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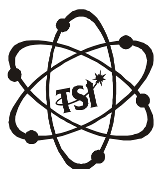

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Optimization of the synthesis parameters ß-fructofuranosidase extracellular for strain isolated Candida guilliermondii brine olives

M.Sobh[1], A.Chaouch[1], A.Echchelh[2*], H.Oudda[3], M.Ouhssine[1]

[1]Laboratory of Biotechnology, Environment and Quality, Faculty of Science, Ibn Tofail University, BP 133, 14000 Kenitra, (MOROCCO)

[2]Laboratory of Electrical Engineering and Energetic, Faculty of Science, Ibn Tofail University, BP 133, 14000 Kenitra, (MOROCCO)

[3]Laboratory Processes of Separation, Department of Chemistry, Faculty of Science, Ibn Tofail University, BP 133, 14000 Kenitra, (MOROCCO)

E-mail: sobh72@yahoo.fr

ABSTRACT

Six strains of lactic acid bacteria and yeasts six strains were isolated from brine olives, carrots and artichokes spontaneous fermentation. One of them has been selected and is Candida guilliermondii. Synthesizes a ß-fructofuranosidase extracellular activity with a maximum of 1488 µM.l-1.min-1 after 24 h of culture at 30 ° C and pH 5 in the presence of 4 g / l sucrose.

And Lactobacillus plantarum synthesizes the bacteriocin.

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KEYWORDS

Brine;
ß-fructofuranosidase;
Candida guilliermondii;
Fermentation;
Olives.

INTRODUCTION

Saccharolytic enzymes are used in the food industry to hydrolyze sucrose into glucose and fructose more soluble. Invertase is also working to produce artificial honey[17]. Invertase is constituted by a wide variety of microorganisms[3,7,9-11,19,23-24].

There are endo and extracellular invertases[7,8]. Invertase endocellular is obtained from yeast (Saccharomyces cerevisiae, S. carlsbergensis) by cell lysis in the presence of toluene[17]. Yeast can be used in conjunction with lactic acid bacteria[21] fermentation plant. The sucrose hydrolysis may be carried out by lactic acid bacteria[5], lacti cacid. product with the favors banning unwanted germs. The hydrolysis of sucrose is complicated because many variables determine the productivity, temperature, pH, agitation, etc..[20]. The present work aims at the isolation and purification from olives fermentation of a strain of yeast with a saccharolytic acidifying power and ß-fructofuranosidase activity levels and optimize its growth conditions and enzyme production for subsequent application in controlled fermentation of olives.

MATERIALS AND METHODS

Isolation and purification of stem

Spontaneous fermentations are carried out by dipping olives, artichokes and olives well sorted and prepared in brines at different concentrations of sodium

chloride (5, 10, 15 and 30%) for the isolation of yeasts. Like any vegetable fermentation, the presence of lactic acid bacteria was noted. Yeasts were isolated on PDA (Potato Dextrose Agar) from brine olives. The yeasts isolated were purified by four successive rounds of subculturing on PDA medium. The purity of yeast is checked under a microscope. Conservation is carried out on PDA agar inclined tubes at 4°C with subculturing every three months.

Selection and identification of strains

The purified yeast acidifying power is estimated by measuring the final pH after incubation at 30°C for 48 hours in a semi-synthetic liquid (3g glucose, 3g yeast extract, 1g $(NH_4)2SO_4$, 1g $MgSO_4$, 1g KH_2PO_4, 1L distilled water) at pH of 5.55 obtained by addition of NaOH and HCl.

A synthetic culture medium solid (3g sucrose, 1g $(NH_4)2SO_4$, 1g $MgSO_4$, 1g KH_2PO_4, 1L of distilled water, 15g/l (agar) sterilized 15 min at 120°C is used for the selection of strains to saccharolytic to promote the use of the carbon source (saccharose) by yeast in the absence of yeast extract. yeast was able to grow on synthetic medium.

SMLV1 yeast was selected for further work. Identification is performed by API 20C AUX gallery (bioMérieux). Other yeasts were cultured on medium acetate (sodium acetate 4g/L, yeast extract 0.1g/ L) solid (20g/L agar) 1 to 3 weeks at 30°C for the equality.

Location fructofuranosidase activity

Cultures were subjected to a semi-synthetic liquid sucrose 3g stopped after 24h of incubation at 30°C. After centrifugation for 20 min at 12,000 g, we obtain the culture medium and a pellet containing the cells. Part of the cells was resuspended in a phosphate buffer (0.1M, pH5) and then lysed by sonication. Another centrifugation at 12,000 g results in a soluble fraction and an insoluble fraction.

The determination of enzyme activity is performed for each fraction using the method of Somogyi[22] and Nelson[14]: the reaction mixture contains 0.1 ml of each fraction (the pellet after three washes in phosphate buffer is suspended again in the buffer), 0.25ml of 0.1 M sucrose and 0.15 ml of phosphate buffer. The reaction mixture was incubated for 10min in a water bath at 40°C. The reaction was stopped by addition of Somogy reagent followed by heating for 15min in a water bath at 100°C[25] to precipitate reducing sugars. 1 ml of Nelson reagent, revealing color is added after cooling and the absorbance of each sample was read at 540nm using the indicator Without sucrose. The amount of reducing sugars was determined by reference to a standard curve from a solution containing 0.3g/l glucose and 0.3g/l of fructose and enzyme activity is expressed in$\mu M.l^{-1}min^{-1}$.

Optimization of cell growth and enzyme production

Subsequently, a semi-synthetic fluid (sucrose 4g, 3g yeast extract, 1g (NH_4) $2SO_4$, 1g $MgSO_4$, 1g KH_2PO_4, 1L distilled water) is used to highlight the optimal conditions for growth of yeast and biosynthetic enzyme. Incubation is carried out for 24h at 30°C at pH 5.5 by default. Various conditions were tested:

- Concentration of sucrose (1, 2, 3, 4, 5 and 7g/l);
- Carbon source to 4g/l (glucose, galactose, starch, lactose, mannitol) in the absence of sucrose in addition to a test sample ontaining sucrose 4g/l;
- PH (3, 3.5, 4, 4.5 and 5), the enzyme activity is controlled to 24 hours and the pH and biomass (absorbance at 600 nm) at 48h;
- Inorganic nitrogen source to 1g/l ($NH_4H_2PO_4$, $NaNO_3$, (NH4) $2SO_4$) or organic 3g/l (peptone, yeast extract)
- Temperature (30, 35, 40, 45 and 50) with monitoring the growth and enzyme production.

RESULTS

Isolation and purification of stem

Among the six yeasts isolated, the lowest pH was obtained with SMLV1, which is Candida guilliermondii. It is capable of lowering the pH from 5 to 4.25 after 48 h of incubation in the semi synthetic and has good growth on solid synthetic medium with 3g/l sucrose.

Localization of the enzyme activity

The enzymatic activity is found primarily in the culture medium (TABLE 2).

Optimization of cell growth and enzyme production

Enzyme activity increases with the concentration of sucrose to a maximum of 4g/L (TABLE 3).

TABLE 1: Acidifying power of six strains of yeasts isolated from brine olives after 48 h incubation at 30 ° C in a semi-synthetic liquid growth on solid synthetic medium.

Strain	Determination	synthetic medium liquid 3 g / l glucose 3g/l yeast extract pH initial	pH at 48 h	synthetic medium solid 3 g/l sucrose 0 g / l yeast extract growth (+) non-development (-)
SMLV1	Candida guilliermondii	5.55	4.25	+
L2	Candida sp	5,55	4,88	-
L3	Candida sp	5,55	5,28	-
L4	Candida sp	5,55	5,35	-
L5	Candida sp	5,55	5,44	-
L6	Candida sp	5,55	5,72	-

TABLE 2 : Localization of beta-fructofuranosidase activity after culture of the strain of Candida guilliermondii L2 24 h at 30°C in a liquid semi-synthetic medium with 3g / l of sucrose.

Fraction	Enzyme activity (µM.l^{-1}.min^{-1})
Suspension at 1200 g	1,047
Pellet at 12,000 g	36
Soluble fraction after sonication of the pellet	85
Insoluble fraction after sonication of the pellet	83

TABLE 3 : Influence of sucrose concentration on cell growth and activity ß-fructofuranosidase L2 strain of Candida guilliermondii after 24 h at 30°C in liquid semisynthetic medium.

Sucrose (g/L)	$A_{24\,h}$ 600 nm	pH initial	pH To 48h	Activity enzymatic to 24 h (MM.l^{-1}.min^{-1})
1	1,65	5,55	4,82	85
2	1.67	5.55	4.75	897
3	1.65	5.55	4.36	1,243
4	2.85	5.55	4.24	1,488
5	1.77	5.55	4.30	1,396
7	2,11	5,55	4,72	877

The enzymatic activity is almost zero for simple sugars (TABLE 4).

TABLE 4 : Influence of the nature of the carbon source on the strain of SMLV1 Candida guilliermondii on semi-synthetic fluid at 30 ° C.

carbon source	$A_{24\,h}$	enzyme activity (MM.l^{-1}.min^{-1}) to 24 h
Sucrose	2.62	1327
galactose	1.85	42
Starch	0.96	34
Lactose	1.97	85
Mannitol	0.98	73
Glucose	1,67	68

The optimum pH of the enzyme production is 5 (TABLE 5).

TABLE 5 : Influence of pH on the strain of Candida guilliermondii SMLV1 on semi-synthetic liquid at 30°C.

pH initial	pH to 48 h	$A_{24\,h}$ 600 nm	Enzyme activity (MM.l^{-1}.min^{-1}) to 24 h
3	5.27	0,57	654
3.5	5.46	1.56	773
4	4.76	1.77	792
4.5	4.74	1.88	878
5	4,23	2,57	1174

Gives good growth with a high enzymatic activity in the presence of (NH4) 2SO4 (TABLE 6). The optimum growth and the maximum activity is obtained at 30 ° C.

TABLE 6 : Influence of nitrogen source and temperature on the strain of Candida guilliermondii SMLV1 on semi-synthetic fluid.

	$A_{24\,h}$ 600 nm	enzyme activity (MM.l^{-1}.min^{-1})
Nitrogen source		
peptone	1.25	867
Yeast extract	1.17	834
(NH4) 2SO4	2.54	1,337
NaNO3	0.75	735
NH4H2PO4	0.94	832
Peptone + (NH4) 2SO4	1.32	678
Temperature		
30 ° C	2.67	1,310
35 ° C	0.98	120
40 ° C	1.26	735
45 ° C	1.26	862
50 ° C	1.34	472

DISCUSSION AND CONCLUSION

Several species of yeasts were isolated from olives fermentation. Indeed, the yeast in their order of suc-

cession acidotolérance and adaptation in the fermentation of olives.

Among the six strains isolated brine olives, only one strain could grow in a synthetic medium containing sucrose and lacking yeast extract. It is Candida guilliermondii.

The enzyme activity is mainly in the culture medium, suggesting that beta-fructofuranosidase elaborated mainly extracellular.

The optimization of growth parameters SMLV1 strain of Candida guilliermondii has achieved enzymatic activity of 1488 $\mu M.l^{-1}.min^{-1}$.

Beyond 4g/l, sucrose is inhibiting the growth and development of the saccharolytic activity in agreement with other authors[2,12]. Receivers enzyme could be saturated. To a lower concentration, the yeast grow less because less substrate is available to them.

Unlike sucrose, the enzyme activity is almost zero for simple sugars. We conclude that it is an enzyme induced.

The optimum pH of the enzyme production of 5 is consistent with the literature data. Yeasts grow in a wide pH range of 2.5 to 5.5 with an optimum value at about 4 to 5[15] and the optimum production of the enzyme is found to pH 4.5 to 5[1,4,26].

Strain Candida guilliermondii appreciate ammonium sulphate and less other nitrogen sources.

Every organism requires a certain temperature for optimal growth. Enzymes are extremely sensitive to environmental conditions, especially temperature and pH[16]. High temperatures cause denaturation of the enzyme[2]. Invertase is denatured at -7°C and 45°C[13] in Saccharomyces.

Candida guilliermondii has an optimum growth and production of fructofuranosidase at 30°C and can be considered a mesophilic yeast.

Fructofuranosidase in the presence of C. guilliermondii could find applications in biotechnology. SMLV1 strain is also tested for controlled fermentation of olives in combination with lactic acid bacteria[6].

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