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APPLICATION OF *CANDIDA VALIDA* AS A PROTEIN SUPPLEMENT

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ABSTRACT

An investigation was carried out on the carbon and nitrogen sources needed for the growth of a yeast, Candida valida *(syn.* Candida mycoderma*), isolated from Ogi, a fermented edible corn product given to babies at weaning. The suitability of this organism as a protein supplement in foods was also determined. The yeast was grown in different carbon sources: glucose, fructose, lactose, maltose, starch, dextrin, mannitol and ethanol, and in different nitrogen sources like urea, amino acids, ammonium nitrate, sodium nitrate and ammonium sulfate, as part of the synthetic basal medium for 7 days and the growth measured using the dry weight method. A significantly high increase in yield was observed when 1% fructose was used and maximum yield was obtained with 3.5 and 0.2% (w/v) of fructose and urea as carbon and nitrogen sources, respectively. Growing the fungus in 0.3% cane molasses, a natural substrate gave a significantly higher increase in yield than in a synthetic medium. As a food supplement, other nutrient contents of this organism such as ash, crude fiber, lipids and carbohydrates were also analyzed in addition to the protein, free amino acids and energy values.*

PRACTICAL APPLICATION

Candida tropicalis is already in use for protein enrichment of major food materials like corn and cassava which have very low protein content (about 1–3%). In addition, the yeast is known to have high lysine and tryptophan

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levels, thus, further increasing the nutritional values of these African staple food. The organism ***Candida valida*** can be incorporated when producing snacks, giving a balanced diet for human consumption. Thus, ***C. valida*** also has high potential when used in these forms as it is indigenous to the already established edible food products "ogi" which has wide acceptance in the African continent.

INTRODUCTION

The United Nations population experts project that there will be 8 billion people living on this planet by 2015 and 10.5 billion by 2110. This means that during the 35-year period (1980–2015), man must produce as much food as we have since the dawn of agriculture about 12,000 years ago. The World Health Organization estimates that 12,000,000 people die of hunger and starvation-related diseases every year, half are children under the age of 5 years (Miller 1985). The current global economic meltdown coupled with the increasing world population has challenged science and technology to master the problem of supplying mankind with sufficient food, particularly protein (Willey *et al*. 2008). Conventional methods made to resolve this by increasing the productivity of agriculture, husbandry and fishery and decreasing losses in the manufacture and storage of raw materials and products have many constraints. Although cereals are the major source of protein, the protein from these is not as high in quality for human food as in meat protein which is still considered to be totally inadequate to meet the expected high protein demand. The use of microorganisms as a protein source appears very attractive and two approaches to such utilization have developed: the use of single cell protein in animal breeding and fishery and the use of microbial protein directly in human nutrition without first feeding it to animals. This latter approach is considered more advisable by some experts because of high protein losses in the production of husbandry products (Kharatyan 1978). Thus, the use of microorganism as a protein supplement appears attractive. Economically, this approach is more advisable because it takes 4.4 kg of fodder protein to produce 1 kg of food protein as milk or eggs and about 20 kg of fodder protein to produce 1 kg of beef protein (Prescott and Dunn 2004). Other advantages of microbial protein include the short generation time of about 0.5–2 h and 1–3 h and 4–12 h for bacteria, yeasts and filamentous fungi to produce a rapid mass increase under optimal conditions (Bohra and Parihar, 2006; Kuforiji *et al*. 2009). Besides having a high protein content containing amino acids, the cell matter is especially rich in most vitamin B groups, and therefore constitutes potential vitamin enrichment for deficient diets (Jay 2005).

Most organisms involved in microbial protein production can utilize lignocellulosic and citrus wastes, sewage, five or six carbon sugars,

disaccharides like molasses, whey and sulfite liquor and polysaccharides from grains, cassava, corn cobs, prawn shells and municipal solid wastes (Chanda and Sibani 1996; Rhishipal and Rosamma 1998). For the utilization of lignocellulose, a pretreatment is necessary, and to the present time, the only economic utilization of this is in mushroom production. These mushrooms contain lignocellulosic enzymes that can break down the complex compounds in these wastes and are cultivated for food in Asia and Africa (Poppe 2000; Kuforiji and Fasidi 2008). Based on specifications, some microorganisms have been accepted as protein supplements, these include; algae – *Chlorella* and *Spirulina*; bacteria – *Bacillus* and *Methylomonas*; yeasts – *Candida* and *Saccharomyces* (Kihlberg 1972; Dubey 2009). Yeasts, having been used since the ancient times in baking, have the most favorable characteristics for use as a major source of food because of its nutritive value, and *Candida tropicalis*, isolated from the rind of Japanese mandarin orange, has been successfully cultivated for single cell production and also for protein enrichment of cassava and corn due to its high lysine and tryptophan content, while *Candida utilis* was incorporated into human food during the two World Wars (Azoulay *et al*. 1980). Generally, safety problems do not seem to provide any obstacles toward the use of these organisms (Nester *et al*. 2007).

Thus the objectives of this study are:

(1) to isolate and identify an appropriate organism of high protein content from indigenous babies' weaning food produced from fermented corn (Ogi).
(2) to determine the best carbon and nitrogen sources as primary nutrient sources for growth of the identified organism (synthetic medium).
(3) to compare the yield of the organism in the above condition to that in cane molasses, a natural substrate.
(4) to determine the proximate composition of the identified organism in synthetic medium and cane molasses for their suitability as supplement in foods.

MATERIALS AND METHODS

Isolation and Identification of the Yeast

Candida valida was isolated from babies' weaning food produced from fermented corn (Ogi), characterized and identified using standard mycological methods (Smith 1969; Proctor 1976).

Carbon Nutrition

The carbon nutrition study of the yeast was determined. The basal growth medium consisted of $(NH_4)_2SO_4$ 2.0 g; $MgSO_4.7H_2O$ 0.2 g; NaCl 5.0 g;

$FeSO_4$ 0.01 g; K_2HPO_4 0.5 g and distilled water to 1 L mark. Ten grams 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 (Staples 1976).

For this organism, fructose proved to be the best carbon source; hence, different concentrations of fructose were prepared by dissolving 2.0–6.0 g of fructose in 100 mL of the basal medium. The mixture was sterilized at 110C for 10 min.

Nitrogen Nutrition

The nitrogen nutrition was determined by dissolving 0.4 g of the total nitrogen in urea, ammonium sulfate, ammonium nitrate, asparagine, glutamine, methionine and other amino acids in 1 L of the basal medium (excluding ammonium sulfate). 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 0.01–0.4 g in 100 mL of the basal medium for microorganism using different carbon sources. The mixture was sterilized at 110 for 1 h. It was cooled, and 10 mL of the yeast suspension was inoculated into the medium.

In all cases, the period of incubation was 7 days at 28C.

Chemical Analysis of the Yeast Samples;

Moisture Content. Moisture was determined after drying the sample at 105C for 24 h (Staples 1976).

Ash and Crude Fiber. The ash content of the yeast sample was determined by incinerating the dried fungus of known weight at 600C for 12 h in a Gallenkamp furnace.

The crude fiber was determined by treating the defatted yeast sample with 0.112 M H_2SO_4 and 0.313 M NaOH (Pearson 1976).

Total Lipids. The lipid content in 2 g of each sample was determined as in AOAC (1980).

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 (Nielsen 2002).

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 (1980).

Free Amino Acids. This was determined by first hydrolyzing the samples in 6 M HCl and incubating in vacuo at 110C for 2 h. A standard curve using serially diluted leucine was obtained (Barker 1971).

Energy Values of *C. Valida*. 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 kJg^{-1}, respectively (Europa 1990).

Statistical Analysis

Samples were in three replicates and the means were quoted with their standard errors. Statistical analyses were carried out using analysis of variance (Sokal and Rohlf 1995).

RESULT AND DISCUSSION

C. valida isolated from babies' weaning food product made from fermented corn, Ogi, 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 0.14 times was obtained for starch (Fig. 1). The sample statistics of F distribution (FS) showed that the carbon sources had a significant effect on growth of the organism (Sokal and Rohlf 1995). 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 (Sols *et al*. 1971). Before the utilization of starch, however, there must be the enzyme amylase to hydrolyze it. This may account for the minimal growth in the medium containing starch as a carbon source. 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 *C. valida* increased maximally at a concentration of 3.5%. 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" (Sols *et al*. 1971).

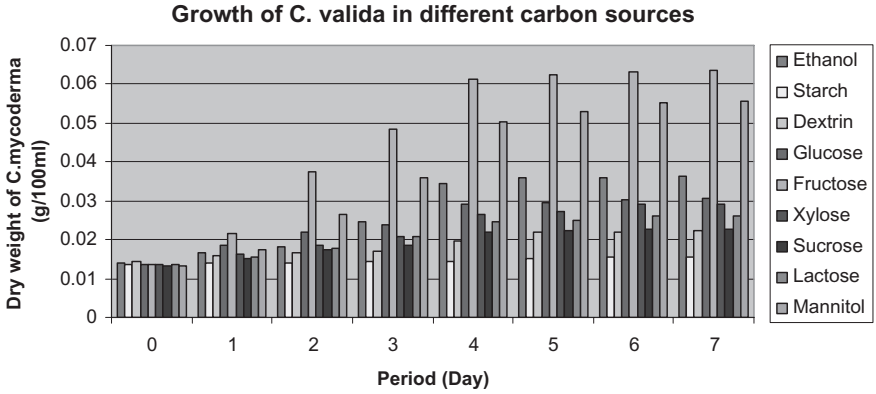

FIG. 1. VALUES ARE MEANS OF THREE REPLICATES $F_{0.05} = 0.010$; F = $_{0.01}$= 0.013
Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively.

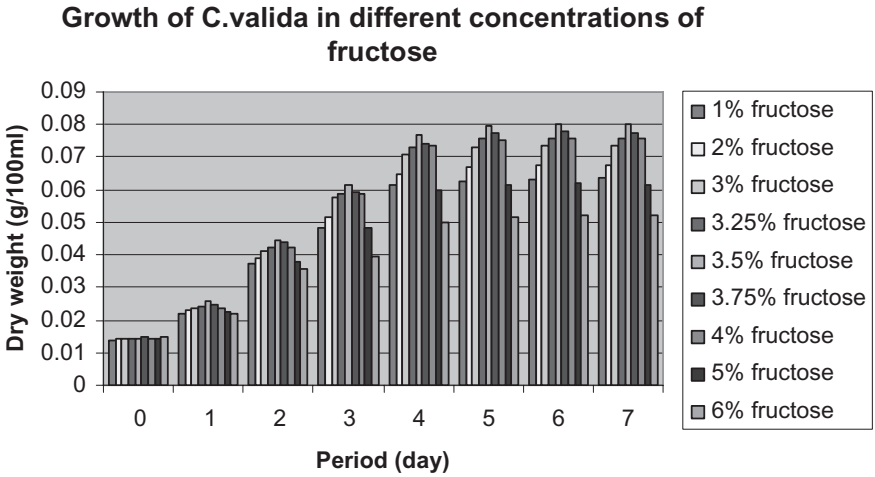

FIG. 2. VALUES ARE MEANS OF THREE REPLICATES $F_{0.05} = 0.023$; $F_{0.01} = 0.031$
Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively.

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 (Fasidi and Olorunmaiye 1994). *C. valida* exhibited the highest growth rate (about 31.5 times increase) in urea at a concentration of 0.2% followed by a decrease when higher concentrations of 0.3 and 0.4% urea were used (Figs. 3 and 4). Urea, in addition to being a nitrogen source, also contributes carbon and energy sources. Morris

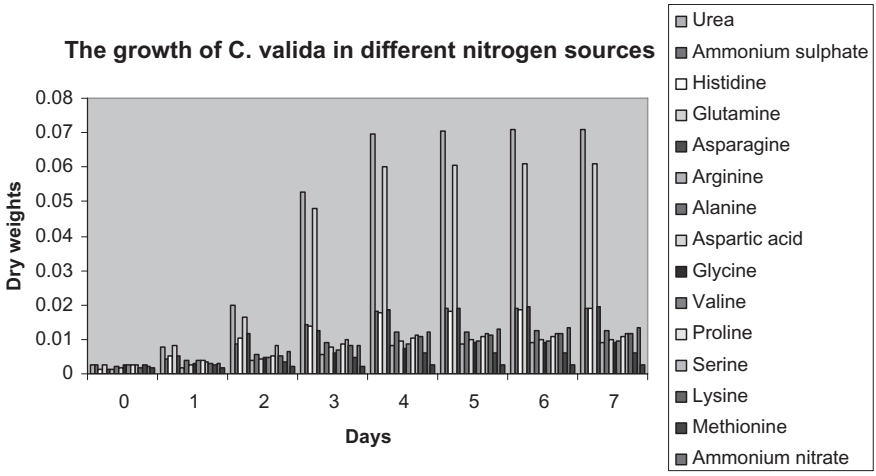

FIG. 3. VALUES ARE MEANS OF TRIPLICATE DETERMINATION
$F_{0.05} = 0.011$; $F_{0.01} = 0.015$. Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively.

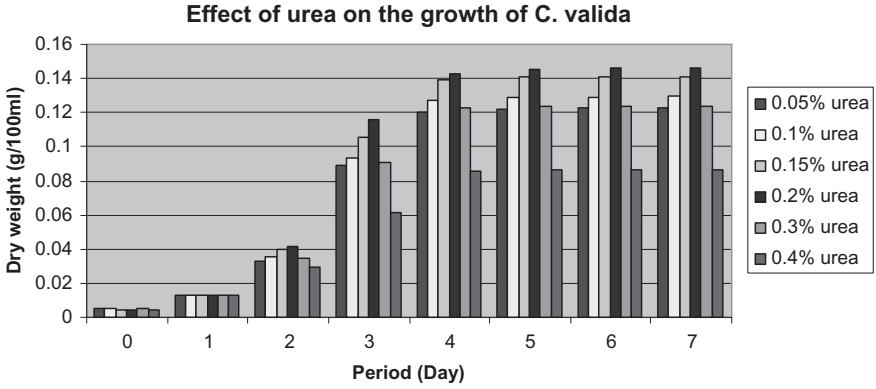

FIG. 4. VALUES ARE MEANS OF THREE REPLICATES
$F_{0.05} = 0.056$; $F_{0.01} = 0.074$. Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively.

(1958), 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.

Pure chemicals are generally not used for "single cell protein" production on an industrial scale because of the high cost of materials. Natural

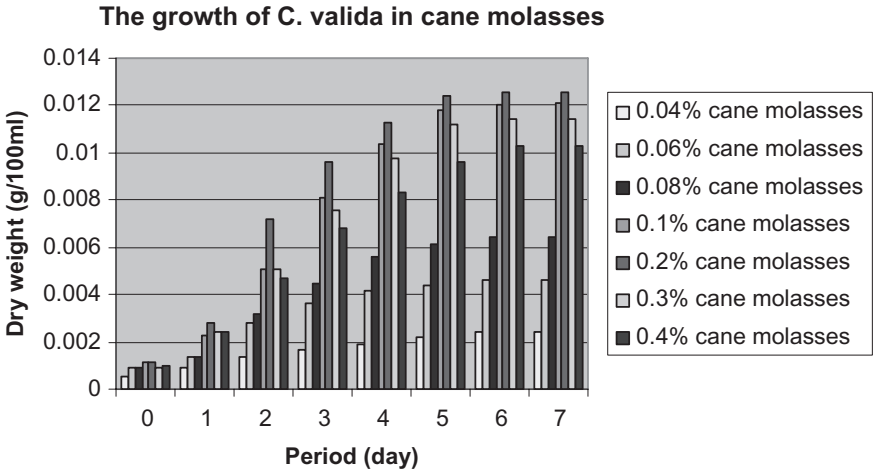

FIG. 5. VALUES ARE MEANS OF TRIPLICATE DETERMINATION
$F_{0.05} = 0.003$; $F_{0.01} = 0.004$. Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively.

substrates like cane molasses are commonly used (Madigan *et al*. 2009). There was an increase in dry weight of the organisms as the concentration in cane molasses was increased from 0.01 to 0.3% (Fig. 5). This was followed by a decrease when a higher concentration of 0.4% was used. *C. valida* showed the greatest and least increase of about 11.7 and 9.3 times at concentrations of 0.3 and 0.4%, respectively (Fig. 5). When the dry weight of *C. valida* grown in 0.3% of cane molasses were converted to an equivalent of 1% (i.e., the dry weights obtained multiplied by 3.3), *C. valida* was found to have initial dry weight of 0.015 g/100 mL (day 0) and final dry weight of 0.0705 g/100 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).

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 analyzed (Table 1). Most microorganisms contain between 7 and 12% nitrogen on a dry weight basis which after correction for nitrogen from purines, pyrimidines, etc. indicate a true protein content which is higher than that for most common foodstuffs (Malkhas'yan *et al*. 1982). 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 (Peppler 1970). Cane molasses as a substrate

TABLE 1.
PROXIMATE COMPOSITION OF *CANDIDA VALIDA* IN A SYNTHETIC MEDIUM AND IN CANE MOLASSES

Growth medium	Moisture (%)	Ash	Crude fiber	Lipids	Protein	Carbohydrate	Energy value (kJg^{-1})	Free amino acid (g/L)
Synthetic	4.7	2.0	3.7	4.4	42.6	28.8*	1,376.6*	46×10^{-3}
Cane molasses	4.9	3.0	4.0	3.9	44.3*	26.9	1,354.7	42×10^{-3}

Values of ash, crude fiber, lipids, protein and carbohydrate are in% dry weight.
Values are means of triplicate determinations. $F_{0.05} = 1.16$.
* Mean ± SE differences higher than $F_{0.05}$ are significant at 5% level.

gave a higher protein content which may be due to the presence of urea and other nitrogen sources in it. 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 (Kihlberg 1972). 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 1,376.6 and 1,354.7 kJg^{-1} in synthetic medium and in cane molasses, respectively (Table 1). Kuforiji and Aboaba (2009) reported that *C. tropicalis* had higher energy values of 1,504.81 and 1,373.91 kJ/g in synthetic medium and in cane molasses, respectively, when compared with *C. valida*. The low lipid content implies that yeast is nonfattening, and the crude fiber content suggests 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 fiber-depleted diets, while high fiber ingestion may decrease the digestibility of dietary protein, minerals, fats and energy because of their absorptive properties (Amen and Spiller 1978). Miller (1978) 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. valida* were below the acceptable levels in both media, while the carbohydrate and lipid contents were within 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. However, natural substrates like cane molasses generally have extraneous materials present in them which are sometimes responsible for the final and distinctive property of the product in addition to the carbon and nitrogen sources present in them. These also contribute vitamins or growth factors which include biotin, thiamine and pyridoxine as well as amino acids such as glutamic acid, asparagine, serine, alanine, valine and aminobutyric acid, thus supplying some of the nutrients needed for the growth of these organisms (Madigan *et al*. 2009). In all, the yeast *C. valida* 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 yeasts to obtain the needed components at affordable price (Kuforiji and Fasidi 2008). Azoulay *et al*. (1980) reported

that *C. tropicalis* has been used for protein enrichment of cassava and corn with the resultant mixture having protein content of above 20%. This represents a balanced diet for either animal or human food.

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