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Efficient Anaerobic Fermentation of Simple Sugars by Yeast Fuels Resistance Candida spp. Infections to Eradication by Drugs

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Abstract: Problem statement: Human systemic Candida infections had proved difficult to eradicate by the medical health care system. Some practitioners and scholars see the problem as being due to drug resistance. For example an author wrote that 'secondary drug resistance is clearly being encountered in one setting, oropharyngeal candidiasis in patients with advanced Human Immunodeficiency Virus type 1 (HIV-1) infection usually following years or months of azole therapy. **Approach:** This research work understudied the nutritional strategies of yeast colonies to serve as a guide to understanding the survival strategies of Candida colonies in human Candidiasis. The aim of the research was to make some impute into more effective ways of eradicating human Candida infections. Ethanol was produced biologically by fermentation of sugar by micro-organisms. The yeast Saccaromyces cerevisiae metabolises complex carbohydrates like starch in the absence of oxygen to ethanol, carbon dioxide and water. This study compared the average ethanol yield of hydrolyzed and unhydrolyzed gelatinized cassava starch fermented by Saccharomyces cerevisiae. The starch was hydrolyzed by α and β-amylase enzymes. Fermentation of the starch was done with a 1% innoculums of a 12 h culture of saccharomyces cerevisiae incubated for 48 h under anaerobic conditions. **Results:** The results of the study showed that there was no starch hydrolysis in the absence of α and β-amylase enzymes. Starch hydrolysis in the presence of α and β-amylase enzyme took 1 h. There was no starch fermentation in the absence of saccharomyces cerevisiae. The ethanol yield of starch which had been hydrolyzed by α and β-amylases prior to fermentation by saccharomyces cerevisae was 28 times higher than the ethanol yield of starch which had not been previously hydrolyzed by α and β-amylases. These results of the study suggest that yeast infections in human and animal tissues produce 28 times more ethanol yield from the glucose present in the host tissues (for tissue respiration) than they would produce from the fermentation of unhydrolyzed starch outside the body tissues of the host (like from undigested starchy food trapped in the mouth and throat by oropharyngeal Candida infections). **Conclusion:** The findings of the study enables us to conclude that this innate ability of yeast species to easily produce large yields of ethanol from anaerobic fermentation of simple sugars like glucose creates a competitive advantage which enhances their continuous survival in systemic human body tissues where glucose available for host tissue respiration is ever present. The efficient eradication of such yeast infections in human victims (and animals) should incorporate ways of diminishing the availability of excess hydrolyzed sugars in the host tissues (which the yeast colonies easily survive on). The escalating effect of stress (including oxidative stress) on Candidiasis lnfection proliferation should also be communicated to systemic Candidiasis patients.

Key words: Efficient eradication, additional distilled, gelatinized starch, amylopectin content, determined independently, hundred milliliters

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INTRODUCTION

Amphotericin B is regarded as the most efficacious drug for the treatment of Candidiasis yet the following two statements illustrate the frustration of the medical health care system over the resistance of Candidiasis to eradication by Amphotericin B:

- The rarity of significant Amphotericin B resistance arising during therapy has left it unclear whether or not ergosterol-deficient mutants retain sufficient pathogenicity to survive in deep tissue
- Topical Amphotericin B is useful only only in cutaneous Candidiasis. Oral tablets are available commercially in Europe for decreasing colonization of the intestine by Candida

This research study aims at under-studing the nutritional and thus survival stratagies of the yeast Saccharomyces cerevisiae as a pointer to the nutritional/survival strategies of Candida colonies which are partly fungus and partly yeast morphologically and physiologically.

Ethanol has been in use as a fuel in the United States and Europe since the 1800s. Henry Ford designed a car to run on a mixture of gasoline and Ethanol which he called the fuel of the future
Ethanol keeps the engine running smoothly without the need for lead or other chemical additives .

Cassava has been shown to be a good source of ethanol production because it has a high amylase/amylopectin ratio (Dubey, 2001) The amylase content of cassava starch was determined independently by Jaleel *et al*. (1988) to be 16.67%. Amylose content accounts for the different properties of starch gels and the generation of different oligosaccharides from starch. The amylopectin content of starch is responsible for the higher facility for water entrance in cassava starch which causes the starch to swell and become more susceptible to enzymatic hydrolysis.

Different processes have been formulated for production of Ethanol from cassava starch by liquid and solid substrate fermentation of the cassava starch after hydrolysis of the starch (Jaleel *et al*., 1988). Since different yeast colonies ferment starch, this study investigated the ethanol yield of gelatinized starch fermented by Saccharomyces cerevisiae subsequent to enzymatic hydrolysis by α and β-amylase enzymes and in the absence of such hydrolysis.

MATERIALS AND METHODS

Pure starch was produced from the grated pulp of peeled mature wholesome cassava roots and stored in a freezer at 0°C after warer had been squeezed out of it. All operations were carried out under aseptic conditions.

Alpha amylase (α-amylase) [Termamyl 120 l NOVO NORDISK] and beta amylase (β-amylase) were obtained in separate sterile bottles from Jos International Breweries Production Unit, Jos and Plateau State, Nigeria. The two enzymes were stored at temperatures below 10°C throughout the period of the study.

Pure culture of Saccharomyces cerevisiae was obtained from the Diagnostic and Microbiology laboratory of Veterinary Research Institute,Vom, Plateau State, Nigeria. The Saccharomyces cerevisiae used in the study was prepared by inoculation on 20ml peptone water at room temperature.

Preparation of the cassava starch for the ethanol production experiments: The cassava starch solution used for the experiments was prepared by dissolving 50 g of the cassava starch prepared for the study in a clean beaker with 100 mL of distilled water by stirring it into the distilled water to form slurry. Additional 850 mL of gently boiling water was added to the slurry while stirring vigorously to gelatinize the starch. The gelatinized starch was made up to one liter with additional distilled water to give a 50g L^{-1} gelatinized starch.

Experiment A: Treatment of starch solution with α and β-amylase enzymes: Two hundred milliliters (200 mL) of the 50 g L^{-1} gelatinized starch solution of pH 5.5-7.5 was put in a 500 mL beaker and was steadied in a water bath at 70°C. Alpha amylase (1 mL) was added to the solution and was allowed to act for 30 min. The temperature of the solution was then reduced to 60°C and 1mL of β-amylase enzyme was added to the starch solution to act for 30 min. The resulting solution was then tested for the presence of starch.

Experiment B (Control experiment 1): Two hundred milliliters (200 mL) of the 50 g L^{-1} gelatinized starch solution of pH 5.5-7.5 was put in a 500 mL beaker and kept steady in a water bath at 70°C. Nothing was added to the solution and it was allowed to stand for 30 min. The temperature of the solution was then reduced to 60°C and nothing was added to the starch solution but it was allowed to stand for 30 min. The resulting solution was then tested for the presence of starch.

Experiment C (Control Experiment 2): Two hundred milliliters (200 mL) of the 50 gL^{-1} gelatinized starch solution of pH 5.5-7.5 was put in a 500 mL beaker steadied in a water bath at 70°C. Nothing was added to the solution and it was allowed to stand for 30 min. The

temperature of the solution was then reduced to 60°C and nothing was added to the starch solution again but it was allowed to stand for 30 min. The resulting solution was then tested for the presence of starch.

Experiment D: Fermentation of the starch solutions of experiment A with Saccharomyces cerevisiae.

Fermentation was carried out by putting three 200 mL samples (Abouzied and Reddy, 1986) of the hydrolysed starch in 500 mL conical flasks with a 1% innoculum from the 12 h culture of Saccharomyces cerevisiae at 30°C. The flasks were each covered tightly with a one-hole rubber stopper to which a pipette was plunged in and connected to a rubber tube that was placed under bromothymol reagent to check for carbon dioxide production (anaerobic conditions). The samples were incubated for 48 h.

Experiment E: Fermentation of the starch solutions of experiment B with Saccharomyces cerevisiae.

Fermentation was carried out by putting three 200 mL samples (Abouzied and Reddy, 1986) of the unhydrolysed starch of experiment B in 500 mL conical flasks with a 1% innoculum from the 12 h culture of Saccharomyces cerevisiae at 30°C. The flasks were each covered tightly with a one-hole rubber stopper to which a pipette was plunged in and connected to a rubber tube that was placed under bromothymol reagent to check for carbon dioxide production (anaerobic conditions). The samples were incubated for48 h.

Experiment F (Control experiment 3): Fermentation of the starch solutions of experiment C without Saccharomyces cerevisiae.

Three two hundred milliliter (200 mL) sample (Abouzied and Reddy, 1986) of the unhydrolysed starch of experiment C was put in 500 mL conical flasks without a 1% innoculum from the 12 h culture of Saccharomyces cerevisiae at 30°C (Dombek and Ingram, 1987). Each of the flasks was covered tightly with a one-hole rubber stopper to which a pipette was plunged in and connected to a rubber tube that was placed under bromothymol reagent to check for carbon dioxide production (anaerobic conditions). The sample was incubated for 48 h.

Number of experiments conducted: The results of each of experiments A, B, C, D, E and F were recorded.

Test for starch: Four to five drops of a sample were put on a clean cover glass. Two drops of iodine reagent were added to the sample on the cover glass. The turning of the sample to blue-black on immediate contact with iodine indicated the presence of starch.

Test for ethanol: Two drops of $KMnO_4$ were added to 5 mL of the test solution in a test tube. An equal volume of H_2SO_4 was added to the solution in the test tube and the solution heated till boiling.

RESULTS

The giving off of a smell like that of acetaldehyde by the solution on boiling was a positive indication of the presence of ethanol.

Determination of Ethanol Content of Samples obtained in Experiment D, E, F: After fermentation of the samples in experiment D, E and F, the samples were each filtered with number 5 filter paper and 100 mL of the filtrate of each sample was used to determine the specific gravity of that sample.

Determination of the specific gravity of the samples: The 100 mL filtered sample from the result of experiment D, E and F was heated in a water bath at 80°C until the ethanol evaporated; passed through a distillation column and collected in a density bottle.

The ethanol in the density bottle was made up to 100 mL with distilled water and placed in a water bath at 20°C. After the solution in the density bottle had attained 20°C, it was weighed on an electronic weighing balance and the weight recorded.

The specific gravity of the ethanol sample was calculated thus.

Weight of the sample:

Weight of equal volume of distilled water: The weight of the empty density bottle was subtracted from the weight of the density bottle and the sample. The weight of the distilled water alone was similarly obtained by subtracting the weight of the empty bottle from the weight of the distilled water and the empty bottle.

Determination of the ethanol content of the sample: The ethanol content of the test results of experiment D, E and F were determined by reading off the result obtained for the specific gravity of experiment D, E and F samples on a table of 'the relations between specific gravity and 20/20°C alcohol content of mixtures of ethyl alcohol and water' obtained from figures in table 20, page 34 of methods of analysis,

association of official agricultural chemicals, Washington D.C., 1930.

Results: There was starch hydrolysis in the test sample of experiment A in a time of 1 h as the resultant sample at the end of experiment a tested negative for the presence of starch.

There was no starch hydrolysis in the test samples of experiment B and C as the resultant samples at the end of experiment B and C tested positive for the presence of starch.

Ethanol was produced by experiments D and E as the resultant samples at the end of experiment D and E tested positive for the presence of ethanol.

Ethanol was not produced by experiments F as the resultant sample at the end of experiment F tested negative for the presence of ethanol.

Comparism of the percentage ethanol yield by volume of experiments D and E: The ethanol yields (% by volume) of three samples of hydrolysed starch (of experiment A) fermented by Saccharomyces cerevisiae) were 8.76, 8.64 and 8.70 respectively.

The ethanol yields (% by volume) of three samples of unhydrolysed starch (of experiment B) fermented by Saccharomyces cerevisiae) were 0.36, 0.28 and 0.28 respectively. A comparism of the ethanol yields of hydrolyzed and unhydrolyzed gelatinized cassava starch fermented by Saccharomyces cerevisiae in this study is shown graphically in Fig. 1.

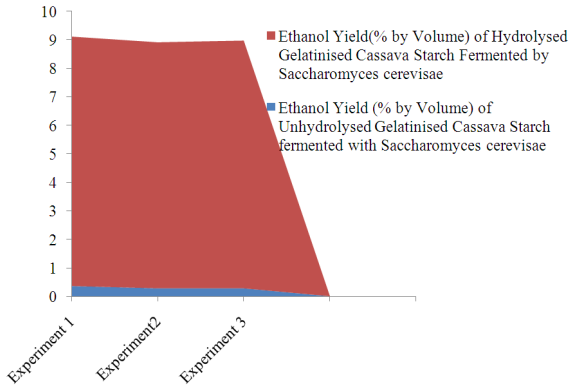

Fig. 1: Hydrolysis of gelatinized cassava starch with α and β-amylase enzymes (which are similar to the enzymes that break down cooked starch in the digestive system of man to a final product of glucose) aused a 280% increase in the Ethanol yield of the starch from its fermentation by Saccharomyces cerevisae (yeast cells)

DISCUSSION:

From the mean of the figures of the ethanol yields obtained in experiment D and E and from the graphical comparism of these figures in Fig. 1, hydrolysis of the gelatinized starch increased the ethanol yield of the Saccharomyces cerevisiae anaerobic fermentation of the gelatinized starch by 280%. This result illustrates the high efficiency of yeast anaerobic fermentation of hydrolyzed gelatinized (cooked) starch.

Hydrolysis of gelatinized starch is similar to digestion of cooked starch in the human digestive system. Both processes produce glucose as the end product of the breakdown of high energy complex carbohydrates like starch. This study thus illustrated that yeast colonies like Candida infection colonies residing in systemic body organs (which can provide anaerobic media in situations of oxygen lack in the body like during stress) can efficiently ferment the glucose available in the energy generation systems of the host cells to produce large quantities of ethanol for their own metabolism.

Starch molecules are glucose polymers linked together by α-1, 4 and α-1, 6 glucosidic bonds (unlike the β-1, 4 glucosidic bonds of cellulose). Because of the presence of these two types of bonds in the starch molecule, different structures are possible for the starch molecule. An unbranched chain polymer of 500-2000 glucose subunits with only the alpha-1, 4-glucosidicbonds is called amylose. The presence of α-1, 6-glucosidic linkages results in a branched glucose polymer called amylopectin. The major chemical difference that exists in starch from various sources is in the ratio of amylose to amylopectin (Bailey and Ollis, 1986).

Enzymatic hydrolysis of starch has produced sugars and malt dextrins with high yield (Bailey and Ollis, 1986; Nagi *et al*., 1999). The efficiency of starch hydrolysis to produce sugars and malto-oligosaccharides depend on α-amylase action. Alpha-amylase acts at random locations along the starch chain to break down long chain carbohydrates to ultimately yield maltotriose and maltose from amylose or maltose; and to produce glucose and dextrins from amylopectin

Beta-amylase works from the non-reducing end to catalyze the hydrolysis of the second α-1, 4-glycosidic bond, cleaving off two glucose units (maltose) at a time

The results of this study demonstrated that saccharomyces cerevisiae (yeast) could produce ethanol from both hydrolyzed and unhydrolyzed gelatinized [cooked] starch This can be extrapolated to mean that yeast infections in the human body can ferment

unhydrolyzed starch like the starch of undigested carbohydrate foods present in the mouyh or traped along the pharynx by oropharyngeal Candidiasis.

What was more disturbing was the finding that the Saccharomyces cerevisiae fermentation had 280% increase in its ethanol production from the same quantity of starch when the starch was hydrolyzed into sugars like glucose by α and β-amylase enzymes. This result suggests that pathologic yeast infections like Candida spp. infections produce large volumes of ethanol from the glucose assimilated into body cells (after the absorption of digested dietary carbohydrates) especially under situations of oxygen lack or oxygen insufficiency that may exist when the body is experiencing physiological stress. No wonder the rapid and exponential spread of Candida infections in Aids and immune-compromised patients suffering from other diseases and body disorders like stroke.

As has been pointed out in this study, ethanol is an efficient fuel source. Thus yeast infections of man and other animals can utilize the large ethanol yields they obtain from fermentation of glucose and other monosaccharides or disaccharides to generate energy for their body activities, the most important of which is self proliferation.

Ethanol reacts with ammonia to produce ethylamine which the yeast infection can use to produce chemotherapeutic and other body metabolism moderators including surfactants as is done in chemical manufacturing industries.

Host tissues require oxygen for tissue respiration to generate energy from glucose but yeast cells produce ethanol from glucose anaerobically. This suggests that pathogenic yeast cells like Candida infections utilize glucose in the host's tissue cells more efficiently than their host tissues. This explains why yeast infections like Candida spp. infections are highly resistant to complete eradication by drugs as glucose generated from digestion of dietary high energy foods like starches are ever present in host tissue cells(especially immediately after meals) for host tissue energy generation.

Antibiotic assay was done by using agar dilution method which confirms the importance of the presence of sugars in microbial agent elimination. When to pathogenic micro-organisms have to compete for the same food sourse or feed synergistically on the same food source, the establishment of one organism will be inhibited or enhanced by the existence of the other in the same environment (Kuo *et al*., 2005; Kugelberg *et al*., 2005).

Nigella sativa seed which has demonstrated numerous antimicrobial anti-oxidant, immunomodulatory and therapeutic effects (Abu-Al-Basalc, 2009; Hanafy and Hatem, 1991; El-Wakil, 2007; Salem, 2005; Siebenhaar *et al*., 2007) enhanced glucose-induced induced insulin release from rat isolated Islets of langahans (Rchid *et al*., 2004), which highlights the pivotal role of simple sugars in disease infectivity.

CONCLUSION

The study concludes that the fact that yeast species can easily anaerobically produce large yields of ethanol from fermentation of simple sugars like glucose creates an enabling environment for their efficient survival in human body cells where they survive efficiently on the glucose available for host cell energy generation. Successful eradication of such yeast infections in human hosts (especially those of carbohydrate or high calorie diet culture) should incorporate a reduction of calorie intake and a reduction of the patient's exposure to stress and stressors.

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