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# Potentials of Wild Strain *Saccharomyces cerevisiae* in Ethanol Production

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**Abstract:** Yeast strain isolated from rotten Irish potato was identified as *Saccharomyces cerevisiae*. It was characterized biochemically and morphologically and found to be identical with the standard strain of *S. cerevisiae*. The isolate potential for ethanol production from Cassava starch was assayed and found to produce ethanol from starch under natural environmental conditions. The optimal pH was found to 3.5-3.7 with a directly proportional increase in ethanol and glucose concentration (Mean ± SD). This wild strain of *S. cerevisiae* possessed ability to produce starch degrading enzymes, thus salvaging the cost of purchasing starch degrading enzymes in ethanol production. This research paper was to hypothesize that certain wild strain of *S. cerevisiae* could posses the ability to produce starch degrading enzymes in their natural environment and as such could produce both glucose and ethanol.

**Key words:** *S. cerevisiae* · Wild strain · Cassava · Ethanol

## INTRODUCTION

There is a great need for alternative source of energy to substitute petroleum based fuel for both energy and climate change considerations. Cassava (*Manihot esculenta*) is an efficient carbohydrate crop [1, 2]. Cassava has been documented as one of the potential bioethanol crop [3]. Cassava is a cheap substrate that is easily available in tropical countries such as Africa [4]. Microorganism like *Saccharomyces cerevisiae* among others has attracted considerable attention in recent years for the production of ethanol from renewable agricultural resources [5-10]. *S. cerevisiae* ability to produce a high concentration of ethanol is commonly utilized in ethanol production [11]. *S. cerevisiae* has the potential as ethanol fermenting yeast from glucose, but said to be ineffective in producing ethanol from starch since it was documented that they lack starch-decomposing enzymes such as α-amylase and glucoamylase [12]. It has also been documented that it is necessary to add starch decomposing enzymes in a bioreactor before *S. cerevisiae* can utilize starch as a carbon source [13, 14].

This paper revealed a wild strain of organism causing soft rot in Irish potato and investigated for its ability to degrade starch for ethanol production and had been identified as *S. cerevisiae* which is capable of fermenting cassava starch to ethanol without the addition of starch degrading enzymes at room temperature and without external alteration or adjustment of pH. The organism was observed to carry out fermentation as it would have done in its natural environment. The study had not only revealed the potential of this wild strain of *S. cerevisiae* in ethanol production from cassava, but also promises a high impact in industrial ethanol production through cost effectiveness by salvaging the high amount of money spent on the production or purchase of starch degrading enzymes. Its ability to carry out fermentation at room temperature will significantly reduce the high cost of cooling in fermentation industry. This strain presents a promising organism for further study and re-engineering for effective use in bioethanol industry.

## MATERIALS AND METHODS

**Isolation of Yeast Strain:** The yeast was isolated from rotten Irish-potato obtained from Maiduguri main market in Borno state, Nigeria. The rotten Irish-potato was sliced and inoculated on potato dextrose agar (PDA): 200g of potato tuber was peeled, sliced and placed in 1000ml beaker containing 500ml of distilled water and allowed to boil for 45 minutes. The extract was decanted into another beaker and 20g of dextrose was dissolved in it with 15g agar powder and stirred thoroughly, the total volume was about 850ml. the media was then transferred to 1000ml

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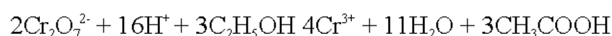
conical flask corked with clean and sterile cotton wool and aluminium foil and then autoclaved at 121°C for 15 minutes. Small amount of lactic acid and 50 mg chloramphenicol was added to the media to inhibit bacterial growth. Plates were incubated at 28°C for 48 hours to obtain a mixed culture. Pure culture was later obtained by subculturing distinct colonies on freshly prepared PDA, incubated at 28°C for another 48 hours.

**Identification of Yeast Strain:** Identification of the yeast based on morphology and physiological characteristics were carried out using standard yeast identification technique [15] along side with the methodology reported by Yarrow [16] and Yabaya and Jatau [17] with slight modification. Maximum growth temperature was determined in a yeast peptone dextrose broth using metal block baths (ISOCAL-6, Isotech, Southport, England). The utilization of the various carbon sources and other physiological characteristics were determined using a YT microplate™ (Biolog, Hayward, CA, USA) according to the manufacturer's instruction.

**Sample Preparation:** Cassava (*Manihot esculenta*) obtained from Maiduguri Monday market was peeled, dried and grounded into powder and were measured in 5, 10, 15, 20 and 25 in two replication into 150ml conical flask. 100mls of water was then added to each flask giving a concentration of 5/100, 10/100, 15/100, 20/100 and 25g/100ml of sample respectively. These concentrations were boiled in an autoclave at 121°C for 15 minutes, the process that sterilizes and also acts as pre-treatment and hydrolysis of the starch granules for microbial enzymes activities.

**Batch Fermentation:** Yeast isolates (5% inoculum size) were inoculated into the respective reaction flask, except the control and observed for fermentation product for 192 hours. The initial media pH ranged from 6.1-6.5. There was no pH adjustment but the pH of the medium continues to fluctuate due to production of acid as a by-product. Glucose and ethanol concentration were observed along with the decreasing pH values to ascertain the value at which the organism best carry out fermentation of starch under natural environmental condition. The temperature was at room temperature. The fermentation vessels covered with cotton wool and Aluminium foil were shaken periodically with the use of manual agitator. The medium contained optimal concentration of $(NH_4)_2SO_4$, $KH_2PO_4$, $MgSO_4.7H_2O$ as sources of Nitrogen, Potassium and Magnesium, respectively. There was no addition of α-amylase and glucoamylase, an aliquot of dextrose was added as a starter or boster to initiate the fermentation of glucose. Glucose and ethanol contents were assayed 24 hourly after the 72h of inoculation. The ability of the yeast strain to ferment cassava without the addition of starch degrading enzymes was studied without pH adjustment at room temperature to ascertain its potential in ethanol production under natural environmental condition.

**Analytical Methods:** The concentration of reducing sugar (glucose) was analyzed colorimetricaly by the method of Miller [18] using glucose standard. The ethanolic concentrations were analyzed by redox titration methods. The ethanol was oxidized to ethanoic acid by reacting it with excess of potassium dichromate in acid.



$$2Cr_2O_7^{2-} + 16H^+ + 3C_2H_5OH \; 4Cr^{3+} + 11H_2O + 3CH_3COOH$$

The amount of unreacted dichromate was then determined by adding potassium iodide solution which is also oxidized by the potassium dichromate forming iodine.

$Cr_2O_7^{2-} + 14H^+ + 6I^- \quad 2Cr^{3+} 3I_2 + 7H_2O$ the iodine was then titrated with standard solution of sodium thiosulfate and the titration results are used to calculate the ethanol content of the sample: $2S_2O_3^{2-} + I_2 \; S_4O_6^{2-} + 2I^-$.

## RESULTS

The yeast strain pure isolates had a cream-white colour on PDA after incubating at 28°C for 48hours. Gram stain reaction showed a G+ oval shaped cell. Microscopy (Bright field microscope: Olympus CH-2) revealed a yeast like colonies that reproduce by budding and was about 5.8-6.6 × 9.0-15.5$\mu$m. Acospores are present. Physiological characteristics based on certain markers showed that it utilized D-glucose, sucrose, maltose raffinose and maltotrios and had inability to utilize lactose, trehalose and galactose as carbon sources (Table1).

Table 1: Physiological and Biochemical Characteristics of Yeast strain

| Characteristic | Result |
|---|---|
| A.Carbon Utilization | |
| D-Glucose | + |
| Sucrose | + |
| Lactose | - |
| Maltose | + |
| Trehalose | - |
| Raffinose | + |
| Galactose | - |
| Maltotriose | + |
| B.Nitrate Assimilation | |
| $KNO_3$ | - |
| $NH_4SO_4$ | + |
| C.Ascospore | + |
| D.Growth at Elevated Temperature | |
| 20°C | + |
| 25°C | + |
| 30°C | + |
| 35°C | + |
| 40°C | + |
| 45°C | - |

+ = Positive, - = Negative

Table 2: Glucose concentration with varying concentration of biomass over Fermentation Time

| | Glucose (g/cm³) | | | | | |
|---|---|---|---|---|---|---|
| Biomass (g/dl) | 5 | 10 | 15 | 20 | 25 | Control |
| 24h | 2.55 | 2.69 | 2.80 | 3.07 | 3.26 | 0.35 |
| 48h | 2.82 | 3.13 | 3.96 | 4.52 | 4.86 | 0.48 |
| 72h | 3.04 | 4.45 | 4.97 | 5.27 | 5.41 | 0.52 |
| 96h | 3.23 | 4.62 | 5.04 | 5.31 | 5.56 | 0.51 |
| 120h | 3.33 | 3.42 | 5.13 | 5.36 | 5.57 | 0.45 |
| 144h | 4.22 | 4.29 | 5.30 | 4.47 | 5.54 | 0.54 |
| 168h | 3.68 | 3.91 | 5.33 | 5.42 | 5.53 | 0.50 |
| 192h | 2.91 | 3.85 | 4.90 | 5.22` | 5.28 | 0.48 |

Glucose and ethanol concentration (Mean ± SD) increases with biomass concentration (Table3).

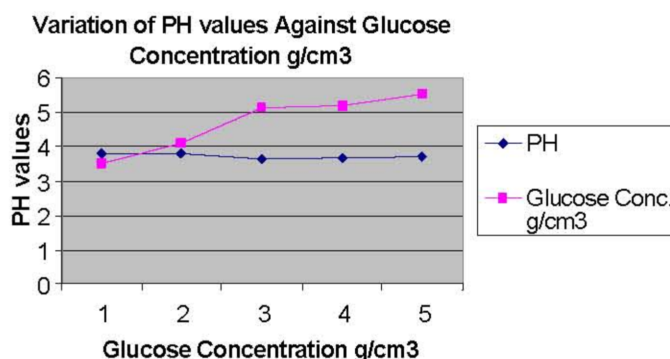



Fig. 1:

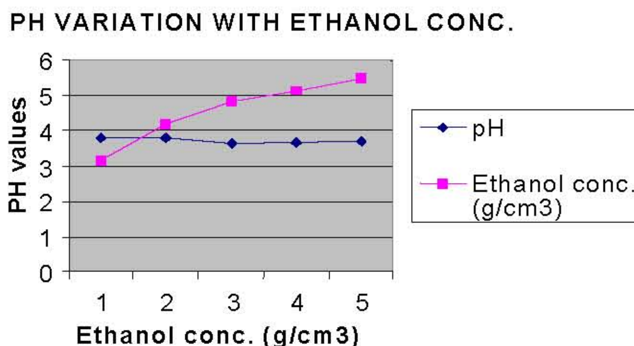



Fig. 2:

The yeast strain utilized ammonium sulfate as nitrate source, but showed inability to utilize potassium nitrate. It exhibited growth at 20-40°C (Table 1). Glucose concentrations in various biomass over fermentation time is optimal at 144h in 5 and 10g/dl, 168h in 15g/dl and 120h in 20 and 25g/dl after which they began to flunctuate, however a gradual increase in glucose concentration was observed in various concentration of biomass over a given fermentation time (Table 2).

Ethanol concentration was slightly higher than that of glucose. Both glucose and ethanol concentration increases with decrease in pH, with optimal pH range of 3.5-3.7 (Figs.1 and 2).

## DISCUSSION

Biochemical and morphological analysis identified the yeast strain as *S. cerevisiae* [17, 19]. Decrease in glucose concentration after 144h in 5 and 10g/dl, 168h in 15g/dl and 120h in 20 and 25g/dl showed that the organism logarithmic phase differs in various substrate concentrations which in turn determine the number of viable cells carrying out the hydrolysis. Higher amount of glucose concentration in the fermentation flask as compared to controls showed the organism ability to produce glucose from Cassava which had proved their ability to produce α-amylase and glucoamylase enzymes

Table 3: Glucose and ethanol concentration (Mean ± SD) with varying concentration of biomass

| Biomass (g/dl) | Glucose (g/cm$^3$) | Ethanol (g/cm$^3$) |
|---|---|---|
| 5 | 3.22 ± 0.53 | 3.16 ± 0.25 |
| 10 | 3.80 ± 0.67 | 4.20 ± 0.60 |
| 15 | 4.68 ± 0.87 | 4.84 ± 0.18 |
| 20 | 4.96 ± 0.82 | 5.15 ± 0.18 |
| 25 | 4.91 ± 1.06 | 5.50 ± 0.24 |

contrary to documented envidence that starch degrading enzymes must be added to the bioreactor before *S. cerevisiae* can effectively produce glucose [13,14]. Small amount of glucose as detected in the control was due to aliquots of glucose added to the substrate as booster and due to partial hydrolysis by heat during sterilization of the substrate. Slight increase in the mean ± SD values of ethanol concentration compared to glucose concentration (Table3) was due to immediate utilization of the glucose as direct substrate to ethanol production. Production of acid as by-product must have accounted for the continual decrease in the pH value which was a regulatory mechanism to determine how flunctuation in pH affects the activity of this wild strain of *S. cerevisiae*. High amount of glucose and ethanol was then obtained at pH range of 3.5-3.7 which by implication showed the optimal pH at which this wild strain of *S. cerevisiae* could carry out fermentation in its natural environment.

In concluaion, this research paper hypothesized that certain wild strain of *S. cerevisiae* could posses the ability to produce starch degrading enzymes in their natural environment and as such could produce both glucose and ethanol.

## REFERENCES


1. Wang, W., 2002. Cassava production for industrial utilization in China-Present and future perspective (online); http://www.ciat.cgiar.org/asia-cassava/pdf/proceedings-workshops;02/33.pdf;2002 (accessed 29.12.08)
2. Bokanga, M., 1996. Biotechnology and Cassava proceedings in Africa. IITA Res., 12: 14-18.
3. Lewis, H.Z., G.B. Runion, M. Tomecek, A.P. Stephen, H.A. Torbet and R. Sicher, 2009. An evaluation of Cassava, sweet potato and field corn as potential carbohydrate sources for bioethanol production in Alabama and Maryland. Biomass and Bioenergy, 33: 1503-1508.
4. Brauman, A., S. Keleke, M. Malonga, E. Miambi and F. Ampe, 1996. Microbiological and Biochemical characterization of Cassava rating, traditional lactic acid fermentation for foo-foo (Cassava flour) production. Appl Environ Microbiol., 62: 2854-2858.
5. Deshpande, V., H. Sivaraman and M. Rao, 1983. Simultaneous saccharification and fermentation of cellulose to ethanol using Penicillium funiculosum cellulase and free or immobilized Saccharomyce suvarum cells. Biotechnol. Bioeng, 25: 1679-1684.
6. Krishnan, M.S., F. Taylor, B.H. Davison and N.P. Nghiem, 2000. Economic analysis of fuel ethanol production from corn starch using fluidized-bed bioreactors. Bioresour. Technol., 75: 99-105.
7. Limtong, S., C. Sringiewa and W. Yongmanitchai, 2007. Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*. Bioresour Technol., 98: 3367-3374.
8. Pan,Y.C. and W.C. Lee, 2005. Production of high-purity isomalto- oligosaccharides syrup by the enzymatic conversion of transglucosidase and fermentation of yeast cells. Biotechnol Bioeng, 30: 797-804.
9. Rhee, S.K., G.M. Lee, C.H. Kim, Z. Abidin and M.H. Han, 1986. Simultaneous sago starch hydrolysis and ethanol production by Zymomonas mobilis and glucoamylase. Biotechnol Bioeng Symp., 17: 481-493.
10. Verna, G., P. Nigam, D. Singh and K. Chaudhary, 2000. Bioconversion of starch to ethanol in a single-step process by coculture of amylolytic yeast and *Saccharomyces cerevisiae*. Bioresour Technol., 72: 261-266.
11. Yazawa, H., H. Iwahashi and H. Uemura, 2007. Disruption of URA7 and GAL6 improves the ethanol tolerance and fermentation capacity of *Saccharomyces cerevisiae*. Yeast, 24: 551-560.
12. Gupta, R., P Gigras, H. Mohapatra, V.K. Goswami and B. Chauhan, 2003. Microbial -amylases: a biotechnological perspective. Process Biochem., 38: 1599-1616.
13. Altintas, M.M., K.O. Ülgen, B. Kirdar, Z.I. Önsan amd S.G. Oliver, 2003. Optimal substrate feeding policy for fed-batch cultures of *S. cerevisiae* expressing bifunctional fusion protein displaying amylolytic activities. Enz. Microbial. Technol., 33: 262-269.

14. Nakamura, Y., T. Sawada and A. Komatsu, 2002. Ethanol production from raw starch by recombinant yeast having saccharification and fermentation activities. J. Chem. Technol. Biotechnol., 77: 1101-1106.
15. Mossel, D.A.A., J.E.L. Lorry, C.B. Struik and R.M. Baird, 1995. Essentials of Microbiology of foods. John Willey and sons Ltd, pp: 44.
16. Yarrow, D., 1998. Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, J.W, Fell, editors. The yeasts: a taxonomic study. Amsterdam: Elsevier, Science. B.V., pp: 77-100.
17. Yabaya, A. and E.D. Jatau, 2009. Investigating wild yeast baking potentials. Middle-East Journal of Scientific Res., 4: 320-322
18. Miller, G.L., 1969. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.
19. Gi-Wook, C., U. Hyun-Ju, K. Yule, K. Hyum-Woo, K. Mina, C. Bong-Woo and K. Yang-Hoon, 2010. Isolation and characterization of two soil derived yeast for bioethanol production on Cassava starch. Biomass and Bioenergy, 34: 1223-1231.