J. Natn. Sci. Foundation Sri Lanka 2000 28(1): 79-86

CHARACTERISTICS OF TWO YEAST STRAINS (*CANDIDA TROPICALIS*) ISOLATED FROM *CARYOTA URENS* (KITHUL) TODDY FOR SINGLE CELL PROTEIN PRODUCTION

S. CHANDRANI WIJEYARATNE* and A. N. JAYATHILAKE
Department of Botany, University of Sri Jayewardenepura, Nugegoda

(Received: 03 October 1999 ; accepted: 03 March 2000)

Abstract: Characteristics of two strains of yeast, identified as *Candida tropicalis*, strains NCYC 2705 and NCYC 2699 isolated from *Caryota urens* toddy were studied for their possible utilization in single cell protein (SCP) production. Having a moisture content of 7%, ash content of 8% and crude protein content more than 45 %, these two strains conform to official specification for food yeast of U.S. Further these two strains contain essential amino acids amounting to 82 % and 72 % of the total amino acid content. One of the salient features of the amino acid contents of these two strains of *C. tropicalis* was the high content of cysteic acid which is normally found in low amounts in most of the other microorganisms. Substantial high levels of vitamins especially thiamine and riboflavin in these two *C. tropicalis* strains make them suitable candidates for SCP production.

Key words: *Candida tropicalis, Caryota urens*, Single cell protein.

INTRODUCTION

Increasing human population and limitation in cultivable land with dwindling natural resources have made it necessary to look into alternative sources for food and feed. Protein deficiency and malnutrition are consequences of limited food supplies. For centuries microorganisms have been an indirect and direct source of protein in the diet of humans. Filamentous fungi and yeast are widely used in the traditional food technologies worldwide to modify the dietary staples. Modification includes improved taste, texture and digestibility with or without any net increase in the protein content of the foodstuff. e.g. yogurt, curd, fermented fish and meat etc.

Several fungi, i.e. *Fusarium oxysporum* var. *lini*[1] and *Chetomium cellulolyticum*,[2] algae i.e. *Chlorella and Spirulina*, Yeast, i.e. *Candida lipotytica* and *Saccharomyces cerevisiae*[3] and phototrophic bacteria such as *Rhodospirilum* spp.[3] had been explored for single cell protein (SCP) production. Nutritional quality and absence of toxicity are the primary criteria that should be satisfied before any organism can be used in SCP production. Certain yeast, bacteria and algae species have been found to meet these criteria with high protein content, high amounts of essential amino acids, vitamins and low amounts of nucleic acids. Such microorganisms also possess other characters such as high growth rate, high yield from consumed sugar and acceptable content of vitamin B-12 and carotenoids etc. to consider them for scale-up operations.

* Corresponding author

In this study two *Candida tropicalis* strains (NCYC- 2699 and NCYC-2705) isolated from fermenting phloem sap of *Caryota urens* (Kithul) were investigated for their potential use in SCP production. These cultures were accessioned into the National Collection of Yeast Cultures (NCYC) at Norwich, UK, as they were different from other *C. tropicalis* strains in the collection. As the molasses is a cheap byproduct of the sugar industry in Sri Lanka, this is a suitable substrate in SCP production. In this study, molasses was used as the carbon source for growth of yeast.

METHODS AND MATERIALS

Characteristics of yeast strains used: C. tropicalis, strain NCYC –2705, Cells oval to long-oval (3-5) x (4-10) μ in YM broth and (2-8) x (2-9) μ in YM agar, form Candida type pseudomycelium in both CMC and PDA media with few blastospores. *C. tropicalis*, strain NCYC-2699, cells oval to long oval in (4-8) x (7-10) μ in YM broth and (4-8) x (5-9) μ in YM agar, form well developed mycotoruloides type pseudomycelium with many blastospores in both CMA and PDA media.

Production of Yeast cells for analyses: Yeast cells were grown in flasks containing yeast extract, peptone, dextrose (YPD) medium. The cultures were aerated using a shaker at a speed of 200 rpm for three days. The cell mass was harvested by centrifugation and freeze dried after washing with water to remove the contaminated medium.

In all the following determination procedures, the final result obtained was the mean value of three replicates.

Determination of moisture content of cells: One gram of freeze dried sample was weighed into a crucible and placed in an oven at 105° C for 4 to 5 h. After drying, the samples were cooled in a desiccater and weighed. Drying and cooling was done several times, until a constant weight was obtained.

Determination of ash content: To determine the ash content 2.00 g of sample was weighed into a porcelain crucible with a lid and ignited in a muffle furnace at 550°C until gray ash was obtained. The crucible with ash was left to cool in a desiccator and weighed. The sample was transferred back into the furnace and left for further 1/2 an hour. Then cooled and weighed again. This procedure was repeated until a constant weight was obtained.

Determination of total nitrogen and protein content: The freeze- dried cells were used to determine the total nitrogen content using micro-Kjeldahal method as described in AOAC[4]. Crude protein content of the cells was determined by multiplying the total nitrogen by the factor 6.25.

Growth at different temperatures: The two selected strains were tested for their ability to grow at different temperatures 30°, 35° and 40° C using the synthetic medium.[5] A known amount of cells (1x10 ^{2} cells/ml) were inoculated and allowed to grow statically at different temperatures as above. The growth of these cultures was estimated by measuring the optical density at 660 nm at different intervals of time.

Growth in molasses medium: The molasses was diluted three-fold to give approximately 20% fermentable sugars and added 0.5% magnesium sulphate and 0.1 % urea. The pH was adjusted to 4.8 before autoclaving. Flasks containing 100ml of the above medium were inoculated with a loop-full of yeast cells and incubated at room temperature for three days on a shaker at 200 rpm. After three days, the medium was filtered and dry mass of cells was determined.

Determination of amino acid content: Amino acid content was determined using Phenylthiocarbamyl (PTC) derivative of samples by HPLC as described by Bidlingmeyer *et al.*[6]

Determination of Thiamine and Riboflavin contents: Thiamine and riboflavin contents were determined by the HPLC method developed at the Laboratory of Food Chemistry, Agricultural Research Center of Finland by Hagg.[7]

RESULTS

Moisture content: Both strains of *C. tropicalis* contained about 6-7% moisture (Table 1).

Ash content: Both strains of *C. tropicalis* had about 7% ash. (Table 1).

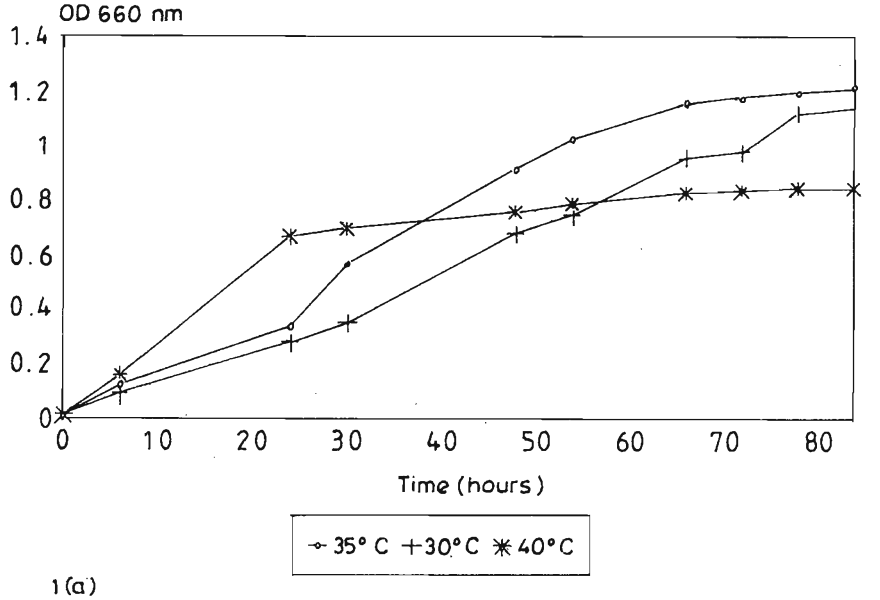
Total nitrogen and protein contents: Both strains of *C. tropicalis* contained 9% total nitrogen and more than 50% crude protein on dry weight basis. Strain NYCY 2699 had a higher crude protein content of 57.7 % on dry weight basis. (Table 1).

Table 1: Moisture, Ash, Total nitrogen and Crude Portein contents of two *C. tropicalis* strains NCYC 2705 and NCYC 2699.

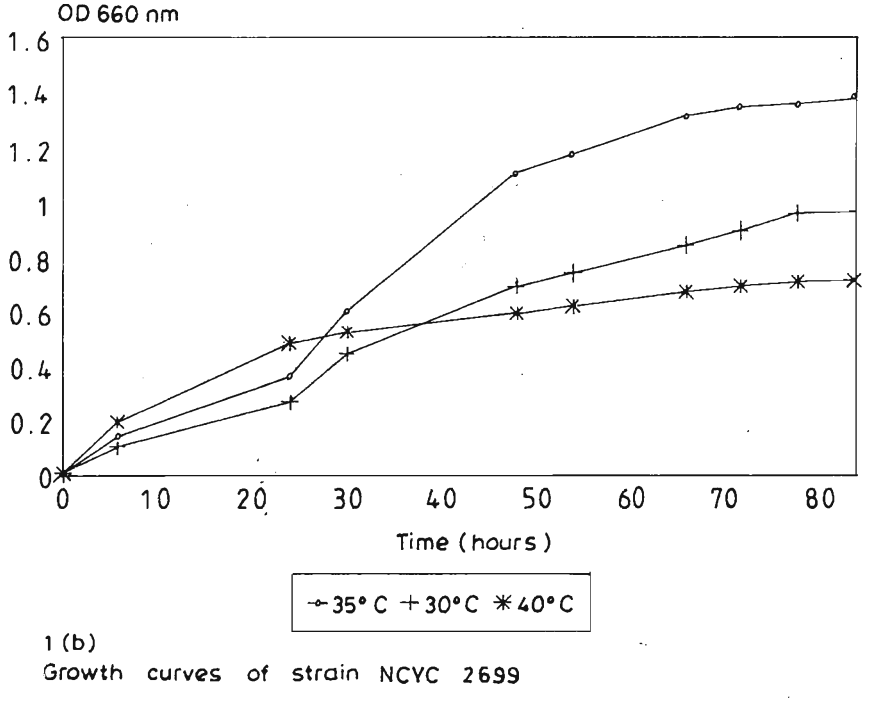
Strain No	Moisture content* % dry wt.	Ash content* % dry wt.	Total nitrogen* % dry wt.	Protein content* % dry wt.
NCYC 2705	6.58	7.12	9.10	56.9
NCYC 2699	7.33	6.92	9.23	57.7

* Mean value of three replicates

Growth at different temperatures: Figs. 1a and 1 b show the growth curves of two strains under this study at different temperatures. Both cultures showed good growth at 35° C. At 40° C both cultures showed a higher growth rate initially but this decreased later.



1 (a)
Growth curves of strain NCYC 2705



1 (b)
Growth curves of strain NCYC 2699

Figure 1: Growth curves of two yeast strains at different temperatures.

Amino acid content: Percentage composition of amino acid of two strains of *C. tropicalis* under this study, calculated from chromatographs are given in Table 2. Both these strains contained appreciable amounts of cysteic acid , 8.9% in strain NCYC 2705 and 10.2% in strain NCYC 2699. Glutamic acid, theronine and isoleucine were found in higher amounts in strain NCYC 2699 than in strain NCYC 2705, while serine, histidine, alanine, valine and cystine were found in higher amounts in strain NCYC 2705 than in strain NCYC 2699. Strain NCYC 2705 had aspartic, tyrosine, methionine and lysine in amounts less than (1%) while strain NCYC 2699 had lesser amounts of serine, proline and leucine.

Table 2: Percentage Composition of Amino Acid of two *C. tropicalis* strains NCYC 2705 and NCYC 2699

Amino Acid	% Composition of Amino Acid	
	NCYC 2705	NCYC 2699
Cysteic acid	8.9	10.2
Aspartic acid	0.27	2.2
Glutamic acid	4.7	8.7
Serine	4.5	0.92
Glycine	1.6	1.9
Histidine	4.7	1.1
Arginine	1.7	2.1
Threonin	2.8	6.1
Alanine	4.1	2.7
Proline	2.3	0.27
Tyrosine	0.16	1.2
Valine	2.5	1.7
Methionine	0.42	1.6
Cysteine	5.2	2.8
Iso-leucine	5.5	7.1
Leucine	1.5	0.85
Phenylalanine	3.7	2.6
Lysine	0.58	1.6

Vitamin B content: Both strains contained 0.1 to 0.2 mg of riboflavin per 1.0 g of cells on dry weight basis. The thiamine and riboflavin contents of the two strains are given in the Table 3.

Table 3: Riboflavin and Thiamine contents of *C. tropicalis* NCYC 2705 and NCYC 2699

Strain No.	Riboflavin mg/g	Thiamine mg/g
NCYC 2705	0.213	0.178
NCYC 2699	0.175	0.10

DISCUSSION

Use of microbes in the production of protein gives many advantages over the conventional methods due to following reasons. Microbes have shorter generation time, allow easy genetic transformation, utilize many substrates, have no requirement for arable land or any particular season to grow, and have the possibility of continuoes production anywhere in the world. The cell yield (g dry weight cells/g) varies according to the substrate and the yeast species employed. The theoretical yield to be expected from one gram of glucose is approximately 0.45 - 0.5 g of cells, i.e. 45 – 50% of substrate is converted to cells under aerobic metabolism. Both strains of *C. tropicalis* used in this study yielded 0.2 g /g of fermentable sugars in molasses medium. This is a moderate amount which could be improved by mechanical aeration and supplementing the medium with vitamins etc.

According to official specification for food yeast in U.S, a yeast species to be used in SCP production should have the following requirements: maximum moisture content of 7%, a maximum ash content of 8%, and a minimum crude protein content of 45%.[8] Single cell proteins produced from these strains of *C. tropicalis* conform very well to the above-mentioned standards.

Protein content is an important criterion that is used to determine the suitability of microorganisms for SCP production. Fungi when grown at higher growth rate normally have higher protein contents as well as increased levels of nucleic acids. As far as potential use of SCP in human feeding is concerned, the nucleic acid is undesirable because their metabolism leads to unacceptably high levels of uric acid.[9] Two *Candida* strains studied had high protein content (55% on dry weight basis) and this may include some non-protein nitrogen also. However, it is possible to limit the amount of nucleic acid formed (especially RNA) by controlling the growth rate of the culture.

The nutritional value of a protein is dependent on its amino acid pattern and is judged to be better the more closely it resembles the amino acid content of whole egg. There are nine essential amino acids, valine, methionine, phenylalanine, iso-leucine, leucine, histidine, threonin, tryptophan and lysine .[9] The strain NCYC 2699 contained 41% essential amino acids while NCYC 2705 contained 42% essential amino acids from the total amino acid content of each of the strains. In SCP sources sulphur-containing amino acids are usually limiting.[11] However in the two strains studied cysteine content was fairly high when compared with other published results, although methionine content was low.

In addition to valuable proteins, these two yeast strains contained substantial levels of vitamins, especially those of the B group. Thiamine and riboflavin are water soluble vitamins which are needed for carbohydrate metabolism and protein metabolism respectively. The two strains of yeast contained about 200 μg of riboflavin /g and 100 μg of thiamine / g of dried cells which is adequate to fulfill the daily requirement. The values are substantially higher than those reported for *C. utilis* grown on sulphur waste liquor, viz 45 μg and 50 μg /g dry weight of cells.[11]

These results reveal that the two strains of *C. tropicalis* used in this study, if grown more efficiently using molasses as the substrate, are promising yeast strains for the production of single cell protein. As such these strains could be recommended for the production of animal feeds using cheaper substrate such as molasses.

Acknowledgement

This work was supported by the University of Sri Jayewardenepura. The authors are grateful to Dr S. S. E. Ranawana of the Veterinary Research Institute, Gannoruwa for providing the HPLC facilities for amino acid and vitamin analyses and the National Collection of Yeast Cultures, Norwich , UK, for identifying yeast cultures.

References

1. Silva M. E. S. T. & Nicoli J. R. (1985). Production of nutritive value of single cell protein from *Fusarium oxysporum* var. *lini* grown in vinasse. *Journal of Fermentation Technology* **63**(1): 91-94.

2. Moo-Young M., Chahal D. S. & Vlach D. (1978). Single cell protein from various chemically pretreated wood substrate using *Chetomium cellulolyticum*. *Biotechnology and Bioengineering* **xx**, 107-118

3. Sasikala CH. & Ramana CH. V. (1995). Biotechnological potential of anoxgenic phototrophic bacteria. *Advances of Applied Microbiology* 4: 173 –235,

4. Offcial Methods of Analysis, *Association of Analytical Chemist*, 4th edition, (1984). Published by Association of Official Analytical Chemists, Inc. Virginia, U. S. A.

5. Lodder J. (1984). *"The Yeast" A taxonomic study.* 3rd Edition pp 1385, North Holland Publishing Company, Amsterdam.

6. Bidlingmeyer Brian A., Cohen Steven A. & Tarvin Thomas L. (1984). Rapid analysis of amino acids using pre-column derivative. *Journal of Chromatography* **336**: 93-104.

7. Hagg Margareta & Kumpulainen Jorma (1994). Thiamine and riboflavin contents of Finnish breads and their corresponding flours. *Journal of Food Composition and Analysis* **7**: 94-101,

8. National Formulary (NF XII), American Pharmaceutical Association, Washington D. C.

9. Edozien J. C., Udo U. V., Young V. R., & Scrimshaw N. S. (1970). Effects of high levels of yeast feeding on uric acid metabolism of young men. *Nature*, London, **228**: 180.

10. Concise Encyclopedia of Biochemistry (German- Brockhaus ABC Biochemie) Translated and revised by Thomas Scott & Mary Brewer, Published by Walter de Gruyter, Berlin.

11. Mateles Richard I. (1979). The physiology of single cell protein (SCP) production. In: *Microbial Technology: Current State, Future Prospects.* (Eds. A.T.Bull, D.C.Ellwood and C. Ratledge) Twenty-ninth symposium of the Society for General Microbiology.