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Kinetic models application on glycerol production from glucose by the marine yeast *Candida orthopsilosis*

Abdel-Hamied M. Rasmey*, Akram A. Aboseidah, El-Shahat M. Khalaf, Mohamed H. Ahmed and Heba Hawary
Botany and Microbiology Department, Faculty of Science, Suez University, Suez, Egypt.
*Corresponding author: am_rasmey@yahoo.com

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ABSTRACT

This study aims to investigate the kinetic parameters of carbon source effect on biomass and glycerol production by *Candida orthopsilosis* HH52 isolated from marine sediment. The yeast isolate HH52 was selected among five tested marine isolates as the most efficient producer of glycerol (64.42 gl^{-1}). Based on the phenotypic and genotypic identification, this isolate was identified as *Candida orthopsilosis* and was deposited in the GenBank database under the accession number MK156301. The maximum specific glycerol production rate (v_{max}) was 0.101 gg^{-1} (glycerol /biomass) at 225 gl^{-1}glucose, whereas the maximum specific growth rate (μ_{max}) was 0.211 hr^{-1} at 150 gl^{-1} glucose. The glycerol ($Y_{P/S}$) and biomass ($Y_{X/S}$) yield coefficients were 0.3576 and 0.1128 gg^{-1}, respectively. These findings suggest the possibility of using *Candida orthopsilosis* HH52 as a promising source for glycerol production.

INTRODUCTION

Glycerol is a polyolalcohol with unique properties make it has different uses such as a hygroscopic agent, softening agent, sweetening agent, emollient, lubricant, refrigerant, preservative, and many others (Pagliaro, 2017). Glycerol is also an intermediate in different industries such as food, paint, pharmaceutical, cosmetic, soap, toothpaste (Fan *et al*., 2010). Moreover, glycerol could be used as a raw material for the production of appreciated chemicals such as biodiesel, butanol, citric acid, acrolein and hydrogen (Mirończuk *et al*., 2016). Glycerol is chemically synthesized from propylene synthesis or from soap and fatty acids industries as a by-product (Bagnato *et al*., 2017). However, production of glycerol by yeast fermentation represents a promising alternative way to the chemical synthesis due to its simplicity and low cost (Sahoo and Agarwel, 2001; Wang *et al.*, 2001; Grembecka, 2015). Glycerol production using halotolerant yeast genera such as *Pichia*, *Candida*, *Debaryomyces* and *Zygosaccharomyces* received more attention during the recent years (Liu *et al.*, 2003; Chen *et al.*, 2008). Glycerol is involving in many vital physiological roles during marine yeast fermentation. Under osmotic stress condition glycerol is accumulated as osmolyte to re-adjust osmotic gradient across the cell membrane and maintain cellular functions (Kayingo *et al*., 2001; Tomaszewska *et al.*, 2012; Amin *et al.*, 2018). Furthermore, glycerol serves in keeping cytosolic redox balance that the surplus NADH can be reoxidized via

glycerol synthesis by acting as the reaction cofactor (Ansell *et al.*, 1997; Zhao *et al.*, 2015). Higher glycerol yields have been obtained from species that show a relatively strong tolerance for high concentration of sugars and salts (Loray *et al*., 1995; Patil, 2013). Selection of yeast strains and optimization of fermentation conditions such especially the carbon source are critical parameters for glycerol overproduction (Ehsani *et al*., 2009; Orlic *et al.*,2010; Mirończuk *et al*., 2016; Singh *et al*., 2016; Hawary *et al.*, 2019). In this study, screening of some marine yeast isolates for glycerol production was performed. Characterization and molecular identification of the most efficient isolate was investigated. Furthermore, kinetics of growth and glycerol biosynthesis of the selected isolate *Candida orthopsilosis* HH52 were determined.

MATERIALS AND METHODS

Microorganisms and inocula preparation:

The used five marine yeasts in the present study were isolated and selected as highly glycerol producers from marine sediments and seaweeds by the same authors in a previous study (unpublished). The yeasts were kept on yeast malt extract (YM) agar slants at 4^{o}C. YM agar medium is composed of (gl^{-1} seawater): yeast extract, 3.0; malt extract, 3.0; peptone, 5.0; glucose, 10.0 and agar-agar, 20.0, and adjusted to pH 6.5with 0.1 N HCL & 0.1 N NaOH**.** Yeast inocula were prepared by inoculating a loopfull of 48 hours old culture into 50 ml sterilized YM broth and incubated for 48 hours at 30 ^{o}C on a rotary shaker (150 rpm).

Fermentation process:

The fermentation was performed in 100 ml volume glass bottles each containing 50 ml of fermentation medium inoculated with 20 % of yeast inocula (10^{6} cells /ml) and incubated at aerobic conditions for 96 hours on a rotary shaker (150 rpm) at 30 ^{o}C. The glycerol fermentation medium contained (gl^{-1} distilled water): glucose, 180; peptone, 1; yeast extract, 1; KH_2PO_4, 1; $MgSO_4$, 0.5 and NaCl, 58.44 (1M). The medium pH was adjusted to 6 ± 0.2 using 0.1 N NaOH and 0.1 N HCl.

Dry biomass determination:

The dry biomass (gl^{-1}) was determined by collecting the cells from the fermentation medium and drying them at 85˚C for 24 hr in an oven and then weighted (Shoaib *et al.*, 2018).

Glycerol extraction and determination:

Yeast cultures were centrifuged for 10 minutes at 4000 g. The intracellular glycerol was measured using the cell pellets, whereas the supernatant was used to determine the extracellular glycerol production after centrifugation of the culture at 4000 g for 10 minutes. The cell pellets were washed twice with phosphate buffered saline (PBS) contains (M): 0.0027 KCl, 0.137 NaCl, 0.0018 KH_2PO_4 and0.01 Na_2HPO_4. After washing, 3 mL 0.1 $moll^{-1}$ TRIS/HCL buffer (pH 7.7) containing 2 $mmoll^{-1}$ EDTA were added to the cell pellets and boiled for 5 minutes then centrifuged at 11000 g for 10 minutes to remove the cell debris. Glycerol was determined using sodium periodate method (Kuhn *et al.*, 2015).

Phenotypic and genotypic identification of the marine yeast HH52:

Phenotypic characterization:

The marine yeast isolate HH52 was morphologically characterized on four different types of media. The color, appearance, elevation, texture, and margin of the colonies were recorded onto YM agar. The cell shape, budding type, formation of pellicle, asci and ascospores were examined onto sodium acetate agar medium

(McClary *et al.*, 1959; Sulieman, *et al.*, 2015) and into 5% malt broth (Wickerham, 1951; Ali and Khan, 2014). The pseudohyphae were observed on cornmeal agar using the coverslip method (Lodder and kreger-van Rij, 1952).

Biochemical characteristics were determined such as diazonium Blue B (DBB) test, citrate utilization, indole production, Voges-Proskauer (VP), methyl red (MR), growth in osmotic and vitamin free medium, fermentation and assimilation of various carbon compounds. The isolate was tested also for production of cellulase, amylase, and urease. Moreover, the halo-tolerance and temperature profiles of the isolate were determined (Rasmey *et al.*, 2018).

Genotypic identification:

Genomic DNA was extracted using chloroform-extraction and ethanol-precipitation method.The primers used: ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3'. PCR reactions were carried out in a final volume of 100 µL with the following reagent concentrations: dNTP mixture (200 µM each), forward and reverse primers (0.2 µM each); Taq buffer (1x); Taq DNA polymerase (2.5 U/100 µL); 50ng of gDNA template and the total volume of the PCR reaction was adjusted to 100 µL with nuclease-free water. The complete reaction mixture was incubated and performed at an automated MJ research thermal cycler (USA). PCR amplification reaction conditions included an initial denaturation at 95 ^{o}C for 3 minutes, followed by 35 cycles: 95 ^{o}C for 30 sec, annealing at 55 ^{o}C for 30 sec and extension 72 ^{o}C for 60 sec. A final extension step was conducted at 72 ^{o}C for 5 min. All PCR amplicons were analyzed using gel electrophoresis through 1% agarose gels in 1x Tris-acetate-EDTA (TAE) buffer (40 mM Tris, 20 mM Acetate and 1mM EDTA, pH 8.0). The PCR amplicons sizes were visualized under UV light after staining with ethidium bromide (5 µg/ml) and estimated against a DNA ladder (Applied Biotechnology Co, Egypt) and the purified PCR products were sent for sequencing at Solgent Co Ltd, South Korea. The purified PCR products were cycle sequencing with didesoxy mediated chain-termination (Sanger *et al.*, 1977). The sequences resulted were trimmed and assembled in Geneious software (Biomatters). Then, the full length trimmed sequences obtained were identified and matched with previously published sequences available in NCBI using basic local alignment tool (BLAST) at the NCBI website: http:// www.ncbi.nlm.nih.gov/ BLAST/ to assess the degree of DNA similarity.

Kinetics study:

The effect of different carbon sources (glucose, fructose, sucrose, lactose and maltose) at 18 % was investigated individually to obtain the maximum yield of glycerol. Moreover, the determination of the microbial growth and glycerol production kinetics using different glucose concentrations (50 – 250 gl^{-1}) at different fermentation times was performed.

The extracellular glycerol concentrations (P_{ex}), intracellular glycerol concentrations (P_{in}), total glycerol concentrations (P_T), dry biomass (X_T), glycerol coefficient yield ($Y_{P/S}$), biomass coefficient yield ($Y_{X/S}$), specific growth rate (μ_x) and specific glycerol production rate (V_g) were detected according the following equations. S_{Go} is the initial sugar concentration. The specific growth rate values were calculated from the logarithmic plots of the dry biomass data with the fermentation time.

$$Y_{p/s}\ (g/g) = X_T/S_{G0} \qquad [1]$$

$$Y_{x/s}\ (g/g) = P_T/S_{G0} \qquad [2]$$

$$\mu_x = dX/dt \qquad [3]$$

V_g ($gg^{-1}hr^{-1}$) = dP/Xdt [4]

Statistical analysis:

Non-linear regression analysis was performed using SPSS 10 statistical package program. Glycerol production and biomass mean values were compared at 5% significance level using Tukey's test.

RESULTS AND DISCUSSION

Screening and identification:

Data in Figure 1 indicated variability among the tested yeasts concerning to their efficiency in producing glycerol. In the current study, the maximum glycerol production 64.42 gl^{-1} was achieved by the isolate HH52 with volumetric glycerol productivity 0.8791 $gl^{-1}hr^{-1}$, whereas the minimum glycerol production 52.25 gl^{-1} was achieved by the isolate HH108 with glycerol productivity 0.7256 $gl^{-1}hr^{-1}$. These results were inconsistent with Hohmann (2002) who indicated that osmoadaptation is an intrinsic property of the yeast strain. According to Thome (2007**)** the ability of marine yeasts to adapt a low water activity (a_w) with an external high osmotic pressure can vary from species to a species but generally based on the amount of osmolytes that accumulated in the cells. The main reason why marine yeasts accumulate glycerol is its protective properties against hyperosmotic stress e.g. salt stress (Klein *et al.*, 2017). However, an excess of the synthesized glycerol may exit by diffusion across the plasma membrane into the surrounding medium to maintain the intracellular energy (Bubnová *et al*., 2014).

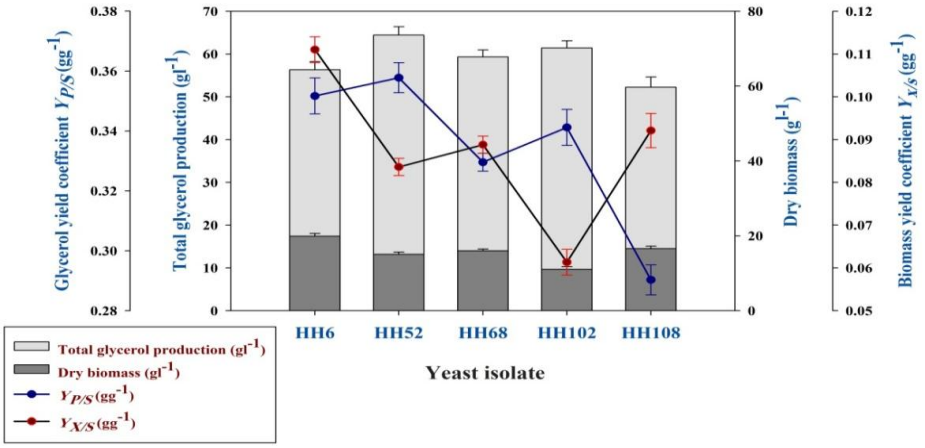

Fig. 1: Glycerol and dry biomass production by the selected five marine yeast isolates.

The morphological characteristics of the isolate HH52 are described in Table 1 and shown in Figure 2. The colony on YM agar was white-colored, butryous, smooth, glabrous and raised. The cells morphological characters indicated that the cell shape was sub-globose to ovoidal with some larger elongated forms observed, budding was monopolar, bipolar and multilateral.

Table 1: Morphological characteristics of the marine yeast HH52.

Medium	Character	Observation
YM agar	Colony color	White
	Nature	Butryous
	Appearance	Glabrous, smooth
	Elevation	Raised
	Margin	Entire
Sodium acetate agar	Cell shape	Subglobose to ovoidal with some larger elongated forms present
	Budding	Monobloar
	Sporulation	Vegetative cells are transformed into asci containing two or four or eight ascospores
5% Malt broth	Cell shape	Subglobose to ovoidal
	Budding	Monoploar or bipolar or multilateral
	Sporulation	Cylindrical asci are formed with four ascospores
	Pellicle formation	Formed and thick folded
Cornmeal agar	Pseudohyphae formation	Pseudohyphae are branched and abundantly formed bearing chains of ovoidal blastoconidia.

Vegetative cells are transformed into subglobose and cylindrical asci containing two or four or eight ascospores. Pseudohyphae are branched and abundantly formed bearing chains of ovoidal blastoconidia.

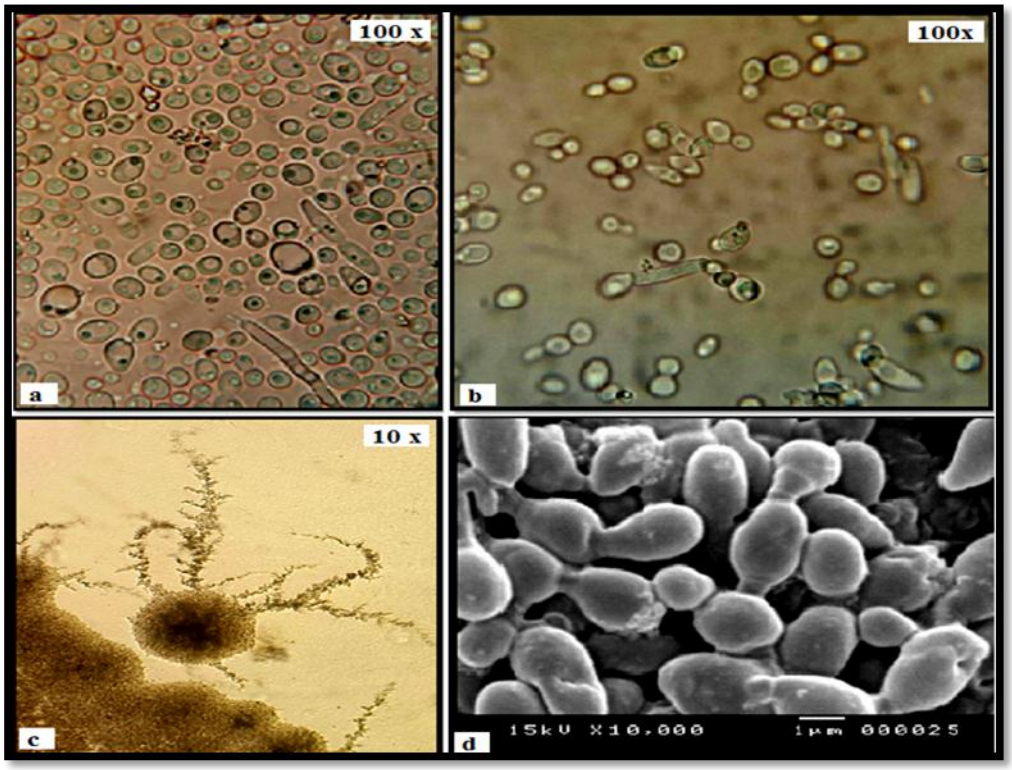

Fig. 2: Morphological characteristics of the yeast HH52; a) Cells are subglobose to ovoidal, asci containing two or four or eight ascospores on 5% malt, b) Vegetative cells are transformed into subglobose and cylindrical asci on sodium acetate agar medium, c) Pseudohyphae are branched and abundantly formed bearing chains of ovoidal blastoconidia on cornmeal agar (10 x) and d) Scanning electron microscope (SEM) of subglobose budding yeast cells on 5% malt broth (10.000 x).

The biochemical and physiological characteristics (Table 2) indicated that the isolate HH52 is ascomycetetous yeast based on its negative reaction to diazonium blue B test (DBB). This test is carried out to determine whether asexual yeast a basidiomycetous genus (+ve reaction) or to belongs to ascomycetous yeasts (-ve

reaction) (Ghindea *et al.*, 2009; Kurtzman and Fell, 1998). The isolate was positive to to voges-proskauer (VP) and indole tests but negative to methyl red (MR). It was able to utilize citrate, glucose, sucrose, maltose, D- xylose, L- arabinose, cellulose, starch, and urea, but not cellobiose, erythritol and nitrate. It couldn't ferment lactose and galactose but can assimilate them.

Table 2: Biochemical and physiological characteristics of the marine yeast HH5.

Characteristic	Result
Diazonium Blue B (DBB)	-
Indole	+
Methyl red (MR)	-
Voges-proskauer (VP)	+
Citrate	+
Urease	+
Starch hydroysis	+
Celluolse hydrolysis	+
50% glucose	+
Vitamin free	+
Fermentation:	
Glucose	+
Sucrose	+
Lactose	-
Maltose	+
Galactose	-
Assimilation:	
Glucose	+
Sucrose	+
Lactose	+
Maltose	+
Galactose	+
Erythritol	-
D- Xylose	+
L- Arabinose	+
Soluble starch	+
Cellobiose	-
Nitrate	-
Glycerol	+
Halo-tolerance profile:	
0.5 M NaCl	+
2 M NaCl	+
4 M NaCl	+
Temperature profile:	
4^{o}C	-
8^{o}C	+
25^{o}C	+
30^{o}C	+
42^{o}C	+

Results showed that this non-conventional isolate could grow under extreme environmental stress conditions such as high osmotic pressure (50% glucose) and vitamin free medium. It could tolerate sodium chloride concentrations up to 4.0 M and grow well at temperature range 8-42 ^{o}C.

Genotypic identification of the marine yeast HH52 was relying on phylogenetic analysis. By the comparison of ITS-rDNA gene sequence (600 bp) of the selected isolate and ITS-rDNA gene sequences of the GenBank database, the isolate was genetically identified as *Candida orthopsilosis* with a similarity of 100% with

Candida orthopsilosis LC389311. The novel marine isolate was deposited under the accession number MK156301in the GeneBank. The isolate taxonomic position was family Debaryomycetaceae, order Saccharomycetales, subphylum Saccharomycotina in the phylum Ascomycota. According to Tavanti *et al*. (2005), *C. orthopsilosis* HH52 is closely related to the pathogen yeast *Candida parapsilosis* and proposed as separate species from *C. parapsilosis*. However, whereas *C. parapsilosis* is one of the most common causes of diseases in immunosuppressed individuals, *C. orthopsilosis* is rarely associated with infection (Riccombeni *et al.,* 2012).

Kinetics of carbon source effect on glycerol production by *C. orthopsilosis* HH52:

Data presented in Figure 3 showed that *C. orthopsilosis* HH52 could accumulate glycerol at variable concentrations using different carbons, the reason could be that various carbon sources used in fermentation might exhibit different degrees of osmotic pressure on the yeast cells (Leandro *et al.*, 2011).

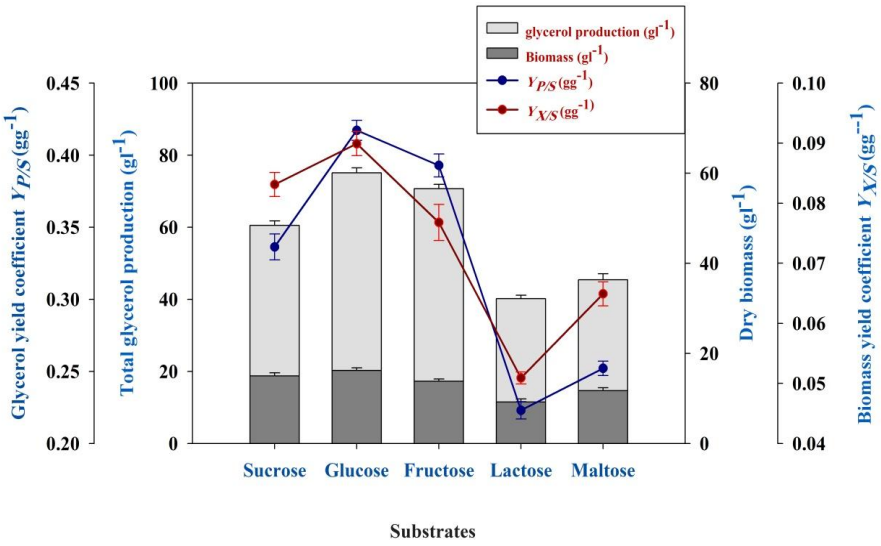

Fig. 3: Glycerol and dry biomass production by *C. orthopsilosis* HH52 on different carbon sources.

Glycerol synthesis is essential for yeast cells to recompense an osmotic stress which is carbon source dependent (Ansell, 1997; Babazadeh *et al*., 2017). The current study indicated that the monosaccharides hexoses such as glucose and fructose exhibit the highest glycerol production yield. The maximum glycerol and dry biomass yields were 0.4172 and 0.0899 gg^{-1}, respectively in case of glucose as a sole carbon source in the fermentation medium. Orlic *et al.* (2010) revealed that glycerol accumulation was higher in glucose-rich medium than in medium supplemented with maltose, the reason might be that glucose can directly enter the glycolytic pathway and more DHAP and NADH were generated.

Kinetic models are crucial to provide meaningful and quantitative interpretation of experimental results and understanding many phenomena in biotechnological processes (Okpokwasili and Nweke, 2005). In shake flask experiments, the influence of different initial glucose concentrations (50 - 250 gl^{-1}) at specific time intervals were investigated. Variations in biomass and glycerol concentrations of the isolate during fermentation time are shown in Figures 4, 5.

The microbial kinetics (Figure 6) are based on the work of Monod (1949). The Monod model equation describes the specific growth rate of a microbial cell as the function of a defined nutrient environment as following:

$$\mu = \frac{\mu_{max} + S_{G0}}{ks + S_{G0}} \qquad [9]$$

Where μ_{max} is the maximum specific growth rate, S_{Go} is initial glucose concentration, K_s is the Monod constant of substrate utilization.

The maximum specific glycerol production rate (v_{max}) was obtained at an initial glucose concentration of 225 gl^{-1} and calculated as 0.101 g gly g^{-1} yeast cells. While the maximum specific growth rate (μ_{max}) was obtained at an initial concentration of glucose (150 gl^{-1}) and calculated as 0.211 hr^{-1}. Moreover, the glycerol yield coefficient ($Y_{P/S}$) and biomass yield ($Y_{X/S}$) coefficients were 0.3576 and 0.1128 gg^{-1}, respectively. The maximum specific glycerol production and growth rates were correlated statistically using the non-linear regression method as follows:

$$\mu = \frac{0.211 + S_{G0}}{40.22 + S_{G0}} \quad , R^2 = 0.840$$

$$v = \frac{0.101 + S_{G0}}{85.55 + S_{G0}} \quad , R^2 = 0.917$$

Our results were in agreement with Petrovska *et al.* (1999) who revealed that glycerol synthesis is stress dependent that impacts on the type and concentration of sugars. High substrate concentrations create high osmotic stress which increases glycerol 3- phosphate dehydrogenase thus force the yeast cells to accumulate osmolytes such as glycerol protecting the cell against lysis. Furthermore, a rich glucose medium, the so-called Crabtree effect in yeast has happened meaning that under such conditions even at aerobic conditions, cells perform fermentation instead of (or together with) respiration (Marques, 2016). When respiration is repressed, reducing the capability of the organism to reoxidize surplus amounts of NADH formed in biosynthetic reactions (Nissen *et al*., 2000). However, a dramatic decline at the specific growth and glycerol production rates was reported above the optimum glucose concentration; this may result from the inhibitory effect of glucose (Yalçin and Özbaş, 2005). According to Sivasakaran *et al.* (2014), glycerol synthesis by osmotolerant yeasts increases as the glucose concentration is raised until the optimum concentration, after which production decreases.

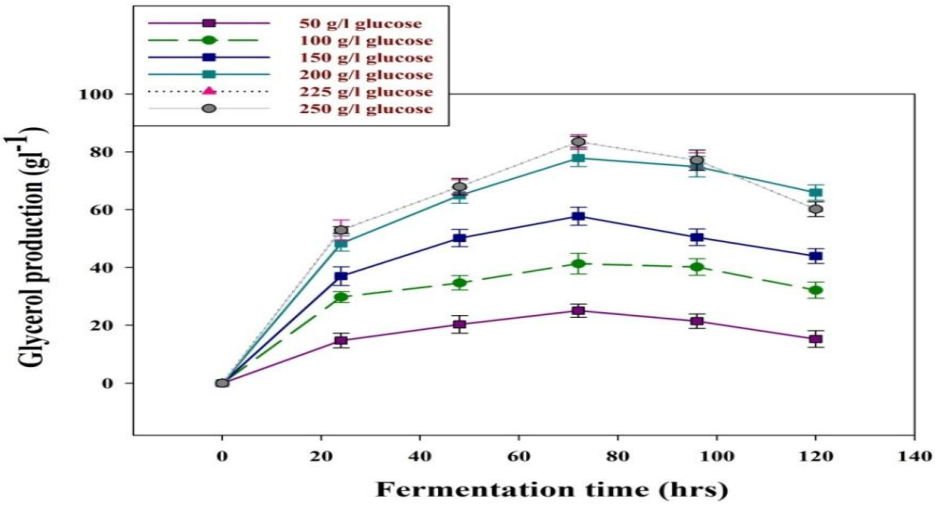

Fig. 4: Variation in glycerol production by *C. orthopsilosis* HH52 on glucose during different fermentation periods.

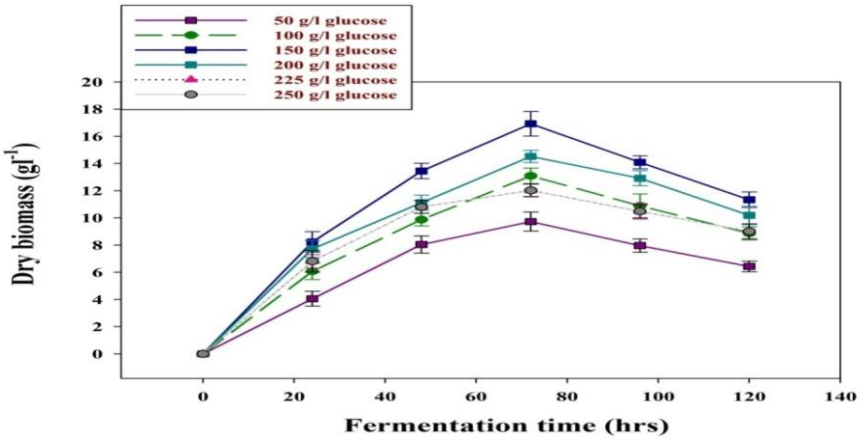

Fig. 5: Variation in dry biomass by *C. orthopsilosis* HH52 on glucose during different fermentation periods.

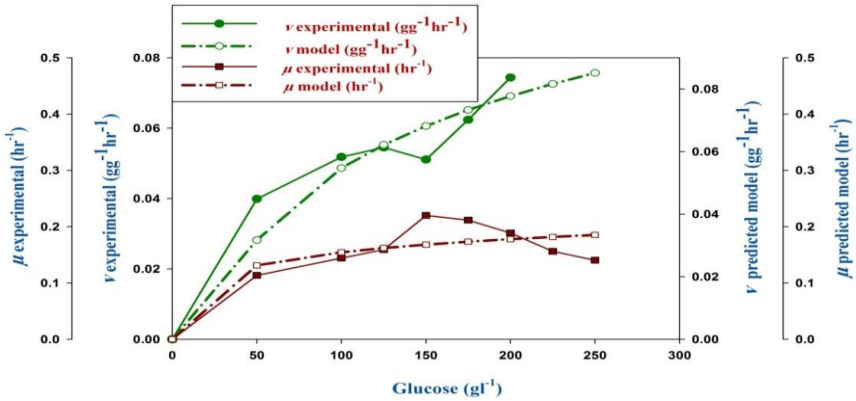

Fig. 6: The experimental and predicted specific growth and glycerol production rates of *C. orthopsilosis* HH52 in Monod kinetic model.

CONCLUSION

Marine yeasts have extraordinary tolerance to extreme environments provides them a unique potential for synthesis of significant biomolecules such as glycerol. The type of carbon source and its initial concentration significantly affects yeast growth and glycerol production. Glucose is the most suitable substrate for yeast growth and glycerol production by *Candida orthopsilosis* HH52. The maximum specific glycerol production rate (v_{max}) was obtained at an initial glucose concentration of 225 gl^{-1}, whereas the maximum specific growth rate (μ_{max}) was attainted at an initial concentration of glucose (150 gl^{-1}).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Ali, N. and Khan, M.N. (2014). Screening, identification and characterization of alcohol tolerant potential bioethanol producing yeasts. Curr. Res. Microbiol. Biotechnol., 2(1): 316-324.

Amin, H.F.; Ahmed, O.M.; Rasmey, A.M.; Younis, A. and Bekhitd, A.A. (2018). Effect of technological processing on the safety of Indian mackerel

(*Rastrelliger kangurata*) from Suez, Egypt. Egypt. J. Aquat. Biol. Fish., 22(5): 283-294.

Ansell, R.; Granath, K.; Hohmann S.; Thevelein J.M. and Adler, L. (1997). The two isoenzymes for yeast NAD+ − dependent glycerol 3-phosphate dehydrogenase encoded by GPD1 and GPD2 have distinct roles in osmoadaptation and redox regulation. EMBO J., 16: 2179-2187.

Babazadeh, R.; Lahtvee, P.J.; Adiels, C.B.; Goksör, M.; Nielsen, J.B. and Hohmann, S. (2017). The yeast osmostress response is carbon source dependent. Sci. Rep., 7: 990.

Bagnato, G.; Iulianelli, A.; Sanna, A. and Basile, A. (2017). Glycerol Production and Transformation: A Critical Review with Particular Emphasis on Glycerol Reforming Reaction for Producing Hydrogen in Conventional and Membrane Reactors. Membr., 7(2): 17.

Bubnová, M.; Zemančíková, J. and Sychrová, H. (2014). Osmotolerant yeast species differ in basic physiological parameters and in tolerance of non-osmotic stresses. Yeast., 31: 309-321.

Chen, X.Z.; Fang, H.Y. and Rao, Z. M. (2008). Cloning and characterization of a NAD+-dependent glycerol-3-phosphate dehydrogenase gene from *Candida glycerinogenes*, an industrial glycerol producer. FEMS Yeast Res., 8(5): 725-734.

Ehsani, M.; Fernández, M.R.; Biosca, J.A.; Julien, A. and Dequin, S. (2009). Engineering of 2,3-butanediol dehydrogenase to reduce acetoin formation by glycerol-overproducing, low-alcohol *Saccharomyces cerevisiae*. Appl. Environ. Microbiol., 75: 3196-3205.

Fan, X.; Burton, R. and Zhou, Y. (2010). Glycerol (Byproduct of Biodiesel Production) as a Source for Fuels and Chemicals - Mini Review. Open Fuel Energ. Sci., 3:17-22.

Ghindea, R.; Vassu, T.; Stoica, I.; Tanase, A.M. and Csutak, O. (2009). Preliminary taxonomic studies on yeast strains isolated from dairy products. Rom. Biotechnol. Lett., 14(1):4170-4179.

Grembecka, M. (2015). Sugar alcohols-their role in the modern world of sweeteners: a review. Eur. Food Res. Technol., 241:1-14.

Hawary, H.; Rasmey, A. M.; Aboseidah, A. A., El-Morsi, E. S. and Hafez, M. (2019). Enhancement of glycerol production by UV-mutagenesis of the marine yeast Wickerhamomyces anomalus HH16: kinetics and optimization of the fermentation process. 3 Biotech, 9(12): 446.

Hohmann, S. (2002). Osmotic stress signaling and osmoadaptation in yeasts. Microbiol. Mol. Biol. Rev., 66: 300-372.

Kayingo, G.; Kilian, S.G. and Prior, B.A. (2001). Conservation and release of osmolytes by yeast during hypo-osmotic stress. Arch. Microbiol., 177: 29-35.

Klein, M.; Swinnen, S.; Thevelein, J.M. and Nevoigt, E. (2017). Glycerol metabolism and transport in yeast and fungi: established knowledge and ambiguities. Environ. Microbiol., 19(3): 878-893.

Kuhn, J.; Müller, H.; Salzig, D. and Czermak, P. (2015). A rapid method for an offline glycerol determination during microbial fermentation. Electron. J. Biotechnol., 18(3):252-255.

Kurtzman, C.P. and Fell, J.W. (1998). The yeasts-a taxonomic study (Kurtzman, C.P. and Fell, J.W. eds.), 4th ed. Elsevier Science Publ. B. V. Amsterdam, the Netherlands, 149(3): 1055.

Leandro, M.J.; Sychrova, H.; Prista, C. and Loureiro-Dias, M.C. (2011). The osmotolerant fructophilic yeast *Zygosaccharomyces rouxii* employs two plasma-membrane fructose uptake systems belonging to a new family of yeast sugar transporters. Microbiol., 157(2): 601-608.

Liu, H.J.; Liu, D.H. and Zhong, J.J. (2003). Novel fermentation strategy for enhancing glycerol production by *Candida krusei.* Biotechnol. Prog., 19(5): 1615-1619.

Lodder, J. and Kreger-Van Rij, N.J.W. (1952). The yeasts: A taxonomic study (Lodder, J. Kreger-Van Rij, N.J.W eds.) Amsterdam: North-Holland Pub New York: Interscience, pp 713.

Loray, M.A.; Spencer, J.F.T.; Spencer, D.M. and Figueroa L.I.C. (1995). Hybrids obtained by protoplast fusion with a salt-tolerant yeast. J. Ind. Microbiol., 14:508-513.

Marques, W.L.; Raghavendran, V.; Stambuk, B.U.; Gombert, A.K. (2016). Sucrose and *Saccharomyces cerevisiae*: a relationship most sweet. FEMS Yeast Res., 6(1): fov107.

McClary, D.O.; Nulty, W.L. and Miller, G.R. (1959). Effect of potassium versus sodium in the sporulation of *Saccharomyces*. J. Bacteriol., 78: 362-368.

Mirończuk, A.M.; Rzechonek, D.A.; Biegalska, A.; Rakicka, M. and Dobrowolski, A. (2016). A novel strain of *Yarrowia lipolytica* as a platform for value‐added product synthesis from glycerol Biotechnol. Biofuels, 9:180

Monod, J. (1949). The Growth of Bacterial Cultures. Annu. Rev. Microbiol., 3:371-394.

Nissen, T.; Hamann, C.W.; Kielland-Brandt, M.C.; Nielsen, J. and Villadsen, J. (2000). Anaerobic and aerobic batch cultivations of *Saccharomyces cerevisiae* mutants impaired in glycerol synthesis. Yeast, 16: 463-474.

Okpokwasili, G.C. and Nweke, C.O. (2005). Microbial growth and substrate utilization kinetics. Afr. J. Biotechnol., 5(4): 305-317.

Orlic, S.; Arroyo-Lopez, F.N.; Huic-Babic, K.; Lucilla, I.; Querol, A. and Barrio, E. (2010). A comparative study of the wine fermentation performance of *Saccharomyces paradoxus* under different nitrogen concentrations and glucose/fructose ratios. J. Appl. Microbiol., 108:73-80.

Pagliaro, M. (2017). Chapter 5 - Glycerol: A Key Platform Chemical of the Forthcoming Bioeconomy. In: Glycerol: The Renewable Platform Chemical. Elsevier Science Publ, B. V. Amsterdam, the Netherlands, pp. 109-132.

Patil, S.V. (2013). Kinetics, Simulation and Scale up of Process for Fermentative Production of Glycerol and Arabitol Using *Hansenula anomala*. PhD thesis in biotechnology. University of Pune. India, pp 258.

Petrovska, B.; Winkelhausen, E. and Kuzmanova, S. (1999). Glycerol production by yeasts under osmotic and sulfite stress. Can. J. Microbiol., 45:695-699.

Rasmey, A. M.; Hawary, H.; Wahid, O. A. and Abo-Seidah, A. A. (2018). Enhancing Bioethanol Production from Sugarcane Molasses by *Saccharomyces cerevisiae* Y17. Egypt. J. Bot., 58(3): 547-561.

Riccombeni, A.; Vidanes, G.; Proux-We´ra, E.; Wolfe, K.H. and Butler, G. (2012). Sequence and Analysis of the Genome of the Pathogenic Yeast *Candida orthopsilosis.* PLoS ONE, 7(4): 4-e35750.

Sahoo, D.K. and Agarwal, G.P. (2001). An investigation on glycerol biosynthesis by an osmophilic yeast in a bioreactor. Process Biochem., 36**:** 839-846.

Sanger, F. Nicklen, S. and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, 74: 5463–5467.

Shoaib, A.; Bhran, A.; Rasmey, A. H. and Mikky, Y. (2018). Optimization of cultural conditions for lipid accumulation by *Aspergillus wentii* Ras101 and its transesterification to biodiesel: application of response surface methodology. 3 Biotech., 8(10): 417.

Singh, V.; Haque, S.; Niwas, R.; Srivastava, A.; Pasupuleti, M. and Tripathi, C.K.M. (2016). Strategies for Fermentation Medium Optimization: An In-Depth Review**.** Front. Microbiol., 7: 2087.

Sivasankaran, C.; Ramanujam, P.K.; Shanmugam, S.; Sathendra, R.; Balasubramanian, B. and Mani, J. (2014). Comparative Study on *Candida Sp.* for the Production of Glycerol. Chem. Tech., 6(12): 5058-5063.

Sulieman, A.; Esra, A.M. and Abdelgadir, W.S. (2015). Isolation and Identification of Yeasts from the Different Stages of *Hulu-mur* Fermentation. J. Microbiol. Res., 5(2):71-76.

Tavanti, A.; Davidson, A.D.; Gow, N.A.; Maiden, M.C. and Odds, F.C. (2005). *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* Groups II and III. J. Clin. Microbiol., 43: 284–292.

Thome, P.E. (2007). Cell wall involvement in the glycerol response to high osmolarity in the halotolerant yeast *Debaryomyces hansenii*. Antonie Leeuwenhoek, 91:229-235.

Tomaszewska, L.; Rywińska, A. and Gładkowski, W. (2012). Production of erythritol and mannitol by *Yarrowia lipolytica* yeast in media containing glycerol. J. Ind. Microbiol. Biotechnol., 39(9): 1333–1343.

Wang, Z.X.; Zhuge, J.; Fang, H. and Prior, B.A. (2001). Glycerol production by microbial fermentation: a review. Biotechnol. Adv., 19: 201-223.

Wickerham, L.J. (1951). Taxonomy of yeasts (Wickerham, L.J. ed.), Tech. Bull. U.S. Department of Agriculture., 1029:1-55.

Yalçin, S.K. and Özbas, Z.Y. (2005). Determination of growth and glycerol production kinetics of a wine yeast strain *Saccharomyces cerevisiae* Kalecik1 in different substrate media. World J. Microb. Biot., 21: 1303-1310.

Zhao, X.; Procopio, S. and Becker, T. (2015). Flavor impacts of glycerol in the processing of yeast fermented beverages: a review. J. Food Sci. Technol., 52(12): 7588-7598.