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Coculture of *Saccharomyces Cerevisiae*$_{C8-5}$and *Candida Tropicalis*$_{C0-7}$use foramylase production on Starch Products Medium

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Abstract: -This study was conducted to evaluate the capacity of *Saccharomyces cerevisiae* and *Candida tropicalis* to product amylase on agricultural product starch.Three starch samples were collected from different local products such as cassava, millet and corn.All isolated colonies of microorganisms were screened by amylase production on solid medium with iodine used solution for revelation. The effect of pH andsubstrate concentration were investigated in solid medium. Then the cell proteins, pH and amylase activity were followed during three days of liquid fermentation at 30°C. The results indicated that the best hydrolysis zones were obtained for using the corn starch(14 ± 1.32 mm; 9± 1mm)and millet starch (10±0.5 mm; 8 ± 1.32 mm) at 1% (w/v) respectively for *Saccharomyces cerevisiae* and *Candida tropicalis*. In liquid medium of fermentation, we noticed that highest amylase activity of microorganisms were obtained after 24 hours of fermentation with millet starch 1% (w/v) and the activity decreased whenincubation time increase. The activity were respectively 130.94 UI/h at pH 6.93 for *Saccharomyces cerevisiae* and 130 UI/h at 7.13 for *Candida tropicalis.*When these two yeasts were used as starters in pure culture and co-culture at proportion of 1:1 and 2:1 (cell/cell), amylase activity increased during the first 24 hours and decreased until at the end of fermentation whiles the pH increase during all incubation time. The best amylase activity was obtained with coculture *C. tropicalis + S. cerevisiae*(1:1). The value was 150.73 UI/h at pH 6.94 with 1% of millet starch.

Keywords: amylase activity, C*andidatropicalis*, coculture, *Saccharomycescerevisiae*

I. INTRODUCTION

Recent discoveries on the use of microorganisms as sources of industrially relavent enzymes have led to an increased in the application of microbial enzymes in various industrial processes [1].The major advantage of using microorganisms for production of amylases is in economical bulk production capacity and microbes are also easy to was prepared by the addition of sterile distilled water in manipulate to obtain enzymes of desired characteristics [2]. Amylases are enzymes that break down starch or Glycogen [3].Because most of the yeasts from nature are not harmful as compared to bacteria, interest in yeasts with potential use in biotechnological processes has increased in recent years [4].The Microbial amylase accounts for about 30% of the world's enzyme production [5]. Today, amylases are available commercially in the large number and they have almost completely replaced chemical hydrolysis of starch processing and reduce the production of chemicals used in carbohydrate hydrolysis[6]. Amylases stand out as a class of enzymes, which are useful applications in the food, brewing, textile, detergent and pharmaceutical industries [3].However the competitiveness of enzymes compared to the chemical products is limited by their highest production cost. The choice of the suitable fermentation medium is essential for the microorganisms, as well for their growth and the enzymes production [7]. Indeed the production of microbial amylase was improved considerably by the addition of various sources of carbon such as the starch [8].Starch is the best substrate for production of yeast cells in a large scale and easily available raw material in most regions of the world[4]. The starch will affect not only the microorganism's growth, but also the appearance of amylases and also the conversion speed of the carbohydrates[7]. In Ivory Coast several agricultural products (corn, millet, cassava, sorghum…) generally intended for human consumption and production of fermented food (*attiéké*, *tchapalo*…) could be used for amylase production. The exploitation of technological properties of micro-organisms required in addition to one good medium of micro-organism'sgrowth, a cheap substrate. Thanks to their high content of starch, the agricultural products could constitute a true substitute of synthetic starch for amylase production. The purpose of this study was to investigate the technological properties particularly amylase production by pure strains of *Saccharomycescerevisiae* and *Candidatropicalis*starting from a fermentation mediumcontaining corn starch and

millet starch like carbon source. In this study two microorganism's strains were constituted in coculture in order to evaluate their influence on amylase production.

II. 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 [9]. 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 %.

2.2.Amylase screening

In this part, the amylase activity is observed by appearance of hydrolysis zones of microorganisms using Bataichemethod. [10]The amylase medium used contains: peptone of casein 9 g/l, yeast extract 9 g/l, agar 13.5 g/l and starch (corn, cassava, milet) 12 g/l. Each young microorganism colony (24 hours) is demounting by spot of 3 mm on amylase medium. The plates were incubated at 30°C during 48 hours.

The apparition of clear hydrolysis zone after addition of Lugol's Iodine solution revelated the amylase activity of microorganism.Amylase activity was determined by measurement of hydrolysis zone according to following formula: (D – d).

D: total diameter of hydrolysis zone and **d** is a spot diameter

2.2.1. Effect of pH

The amylase medium was prepared with varying pH values (4.5; 5; initial pH; 7) to investigate effect of pH on amylase production.

2.2.2. Effect of substrate concentration

To study effect of substrate concentration, the amylase medium was prepared with varying starch concentration (1%; 1.2%; 1.5%; 2%) (W/v).

2.2.3. Effect of incubation time

The effect of incubation time was observed in liquid medium of fermentation. For that, samples of 8 ml of fermented medium were collected each 12 hours during 72 hoursof fermentation.

2.3. Inoculum preparation

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

2.4. Amylaseproduction

In Erlenmeyer of 250 ml containing 54 ml of liquidfermentation medium constituted ofyeast extract 10 g/l, peptone 10 g/l, starch (corn, milet) 10 g/l and chloramphenicol 0,5 g/l were inoculatedwith 6 ml of inoculum. For cocultures,inoculumvolumewas 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 72 hours. At 0h, 12 h, 24 h, 36 h, 48 h, 60 h and 72 h, samples of 8 ml were collected for amylase activity, proteins and pH assay. The samples were centrifuged at 5000 rpm at 4°C for 20 mn. The supernatants were collected and amylase assay was carried out using Dinitro Salicylic acid method.

2.5. Amylase assay

The supernatant obtained constitutes the enzymatic extract.The reaction mixture containing 125µl of 1% starch (corn, millet) (w/v) in 0.1 M acetate buffer (pH: 5.6) and75 µl of crude enzyme solution was incubated in water bath maintained at 40°C for 30 min. The reaction was stopped by adding 300 µl of 3,5-Dinitrosalicylic acid solution. This mixture was heated in a boiling water bath during 5 mn and cooled at room temperature to develop brown colour. 2 ml of deionized water was added to this solution. The absorbance was measured at 540 nm with a spectrophotometer.One unit of amylaseactivity was defined as number of µmoles of glucose liberated by 1 mL of enzyme solution per minute.

2.6. Protein assay

The proteins proportioning was made according toLowry *etal*[11]. Five solutions were prepared: solution A: folin-ciocaltor reagent diluted at third in NaOH 0.1 N; solution B: potassium carbonate (2% w/v) in NaOH 0.1 N; solution C1: copper sulfate (0.5% w/v) in distilled water; solution C2: tartrate of sodium and potassium (1% w/v) in distilled water. The solution D is made up of 100 µL of solution C1, 100 µL of solution C2 and 10 mL of solution B.The proportioning is carried out with 20 µL of protein preparation added 2 mL of solution D. The mixture is agitated and incubated during 10 minutes at room temperature. Then, 200 µL of solution A are added there. The reactional medium is agitated and let rest during 30 minutes in darkness.
The optical density is measured with spectrophotometer with 660 nm against a witness and the quantity of proteins is given thanks to a calibration line obtained with a bovine serum albumin solution (0.2 mg/ml).

2.7. 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 withlevel of significance 5%.

III. RESULTS

In this study, effect of pH and starch concentration were followed in solid medium. The follow-up of pH,amylase activity and proteins production was done during the fermentation in liquid medium.

3.1. Effect of starch concentration

The effect of starch concentration was observed by measuring diameter of hydrolysiszone. For that,medium was supplemented with 1%, 1.2%, 1.5% and 2% of cassava starch,milletstarch andcornstarch. After incubation at 30°C during 48 hours, the results showed a variation of hydrolysis diameters for each microorganism according to substrate concentration (**Table1).***Saccharomycescerevisiae* recorded the highest hydrolysis zone on medium containing 1% of corn starch ($14 \pm 1.32\ mm$) whereas that of *Candidatropicalis* is obtained on medium formulated from milletstarch 1.2% (10 ± 1.32 mm). Generally the besthydrolysis zonesare obtained by using1% of corn starch and1% of millet starch.

Table 1: Hydrolysis diameter (mm) in function ofstarch concentration

	Saccharomyces cerevisiae			*Candida tropicalis*		
Starch concentration	Corn	Millet	Cassava	Corn	Millet	Cassava
1%	14 ± 1.32^{a}	10 ± 0.5^{a}	5 ± 0.87^{a}	9 ± 1^{a}	8 ± 1.32^{ab}	3 ± 0.87^{a}
1,20%	7 ± 0.87^{b}	8 ± 1.32^{ab}	4 ± 0.5^{a}	8 ± 0.87^{a}	10 ± 1.32^{ac}	4 ± 1.52^{ac}
1,50%	10 ± 1^{b}	7 ± 0.87^{b}	4 ± 1^{a}	4 ± 1.32^{b}	6 ± 1.32^{b}	3 ± 0.87^{a}
2%	9 ± 1.53^{b}	7 ± 0.87^{b}	5 ± 1.32^{a}	5 ± 0.87^{b}	7 ± 1.32^{ab}	6 ± 1.32^{bc}

NB :On the same column, the values carrying the same letters do not present a significant difference to the level of

Effect of pH

3.2.Effect of pH

The effect of pH was observed by adjusting pH of various mediums at 4.5; 5; initialpH (initialpH corn = 6.53; Initial pH millet = 6.22) and 7 by maintainingsubstrate concentration at 1%. The statistical analyzes showed any significant difference between amylase activities of two strains on medium containing millet starch. However the hydrolysis diameters varied generally on medium formulated with corn starch. The diameters remained stable toinitial pH with 9 mm for *Candidatropicalis* on two mediums against 7 mm for *Saccharomycescerevisiae*(**Table2**).

Table 2: Hydrolysis diameters (mm) in functionof medium pH

	Saccharomyces cerevisiae		*Candida tropicalis*	
pH	Corn	Millet	Corn	Millet
4,5	6± 0,87 ^{a}	8,5 ± 1 ^{a}	5 ± 0,5 ^{a}	7 ± 0,5 ^{a}
5	6± 0,87 ^{a}	7,5 ± 1 ^{a}	7 ± 1 ^{ab}	8 ± 0,87 ^{a}
initial pH	7± 0,5 ^{ab}	7 ± 1,32 ^{a}	9 ± 1,32 ^{b}	9 ± 1,32 ^{a}
7	9± 0,87 ^{b}	8,5 ± 1,32 ^{a}	7 ± 1,32 ^{ab}	7 ± 1,32 ^{a}

NB :On the same column, the values carrying the same letters do not present a significant difference to the level of 5%

3.3. Effect of incubation time

3.3.1. In presence of monoculture

The observation of curves shows thatpH evolution at 30 °C is similar for two strains during fermentation. pH knows an increase in beginning until the end of fermentation. The pH valuesin two cases are includedbetween 6 and 9. Maximumamylase activities are reached after 24 hours of fermentation in medium containingmillet starch (**Fig 1B**). However amylase activities reached their maximum after 36 hours of fermentation in medium formulated starting from corn starch (**Fig 1A**). These activities decrease thereafter until the end of fermentation. The best activities are recorded on medium formulated withmillet starch. The values are respectively 130.94 µmol/ml/h for*Saccharomycescerevisiae*and 130µmol/ml/h for*Candidatropicalis.* The results were showed in

Fig1.

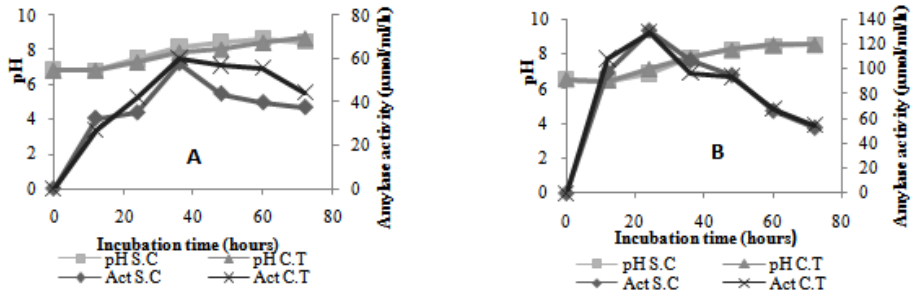

Figure 1 : Evolution of pH and amylase activityduring the incubation timein presence of monoculture (A : cornstarch ; B : milletstarch)

3.3.2. In presence of coculture

Curves evolution is similar at those obtained from monocultures.pH increases during all fermentation. With medium containing corn starch, the highest activities are obtained at 36 hours of fermentation. Maximum amylase activities are observed after 24 hours of fermentation on medium containing millet starch. The best activities are recorded on medium containing millet starch with 150.73 µmol/ml/h for coculture (1:1) and 115.82 µmol/ml/h for coculture (2:1).The results were showed in

Fig2.

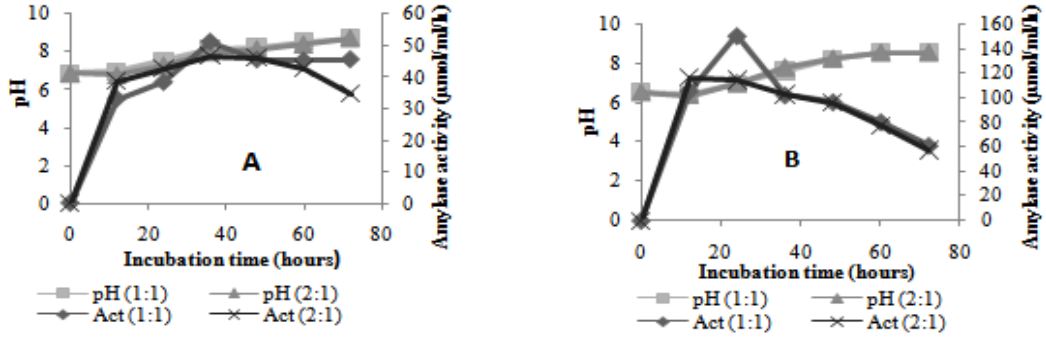

Figure 2: Evolution of pH and amylase activityduring the incubation timein presence of the coculture(A : corn starch; B : milletstarch)

3.4. Proteins production during the fermentation

3.4.1. In presence of monoculture

The production of proteins knows two periods: the first period ranging between 0 and 36 hours. During this period the quantity of proteins increased in two mediums, and, the period ranging between 36 and 72 hours the quantity of proteins decreased before stabilizing towards the end of fermentation (**Fig3**). The best proteins production is recorded with *Candidatropicalis* on medium contained corn starch (**Fig3A**). The value of this production is 23.44 mg/ml

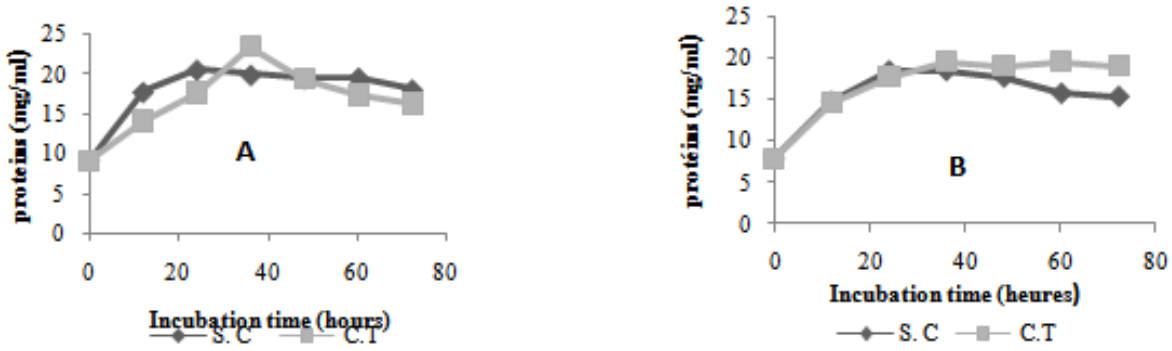

Figure 3 : Evolution of proteinsduring the fermentation in presence of monoculture (A : cornstarch ; B : milletstarch)

3.4.1. In presence of coculture

Evolution of proteins quantity presented two periods. The first 36 hours of fermentation corresponded anincreasing period of proteins and the 36 last hours of fermentation represented a decreasing period. The proteins production is similar with two cocultures on medium containing millet starch (**Fig4B**). This production is different on medium enriched with corn starch. Indeed the proteins production is better with coculture (1: 1) that with coculture (2: 1) (**Fig4A**). The maximum values are respectively 18.94 mg/ml for coculture (1:1) after 36 hours of fermentation and 18.52 mg/ml for coculture (2:1) after 48 hours of fermentation.

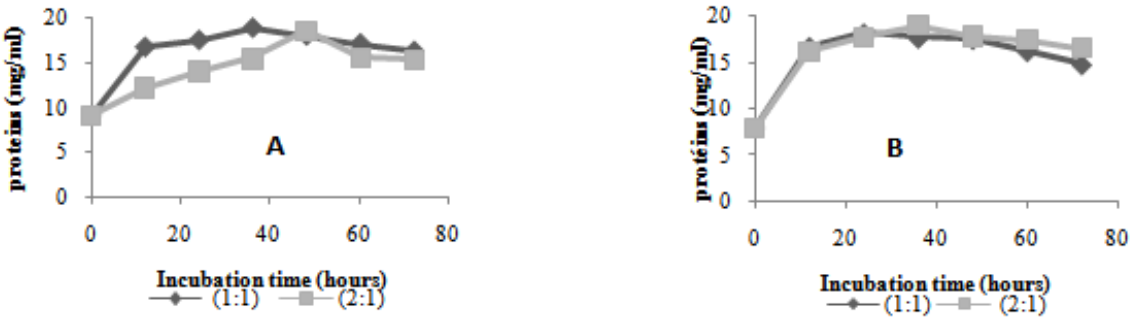

Figure 4 : evolution of proteins during the fermentation in presence of coculture (A : corn starch ; B : millet starch)

IV. DISCUSSION

Duringthis study, twoyeaststrainsisolatedfrom*tchapalo* were tested for their capacities to produce amylase under the influence of certain physicochemicalparameterssuch as pH, incubationtime and substrate concentration. Indeed for achieving high production of α-amylase, it's essential to study the influence of physical and chemicalparameters on α-amylase production [12,13]. According to Chandrashekhar*etal*.[12],the important parameters that govern the Solid State Fermentation process are incubation period, substrate concentration, pH, temperature, nitrogen sources and inorganic nutrients.The effect of substrate concentration on amylase activity of twostrainswasinvestigated and four concentrations of three types starch (millet, corn, cassava) were tested (1%; 1,2%; 1,5% and 2%). The besthydrolysis zones were obtained with corn starch 1% and millet starch 1%. Over 1.5% of substrate concentration, the hydrolysis zonesare reduced. The high starch concentration would inhibit the enzyme's activity. Indeed according to Shidu *etal*. [14], the rate of enzymes decreased when the substrate concentration increase. In addition, Lagzouly*etal*[15] showed that with starch 5%, the glucoamylase activity of *Candidaguilliermondii* decreased. The hydrolysis zonesranging between 5 and 11 mm were obtained by Shruti *etal*.[16] after using1% of starch concentrationinculture medium. A study oflagzouly *etal*. [15] revealed that certain species of *candida* such as*candidatropicalis* had a glucoamylase activity. The hydrolysis zone obtained when this strain is cultivated on medium containing a synthetic starch like carbon source is 2 mm. However, our results are in disagreement with the observations ofChandrashekhar *etal*.[12]. Their study showed that amylase activity of *Bacillussubtilis* increased with the increase ofsubstrate concentration. Indeed the best amylase activity of *B. subtilis* was obtained with 50 g/l of banana waste like substrate.pH values increase at beginning until the end of fermentation. These values were ranging between 6.86 and 8.74 on meduim enriched with corn starch and 6.52 to 8.55 on medium containing millet starch.Among the physical parameters, pH ofgrowth medium plays an important role by inducing morphological change in organism and in enzyme secretion [4].When micro-organisms grow in lower part or over their optimum pH, that could cause a poor microbial growth[3]. In our study, the four types of culture (S.C; C.T ; (1:1) ; (2:1)) were recorded their best activities on meduim which contains millet starch. The pH were respectively 6.93 for *Saccharomycescerevisiae*, 7.13 for *Candidatropicalis*, 6.94 for coculture (1 :1) and 7.01 for coculture (2 :1). Over these values, the amylase activity decreased.Suganyadevi *etal*[1] were showed that maximum yieldof amylase was obtained at pH-7 and the amylase production was 450 U/mg with*Ipomoea batatas.* Varalakshmi *et al*.[17] reported maximum enzyme activity at 75 U /mg of protein at pH- 9.5.

The observation of amylase activity curves showed two phases : a increase phase of amylase activity and a decrease phase of this activity. On level of curves, there is no a latency phase. The substrate is hydrolysed directly by the strains for amylase production.That could be explained by the addition of préculture formulated starting from *Saccharomycescerevisiae* and of *Candidatropicalis*. Indeed the préculture allowed to micro-organism to adapt at conditions of culture medium in order to better hydrolize the substrate. The yeasts cellsbeing in exponential phase of growth or the end could only be maintained in this phase. This represents a very economic aspect for industries. This observation agrees with that done by Lagzouly*etal*.[15] who observed a short latency period after addition of préculture in meduim of glucoamylase production by *Candidaguilliemondii*.Lonsane et Ramesh[18]reported that the enzyme production was initiated about 6 h in media containing 0.2 or 1.0% soluble starch.

In our study, maximumamylase activities on medium containaing millet starch were obtained after 24 hours of fermentation. This result was similar to those of Chandrashekhar *etal*[12]who were recorded the maximum amylase activity (7.26 IU/mL/min) of *Bacillussubtilis* after 24 hours of fermentation.The same observations were madeby Sumathy *etal*[19] during the amylase production by *Bacillussubtilis* using banana peels.After 24 hours of incubation,Suman and Ramesh[20]were obtained the maximum amylase activity of *Bacillus*sp. On medium which contains corn starch, maximum activities of amylase were recorded after 36 hours of fermentation, and these activities are lower than those obtained on medium containing the millet starch. The difference betweem these activities could be attributed at the composition of substrate. The difference in enzyme production could be attributed to certains factor which are associated either with the structure of substrate or with composition of individual substrate [12].However certain authors found maximumamylase activityafter 36 hours of fermentation. It's a case of Harikrishna *etal*.[6]who made 72 hours of fermentation for amylase production by *Bacillussubtilis*.Arkansha et Varsha[5]made also 72 hours of fermentation for amylase production by microorganism isolated fromsoilsample, rottedpotatoandspoiled food waste.The decrease of amylase activity recorded after 24 hours of fermentation on medium with millet starch or after 36 hours of fermentation on medium with corn starch could result in an exhaustion of fermentation medium into nutrients. Indeed during the exponential phase of growth, the microorganisms actively use the substrates to produce the enzymes. The medium not being renewed in nutrients will be impoverished, which will cause a decrease of the enzymatic activity. Leclerc *etal*.[21]meant that this reduction would result from the exhaustion of culture medium in nutrients necessary for the growth of micro-organism and the autolysis of cells. Thedecline in enzyme activity

might be due to denaturation and/or decomposition of α-amylase as a result of interactions with other compounds in fermented medium or due to inactivation by protease secreted into the system[22], but also with the changes of pH which affect the amylase activity negatively [23, 24].

V. CONCLUSION

One of the most effective and successful methods for the discovery of new enzymes is the isolation of microorganisms from natural habitats. This study made it possible to highlight the amylase activity of two strainsof micro-organisms isolated from a traditional beer which is the *tchapalo*. The corn starch and millet starch could be used as carbon sourceforamylase production. That would reduce the synthetic starch dependence and could constituite a veritable substrate for industrial production of enzyme. This study showedthat use of préculture contributed to reduce significantively the latency time of the micro-organisms, which represents a big factor for industrial amylase production.

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REFERENCES

[1] Suganyadevi P., Rinku S., Liji T., Rajila C. Amylase production by *Aspergillusniger* undersubmerged fermentation using ipomoeabatata. *International Journal of AppliedBiology and Pharmaceutical Technology*2012,3: 175-182

[2] Aiyer P. V. Amylases and their applications.*African Journal of Biotechnology*2005, 4(13):1525-1529

[3] Vidyalakshmi R., Paranthaman R., Indhumathi. J. Amylase production on submerged fermentation by *Bacillusspp*. *World Journal of Chemistry* 2009, 4(1): *89-91*

[4] Yalçın H.T., Çorbacı C. Isolation and characterization of amylase producing yeast and improvement of amylase production.*Turkish .Journal ofBiochemistry* 2013, 38(1): 101–108

[5] Akansha K., Varsha N. Production of amylase enzyme by isolated microorganisms and it'sapplication.*International Journal Research of Pharmacy and Biological Sciences* 2013, 3(4): 354 – 360

[6] Harikrishna Y. N., Narasimhulu K., Satish M. Study on theproduction of α-amylase by *Bacillussubtilis*. IOSR *Journal of Engineering* 2012, 2(5): 1053-1055

[7] Bouatenin K.M. JP., Djéni N.T., Kakou A.C., Menan E.H., DjeK.M.Optimisation de la production de l'α-amylase par les microorganismes isolés des ferments traditionnels de manioc provenant de trois zones de production de l'*attiéké* en Côte d'Ivoire.*EuropeanJournalScientific* 2016, 12: 259 – 272

[8] Dubey A. K., Suresh C., Kavitha R., Karanth N. G., Umesh-Kumar S. *FEBS Letters* 2000, 471(2):251-255.

[9] N'guessan K.F., Brou K., Jacques N., Casaregola S., Dje K.M. Identification of yeast during alcoholic fermentation of tchapalo, a traditional sorghum beer from Côte d'Ivoire. *Antonie Van Leeuwenhoek* 2011, 99(4):855–864

[10] BataicheI.Recherche de nouvelles potentialités de *Yarrowialipolytica*, isolé de différents milieux naturels pour des applications biologiquesThèse de doctorat 2014, Université de Constantine 1, 121.

[11] Lowry O.H., N.J. Rosebrough A.L. Farr., R.J. Randall. Proteins measurement with folin-phenol.*JournalofBiology and Chemistry* 1951,48: 17-25.

[12] Chandrashekhar U., Radha I.K., BassapaB.K.Production of α-amylase using banana waste by *Bacillus subtilis* undersolidstatefermentation.*European Journal of experimentalBiology* 2012, 2(4): 1044 - 1052

[13] JianlongW., PingL.Proc.Biochem1998, 33:313-316

[14] Sidhu P., Scharma R., Soni S.K, Gupta J. K. Foliamicrobial 1998, 43: 51-54.

[15] Lagzouli M., Charouf R., Yachioui M., Ouhssine M., Berny H., Jadal M. Optimisation de la croissance et de la production de glucoamylase extracellulaire par *Candida guilliermondii.Bull. Soc. Pharm. Bordeaux* 2007, 146: 251-270.

[16] Shruthi S.D., Mamatha J., Suresh V., Vedamurthy A.B., ShilpiB.Production of α-amylase from Aspergillus flavusundersolid state fermentation with optimum conditions.*InternationalResearch Journal of Pharmacy* 2012, 3(8) : 135-140

[17] Varalakshmi K.N., Kumudini B.S., Nandini B.N, Solomon J., Suhas R., Mahesh B., Kavitha A.P. Production and characterization of α-amylase from *Aspergillusniger* JGI 24 isolated in Bangalore.*Polish Journal of Microbiology* 2009, 58 (1): 29-36

[18] Ramesh M.V., Lonsane B.K. Ability of a solid state fermentation technique to significantly minimisecatabolite repression of amylase production by *Bacilluslicheniformis* M27. *Applied of Microbiology and Biotechnology*1991, 35: 191-193

[19] Sumathy V.J.H., MoiediS.P.Production of -amylase from banana peels with *Bacillus subtilis*using solid state fermentation.*International Journal of Current Microbiology and Applied Sciences*2013, 2(10): 195-206

[20] Suman S., RameshK.Production of a thermostable extracellular amylase from thermophilic*Bacillus* species.*Journal of Pharmaceutical Sciences and Research*. 2010, 2(2):149-154

[21] Leclerc H., Meyer A. et DeianaJ.Cours de microbiologie générale. Nouveauprogramme.*Biosciences et Techniques* 1995 : 73-92.

[22] Ramesh M.V., Lonsane B.K. Solid state fermentation for production of amylase by Bacillus megaterium 16M.*Biotechnology Letters* 1987, 9: 505-508

[23] Ramachandran S., Patel A-K., Nampoothiri M-M., Francis F., Nagy V., Szakacs G., PandeyA.Coconutoil cake--a potentialrawmaterial for the production of alpha-amylase.*Bioresources Technology*. 2004, 93 (2): 169-174.
[24] Nahar S., Hossain F., Ferozal B. et HalimM-A.Production of glucoamylase by *rhizopus* sp. InLiquidculture.*Pakistan Journal of Botany* 2008, 40(4):1693-1698