American Journal of Food Science and Health
Vol. 5, No. 2, 2019, pp. 46-49
http://www.aiscience.org/journal/ajfsh
ISSN: 2381-7216 (Print); ISSN: 2381-7224 (Online)

Technical Sheet of Enhancement α-glucosidase Production from Yeasts Strains (*Saccharomyces cerevisiae* C8-5 and *Candida tropicalis* C0-7)

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Abstract

Since several years, the enzymes were became uncontournables for agrofood, pharmaceutical, cosmetic industries and constitute therefore a market of several millions. However their production from microorganisms and availability remained limited by the highed cost of culture medium particulary the carbon source. In order to ensure the expansion of the enzyme production industry, it is essential to produce enzymes at a lower cost. Furthermore the use of cheap raw materials would be viable, sustainable and economically solution. Thus locally available substrates such as cereals and tubers, would be compatible with the existing low level of technology in most developing countries. The aim of this study was to optimize α-glucosidase production from strains of *Saccharomyces cerevisiae* C8-5 and *Candida tropicalis* C0-7 by using a starches of some agricultural products of Côte d'Ivoire through assessement various physicochemical parameters. Thus, starches from cereals (maize, millet, sorghum), tubers (cassava, yam) have been tested as source of carbon. α-glucosidase activity was assayed by measuring the release of *p*-nitrophenol from the substrate *p*-nitrophenyl- α -D-glucoside (*p*-NPG). The same method (*p*-NPG) was used to assess effect of physicochemicals parameters on α-glucosidase production. It was found that *S. cerevisiae* C8-5 and *C. tropicalis* C0-7 produced maximum α-glucosidase after 48 hours of fermentation at 37°C, pH 7 using corn starch and millet starch (1 %). The more important α-glucosidase activity were obtained with *S. cerevisiae* C8-5 by using 1 % of corn starch (15.96 U/ml).

Keywords

Saccharomyces cerevisiae C8-5, *Candida tropicalis* C0-7, α-glucosidase, Corn Starch, Millet Starch

Received: March 13, 2019 / Accepted: April 25, 2019 / Published online: May 20, 2019

1. Introduction

Fermentation remains one of the old technologies used for food production and preservation [1]. Traditional beverages in particular, sorghum beer (Tchapalo) are experiencing considerable growth among the various populations in Côte d'Ivoire. During the fermentation process, starter strains produce several metabolites such as organic acids, alcohols, volatile compounds and enzymes that improve the final quality of the product. The use of yeast for enzyme production has several advantages, such as moderate temperature for microbial growth, high metabolic diversity and rapid cell growth, and is more energy efficient. This results in shorter fermentation cycles and easy adaptation of microorganisms to different growing conditions [2]. Production of microbial enzymes is a necessary event in the industrial sectors, due to the high and superior performances of enzymes from different microbes, which work well under

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a wide range of varied physical and chemical conditions [3].

Enzyme mediated processes are rapidly gaining interest because of reduced process time, intake of low energy input, cost effective, nontoxic and eco-friendly characteristics [4-5]. Among the enzymes produced, amylolytic enzymes are contributed to the degradation of starch. These enzymes account for 25% to 33% of international market of marketed enzymes and are used in many industrial processes that require partial or total hydrolysis of starch [6].

Among the amylolitic enzymes, α-glucosidase plays an important role in the process of metabolizing starch. Indeed α-glucosidase is the final enzyme involved in the metabolism of starch to glucose, and is used with α-amylase for the saccharification of starch [7]. These starches from cereals (maize, millet, sorghum...), tubers (cassava, yam) and others could be used as a source of carbon for enzyme production and thus to constitute a substitute of synthetic starch whose accessibility remains difficult because of the relatively high price.

The current study was to investigate the enzymatic potentialities of *Saccharomyces cerevisiae* C8-5 and *Candida tropicalis* C0-7 particularly, α-glucosidase production for using corn starch and millet starch. Various physical and chemical parameters of growth medium were optimized to attain the maximum production yield of α-glucosidase under submerged fermentation technique.

2. Materials and Methods

2.1. Yeast Strains and Culture Conditions

Yeast species of *C. tropicalis* and *S. cerevisiae* used as starters in this study were belonged to the culture collection of the Food Technology Department (University of Nangui Abrogoua). They were isolated from traditional sorghum beer from the district of Abidjan (Southern Côte d'Ivoire). They were identified by PCR-RFLP of the ITS region and sequencing of D1/D2 domains of the 26S rRNA gene [8]. Before their growth on solid state medium, yeasts were cultivated on 868 medium with chloramphenicol at 30°C for 24 h. This medium contained (w/v): glucose monohydrate 2 %, yeast extract (Organotechnie, France) 1 %, peptone casein (Organotechnie, France) 1 % and agar (Merck, Germany) 1.5 %.

2.1.1. Inoculum Preparation

A pure colony (24 hours) of each microorganism was inoculated in Erlenmeyer of 250 ml containing 50 ml of medium 863 (glucose 20 g/l, yeast extract 10 g/l, pepton 10 g/l and chloramphenicol 0.5 g/l). These medium were incubated during 12 hours at 28°C.

2.1.2. α-glucosidase Production

The α-glucosidase production medium is composed per liter of 1% peptone, 1% corn starch or millet starch, 0.5% yeast extract, 0.3% K_2HPO_4 and 0%, 1% KH_2PO_4. In Erlenmeyer of 250 ml containing 54 ml of liquid fermentation medium constituted were inoculated with 6 ml of inoculum. For cocultures, inoculum volume was set out again according the ratios between the microorganisms. For each starch source, four fermentation media were constituted as follows: (1) individual pure fermentation medium with *C. tropicalis* and *S. cerevisiae*; (2) mixed fermentation media of both yeast strains, respectively, in ratios of 2:1 and 1:1 (cell/cell). These media were incubated at 30°C in orbital shaker (shaking incubator) set in 150 rpm during 96 hours. At 0 h, 24 h, 48 h, 72 h and 96 h, samples of 8 ml were collected for *αglucosidase activity* and pH assay. The samples were centrifuged at 5000 rpm at 4°C for 20 mn. The supernatants were collected and α-glucosidase assay was carried out using *p*-Nitrophenol -α-D- glucopyranoside (*p*-NPG) method.

2.2. α-glucosidase Assay

α-Glucosidase activity was assayed by measuring the release of *p*-nitrophenol from the substrate *p*-nitrophenyl- α -D-glucoside (pNPG). An assay mixture (0.25 ml) consisting of a 0.1 M phosphate-potassium (pH 7.0), 15 mM of p-NPG and enzyme solution. After incubation for 20 min at 40°C, the reaction was stopped by the addition of Na_2CO_3 at a concentration of 0.1 M, and absorbance of the reaction mixture was measured at 410 nm. One unit of the enzyme activity was defined as the amount of enzyme liberating 1 µmol of pnitrophenol per minute.

2.3. Effect of Physicochemicals Parameters on α-glucosidase Production

2.3.1. Effect of Incubation Time

The effect of incubation time on enzyme production was investigated by checking the enzyme activity on 0 h, 24 h, 48 h, 72 h and 96 h of incubation of fermentation media (pH 7) in orbital shaker (Shaking incubator, Biobase) at 30°C set in 150 rpm. α-glucosidase activity was assayed by measuring the release of *p*-nitrophenol from the substrate *p*-nitrophenyl- α -D-glucoside (*p*-NPG).

2.3.2. Effect of Incubation Temperature

The effect of incubation temperature on enzyme production was investigated by fermentation in different substrates and incubated at 28°C, 30°C, 37°C and 40°C at pH 7 in orbital shaker (shaking incubator, Biobase) set in 150 rpm for 48 hours. α-glucosidase activity was assayed by measuring the release of *p*-nitrophenol from the substrate *p*-nitrophenyl- α -D-glucoside (*p*-NPG).

2.3.3. Effect of Medium pH

The effect of pH on enzyme production was investigated by adjusting the pH of differents fermentation media to 4, 5, 6, 7, 8, 9 and 10. The media were incubated at 30°C in orbital shaker (shaking incubator, Biobase) set in 150 rpm for 48 hours. α-glucosidase activity was assayed by measuring the release of *p*-nitrophenol from the substrate *p*-nitrophenyl- α -D-glucoside (*p*-NPG).

2.4. Statistical Assay

The results obtained during this study were the subject of a statistical processing with software R version 3.2.2. The averages obtained from three values were compared by variance analysis (ANOVA), then by Turkey test with level of significance 5%.

3. Results and Discussion

The maximum activities were respectively to 5.592 ± 0.18 U/mL, 3.00 ± 0.146 U/mL, 9.147 ± 0.098 U/mL and 4.334 ± 0.22 U/mL for *S. cerevisiae* C8-5, *C. tropicalis* C0-7, thecocultures (1:1) and (2:1) in medium formulated by 1 % of corn starch. In medium formulated by 1 % of millet starch, important activities were obtained by a pure culture of *S. cerevisiae* C8-5 after 48 hours of fermentation, the activity value was 6.37 ± 0.53 U/mL. The cocultures (1:1) and (2:1) are enregistred their important activities at 72 hours of fermentation with a respective values of 4.88 ± 0.34 U/mL and 4.75 ± 0.54 U/mL (Table 1). The most important activities were obtained in a pH range between 6.75 and 7.2, whether its corn starch or millet starch that is used as a carbon source. This observation is similar than Muhammad et al. [9] and Muhammad et al.[10] studies. All cultures recorded their highest activity at 37 ° C and were respectively 5.67 U/mL, 3.75 U/mL, 3.85 U/mL and 3.14 U/mL for *S. cerevisiae* C8-5, *C. tropicalis* C0-7, cocultures (1:1) and (2:1) in medium formulated with 1 % of millet. In production medium containing 1 % of corn starch, the most important activities were recorded with the pure cultures of *S. cerevisiae* C8-5 and *C. tropicalis* C0-7 with respective activity values of 10.924 U / mL and 8.148 U / mL at 37°C (Table2). The optimum temperature for microbial growth usually varies from one microorganism to another and depends on mesophilic or thermophilic nature of microorganism [11]. A similar result was observed with the use of *Bacillus licheniformis* KIBGE-IK4 for maximum production of maltase at 37°C by Muhammad et al. [9]. The activities of the strain cultures increase from pH 4 to pH 6 to reach their maximum at pH 7. These maximums are 6.587 U/mL, 4.903 U/mL, 4.075 U/mL and 3.875 U/mL respectively for *S. cerevisiae* C8-5, *C. tropicalis* C0-7, cocultures (1:1) and (2:1) in medium formulated with 1 % of millet starch. In medium containing 1 % of corn starch, the important activities were obtained at pH 7 and are respectively 9.764 U/mL, 5.835 U/mL, 4.578 U/mL and 4.352 U/mL for *S. cerevisiae* C8-5, *C. tropicalis* C07, cocultures (1:1) and (2:1) (Table 3). The results of our study showed that the production of α-glucosidase was better ensured when fermentation media were brought to pH 7, regardless of the carbon source used. Most bacterial and fungal strains can be grown at pH 6.0-7.0 for growth and enzyme production [7].

Table 1. Effect of time incubation on α-glucosidase activity (U/mL).

			0	24	48	72	96
1% of corn starch	SC	Enzyme activity (U/mL)	0 ± 0 [a]	2.17 ± 0.21 [b]	5.59 ± 0.18 [c]	3.65 ± 0,58 [d]	3.41 ± 0.32 [d]
		pH	7 ± 0	6.7 ± 0.05	6.83 ± 0.02	7.45 ± 0.06	7.87 ± 0.03
	CT	Enzyme activity (U/mL)	0 ± 0 [a]	2.27 ± 0.23[b]	3 ± 0.146 [c]	2.05 ± 0.3 [b]	1.75 ± 0.18 [d]
		pH	7 ± 0	6.85 ± 0.01	6.95 ± 0.03	7.23 ± 0.02	7.64 ± 0.03
	1:1	Enzyme activity (U/mL)	0 ± 0 [a]	2.43 ± 0.24 [b]	9.14 ± 0.09 [c]	2.71 ± 0.31 [d]	2.03 ± 0.07 [e]
		pH	7 ± 0	6.64 ± 0.02	6.76 ± 0.01	7.03 ± 0.01	7.26 ± 0.01
	2:1	Enzyme activity (U/mL)	0 ± 0 [a]	1.72 ± 0.27 [b]	2.27 ± 0.32[c]	3.79 ± 0.4 [d]	2.01 ± 0.2 [c]
		pH	7 ± 0	7.1 ± 0.01	7.28 ± 0.02	7.46 ± 0.02	7.68 ± 0.03
1% of millet starch	SC	Enzyme activity (U/mL)	0 ± 0 [a]	3.64 ± 0.65 [b]	6.37 ± 0.53 [c]	4.56 ± 0.45 [d]	3.75 ± 0.08 b
		pH	7 ± 0	6.62 ± 1.05	6.94 ± 0.97	7.14 ± 1.02	7.47 ± 0.89
	CT	Enzyme activity (U/mL)	0 ± 0 [a]	3.45 ± 0.76 [b]	4.84 ± 0.87 [c]	4.27 ± 0.57 [d]	3.55 ± 0.35 [b]
		pH	7 ± 0	6.53 ± 0.78	6.76 ± 0.89	6.98 ± 0.98	7.23 ± 0.75
	1:1	Enzyme activity (U/mL)	0 ± 0 [a]	3.38 ± 0.33 [b]	4.12 ± 0.65 [c]	4.88 ± 0.34 [d]	3.78 ± 0.27 [e]
		pH	7 ± 0	6.48 ± 0.63	6.71 ± 0.75	7.05 ± 0.88	7.23 ± 1.12
	2:1	Enzyme activity (U/mL)	0 ± 0 [a]	3.33 ± 0.47 [b]	3.89 ± 0.43 [c]	4.75 ± 0.54 [d]	3.67 ± 0.58 [c]
		pH	7 ± 0	6.56 ± 0.95	6.86 ± 0.87	7.12 ± 0.67	7.48 ± 0.88

On the same line and for each type starch, the values with the same letters do not present a significant difference to the level of 5 %

Table 2. Effect of temperature on α-glucosidase activity (U/mL).

		28°C	30°C	37°C	40°C
1% of corn starch	SC	2.13±0.14[a]	2.61±0.13[b]	10.92±0.66[c]	7.56±0.1[d]
	CT	2.17±0.21[a]	3.33±0.2[b]	8,148±0.96[c]	6.98±0.54[d]

		28°C	30°C	37°C	40°C
1% of millet starch	(1:1)	2.05±0.12[a]	3±0.29[b]	3,814±0.27[c]	2.76±0.17[b]
	(2:1)	2.25±0.20[a]	2.35±0.13[a]	2,976±0.17[b]	2.42±0.38[a]
	SC	2.36±0.07[a]	3.49±0.08[b]	5.67±0.06[c]	3.86±0.05[d]
	CT	1.87±0.08[a]	2.65±0.06[b]	3.75±0.05[c]	2.95±0.07[b]
	(1:1)	1.36±0.06[a]	3.25±0.07[b]	3.85±0.06[c]	3.17±0.07[b]
	(2:1)	1.46±0.08[a]	2.87±0.06[b]	3.14±0.07[c]	2.57±0.09[b]

In the same column and for each type starch, the values with the same letters do not present a significant difference to the level of 5 %

Table 3. Effect of pH on α-glucosidase activity (U/mL).

		4	5	6	7	8	9	10
1% of corn starch	SC	1.84±0.07[a]	3.94±0.05[b]	6.23±0.09[c]	9.76±0.07[d]	7.02±0.03[e]	2.78±0.09[f]	0,90±0.07[g]
	CT	0,94±0.03[a]	1.84±0.03[b]	3.89±0.02[c]	5.83±0.02[d]	3.85±0.09[c]	1.97±0.02[b]	0.78±0.03[a]
	(1:1)	1.64±0.05[a]	2.533±0.03[b]	2.97±0.03[c]	4.57±0.05[d]	2.98±0.05[c]	1.45±0.05[a]	1.08±0.04[e]
	(2:1)	0.76±0.05[a]	1.834±0.02[b]	2.68±0.05[c]	4.35±0.07[d]	2.63±0.06[c]	1.86±0.04[b]	1.16±0.07[e]
1% of millet starch	SC	1.27±0.04[a]	2.38±0.09[b]	3.56±0.07[c]	6.58±0.04[d]	4.49±0.07[e]	1.14±0.05[f]	0.76±0.09[g]
	CT	0.83±0.04[a]	1.74±0.03[b]	2.41±0.02[c]	4.90±0.05[d]	2.98±0.02[e]	0.95±0.03[a]	0.56±0.04[g]
	(1:1)	0.79±0.05[a]	2.16±0.06[b]	3.89±0.04[c]	4.07±0.02[d]	2.54±0.04[e]	1.15±0.07[f]	0.56±0.03[g]
	(2:1)	0.87±0.03[a]	1.54±0.03[b]	2.59±0.02[c]	3.87±0.03[d]	3.06±0.03[e]	1.25±0.09[f]	0.36±0.05[g]

In the same column and for each type starch, the values with the same letters do not present a significant difference to the level of 5 %

4. Conclusion

Our strains are produced amylolytic activity and particularly α-glucosidase activity which has been greater with a pure culture of *S. cerevisiae* C8-5. The corn starch and millet starch could be used as carbon source for α-glucosidase production. That would reduce the synthetic starch dependence and could constituite a veritable substrate for industrial production of enzyme.

References

[1] Holzapfel, W. H. (2002). Appropriate starter culture technology for small-callefermentation in developing countries. International Journal of Food and Microbiology, 75: 197-212.

[2] Oliveira, A. P. A., Silvestre, M. A., AlvesPrado, H. F., Rodrigues, A., Da Paz, M. F., Fonseca, G. G., and Leite. (2015). Bioprospecting of yeasts for amylase production in solid state fermentation and evaluation of the catalytic properties of enzymatic extracts. African Journal of Biotechnology, 14 (14): 1215-1223.

[3] Rajendra, S. Manoj, K. Anshumali, M. and Praveen, K. M. (2016). Microbial enzymes: industrial progress in 21st century. 3 Biotechnology, 6: 174, 15 p

[4] Li, S. Yang, X. and Yang, S. (2012). Technology prospecting on enzymes: application, marketing and engineering. Comput Struct Biotechnol Journal, 2: 1-11.

[5] Choi, J. M. Han, S. S. and Kim, H. S. (2015). Industrial applications of enzyme biocatalysis: current status and future aspect. Biotechnology Advances, 33: 1443-1454.

[6] Özdemir, S. Matpan, F. Güven, K. and Baysal, Z. (2014). Production and characterization of partially purified extracellular thermostable α-amylase by *Bacillus subtilis* in submerged fermentation (SmF). Prep. Biochemical and Biotechnology, 41 (4): 365-38.

[7] Gupta, A. K. and Gautam, S. P. (1993). Purification and properties of an extracellular a-glucosidase from *the thermophilic fungus Malbranchea sulfurea*. Journal of General Microbiology, 139: 963-967.

[8] N'guessan, F. K. Brou, K. Noémie, J. Casaregola, S. and Djè, K. M. (2011). Identification of yeasts during alcoholic fermentation of tchapalo, a traditional sorghum beer from Côte d'Ivoire. Antonie van leeuwenhoek, 99 (4): 855-864.

[9] Muhammad, A. N. Bibi, Z. N. Ansari, A. Zohra, R. R. and UI-Qader, S. A. (2014). Enhanced production of maltase (αglucosidase) from newly isolated strain of *Bacillus licheniformis* KIBGE-IB4. Pakistan Journal of Pharmaceutical Sciences, 27 (5): 1437-144.

[10] Muhammad, A. N. Bibi, Z. N. Asad, K. Haneef, U. R. Muhsin, J. Tour, J. Afsheen, A. and Qader, U.S.A. (2016). Production of α-1,4-glucosidase from *Bacillus licheniformis* KIBGE-IB4 by utilizing sweet potato peel. Environnement Science of Pollution Research, DOI 10.1007/s11356-0168168-x.

[11] Kumar, C. G. and Takagi, H. (1999). Microbial alkaline proteases: from a bioindustrial viewpoint. Biotechnology Advances, 17: 561-594.