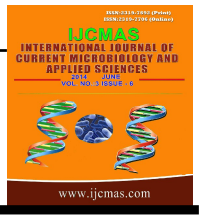
Int.J.Curr.Microbiol.App.Sci (2014) 3(6) 671-684

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 6 (2014) pp. 671-684**
http://www.ijcmas.com



Original Research Article

Isolation and screening of yeast strains involved in performing of traditional production process of cassava bioethanol

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Keywords

Endogenous yeast isolates, cell growth, controlled fermentation, cassava mash, overall ethanol productivity, sugar into ethanol conversion yield

A B S T R A C T

The aim of study has consisted to isolate and select performing yeast strains involved efficiently in the course of ethanol fermentation of cassava (*Manihot esculenta L*) mash with coarsely ground of corn (*Zea mays L*) malt randomly chosen in Brazzaville department, Congo. After culture on specific medium of 0.005% chloramphenicol Sabouraud agar, 40 isolates were obtained then subjected to a sorting on the basis of the colony diameter afferent. Four yeast isolates were sorted, and then identified on base of two performance criteria of fermentation. The yield of conversion of sugars into ethanol during the controlled fermentation by yeasting was highest with the BogY23 isolate (0.74g ethanol/g sugar consumed) compared to the other three isolates (0.62g ethanol /g sugar for BogY1). Just as the overall ethanol productivity was greater with this isolate BogY23 (ratio P_{TE}Y23/ P_{TE}Y1= 1.45/1) and relatively close to an industrial yeast strain YR (ratio P_{TE}YR / P_{TE}Y1 = 2/1) taken as reference and compared to other three indigenous yeast isolates tested (ratio P_{TE}Y1/ P_{TE}Y1=1/1 for BogY1 isolate, P_{TE}Y12/ P_{TE}Y1=1.1/1 for BogY12 and BogY4 isolate).

Introduction

In traditional manufacturing cassava alcohol, ethanol fermentation makes intervene microbial enzymes for the conversion of sugars into various metabolites which include alcohols, esters, aldehydes and organic acids (Diakabana et al., 2007). The microbial strains involved are generally *Saccharomyces* yeast genus, mould and bacteria (Ameyapoh et al., 2006; Diakabana et al, 2008). In

traditional fermentations, microorganisms are brought by raw materials and produce a spontaneous uncontrolled fermentation (Ameyapoh et al., 2006). During these traditional mixed fermentations, different microbial species are in competition, some species dominating other at a given time of fermentation course (Lonvaud-Funel et al., 1988; Gaillardin and Heslot, 1987; Arnaud and Guiraud, 1988; Oyeyiola, 1991; Ameyapoh et al., 2006). So outside of the ethanol fermentation, other fermentations take place in the cassava mash by acting unfavorably on the performance of ethanol production (Diakabana et al., 2007). Increasingly a seeding of pure yeast strains is realized to permit a controlled fermentation (Marc, 1982; Diakabana, 1988; Gosselin et al., 2000; Diakabana et al., 2008). Traditional producers of ethanol cassava are waiting for research various technological improvements which include the control of productivity, product quality and innovation for reducing cost price and increasing of the added value.

The aim of the present study was focused to isolating and evaluating the performance of screened isolates on basis of the conversion of sugars into ethanol and overall ethanol productivity of endogenous yeast strains involved in traditional ethanol fermentation of cassava mash to constitute a working stock.

Materials and Methods

Sample collection

In this work, twelve samples of corn malt intended for traditional production of cassava bioethanol (Diakabana et al., 2008) were collected from twelve different workshops of malting randomly selected in Brazzaville department. These twelve corn malt samples collected were dried at 40°C maximum for two days of sunlight in a solar dryer and stored in laboratory. From 500grams of corn malt of each sample, 6,000grams of a mixture was made to form a working stock kept at laboratory.

Conduct spontaneous fermentation of cassava mash

Each test of spontaneous fermentation of cassava mash, driving at 26 ± 1°C, was realized in triplicate within that four taking of an aliquot of sample were made every two days during incubation, especially at 3hours, 48 hours, 96 hours and 120 hours. After mixing of a mixture of ingredients compound of cassava starch flour and ground maize malt (Figure.1), the volume of the cassava mash was standardized with sufficient water quantity to make 4 liters. The standardized cassava mash taken back to 25-26 °C was introduced into a cylindrical conical micro fermenter of 6litres (Diakabana, 2006). At the beginning of the fermentation and every 24 hours, a sample was taken for analysis.

Preparation of culture media used for the isolation of yeast strains

Preparation of specific medium of Sabouraud agar

The medium of culture of Sabouraud agar enriched with 0.005% chloramphenicol marketed by an Indian firm (HiMedia Laboratories Pvt.Ltd, India) was used for the specific isolation of yeasts involved in spontaneous fermentation of cassava mash induced by corn malt (Diakabana et al., 2008). This medium was prepared by sterilizing an aqueous suspension obtained by dissolving 6.5grams of powdered agar of Sabouraud 0.005% chloramphenicol in 100 ml of distilled water. Sterilization was carried out by

autoclaving the medium in Petri dishes and test tubes at 121°C for 15min.

Preparation of cassava mash agar for sorting of yeast isolates

i) Preparing the dough cooked cassava

125grams of cassava flour were dissolved in 170ml of distilled water. Starch milk obtained was gradually versed into a pot containing 170ml of boiling water, while regularly homogenizing the mixture until complete cooking. The paste of cassava flour obtained was cooled at room temperature to 60 °C.

ii) Preparation of cassava mash

At the cooked paste of cassava flour to 60 ° C contained in a pot, 70grams of ground corn malt were added. The thick mixture was regularly homogenized at this temperature until liquefaction after 20-30minutes. Hot water at 76-80°C was added on in sufficient quantity to make 800ml of volume. The mash obtained was then clarified by means of a funnel with filter paper. The soluble extract of filtrate measuring 7°Brix was concentrated at 8.2 °Brix by boiling and the pH adjusted to 5.6 ± 0.1 with NaOH or HCl. This solution at 8.2 ± 0.2 °Brix was sterilized by autoclaving at 121°C for 15minutes then cooled and stored at about 0°C.

iii) Preparation of agar of cassava mash

At 100ml of clear wort cassava titrating 8.2°Brix, 6grams of agar powder marketed by Fisher Scientific have been added. The mixture was heated and stirred regularly. The culture medium of cassava must agar was sterilized by autoclaving at 121°C for 15min. It was then succumbed at hot into Petri dishes, cooled to room temperature and then stored in a refrigerator at about 0°C in waiting for seeding

Isolation of yeast strains from cassava mash

The isolation of the yeast strains in the course of the four stages of spontaneous fermentation of the cassava mash was realized by using selective medium agar of Sabouraud 0.005% chloramphenicol (Ameyapoh et al., 2006) and cassava must agar.

From three hours time and every 48 hours during the spontaneous fermentation, 10ml of sample of cassava mash were aseptically collected in sterile test tubes plugged with cotton-wool. The solid media used for culture have been planted on surface for the isolation of microorganisms. The method of quadrants was used (Ramalingam and Ramasamy, 2013). After drawing four quadrants of size progressively increasing on the extern bottom of the Petri dish containing agar, a drop of suspension of microorganisms was deposited at the intersection of the two first quadrants1 and 2 near the edge of the box, and the surfaces of the three first quadrants were smaller and that of the fourth largest. Using the button of a sterile Pasteur pipette, streaks enough spaced were performed to carry along a few suspension which was spread on the two first quadrants 1 and 2.

This instrument of spreading has been flamed on Bunsen burner and cooled a few moments. The second quadrant was then spread by streaking on the third quadrant; the instrument has been flamed and cooled. The third quadrant was spread by streaking on the fourth quadrant. The culture realized in duplicate was incubated at 28-30°C for three to five days in an incubator.

Transplanting yeast isolates on solid medium and screening test

For a better isolation of yeast strains, colonies identified and sufficiently separated from adjacent were subcultured twice on solid medium by the method of quadrants. The screening test of isolate was affected by measuring diameter after a delay of 48hours of culture incubation realized at 28±1°C. Diameter of isolate was evaluated in millimeter by using stereomicroscope Zeiss DV4 with micrometric ocular for double scale of measurements spread for magnification 8X and 32X. Colonies of same diameter and color, with same cell form of yeast were considering as identical.

Transplanting yeast isolates on agar slope cultures and conservation

Agar slope (8-10ml) was achieved by keeping the test tube, during cooling in an inclined position by angle of 30-45°C compared with the horizontal plane (Take and Kharat, 2012). The agar contained in Petri dishes and the one slope in the tubes test for storing isolates after subcultures were then stored in a refrigerator waiting for seeding.

The isolates were kept in culture streaked onto agar slope (De Clerck, 1963). The technique of buttoned sterile pipette was used to inoculate slants agar. After loading the tip of a sterile pipette buttoned by soaking it in the suspension sorted isolate, yeast cells were transferred aseptically on sloping agar (Take and Kharat, 2012; Dolui et al, 2012) by making grooves tight and by getting back from bottom toward the tube extremity. The medium was incubated at 28-30°C for 48-72h and the tube stored cold at +4°C.

In order to keep live these long time in good biological conditions, the isolated yeast cultures were maintained on solid medium slope (Sabouraud agar, agar must cassava) in tubes plugged with cotton wool after streak culture.

Using of a reference yeast strain

In this study an industrial yeast strain *Saccharomyces cerevisiae*, marketed under the label Saf-instant (SI Lesaffre, France), commonly used in industrial and traditional ethanol fermentation (Diakabana et al, 2008) was employed as reference.

Evaluation of the performance of yeast isolates tested in a real environment

Conduct of directed fermentation of cassava mash

The conduct of the controlled fermentation by adding yeast in cassava mash was realized according to the following diagram (Figure 2).

Determination of soluble extract content and efficiency of conversion of sugars into ethanol

For the determination of sugar content in Brix degrees (wt / wt) during fermentation, the method by refractometry has been used (Diakabana et al., 2013). During the different tests of fermentation of must of cassava, fermentable extract was determined by using the EBC method (1987). The efficiency of conversion of sugars into ethanol Rc was evaluated from the following relationship (Leveau and Bouix, 1988; Engasser, 1988) adapted by Diakabana et al (2013):

Rc = Et / Sc, with: Et = amount of obtained ethanol (g / l), So = initial amount of sugars (g /l), Sc = amount of consumed sugar (g / l), Sr = amount of residual sugar (g / l), Sc = So-Sr, Rc= yield of conversion of sugars into ethanol (g ethanol/g sugar).

Determination of overall ethanol productivity

The happy ethanol fermentation of the must falling on tests performed was evaluated according to the pycnometry method described by Heineken (1986). The overall ethanol productivity P_{TE} was determined by using the following equation (Diakabana et al., 2013):

$$P_{TE} = Et/t_M;$$

With, E_f: the maximum concentration of ethanol (g / l) evaluated at the end of fermentation; t_M: a total fermentation cycle duration (in hours) and P_{TE} (in g ethanol / l.h). The total ethanol productivity was chosen as a performance criterion to evaluate different fermentation trials tested by taking the time as one of economic constraints (Engasser, 1988).

Statistical analysis of results

The statistical method (Larrieu, 1988) modified based on the law of Gauss-Laplace bell was used. The average number of yeast strains having the same morphological characteristics and the same diameter of colony was evaluated. The value of the tested yeast cell concentration is the mean of three measurements by hematocytometer counting with Malassez cell, standard deviation and confidence intervals were determined for a coefficient of variation less than or equal to 0.1% (Diakabana et al, 2008). The mean of sugar content and overall ethanol productivity was determined for a coefficient of variation less than or equal to 1%.

Results and Discussion

Olfactory characteristic of yeast isolates selected

The smell of subcultured yeast isolates in Petri dishes was examined by sniffing the base of the box. She recalled the banana flavor more or less subtle characteristic of each isolate tested.

Morphological characteristics of yeast isolates from cassava mash

After seeding the agar surface with inoculums cassava wort came from spontaneous fermentation, various yeast colonies were grown (Figure 3). Yeast colonies obtained after culture on the agar surface were noted by their white color, differentiated by their form and size of diameter.

Overview of the population of yeasts isolated from cassava mash

The results showed that the isolates were sufficiently grown from 48 hours on chloramphenicol Sabouraud agar medium and 72 hours on cassava mash agar (Results not shown). Microscopic observation in the fresh state of the aqueous suspension of yeast isolates from rinsing the surface of agar showed quite diverse flora yeast (Figure 4). Several types of cells yeast were noted: spherical forms, ovoid, ellipsoidal, single cells, dividing binary cells and pseudo mycelium.

Identification of yeast isolates at different stages of spontaneous fermentation process

Quantitative screening of yeast isolates

By quantitative screening of yeast isolates based on the diameter of the colony, 40 different isolates of yeast appointed from BogY1to BogY40 respectively, were recorded between 3 hours and 120 hours during the development of the spontaneous fermentation of cassava mash. The average size of the colony diameter was 2.75±1.23 mm and very variable from one isolate to another, with a coefficient of variation of 45%, minimum size evaluated at 1.3mm and maximum at 4.5mm (Table 1). Among the 40 yeast colonies observed, four isolates were regularly occurred between 48 hours and 120 hours of running of fermentation and were screened and selected for the study of performance in the production of ethanol from cassava mash. Each of these screened isolates presented its own morphological characteristics: spherical small form cells (colony Ø=2mm) for BogY1, ovoid form cells (colony Ø=2.23 mm) for BogY12 and (colony Ø=2.5 mm) for BogY23, ellipsoidal form cells (colony Ø=4.5 mm) for BogY4 (Results not shown).

Capacity of consumption of sugars tested in stirred synthetic broth medium

The ability of the yeast isolates to consume sugars was tested for their identification. The four yeast isolates grown respectively on glucose, maltose and sucrose in synthetic broth were deemed able to consume these three sugars, but with different speeds. Glucose was consumed more quickly by isolates BogY1, BogY12 and BogY23 from 2hours of incubation over BogY4 (Figure 5). Glucose consumption stopped at 23 hours of incubation (final sugar content = 1.2° Brix with three isolates BogY1, BogY12 and BogY23**).** The profile of curves of maltose consumption was identical with all four isolates (BogY1, BogY12, BogY23 and BogY4) from 5hours of incubation to a limit (maltose content=1.2° Brix) at 47heures (Figure 6). Sucrose slowly disappeared at the bignning between 2hours and7hours, then more rapidly between 7 and 23 hours (final sucrose content = 1.2° Brix) with all isolates BogY1, BogY12, BogY23and BogY4 tested (Figure 7). By testing these three sugars, the results revealed that sucrose was consumed more rapidly and almost completely after 23 hours of incubation with all yeast isolates tested, followed by glucose between 23-31hours and finally by maltose at 47heures.

Characteristics of the cell growth of yeast strains grown in synthetic liquid medium stirred

The cell growth curves established during the incubation of the cultures (Figures 8, 9) revealed the same duration of the lag phase assessed at three hours of time. However, the profiles of the exponential growth phase and the final cell biomass were variable depending on the type of yeast isolate implemented. The cell culture on maltose and sucrose in synthetic broth has permitted to obtain a fairly stable biomass for isolate BogY4 and favored over the growth of the isolate BogY1 (final average biomass= $550x10^6$ yeast cells / ml) compared to other three isolates (Figures 8, 9). Cell culture of yeast isolates in synthetic broth with maltose (Figure8) more favored growth for isolate BogY1 (final biomass= $600x10^6$ yeast cells / ml) compared to the other three isolates BogY12, BogY23, and BogY4 (final

biomass: 70x 10^6 cells / ml for BogY23). It has also been same on sucrose broth medium (Figure 9), but with a relatively dispersed lower final among of biomass for the four isolates tested (final biomass= 550x10^6 cells / ml for isolate BogY1 and 300x10^6 cells / ml for BogY23).

Performance level of yeast isolates tested in fermentation led by adding of yeast in cassava mash - Consumption of soluble extract

The kinetics of disappearance of the soluble extract of cassava mash were developed progressively for all cases of yeast strains tested in presence of an industrial yeast strain used as a reference witness YR (Figure10). All the curves relating to the reduction of the soluble extract of cassava wort during the fermentation led by every of four isolates showed a decreasing speed. The decrease of the extract was slower for the isolate BogY1 (limit reached at 4.2 ° Brix at 115heures of incubation) compared to the other three isolates tested (3.2 ° Brix achieved at 91heures with isolate BogY4) which approach the reference strain YR (limit reached at 2.2 ° Brix at 43heures with YR).

Efficiency of conversion of sugars into ethanol and overall ethanol productivity

Results of efficiency of conversion of sugars into ethanol in cassava mash were lower with isolate BogY1 (Rc=0.62g ethanol/g sugars), but highest with isolate BogY23 (Rc=0.74 g ethanol /g sugars) and relatively close (Rc=0.87 g ethanol /g sugars) to that of industrial control yeast YR (Table 2). Likewise, the overall ethanol productivity was higher for isolate BogY23 (0.87 g ethanol / liter/hour of incubation) and closer to that obtained with the reference yeast strain YR (1.20 g of ethanol/liter/hour of incubation) compared to isolate BogY1 least alcohol tolerance (0.60g ethanol/ l. h) (Table 2).

The implement of inoculums for yeast strains expressed by direct counting in colony forming units CFU / ml or cells/ml is suitable for precise yeast strain (Diakabana, 1988; Ameyapoh et al., 2006; Ganesan and Nellaiappan, 2014). But that manner can give significant variability because size of each yeast strain implemented during a fermentation run is very different according to results obtained. So that for studying optimization of seeding rates of yeast strains better results should be obtained by measuring the mass cell in dry matter per volume as it is used in industrial brewery (De Clerck, 1984; Heineken,1986; EBC, 1987, Engasser, 1988). Using these three tested sugars revealed that sucrose is consumed more quickly, followed by glucose and maltose finally during a fermentation run of cassava wort with addition of each yeast tested separately (Marc, 1982; Stewart, 1985).

For technological transformation of cassava mash in ethanol fermentation, choice of alcoholgene yeast strains is very important to obtain a more efficient process (Gosselin et al., 2000; Stewart et al., 2007; Diakabana et al., 2007 and 2013; Ganesan and Nellaiappan, 2014). Because of its overall ethanol productivity relatively higher compared to other isolates, the isolate BogY23 (P_{TE}Y3= 0.87g / l.h) is closer to the reference strain YR (P_{TE}YR= 1.20g / l.h). Its behavior in the cassava mash needs to be further studied and optimized to increase its share of a fermentation capacity of biomass production, and secondly its ethanol productivity (Marc, 1982; Stewart, 1985; Diakabana, 1988; Ganesan and Nellaiappan, 2014).

Figure.1 Diagram of spontaneous ethanol fermentation in cylindrical-conical tank (Diakabana et al, 2007)

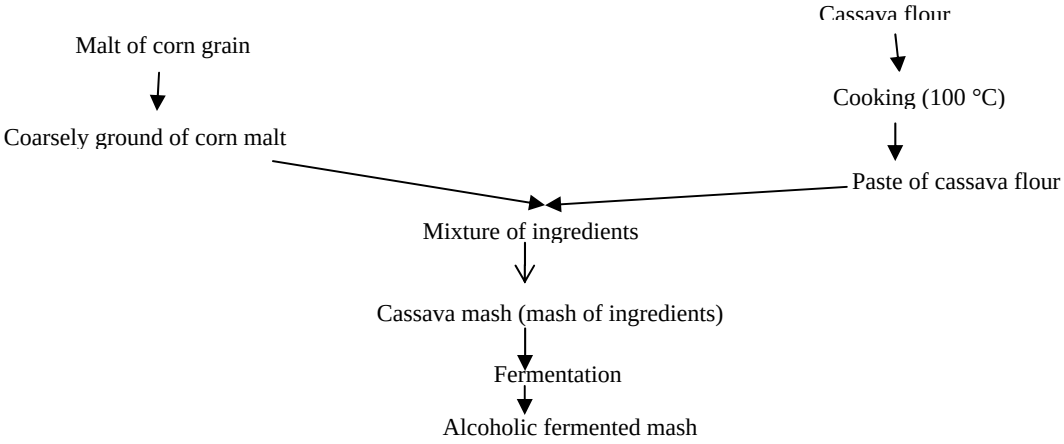

Figure.2 Diagram of ethanol fermentation led by yeasting of cassava mash in cylindrical-conical tank

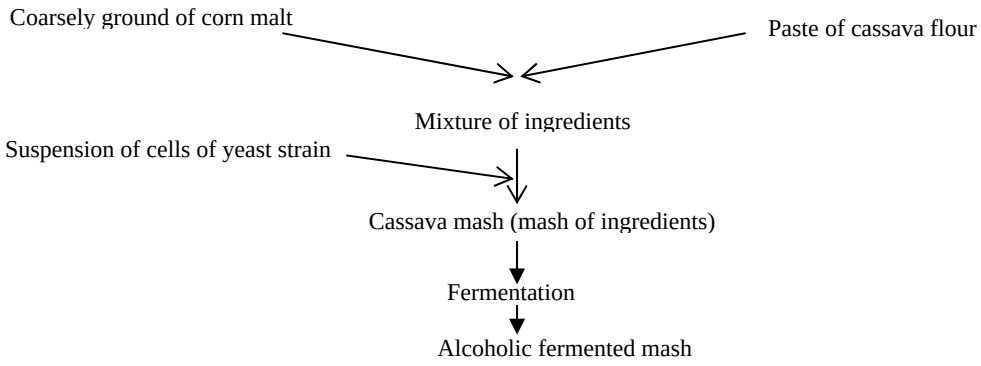

Figure.3 Yeast colonies isolated on Sabouraud 0.005% chloramphenicol agar according to quadrants method.

Figure.4 Morphology of yeast cells observed by the camera ocular microscope, with 40X objective. Aqueous suspension originating from rinse of the surface of agar medium in the Petri box has revealed a fairly diverse flora yeast (pseudo mycelium, spherical forms, ovoid, ellipsoidal, single cells and dividing binary cells).

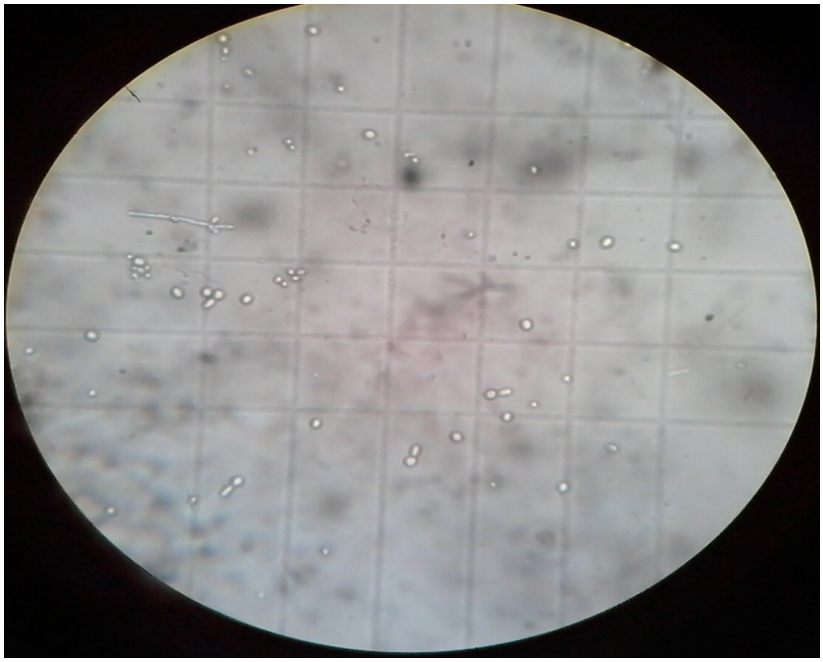

Figure.5 Kinetics of glucose consumption (°Brix) by each of the four isolates yeast (BogY1, BogY2, BogY3 and BogY4) tested during the incubation in synthetic medium broth YEG (Yeast Extract Glucose).

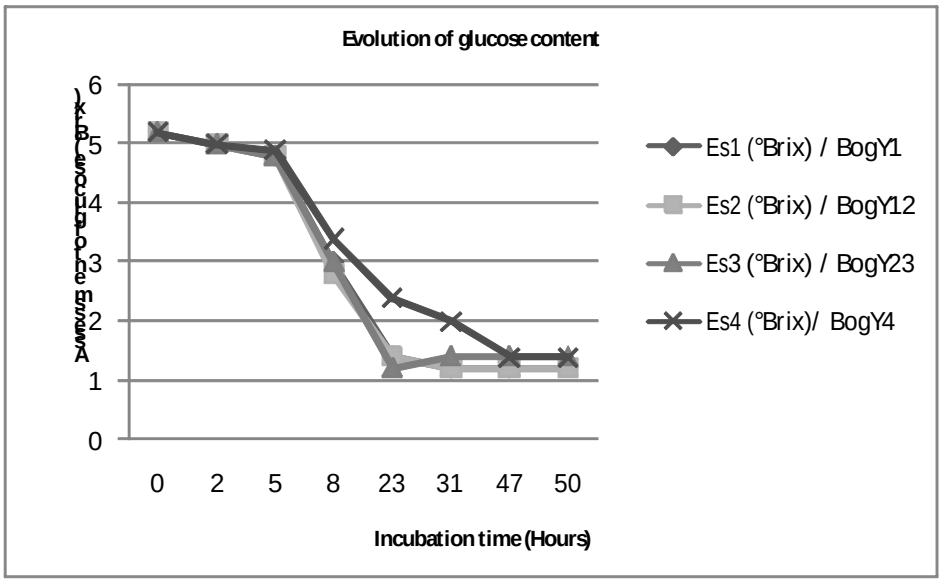

Figure 6. Kinetics of maltose consumption (° Brix) by each of the four isolates of yeast (BogY1 BogY2, BogY3 and BogY4) tested during the incubation in synthetic medium broth YEM (Yeast Extract Maltose).

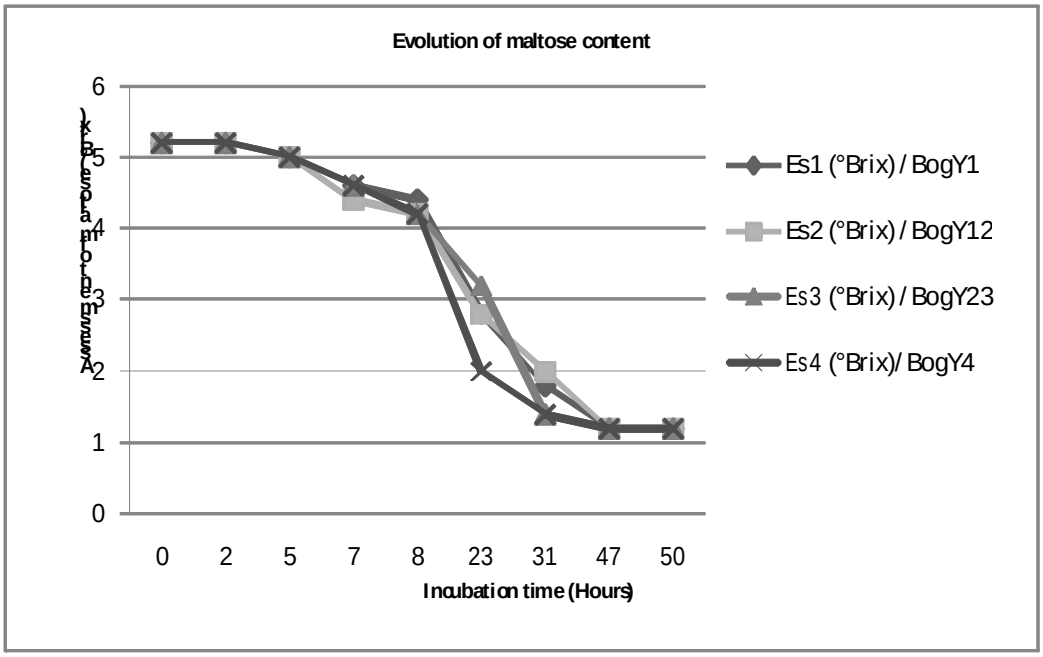

Figure.7 Kinetics of sucrose consumption (° Brix) by each of the four isolates yeast (BogY1, BogY2, BogY3

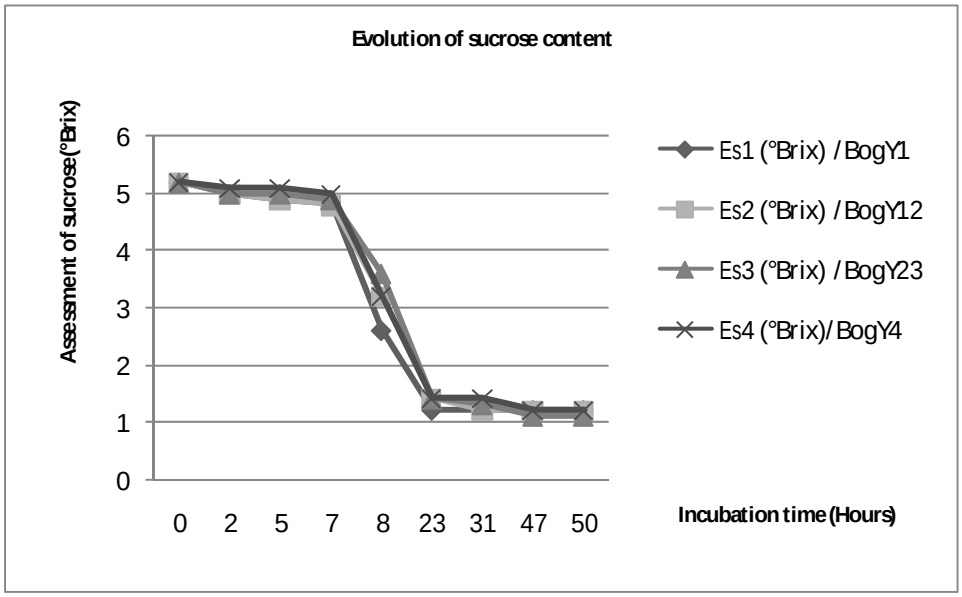

Figure.8 Kinetics of cell growth of each of the four yeast isolates (BogY1, BogY2, BogY3 and BogY4) tested in synthetic medium YEM (Yeast Extract Maltose) during the incubation

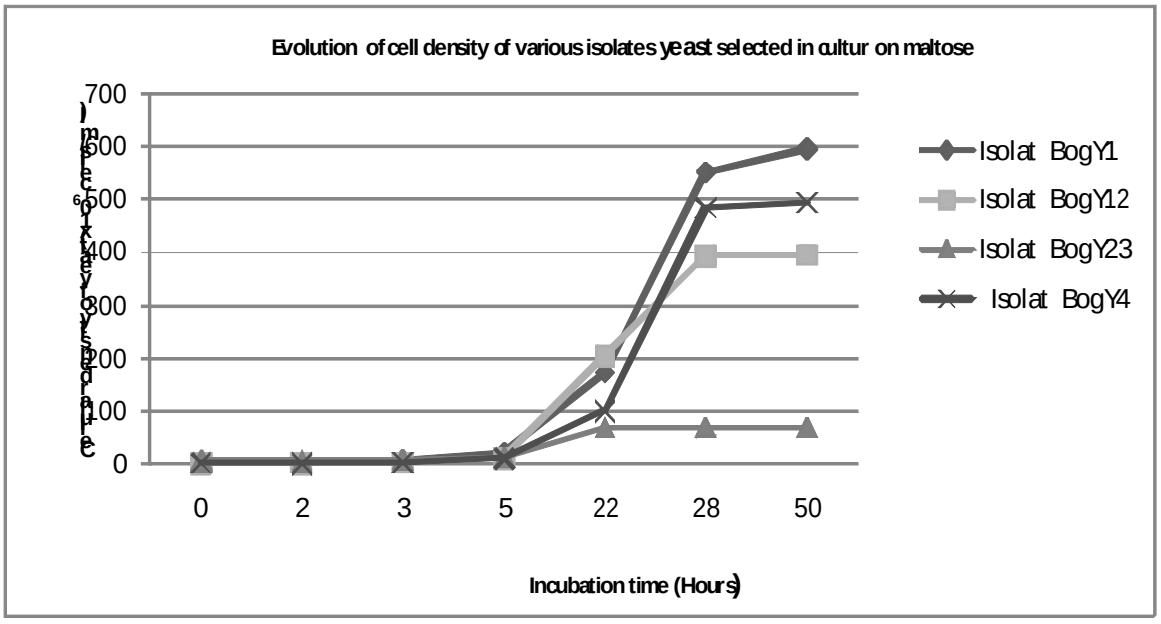

Figure.9 Kinetics of the cell growth of each of the four yeast isolates (BogY1, BogY2, BogY3 and BogY4) tested in **synthetic** broth medium with sucrose YES(Yeast Extract Sucrose) during the incubation

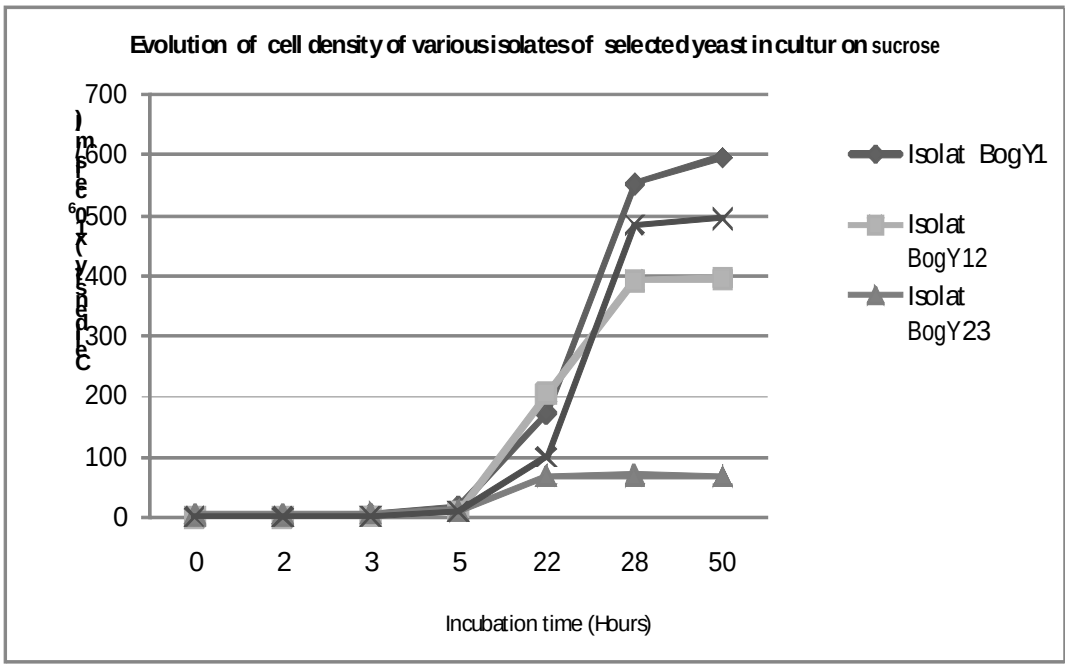

Figure10. Kinetics of consumption of soluble extract Es (° Brix) during the fermentation of cassava wort induced by four different yeast isolates BogY1, BogY12, BogY23 and BogY4 with one yeast strain YR employed as reference.

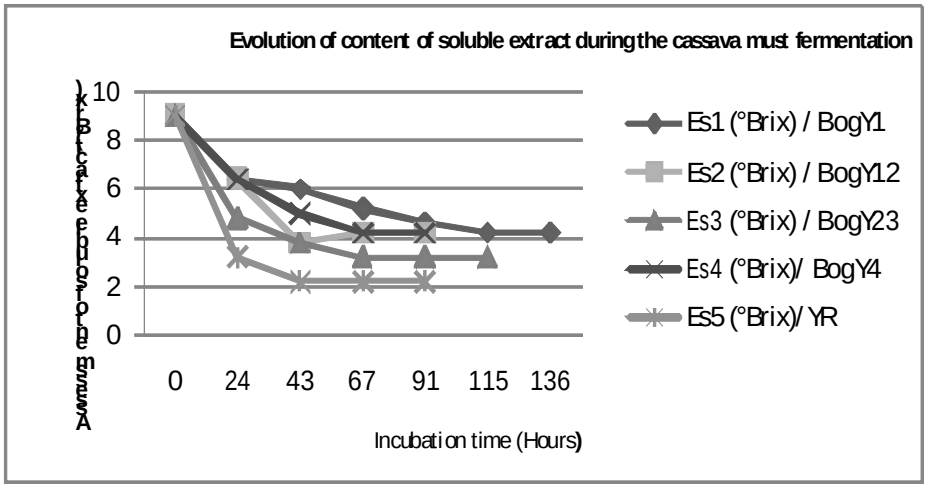

Table.1 Screening quantitative of yeast isolates

Parameter used for the selection	statistical parameters				
	M	σ	CV%	Minimum	Maximum
Diameter of the colony (mm)	2.75	1.23	45.45	1.3	4.5

n= 40 (Results obtained with 40 isolates), M: mean, σ: standard variation); CV%: coefficient of variation in percentage.

Table.2 Evaluation of the performance parameters of various yeast isolates tested during the fermentation of cassava mash led by yeasting

Performance parameters	Code of yeast isolates tested				Reference strain YR
	BogY1	BogY12	BogY23	BogY4	
Rc	0.62	0.68	0.74	0.68	0.87
P_{TE} (g/l.h)	0.60	0.66	0.87	0.66	1.20
Ratio P_{TE}/ P_{TEY1}	1/1	1.1/1	1.45/1	1.1/1	2 /1

Rc: conversion efficiency of sugars into ethanol (g ethanol obtained/g sugars); P_{TE}: Overall hourly productivity of ethanol for each concerned isolate; P_{TE}Y1: Overall hourly productivity of ethanol of isolate BogY1; P_{TE} / P_{TE}Y1: Ratio between overall hourly ethanol productivity of each tested isolate and BogY1 isolate .

References

Ameyapoh, Y., K.Workpor and de Souza C. 2006.Identification and selection of yeast strains for the efficient production of alcohol. Journal of Science, Vol.6 (1): 30-40.

Arnaud, A., and Guiraud J. P. 1988. Microbial biochemistry.In "Biotechnology, Scriban R. coordinator; Techn. Doc. Lavoisier, 3rd Ed Paris: 67-202.

Ayanda, O.I., A. A. Ajayi, G.I. Olasehinde O.T. and Dare (2013).Isolation, characterization and extracellular enzyme detection of microbial isolates from Deteriorated apple (Malus domestica) fruits. Int.J.Biol.Chem.Sci.7 (2): 641-648.

De Clerck, J. 1963.Cours de Brasserie.Volume 2. Vol. II, 2nd Edition. Catholic University of Louvain. Publisher: Chair Jean De Clerck, Belgium.

De Clerck, J. 1984. Yeast. In "Race of Brewing", T.1, Vol.2, 2nd Edn, Catholic University of Louvain, Publisher Chair Jean De Clerck. Belgium 604-635.

Diakabana, P., 1988. Influence of temperature and glucose on the behavior of yeast. M.Sc. Thesis. Catholic University of Louvain (French), Louvain-la-Neuve.

Diakabana, P., 2006. Optimization of Production Process BOGANDA traditional, water spirits of Congo. PhD thesis, University Marien NGOUABI, Brazzaville.

Diakabana, P., D. Louembé and Kobawila S.C. 2007. Biochemical and physico-chemical characteristics of the fermentation of *Boganda*, a brandy of

Congo. Res.J.Biotechnol, 2 (1): 18- 25.

Diakabana, P., C.A. Nyanga-Koumou., S.C. Kobawila, D. Louembé and Derdelinckx G. 2008. Study of characteristic parameters of traditional process in the production of *Boganda*, a brandy of Congo. Int. J. Biol. Chem. Sci. 2 (3): 258-271.

Diakabana, P., S.C. Kobawila, V. Massengo and Louembé D. 2013. Effect of degree of maturation on the kinetics of the fermentation Ethyl mango pulp *Boko* cultivar. Cameroon.J.Experimental Biology.9 (1): 1-8.

Dolui, A.K., Asha Bisht and Kumar A. 2012. HPLC assessment of Production of 6- aminopenicillanic acid by free and alginate locally Immobilized Bacteria isolated soil. Res.J.Biotech. 7 (4): 11-16.

E.B.C., 1987.Analytica-EBC. Engasser, J. M., 1988. Modeling of fermentation process. In Biotechnology, Scriban R. coordinator; Techn. Doc. Lavoisier, 3rd Ed. Paris: 301-323.

Gaillardin, C. and Heslot H. 1987. Yeast. Research.18 :586-601.

Ganesan Sasikala and Nellaiappan Olaganathan Gopal. 2014. Exploration of wild yeast strains for thermotolerance and ethanol production. Res.J.Chem.Environ.**18** (1): 14-22.

Gosselin, Y., and Fels Debourg A. S. 2000. Industrial use of dry yeast in brewing. Bios, No **2,** Congress: 32-38.

Heineken., 1986. Instructions for controling the manufacturing. Amsterdam, Holland.

Larrieu, J., 1988. Use of statistics in quality management. In: Biotechnology, Scriban R. coordinator; Techn. Doc. Lavoisier, 3rd Ed Paris: 661-679.

Jong H.Lee, Sae Lee K. Kwan H.Park, In K.Hwang, Geun H.Ji (1999).Fermentation of rice using amylolytic Bifidobacterium. Int. J.Food Microbiol.50 :155-161.

Leveau Y.J. and Bouix M. 1988.Microbial kinetics. In: Biotechnology, Scriban R. coordinator; Techn. Doc. Lavoisier, 3rd Ed Paris:…..

Lonvaud-Funel, A., J.P. Maseclef, A. Joyeux and Paraskevopoulos Y. 1988. Study of interactions between yeasts and lactic acid bacteria in grape must. Knowledge Vine Wine : 11-24.

Marc, I., 1982. Yeast fermentation: Bibliographic study. Bios.13 (10): 45-58.

Oyeyiola, G.P., 1991. Fermentation to produce *kamu* of millet, a Nigerian starch-cake food. World Journal of Microbiology and Biotechnology: 196-201.

Ramaligam Kowsalya and Ramasamy Gurusamy. 2013.Isolation, screening and Characterization of *Bacillus subtilis* cellulase Producing KG10 from virgin forest of Kovai Kutralam, Coimbatore, India. Res.J.Biotech. 8 (6): 17-24.

Soufleros, E., Bertrand E.C. 1979. Role of the yeast strain in the production of volatile substances during the fermentation of grape juice. Knowledge Vine Wine (13): 181-189.

Stewart, G.G. 1985. New developments in ethanol fermentation. MBAA Technical Quarterly, Vol.22: 119-123.

Stewart, G.G., T. Yonesawa and Martin S.A. 2007. Influence of mashing conditions on fermentation characterstics of all-malt wort used to produce beer or whisky. MBAA, TQ. 44 (4): 256-263.

Take Ajaykumar., M. and Kharat Rajhans G. 2012. Production and characterization of α- amylase isolated from *Aspergillus Niger* by soil state fermentation. Res.J.Biotech. 7 (4): 36-45.