International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

RESEARCH ARTICLE

Selection of yeast strain producing acetic acid

Latifa Elyachioui [1]*, Abdel-ilah Aziane [1] Mohamed Elyachioui[1], and Mohammed Ouhssine[1]

*[1]Laboratory of Biotechnology, Environment and Quality, Faculty of Sciences BP 133, 14000 Kenitra Morocco.
*Author to whom correspondence should be addressed/E-Mail: elyachioui@hotmail.com
Received: May 2017 / Accepted: May 2017/ Published: June 2017

ABSTRACT: A yeast strain E2 was purified from traditional yeast, and retained for its strongly acidifying, fermentative and saccharolytic power. In fact, this strain produces a high concentration of acetic acid 105.85 mg / L revealed by using the H.P.L.C DAD technique during its growth in semi synthetic medium containing sucrose at 5 g /l as only carbon source. The pH of the culture medium increases from 5.58 to 2.76 after 24 hours of culture and to 2.48 after 48 hours of incubation. The initial biomass (D.Oi) increases from the value 0.659 to 5.36 after 24h and to 8.38 after 48h under the same conditions.
Keywords: yeast, acetic acid, acetic bacteria, alcoholic fermentation.

INTRODUCTION

Generally, industrial production of acetic acid is carried out synthetically, by carbonylation of methanol. Bacterial fermentation only affects 10% of production, but it is still important for the manufacture of vinegar, since in most countries the vinegar for food must be of biological origin (Yoneda, Noriyki et al., 2001). World demand for acetic acid is estimated at about 6.5 million tons per year (P.r "Chem. Eng. News, 2005; Suresh Bala, 2003). Acetic acid is a product of the alcoholic fermentation of sugar. It is a volatile acid that its production remains low and less than 1g.L-1. Higher grades may appear in the case of special Wine making (sweet white) or especially in the case of bacterial attack by lactic acid bacteria from sugars or acetic bacteria from alcohol (Divies C., 1989). Acetic bacteria are responsible for the transformation of ethanol synthesized by yeast into acetic acid, the main constituent of vinegar (Boughnou N.,1988). A few studies treat the acetic yeasts are described in the literature.

In the present work, we have been able to select a yeast strain with acidifying, fermenting and saccharolytic power from 28 yeast strains isolated and purified from the various biotopes (lemon, grape, sugarcane and of flour). This strain produces a large amount 105.85 mg /L of acetic acid revealed by using H.P.L.C technique during its growth in semi-synthetic medium containing 5 g /L of sucrose as only carbon source. The strain was subjected to study the various parameters influencing its growth, the production of the sacchrolytic enzyme and the production of acetic acid and to study different parameters as pH, temperature, carbon and nitrogen sources. Production trials have been carried out in a fermented and have been shown that the potential industrial production is promising.

MATERIALS AND METHODS

2.1. Yeasts isolation and identification of yeast strains

The isolation of the yeasts was carried out on a semisynthetic medium containing 5g sucrose, 3g yeast extract, 0,5g MgSO4, 3g KH2PO4, 1g (NH4)2SO4, 2ml of the mineral solution of Cooney & Levine,1972, Sufficient for 1L of distilled water. The solid medium is obtained by adding 15g /l of agar. The media are sterilized during 30min at 105 °C. The carbohydrates are added after filtration on a millipore membrane (0.45 µm) at 40 °C. Only those strains with a high acidifying capacity and whose pH of the culture medium is strongly acidic were retained. The isolated strains are purified after five successive cycles of sub culturing in a semi-synthetic liquid medium at 30 °C and then purified on a semi-synthetic solid medium.

The yeast strains thus purified are then stored at 4 °C. on LB agar (10g bactopeptone,10g NaCl, 20g glucose, 5g yeast extract, 15g agar, Sufficient for 1L distilled water). The isolated strain was preliminary identified by API 20E method, the principle of which is based on the assimilation of sugars. 0.1 ml of the above liquid semi-synthetic medium was placed in each well; after 24 hours of incubation, identification is carried out according to the manufacturer's instructions.

2.2. Monitoring of pH purified yeast strains

The purified yeast strains were grown for 48 hours on a liquid synthetic saccharose medium as a carbon source. The cultures are carried out in 100 ml flask at 30 °C and in the dark with the oxygenation of the medium by using the rotary agitation (105 rpm). pH

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

values were measured by a pH-meter apparatus of the Orion Research type combined electrode. The biomass was followed by measurement of the optical density by spectroscopy method at a wavelength of 600 nm.

2.3. Measurement of the acidifying power of yeast strains

The initial and final acidity assay of the cultured strains on a semi-synthetic saccharose medium was carried out on 2 ml of culture medium using 0.1N sodium hydroxide solution using a Mohr-cruet. In the presence of a drop of a methanol solution of 1% phenolphthalein used as a color indicator.
The acidity is expressed in mg of lactic acid (MW = 90.08 g) per 100 ml of culture.

2.4. Quantification of the production of organic acids by yeast strains

Preparation of sample

The production of the organic acids during the growth of the isolated strain was monitored on a semi-synthetic medium containing sucrose as a carbon source at 5g /l. The pH was set at 5.5 and then, after homogenization, the medium was distributed in well-closed flasks for sterilization at 120 °C for 20 min. The medium is subsequently seeded by the strains and grown at 30 °C with stirring for 24 h. The assay is carried out on the supernatant obtained by centrifuging the culture at 10,000 rpm for 10 min.

2.5. Analysis of the sample

The supernatant was filtered through 0.45 µm membranes and analyzed by high performance liquid chromatography (HPLC) with UV detection (diode arrays) at 210 nm wavelength.
The determination of lactic, acetic and citric organic acids was carried out by the external calibration method with reference to their standards analyzed under the same conditions.

2.6. Study of the parameters influencing the growth of strain E2

2.6.1 Effect of carbon source

The Strain E2 is grown at 30 ° C in several semi-synthetic media each containing a different carbon source. We have prepared media based on monosaccharides; glucose, fructose and galactose disaccharides, lactose, maltose and sucrose; and polysaccharides: starch and inulin. The concentration of dose was set at 5 g /L and the initial pH of the medium was set at 5.5. The evolution of the pH was followed during the growth of the strain isolated in various media at 30 °C for 48 hours.

2.6.2. Effect of sucrose concentration

We prepared semi-synthetic culture media at different concentrations of sucrose: 1g /l; 3g /l; 5g /l; 7g /l and 9g /l and control 0g /l. These media are distributed in flasks and sterilized at 120 ° C for 20min and then seeded by the strain and incubated for 48h at 30 °C with rotary stirring at105 rpm. We followed the variations in biomass, pH and acidity for T(0h), T(24h) and T(48h).

2.6.3. Effect of the pH of the culture medium

The strain E2 was grown on a semi-synthetic medium based on sucrose at 5 g /l on various pH of the medium, namely 4; 5; 5.5; 6; 6.5; 7; 8 and 9. The cultures are carried out in 100 ml flasks containing 20 ml of culture medium at 30° C for 48 hours and in the dark with rotary agitation at 105 rpm. We determined the biomass, the pH of the acidity for T0, T24 h and T48h.

2.6.4. Effect of nitrogen source

Similarly, we studied the effect of the nitrogen source by culture of the strain on a semi-synthetic medium containing a mixture of two sources of nitrogen, organic and inorganic. The inorganic nitrogen sources used at a concentration of 1g /l are (NH4) 2SO4, NH4H2PO4, NaNO3, urea. The sources of inorganic nitrogen used at a concentration of 3 g /L are yeast extract, peptone and urea. The pH of the culture medium was set at 5.5 and the strain was cultured at 30 °C for 48 h.

2.7. Kinetics of acetic acid production and saccharolytic activity

The yeast strain was cultured in a liquid semisynthetic medium (1g (NH4) 2SO4, 0.5g MgSO4, 3g KH2PO4, 3g yeast extract, 2ml Coonney and Levine microelement solution (Cooney C.L. & Levine D.W., 1972), Sufficient for 1L of distilled water), and various sources of carbon:

- Sucrose to 10g/L.
- Glucose 5g/l and fructose 5g/L.
- Fructose 10g/L.
- Glucose 10g/L.

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

The pH of the culture medium is initially set at 5.5 and the culture medium is sterilized at 120 °C for 20 minutes.

The strain growth monitoring in these four culture media was carried out every 2 hours for 24 hours, and the biomass was determined by spectroscopy at a wavelength of 600 nm. The variation in the carbon source concentration was monitored by the Somogyi and Nelson method (Somogyi M. 1952) and Nelson methods (Nelson N. 1944), and the production of acetic acid was carried out every 2 hours by the high performance liquid chromatography technique with UV detection (Diode arrays) at the wavelength 210nm.

2.8. Determination of saccharolytic activity

The saccharolytic activity is determined by assaying the reducing sugars described by Somogyi (Somogyi M. 1952) and Nelson methods (Nelson N. 1944). The reaction medium contains 100 µl of a diluted liquid culture at the tenth and 150µl of phosphate buffer at pH equal to 5.5. The mixture is incubated in 3min water bath at 40 °C. 250 µl of sucrose are then added to 4 g /l and incubated for 10min at 40 °C. The reaction is stopped by adding 2 ml of the Somogyi reagent and the mixture is heated at 100 °C for 15min. 1 ml of Nelson's reagent is added after cooling. The absorbance of each sample is read at 540 nm compared to that of its control, consisting of the same mixture reaction, the sucrose substrate being replaced by phosphate buffer. The zero is made with a tube containing only the reagents. The determination of the amount of reducing sugars is determined from a calibration curve from a stock solution containing a mixture of 0.3g /l of glucose and 0.3g /l of fructose.

RESULTS AND DISCUSSION

28 yeast strains were isolated from the various biotopes (Table 1), on semi synthetic medium, we were able to classify these yeasts in tree classes according to the acidifying power. The first class comprises strongly acidifying strains whose pH can decrease to 3.12 after 24 hours of incubation and to a value lower than 3 after 48 hours of incubation. The second contains strains whose pH is 4 <pH <5 and the third class the pH is greater than 5. The strain E2 classified among the strongly acidifying strains has been retained for the rest of this study.

Among the 28 selected strains yeast, 7 strains were selected in this study because of their high acidifying power. These strains are called EL1, EL2, E1, E2, E3, E4 and E5.

The qualitative and quantitative analysis of the various organic acids determined by UV detection using HPLC technique (Fig. 1) showed that the 7 strains isolated produce organic acids (acetic acid, lactic acid and citric acid) at various concentrations depending on the strains isolated. The strain E2 showed a large amount of the acetic acid production 105.85 mg/l, although this strain was retained for further processing, the other strains are in the process of the advanced study.

3.1. pH effect in the culture medium on the growth of strain E2

The effect of the initial pH of the semi-synthetic medium on the growth of the strain was studied by monitoring the evolution of the biomass determined by D.O using spectrophotometry method at a wavelength of 600 nm at different pH values at 30 °C. The obtained results (Fig. 2) shows that the maximum growth was obtained at pH = 5.5 with a value of 1.340 by D.O method.

3.2. Carbon source effect on strain growth

Under optimum conditions (pH=4.5 at 30 °C), the growth of the strain was monitored in different media containing the monosaccharides (Fig. 3). Glucose galactose and fructose as a source of carbon showed a significant growth as a function of time, in particular on medium containing glucose (the most assimilable sugar by strain E2) and fructose. The pH initially decreases from 5.5 to 2.78 in the case of glucose and 2.86 in the case of fructose and reaches 3.02 and 3 biomass, respectively. As for disaccharides, the growth of the strain is very important in the case of sucrose and maltose with respectively 3.60 and 3.16 of the biomass and 2.87 and 3.02 in the pH value. The results are shown in figure 3.

3.3. Effect of sucrose concentration on the strain growth and with pH

The pH of the culture medium fixed at the start of the culture at 5.5 was greatly reduced to 2.4 at the concentration of 4g /l. FIG. 4 shows the results of this culture (Fig. 4).

The strain was grown in different concentrations of sucrose. The obtained results shows that the biomass increases with increasing concentration of sucrose and reaches a maximum value at the concentration of 4g /l and then decreases from the concentration of 6g /l.

3.4. Effect of nitrogen source

The study of the effect of each source of organic and inorganic nitrogen on the cell culture and on the variation of the pH has been reported in Table 2. According to the results obtained in table 2 the addition of ammonium sulphate and ammonium phosphate as

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

a source of nitrogen to the semi-synthetic medium allowed the biomass to increase, but the pH of the culture medium decrease to the value 5.5.

3.5. Determination of the concentration of acetic acid and the evolution of sugars in the culture medium

3.5.1. Evolution of the production of acetic acid by the yeast strain in a semi-synthetic medium containing 10 g /l of the glucose

In the presence of glucose in the culture medium, the strain exhibits greater and more rapid growth with almost complete degradation of glucose; the production of acetic acid is comparable to the growth of the strain, it reaches 13.05 g /l after 24h of culture with a biomass equal to 9.82 and a glucose concentration equal to 0.63 g /l. (Fig. 5-6).

3.5.2. Evolution of the production of acetic acid by the yeast strain in a semi-synthetic medium containing fructose (10 g /l)

In the presence of fructose in the culture medium as only source of carbon, the yeast strain growth reaches a maximum value of 9.72 after 24 hours of culture with an acetic acid production equal to 10.37 g /l with incomplete fructose degradation which reaches 2.76 g/l towards the end of the culture. The production of acetic acid exceeds and remains comparative growth of the cell after 7 hours of culture (Fig. 7).

3.5.3. Evolution of the production of acetic acid by the yeast strain in a semi-synthetic medium containing sucrose

The production of acetic acid goes in parallel with the growth of the yeast strain and the degradation of the sucrose, it increases gradually to reach a maximum production of 12.01 g/l after 22 hours of cultivation with a biomass of 10.32 and a concentration of Sucrose of 1.95 g/l. (Fig. 8)

3.5.4. Evolution of the production of acetic acid by the yeast strain E2 in a semi-synthetic medium containing 5 g /l of glucose and 5 g /l of fructose

In the presence of two monosaccharides as sources of carbon, 5 g /l glucose and 5 g /l fructose; the yeast strain reaches its maximum growth more rapidly after 20 hours of culture with a biomass of 10.6 g /l. The production of acetic acid is related to cell growth, reaching a maximum value of 13.12 g /l with almost complete degradation of the carbon sources after 24 hours of culture. (Fig. 9)

3.5.5. Evolution of the saccharolytic activity during the growth of the E2 strain

The evolution of the saccharolytic activity of the E2 yeast strain was followed during the growth of the E2 strain. The Fig. 10 shows that the evolution of the saccharolytic activity which increases with the growth of the cell to reach a value of 4973 µM / l / min towards the end of the exponential phase after 14 hours of culture one and a value of 5237 µM / l / min after 24 hours of culture. The production of acetic acid in the culture medium increases with the growth and the appearance of the enzymatic activity to reach concentration 12.03 g /l after 24 hours. (Fig. 10).

CONCLUSION

Generally, the production of small quantity of acetic acid is obtained through by two fermentation process stage. In the first phase the sugar is transformed to the ethanol, a product that accompanies the growth of the various types of yeast on sugar-containing substrates under anaerobic conditions. In the second phase the alcohol is transformed to acetic acid, which in turn is a metabolite accompanying the aerobic growth of acetifying bacteria in ethanol-containing media (M.D. Ould El Hadj et al., 2001). Some strains of lactic bacteria with oxidative metabolism have the ability to form acetic acid from sugar unconsummated during alcoholic fermentation and from L-lactic acid, particularly Oenococcus Oeni (Leguerinel I. et al., 1989).

A few yeasts participate alone in the production of acetic acid without the intervention of the acetifying bacteria. In the present work, we have selected 7 strains of yeast, acidifying and producing different purified organic acids, namely lactic acid, acetic acid and citric acid. The E2 yeast strain exhibited a large amount of acetic acid (105.85 mg /l) during its cultivation in a semi-synthetic saccharose medium as a carbon source. The strain selected showed a high saccharolytic activity estimated at 5237 µM / l / min after 24 hour of culture, the sucrose concentration fixed at the beginning of the culture at a value of 10 g /l decreases to attain 1.76 g /l with high production of acetic acid, with a value of 12.03 g /l. Considering the importance that given, the E2 strain producing acetic acid, was used as leaven in association with lactic acid bacteria to improve the traditional fermentation process of olive, lemon, gherkins and other...

REFERENCES

1. C. Divies, 'Le vinaigre, Microbiologie Alimentaire, les Fermentations Alimentaires', Ed. Lavoisier, Paris, pp. 93-105, 1989
2. Cooney (C.L.), Levine (D.W.) - Microbial utilization of methanol. -Adv. Appl. Microbiol., 1972, 15, 337-365.
3. http://www.garfield.library.upenn.edu/classics1977/A1977DM02500001.pdf

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

4. Leguerinel I., Mafart P., Cleret J.J., Bourgeois C. (1989) Yeast strain and kinetic aspects of the formation of flavor components in cider. Journal of the Institute of Brewing, 95, pp. 405-409.
5. N. Boughnou, 'Essai de Production du Vinaigre à partir des Déchets de Dattes', Thèse Magister, INA, El Harrach, 82 p., 1988.
6. Nelson (N.) - A photometric adaptation of the Somogyi methode for the determination of glucose. - J. Biol. Chem., 1944, 153, 375-380.
7. Production report. Chem. Eng. News (July 11, 2005), 67–76.
8. Qualité Hygiénique et Caractéristiques Physico-Chimiques du Vinaigre Traditionnel de Quelques Variétés de Dattes de la Cuvette de Ouargla*M.D. Ould El Hadj, A.H. Sebihi et O. Siboukeur Rev. Energ. Ren. : Production et Valorisation – Biomasse, (2001) 87-92
9. Somogyi (M.) - Notes on sugar determination. - J. Biol. Chem., 1952, 195(1), 19-23. http://www.jbc.org/cgi/reprint/195/1/19.pdf
10. Suresh, Bala (2003). "Acetic Acid" [archive].CEH Report 602.5000, SRI International.
11. Yoneda, Noriyki; Kusano, Satoru; Yasui, Makoto; Pujado, Peter; Wilcher, Steve (2001). Appl. Catal. A: Gen.221, 253–265.

Table 1: The yeasts selected from different biotopes on the semi-synthetic medium containing sucrose as carbon source at pH = 6.5 and 30 °C.

Biotopes	Selected strains	Semi-synthetic medium based on saccharose [3g		
		pH_f	$D.O._f$	$Ac._f$
Grape juice	**14**	6.22	1.80	0.4
tomato juice	**6**	6.18	3.11	0.5
Olive juice	**8**	5.64	4.81	0.5
Melon juice	**1**	5.57	5.030	0.5
Olive juice	**15**	5.47	4.08	0.5
	9	5.09	5.03	0.5
Pea canned juice	**13**	5.08	4.18	0.5
Traditional yeast based on sound	**V**	4.98	3.775	0.6
Sugar cane press juice	**18**	4.93	4.09	0.6
	7	4.86	5.62	0.6
	16	4.83	4.58	0.6
	12	4.63	5.91	0.6
	10	4.60	6.19	0.6
	11	4.57	5.77	0.6
Tomato juice	**17**	4.42	4.10	0.6
	4	4.42	4.28	0.6
Grape juice	**19**	4.22	4.40	0.6
tomato juice	**3**	4.15	5.15	0.6
Cabbage	**S**	4.08	5.140	0.6
Traditional yeast based on wheat flour	**W**	4.07	4.750	0.6
Traditional yeast based on sound	**N**	4.05	4.500	0.6
	5	4.05	6.77	0.6
Lemon juice	**EL1**	3.23	6.19	0.7
	EL2	3.16	8.30	0.7
Traditional yeast	**E1**	3.12	8.85	0.7
	E2	3.23	5.69	0.7

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

	E3	3.14	9.28	0.7
	E4	3.22	5.07	0.7

pH.$_f$: pH final ; D.O.$_f$: Density Optic final ; Ac.$_f$. : Acidity final

Table 2: Effect of different sources of nitrogen on the strain culture.

Nitrogen source	DO48	pH48	Ac48
(NH4)2SO4+ Ext Yeast	0,38	5,41	0,2
(NH4)2SO4+ Ext Yeast	5,12	6,07	0,2
(NH4)2SO4+ peptone	3,78	4,98	0,25
(NH4)2SO4+ urea	0,08	6,9	0,1
(NH4)2SO4+ NH4H2PO4	4,32	2,94	0,9
(NH4)2SO4+ NaNO3	0,195	5,4	0,2

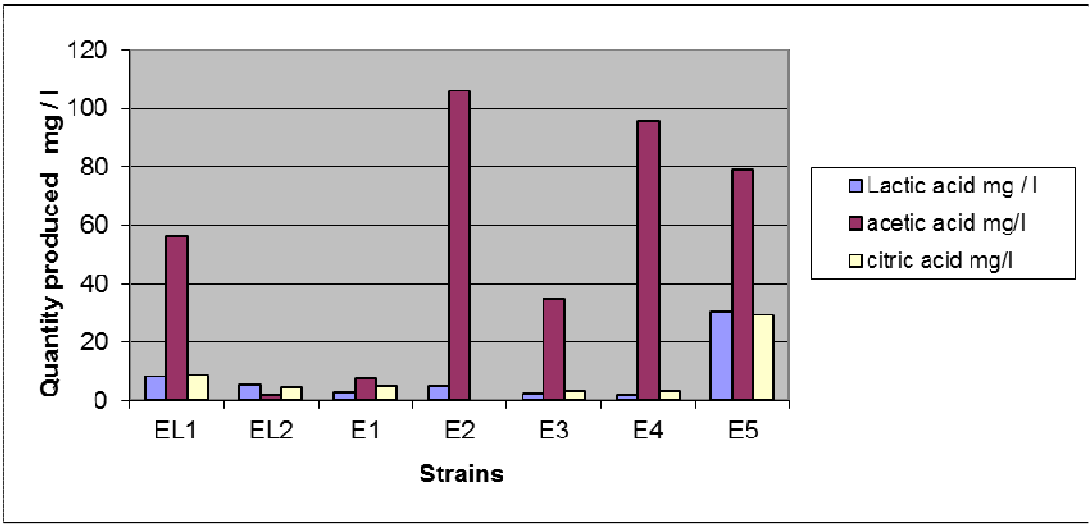

Figure 1: Amount of organic acids produced by the 7 yeast strains grown on sucrose-containing synthetic medium as a source of carbon at 30 °C.

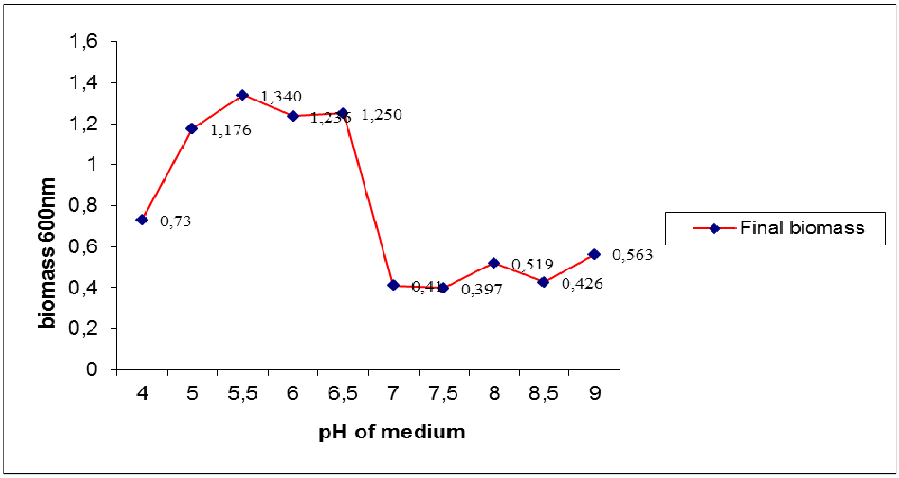

Figure 2: Effect of pH on the evolution of the biomass of strain E2 during its growth in semi-synthetic medium containing sucrose at different initial pH at 30 °C

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

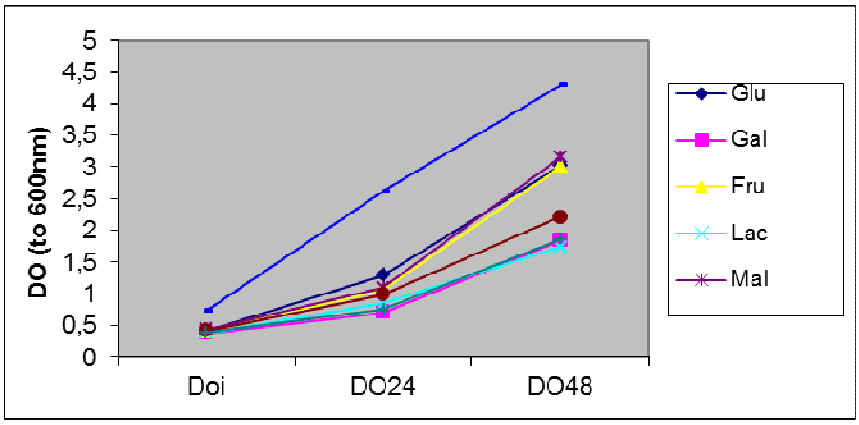

Figure 3: Effect of the carbon source on the growth of the strain

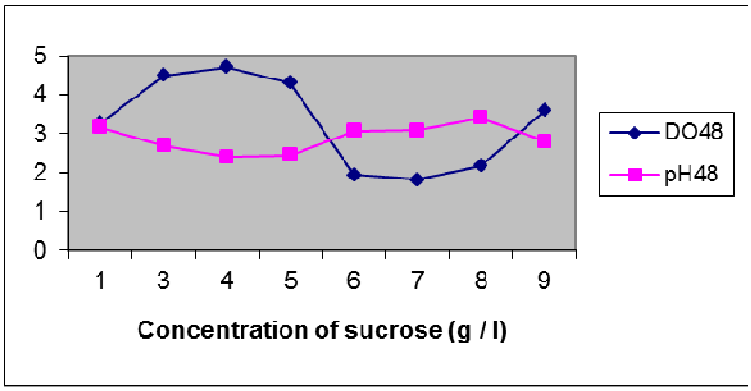

Figure 4: Effect of the concentration of sucrose on the biomass and with pH of various culture media

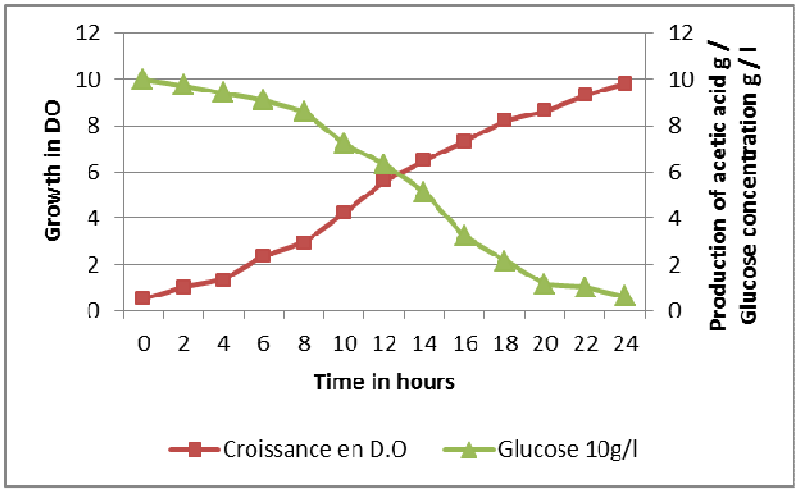

Figure 5: Evolution of glucose degradation during the growth of the yeast strain E2 in a semi-synthetic medium containing 10g /l of glucose.

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

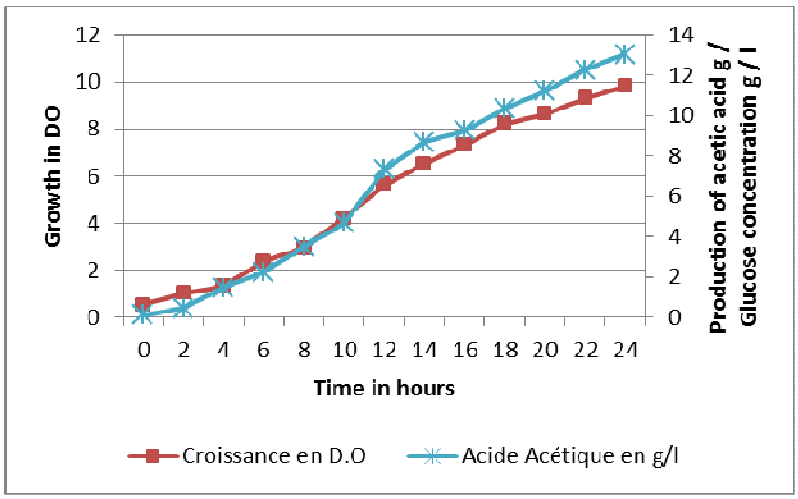

Figure 6: Evolution the production of acetic acid during the growth of the yeast strain E2 in a semi-synthetic medium containing 10g /l of glucose.

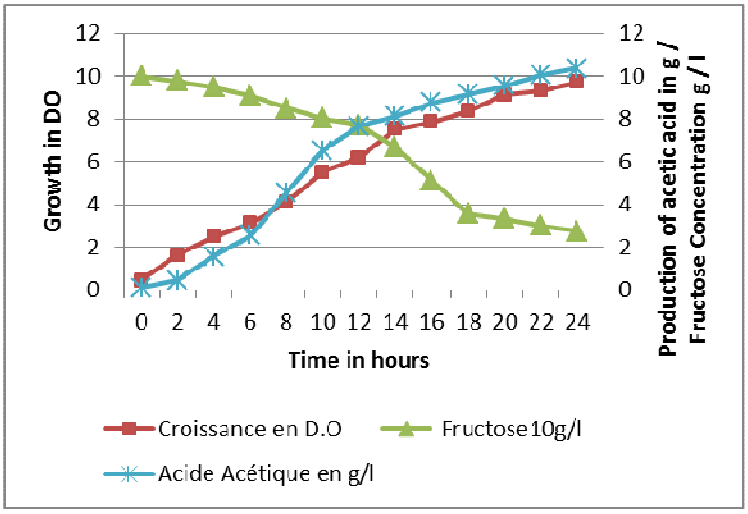

Figure 7: Evolution of the production of acetic acid by the E2 yeast strain in the semi-synthetic medium containing 10 g /l of fructose.

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

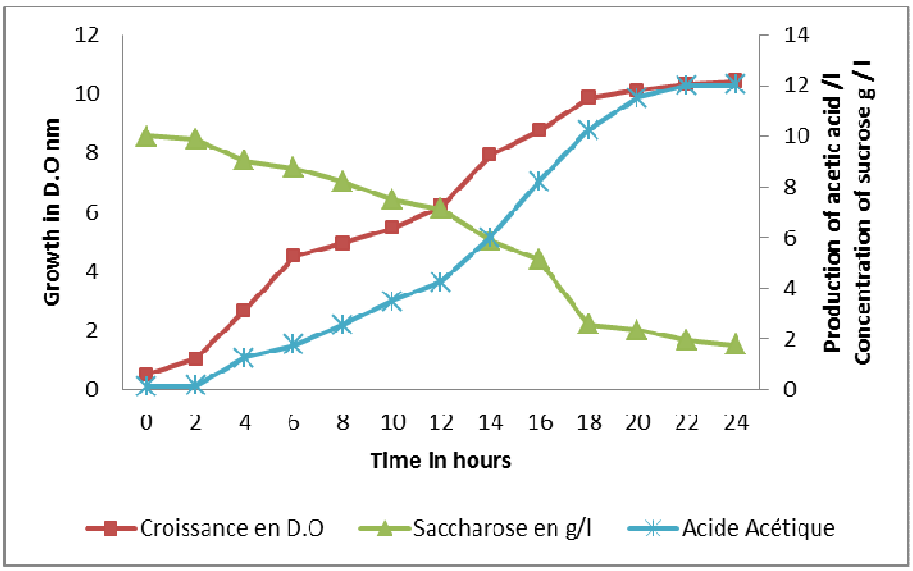

Figure 8: Evolution of the production of acetic acid by the E2 yeast strain in a semi-synthetic medium containing 10 g /l of sucrose at 30 °C.

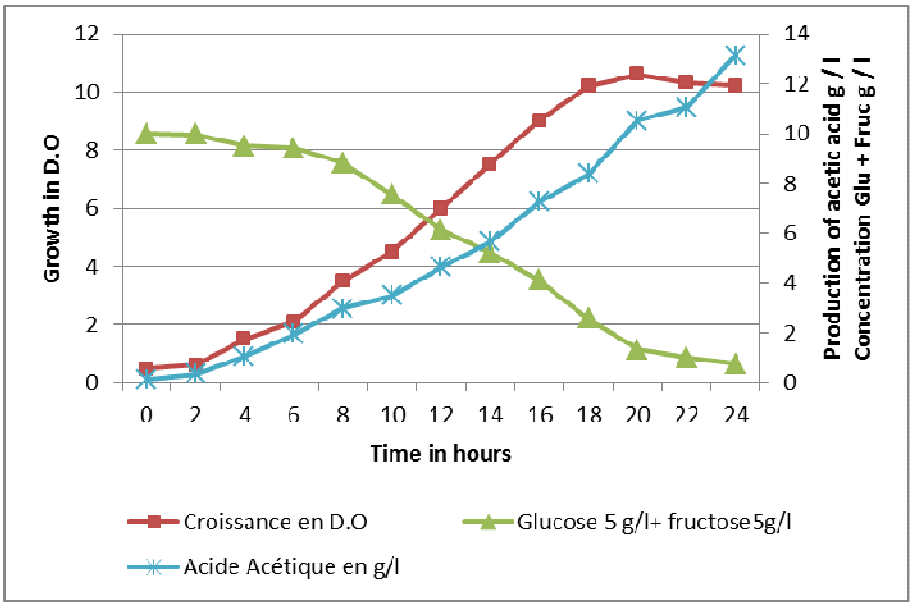

Figure 9: Evolution of the production of acetic acid by the E2 yeast strain in a semi-synthetic medium containing the glucose and the fructose at the same concentration of 5 g /l.

International Journal of Agricultural and Life Sciences- IJALS (2017), Volume 3 (2) pp.178-187
http://dx.doi.org/10.22573/spg.ijals.017.s12200082

Available online at https://www.skyfox.co
ISSN: 2454-6127

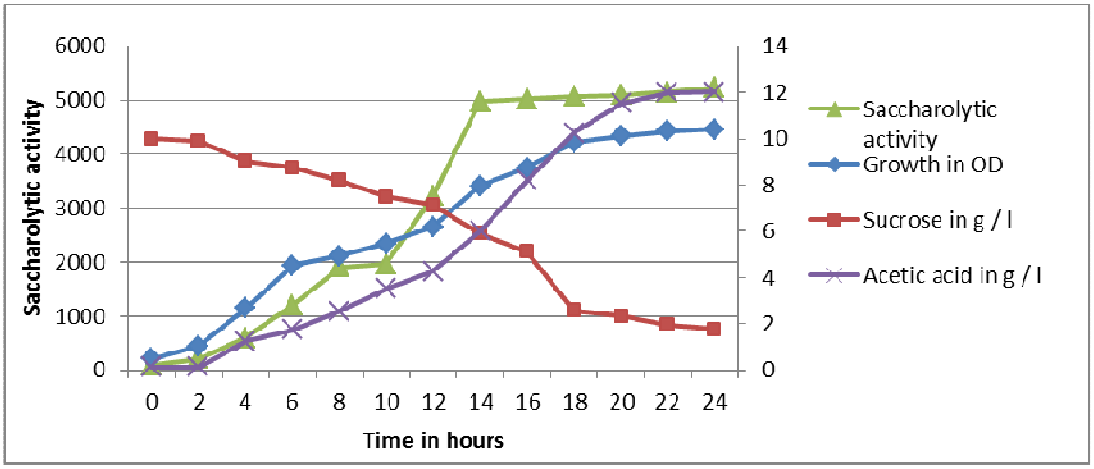

Figure 10: Evolution of the saccharolytic activity and the production of acetic acid during the growth of the E2 strain in the semi-synthetic medium containing 10 g /l of sucrose a pH 5.5 at 30 °C.

How to cite this article
Elyachioui, L., Aziane, A.-i., Elyachioui, M., & Ouhssine, M. (2017). Selection of yeast strain producing acetic acid. Int. J. Agr. Life. Sci, 3(2), 178-187. doi: 10.22573/spg.ijals.017.s12200082.

CONFLICTS OF INTEREST
"The authors declare no conflict of interest".