Assessment of novel yeasts for the production of single cell protein from wasted dates molasses

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Abstract
In the current study non-conventional yeast strains were screened and adapted to produce single-cell protein (SCP) at high productivities and rates from wasted-date molasses. Among the tested yeasts, *Hanseniaspora guilliermondii* JQ690237, *Hanseniaspora uvarum* JQ690236, *Issatchenkia orientalis* JQ690240 and *Cyberlindnera fabiani* JQ690242 emerged as the highest producers of biomass during small scale batch experiments, leading to yields up to 700 g dry biomass/kg of molasses after 48 h of incubation at pH 4.0 and 30 ^{o}C. It was shown that the supplementation of the molasses medium with either organic or inorganic nitrogen sources, enhanced the biotransformation efficiency into single cell protein significantly, with *H. guilliermondii* and *I. orientalis* exhibiting the highest production of biomass with a protein content of up to 54.3%. The scaling up of the process at a 7 L confirmed its efficiency, indicating that new yeasts are promising SCP-producers for possible industrial exploitation of the specific waste towards animal feed.

Introduction
The steadily increasing global population and the subsequent exhaustion of natural resources for animal and human dietary uses have drown the scientific research during the previous decades to seek for new sources of protein production such as microorganisms (Magalhães et al. 2018). As such, single-cell protein (SCP) production technology arose as a promising alternative way to solve the problem of worldwide protein shortage and the focus has shifted to exploit microbes as a potential protein source for humans and as animal feed. Date molasses are produced during dates processing containing high amounts of readily fermentable sugars and other nutrients that can efficiently support yeast growth for SCP production. In the present study molasses from wasted dates were assessed as feedstock for the production of high quantities of single-cell protein by the newly isolated yeasts; *Hanseniaspora guilliermondii*, *H. uvarum*, *Issatchenkia orientalis* and *Cyberlindnera fabiani* from date molasses, focusing on the investigation of the conditions which would favour the overall productivity.

Materials and Methods
Feedstock
The date molasses were produced from spoiled dates from commercial market at Abha city, Kingdom of Saudi Arabia based on the method described by Doma et al. (2013).

Isolation and identification of strains and screening of SCP capacity
Isolates were obtained from different natural sources via the dilution plate method on yeast-malt extract agar (YMA) medium. Screening of the yeast isolates for their single-cell protein (SCP) production efficiency was tested in shaking flasks experiment at 25 °C and 150 rpm for 72 h, with 20%, v:v date molasses (diluted with water) in triplicates. The highest SCP producers were selected were identified by sequencing the variable D1/D2 domain of the large subunit 26S ribosomal DNA. The total yeast genomic DNA was extracted following the protocol of Hesham et al. (2006). The primers: NL1 (5'–GCATATCAATAAGCGGA GGAAAAG-3') and NL4 (5′ GGTCCGTG TTTCAAG ACGG –3') was used to amplify the DNA as described by Kurtzman and Robnett (1998).

Effect of growth conditions on SCP production capacity
The effect of different key parameters effecting yeast growth was investigated aiming to the maximisation of SCP production. Those were incubation time (12, 24, 36, 48, 60, 72, 84 and 96 h), temperature (20, 25, 30, 35 and 40 °C), initial pH values (4.0, 5.0, 6.0 and 7.0), substrate concentration (10, 15, 20, 25 and 30%), nitrogen source (peptone and NH_4CL) and nitrogen concentration (0, 1, 2, 3, 4 and 5 g/l). All experiments were conducted in shaking flasks in triplicate.

Scaling up of SCP production
To validate the scaling up of SCP production process, a fully controlled BioFlo/CelliGen 115 bioreactor (New Brunswick, USA). The reactor was of 7-l capacity and the working volume was 3 l. The sterilized medium (20 %

of date molasses + peptone; 4 g/l) was inoculated with 5% (v:v) of 24 h yeast culture (10^8 cell/ml) obtained from 48-h old culture at 25 °C grown on YPD medium. The temperature of fermentation was maintained at 30 ±1 °C, the pH was regulated at 5.0 and the agitation was 150 rpm. The reactor was maintained under aerobic conditions. Samples were taken after 48 h to estimate the dry weight and protein content of the cells as described above.

Analytical methods

Total dissolved solids (TDS) was quantified according to Standard Methods (APHA, 1995). Dissolved sugars and reducing sugars were quantified according to DuBois et al. (1956) and Miller (1959), respectively. Dry weight of the biomass was calculated as g dry biomass/100 g substrate after dryness in an oven at 80 °C for 24 h following the method of Gao et al. (2007). Protein content (%) was estimated based on the protocol of Zhang et al. (2009) and the crude protein content in the cell was determined according to Strickland and Parsons (1972) of bovine serum albumin as standard.

Statistical Analysis

All statistical analyses were performed using the SPSS 22.0 software (SPSS, 2013). The data were initially examined for their normality of distribution and homogeneity of variance. The significance of variation was assessed using one-way analysis of variance (ANOVA). The least significant difference (LSD) test was used at $P < 0.05$ to identify the significant differences between the means among the treatments and the correlation between the biomass and protein content was estimated.

Results

Characterization of date molasses

Date molasses had the following characteristics: pH, 4.77, moisture, 14.67%, TDS, 77.0% , disolved sugars, 75.0%, reducing sugars, 73.12%, protein, 1.17%. Fat or fibers were not detected.

Effect of growth conditionson SCP production capacity

The optimum incubation time for time for maximum production of biomass and protein content ranged from 48-60 h for the different yeasts, with an apparent strong correlation between biomass and protein whereas the the most appropriate temperature was was 30 °C for all four yeasts. The highest biomass production was detected in case of *I. orientalis* (51.0 g/ 100 g molasses) followed by *H. guilliermondii* (48.2 g/ 100 g molasses), whereas, the highest protein content was detected in case of *H. guilliermondii* (51.5%). Weak acidity (pH 4-5) was the most appropriate for all yeasts to exert their maximum productivity of SCP. At pH 4, *I. orientalis* produced 54.2 g/100 g molasses of biomass and 50.1% of the protein content. *H. guilliermondii* produced 53.0 g/100 g molasses of biomass and 51.7%% of protein content. The productivity of other two yeasts was relatively low compared with either *I. orientalis* or *H. guilliermondii.* The substrate concentration had a strong effect of the growth of the yeast and production of SCP. The most appreciate concentration for all yeast species was 20%. Among the four yeasts, *I. orientalis* produced the highest biomass value (63.5 g/ 100 g molasses). Peptone as nitrogen source, enhanced both the biomass and protein content for all four yeasts whereas the supplementation with NH_4Cl, instead, showed a lower stimulatory effect on the biomass and protein production compared with peptone.

Scaling up of SCP production

The scaling up of SCP production from date molasses (20%), at pH 4, 30 ^{0}C after 48 h of incubation lead to similar biomass and protein content to those obtained from flask experiments. This similarity can be attributed to efficient consumption of sugars which was complete in both flask and scale up experiments.

References

APHA, AWWA, WPCF 1995. Standard Methods for the Examination of Water and Wastewater. M.A. Franson (Ed.) American Public Health Association, Washington, DC.

Doma, M, EL-Shahawy S, Grawish S (2013) PROCESSING OF DATE HONEY. Journal of Food and Dairy Sciences 4 (3):51-64

DuBois, M., Gilles, K., Hamilton, J., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal Chem. 28, 350-356.

Gao, Y., Li, D., Liu, Y. 2012. Production of single cell protein from soy molasses using *Candida tropicalis*. Annals of microbiology 62, 1165-1172

Hesham, A-L, Mohamed H (2011) Molecular genetic identification of yeast strains isolated from Egyptian soils for solubilization of inorganic phosphates and growth promotion of corn plants. J Microbiol Biotechnol 21 (1):55-61

Magalhães, CEB, Souza-Neto MS, Astolfi-Filho S, Matos ITSR (2018) *Candida tropicalis* able to produce yeast single cell protein using sugarcane bagasse hemicellulosic hydrolysate as carbon source. Biotechnology Research and Innovation 2 (1):19-21

Miller, G.L., 1959. Use of dinitrosalicylique acid reagent for determination of reducing sugar. Anal. Chem. 31, 426 – 428.

Strickland, J.D.H., Parsons, T.R. 1972. Kjehldahl method with ninhydrin finish (low levels). A practical handbook of seawater analysis 167, 223-226

Zhang, T, Chi, Z, Sheng, J (2009) A highly thermosensitive and permeable mutant of the marine yeast *Cryptococcus aureus* G7a potentially useful for single-cell protein production and its nutritive components. Marine biotechnology 11, 280-286