRI 45) فى انتاج أعلى تركيزات من الجليسرول (٠.٥١-٠.٥٥ جم/لتر = ٥.٥٤-٥.٩٨ ملي مول). ومن الجدير بالذكر أنه عند وضع الخيوط الفطرية للعزلتين التابعتين لأسبرجيلس فيرسكلر STRI 25، كلافاتس STRI 1 فى الماء المقطر والمعقم لمدة ٢٤ ساعة زادت انتاجية الجليسرول من داخل الخلايا ما يزيد عن عشرة أضعاف فى حالة أسبرجيلس فيرسكلر STRI 25 (63.84)، وما يقرب من خمسة أضعاف فى حالة كلافاتس STRI 1 (28.90 ملي مول).