International Journal of Biomedical and Health Sciences
Vol. 5, No. 1, March 31, 2009
Printed in Nigeria

0794-4748/2009 $12.00 + 0.00

http://www.asopah.org

IJBHS 2009006/5102

The use of *Candida tropicalis* as a source of single cell protein

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(Received February 20, 2009)

ABSTRACT: The biomass formation of *Candida tropicalis*, isolated from Palm wine in different nitrogen and carbon sources was examined using the dry weight method. A significantly high increase in yield was observed when 1 % fructose was used for this organism and maximum yield value was obtained in 4% fructose and 0.075 % urea as carbon and nitrogen sources, respectively. Cane molasses, a natural substrate was also used in growing this organism, and a significantly higher increase in yield was obtained in 0.2% (w/v) of the reducing sugar for *C.tropicalis*.
Analyses of the proximate composition of the fungus revealed that *C. tropicalis* had carbohydrate, protein and lipid values of 31.2, 39.2 and 8.3, respectively, with free amino acid content of 41 x 10-3 g/l in synthetic medium and 29.1, 41.3 and 4.8, respectively in cane molasses. The energy values were also calculated to be 1504.81 and 1373.97 kJ/g, respectively. These results are discussed with reference to the potential nutritional benefits of this organism.

Key Words: Biomass, Yield, Proximate Composition, Nutritional Benefits.

Introduction :

The rapidly increasing world population and the unprecedented climatic change has drastically affected the conventional systems of food production (1). Science and technology is being daily challenged to master the problem of supplying mankind with sufficient food, particularly protein. The quantity of meat protein for human consumption is totally inadequate, thus, the use of unconventional protein sources like cells of algae, bacteria, yeasts and other fungi needs to be revisited. Microorganisms can be used directly as a food source or as a supplement to other foods and are then called Single Cell Protein. Microbial proteins have advantages of short generation time to produce a rapid mass increase. They can also be genetically modified to give variants with the required qualities, and the cell matter being rich in most group B vitamins, constitute a potential vitamin enrichment medium for deficient diets (2).

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Of all the microorganisms studied, yeasts probably have the most favourable characteristics for use as a major source of food . *Candida utilis* was incorporated into human food during the World War 11, while *C. tropicalis* has been successfully cultivated in hydrocarbon media for Single Cell Protein Production (3; 4). Besides a high protein content (45 – 65 % dry weight), yeasts also contain some other nutritive components like lipids, carbohydrates and fibre. However their composition is greatly affected by changes in the medium and culturing conditions (5).

Thus, the objectives of this study are;

i) to isolate and identify appropriate organism of high protein content from indigenous food sources such as palm wine.

ii) to determine the best carbon and nitrogen sources as the primary nutrient for growth.

iii) to compare the growth of the fungus in a synthetic medium as well as in cane molasses, a natural substrate.

iv) to carry out proximate analyses on this organism grown in synthetic medium and cane molasses as to their suitability as food supplement.

Materials and methods:

Candida tropicalis was isolated from Palm wine, characterized and identified using Standard Mycological Methods (6,7,8).

Carbon nutrition

The carbon nutrition study was determined as described by Oguntuyo (9). The basal growth medium consisted of $(NH_{4)}\ _2SO_4$ 2.0 g; $MgSO_4.7H_2O$ 0.2g; NaCl 5.0g; $FeSO_4$ 0.01g ; K_2HPO_4 0.5g and distilled water to 1 litre mark. 10 g of each of the carbon sources; glucose, fructose, mannitol , ethanol , sucrose, dextrin, xylose, maltose, lactose and soluble starch was added to the basal medium in each conical flask. Growth of the organism was assessed using the dry weight method (10).
For this organism, fructose proved to be the best carbon source, hence, different concentrations of fructose (2.0 – 6.0) was dissolved in 100 ml of the basal medium. The mixture was sterilized at 110^{o}C for 10 mins.

Nitrogen nutrition

The nitrogen nutrition was determined by dissolving 0.4 g of the total nitrogen in urea, ammonium sulphate , ammonium nitrate , asparagines, glutamine, methionine and other amino acids in 1 litre of the basal medium (excluding ammonium sulphate). Growth of the organism was assessed using the dry weight method.

Urea proved to be the best nitrogen source, hence the concentration of this was varied from 0.05 -0.4 % to determine that which gave the optimum growth.

Growth of the Yeast in cane molasses- a natural substrate

Different concentrations of cane molasses was prepared by dissolving 0.01- 0.4g in 100ml of the basal medium for microorganism using different carbon sources. The mixture was sterilized at 110^{o}C for 1h. It was cooled and 10ml of the yeast suspension was inoculated into the medium.
In all cases the period of incubation was 7 days at 28^{o}C.

Chemical Analysis of the Yeast Samples;

Moisture content;

Moisture was determined after drying the sample at 105^{o}C for 24 h (10).

Ash and Crude Fibre;

The ash content of the yeast sample was determined by incinerating the dried fungus of known weight at 600^oC for 12h in a Gallenkamp furnace. The crude fibre was determined by treating the defatted yeast sample with 0.112 M H_2SO_4 and 0.313 M NaOH (11).

Total lipids;

The lipid content in 2 g of each sample was determined as in AOAC (12).

Protein

Crude protein was estimated by determination of total nitrogen in 2 g of each sample by the Kjedahl's method, using a factor of 6.25.

Carbohydrates;

The anthrone method was used for the estimation of total carbohydrates. The concentration of glucose was read off from the standard curve of glucose prepared as described by AOAC (12).

Free Amino Acids;

This was determined by first hydrolyzing the samples in 6 M HCl and incubating *in vacuo* at 110^oC for 2h. A standard curve using serially diluted leucine was obtained (13).

Energy values of *C. tropicalis*

The energy values of the yeast were calculated on the basis of their content of crude protein, fat and carbohydrate by using the factors 17, 37 and 17 kJ/g, respectively (14).

Statistical Analysis

This was carried out using Analysis of Variance (ANOVA) (15).

Results and Discussion

Candida tropicalis isolated from palm wine showed increase in dry weight when grown in a synthetic basal medium containing each of the carbon source (Fig. 1). The organism showed the greatest increase of about 3.7 times for fructose, while the least of only 1.2 times was obtained for starch (Fig. 1). Fructose was reported as being readily utilized by yeasts and normally taken up by the constitutive hexose transport system in the same way as glucose (16). Before the utilization of starch, however, there must be the enzyme amylase to hydrolyse it. This may account for the minimal growth in the medium containing starch as a carbon source. Azoulay et al (17) reported that *C. tropicalis* can grow on soluble starch. Organisms tend to grow better at a particular concentration of their requirements, hence the concentration of fructose was varied (Fig.2). The cell yield of *Candida tropicalis* increased maximally at a concentration of 4 %. Higher concentrations had an inhibitory effect on growth of this organism hence there was decrease in the yield of cells which may be attributed to the 'crabtree effect' (16).

For efficient growth in a medium, a utilizable source of the element nitrogen must be present in order that organisms can synthesize amino acids and thus proteins and certain vitamins. *C. tropicalis* exhibited the highest growth rate (about 18.8 times increase) in urea at a concentration of 0.075 % (Figs. 3& 4). Urea in addition to being a nitrogen source also contribute carbon and energy sources. Morris (18) however revealed that there is no optimum amount of nitrogen for a culture since the demand is in the first instance on the carbon supply, thus, any factor may change the apparent optimum concentration of the nitrogen sources.

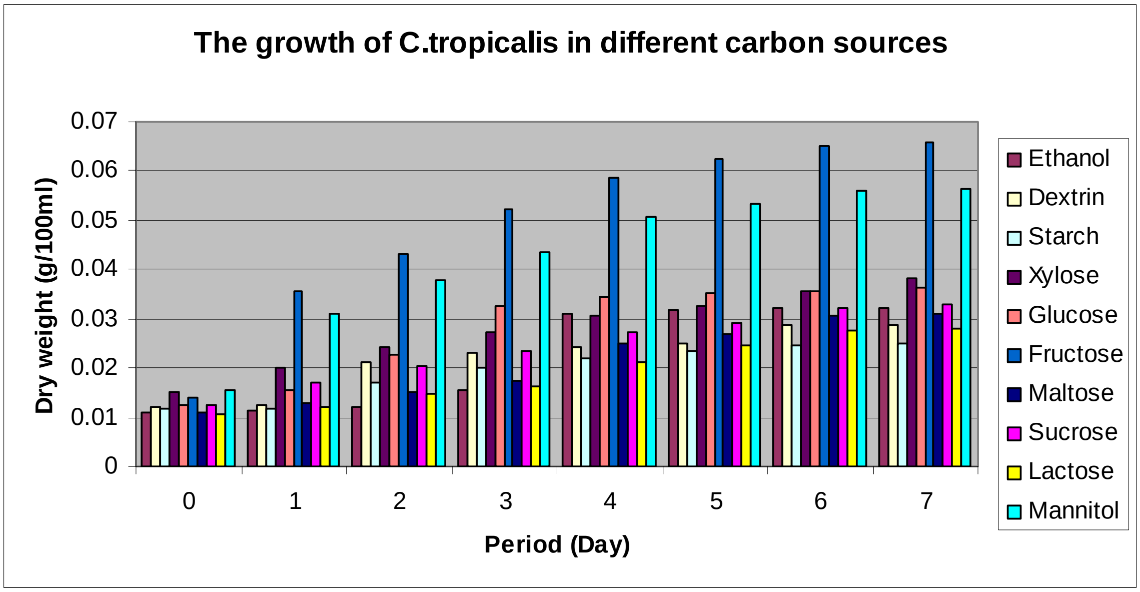

Fig. 1: The growth of *C. tropicalis* on different carbon sources.

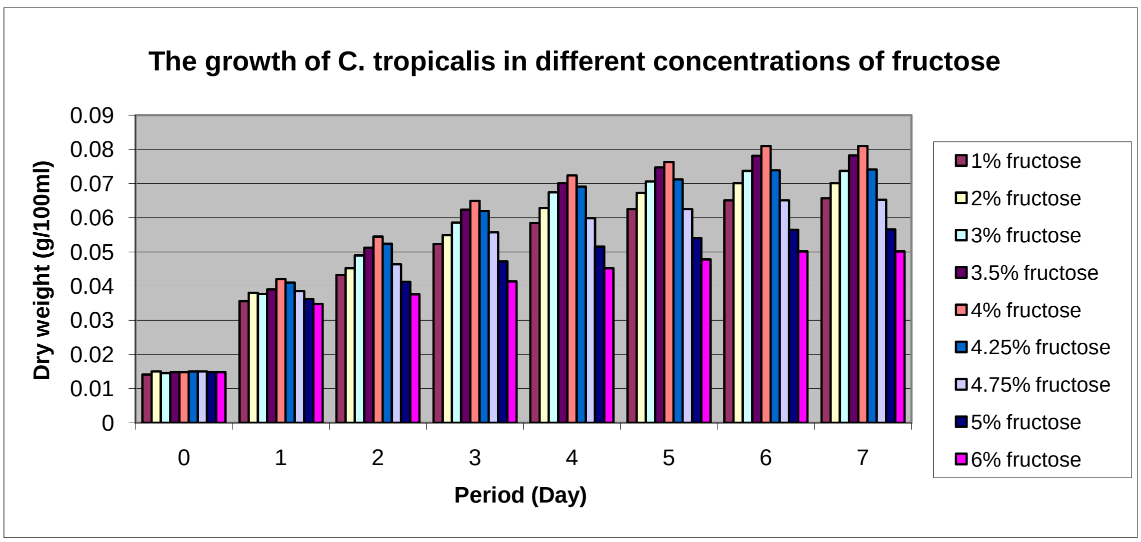

Fig. 2: The growth of *C. tropicalis* in different concentrations of urea.

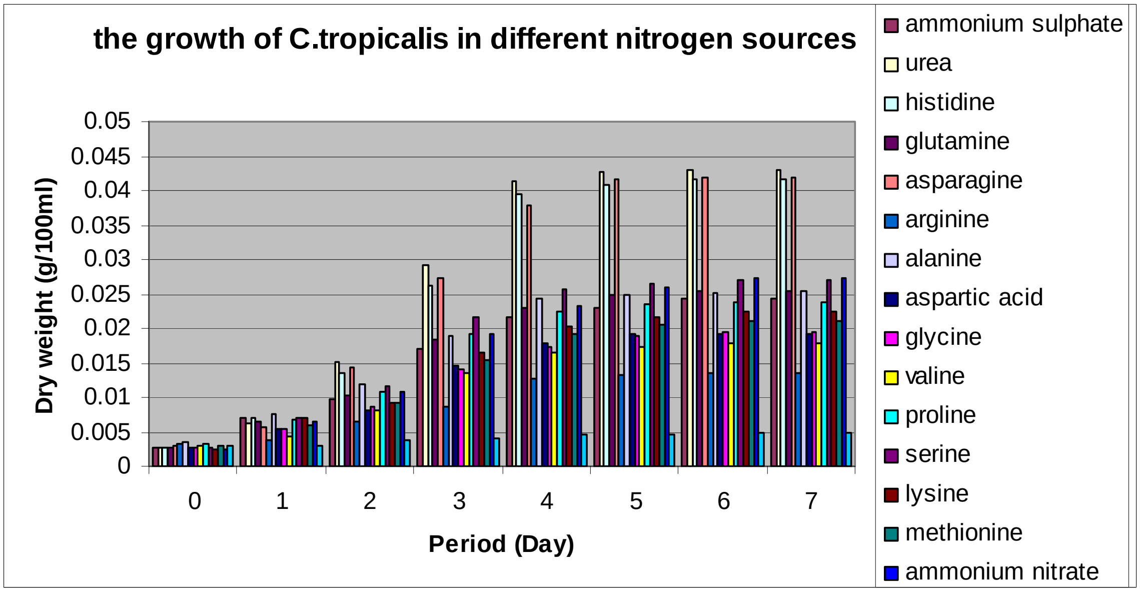

Fig. 3: The growth of *C. tropicalis* on different nitrogen sources.

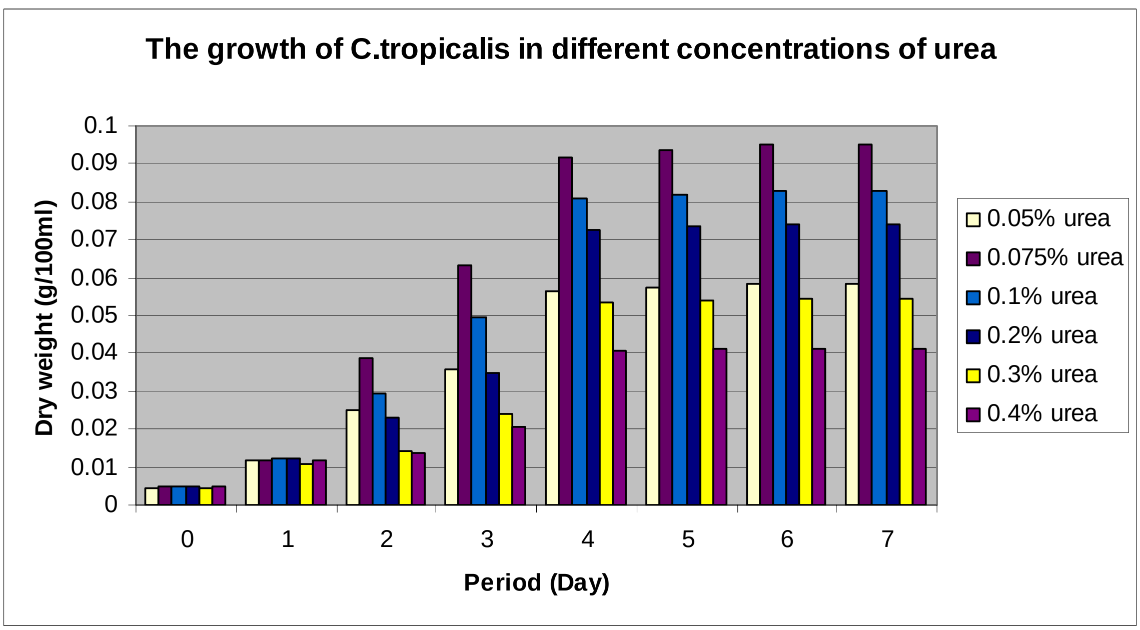

Fig. 4: The growth of *C. tropicalis* in different concentrations of urea.

Pure chemicals are generally not used for ‘Single Cell Protein’ production on an industrial scale because of the high cost of materials. Natural substrates like cane molasses are commonly used. There was an increase in dry weight of the organisms as the reducing sugar concentration in cane molasses was increased from 0.01 -0.3 % (Fig.5). This was followed by a decrease when a higher concentration of 0.4 % was used. *C. tropicalis* showed the greatest and least increase of about 3.7 and 2.5 times at concentrations of 0.2 and 0.4 %, respectively (Fig. 5). When the dry weight of *C. tropicalis* grown in 0.2 % of the reducing sugars in cane molasses were converted to an equivalent of 1 % (i .e. the dry weights obtained multiplied by 3.3, *C. tropicalis* was found to have initial dry weight of 0.003 g /100 ml (day 0) and final dry weight of 0.0376 g / ml (day 7) . Thus, the equivalent growth of the organism with cane molasses was calculated to be higher than the equivalent growth in 1 % fructose (Fig.1).

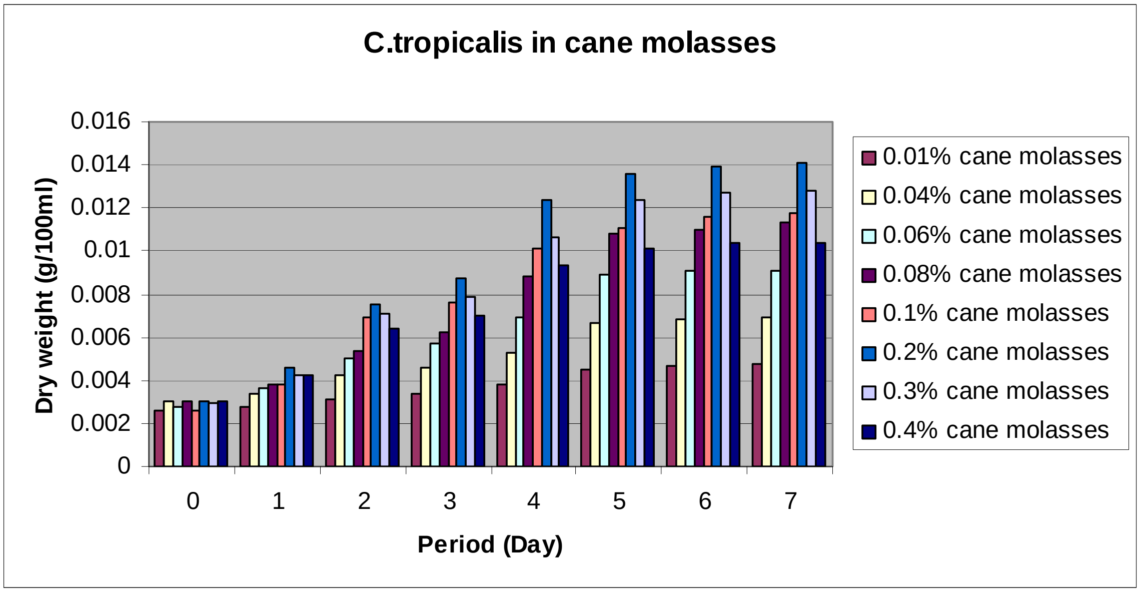

Fig. 5: The growth of *C. tropicalis* in different concentrations of cane molasses.

The use of an organism for food or as a supplement requires that it contains essential nutrients, thus, the proximate composition of the organisms in a synthetic medium and cane molasses were analysed (Table 1).About one half of the dry weight of yeasts generally contain crude protein and this consists of about 80% amino acids,12% nucleic acids and 8% ammonia, with about 7% of the total nitrogen occurring as free amino acids (19). Cane molasses gave a higher protein content which may be due to the presence of urea and other nitrogen sources. The free amino acids can easily be utilized unlike the proteins which are normally associated with yeast cell wall. Furthermore, the nitrogen content on which the protein value was based can best be regarded as only a rough index of the cells nutritive value, since it is known that considerable part of the total microbial cell nitrogen is found in purine and pyrimidine bases of nucleic acids and small amounts, in addition, in glucosamine, choline and so on (20).

Although microorganisms could not be considered as a main source of carbohydrate in food, yet there is a calorific contribution as also expressed by the energy values of 1504.81 and 1373.97 kJ/g in synthetic medium and in cane molasses, respectively (Table 1). The low lipid content implies the yeast is non-fattening and the crude fibre content suggest the roughage ability of this fungus as there is a general agreement among clinical investigators that etiologies of constipation, diverticular diseases of the colon and cardiovascular and lipid metabolism disease may be related to the chronic consumption of fibre depleted diets, while, high fibre ingestion may decrease the digestibility of dietary protein, minerals, fats and energy because of their absorptive properties (21). Miller (22) suggested that yeasts grown as food / feed supplement should have 45 -49 % protein, 4 -7 % fat, 26-37 % carbohydrate and 5-10 % ash on a dry weight basis. It was observed that the protein and ash contents of *C. tropicalis* were below the acceptable levels in all media, while the carbohydrate and lipid contents were with the range. Cane molasses proved to be a better medium for the cultivation of this organism, while, the synthetic medium could be modified to give a better biomass of higher nutritional value. In all, the yeast, *C. tropicalis* has potentials for being used as protein supplement based on the proximate composition. The global economic meltdown portrays increase incidence of malnutrition in developing countries, thus, in addition to sourcing for food rich in proteins at low cost like mushrooms, more efforts must be geared at using yeast to obtain the needed components at affordable price (23). However, more research work needs to be carried out on the toxicity of this organism using laboratory animals.

Table 1: Proximate composition of *Candida tropicalis* grown in a synthetic medium and cane molasses.

Growth medium	Moisture	Ash	Crude fibre	Lipids	Protein	Carbohydrate	Energy Value (kJ/g)	Free amino Acid (g/l)
i) Synthetic	4.95	3.8	3.1	8.33	39.2	31.2	1504.81	41×10^{-3}
ii) Cane molasses	4.71	3.48	3.9	4.77	41.34	29.1	1373.97	30×10^{-3}

Conclusion

Thus, it can be concluded that *C. tropicalis* is potentially suitable as a source of food protein as shown in the proximate composition. *C. tropicalis* has been used for the protein enrichment of cassava and corn with the resultant mixture having protein content of above 20%. This represents a balanced diet for either animal feed or human food (17). Higher yield of this fungus can also be obtained by cultivation on cane molasses instead of synthetic media.

ACKNOWLEDGEMENT: The authors are grateful to Prof. F. O. Olatunji (Chemical Engineering Department, University of Lagos), Dr. D. K. Olukoya and staff of Biological Sciences University of Lagos for various assistance rendered. We also acknowledge the technical assistance of Mr. Aroh, Dr. Onyekwelu and staff of Stored Products Research Institute, Nigeria. The contributions of Mr. K. Akinwumi and Mr. A. Ogunlaja of Bells University of Technology, Ota, in computing are also appreciated.

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