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SCP production from *Candida utilis* isolated from Vellar estuary

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Abstract

In the present study a total of 65 morphologically distinct yeast strains isolated from water (30) and sediment (35) samples collected from Vellar estuary (Lat 11°29'24"N and Long. 79°45' 36" E). From water 30yeast strains and from sediment 35 strains were obtained. The most potent isolate VEW12 was selected for further study and was identified as *C. utilis*. Optimization of growth revealed that parameters such as pH-5, 30°C 0.5% NaCl, 54 hrs incubation, glucose as carbon source (4%), and 0.5% yeast extract as nitrogen source were ideal at which total biomass yield of 4.32g/L. Crude protein, total carbohydrate and total nucleic and content of biomass were respectively 39%, 32%, and 6.5% before nucleic acid reduction whereas after nucleic acid reduction they were found be 43.0, 32.0 and 1.7% respectively. The amino acid composition of *Candida utilis* showed totally 18 amino acids. Among these, 4 amino acids such as Lysine, Phenyl alanine, Leucine and Cystine were found to be high and comparable to FAO (Food and Agricultural Organization) reference protein.

Keywords: SCP, Yeast, *Candida utilis*, Vellar estuary, marine samples.

Introduction

Proteins are present in all living tissues as building block components that provide mechanical support to the body. They are the essence of life processes and are important dietary constituent for the supply of nitrogen as well as sulphur for proper growth and development of all the living things. Its deficiency may lead to a number of health disorders. A large population of the world, especially those who are living below poverty line are suffering from malnutrition. The alarming rate of population growth has increased the demand for food production in third world countries leading to a yawning gap in demand and supply. This situation has created a demand for the formulation of innovative and alternative proteinaceous food sources.

During the sixties, the idea that the single cell protein could help the less developed countries in future food shortages was gaining research interest among scientists in universities and petroleum based industries. The result was the development of SCP technology either for livestock or for human consumption. The term single cell protein refers to dead, dry cells of microorganisms such as yeast, bacteria, fungi and algae. SCP is otherwise known as "microbial protein". SCP may be used as human food supplement or it may be used as animal feed (Singh, 1998). SCP production has the potential for feeding the ever increasing world population at cheaper rates. Najafpur (2007) suggested the potential of SCP to overcome shortage of food in the world. Hence the present study was on isolation of a potential yeast strains from the marine environment for SCP production.

Materials and Methods

Isolation of yeast strains from marine water

Water samples were collected from Vellar estuary, Parangipettai. Samples were kept in ice-box and transported to the laboratory. 1 ml of water sample was transferred to 99 ml diluent blank (50% aged sea water) and serially diluted up to 10^{-6}. After serial dilution the samples were transferred into Sabouraud glucose agar (g/L): Glucose - 40g, Agar-15g, Pancreatic digest of Casein - 5g, Peptic digest of animal tissue - 5g, pH - 5.6± 0.2 at 25°C. 10 ml of Cycloheximide and Chloramphenical Solution were mixed well with above mentioned media after sterilization. Samples were spread on the plates using spread plate technique and incubated for 3 days at room temperature. Distinctly appeared colonies were pure cultured and stored in SGA slants. Potential strains for SCP production were selected based on their growth (OD at 600nm) and protein content.

Identification of yeast strains

The isolated yeast colonies were identified based on colony color, shape, texture, microscopic morphology, physiological, biochemical tests especially various sugar assimilations (Kurtzman and Fell, 1998 and Barnett *et al.*, 2001).

Optimization for growth

The shake-flask culture of potential strain was optimized for the effect of different environmental parameters like incubation period, pH, temperature, salinity and different carbon and nitrogen sources on the growth. While optimizing the pH for the potential strain, SGB was prepared with different pH in the range pH – 4-9 (4, 5, 6, 7, 8 and 9). Then a loopful of culture was inoculated and kept in a shaker (120 rpm) for incubation. The absorbance was measured at 600 nm with an interval of 6 hrs. Similarly, various temperatures like 25°C, 30°C, 35°C, 40°C and 45°C and different salt concentration in the range 0-2.5 % (0%, 0.5%, 1%, 1.5%, 2% and 2.5%). The effect of incubation period on growth of the yeast strain was studied by varying the incubation period from 0 to 60 hrs. Different carbon sources such as glucose, maltose, fructose, sucrose and starch were added in the medium in separate flasks at the concentration of 4% and incubated. Different concentration of ideal carbon source (0.5 – 2.5%) was maintained in the medium and incubated. Different nitrogen sources such as beef extract, yeast extract, peptone, ammonium nitrate, ammonium sulphate and potassium nitrate were added in the medium separately at the concentration of 0.5% in the medium and incubated. Different concentration of yeast extract as nitrogen source (0.1-1.0%) was maintained in the medium and incubated at 35°C. Growth was assessed at 600 nm for every 6 hrs.

Note: All optimization studies were done at 120 rpm.

Reduction on nucleic acid in yeast protein

Reduction of nucleic acid and extraction of protein from yeast cells with sodium hydroxide was carried out according to the method described by Herbert *et al.*, 1971. 20 ml of 1N NaOH was added to 5 g of dried yeast cells. Extraction of protein was performed in a boiling water bath for 10 min. This was then followed by cooling in cold water. The solution was centrifuged and the supernatant was used for the estimation of RNA and DNA determinations.

Mass scale culture in shake flask

The potential strain *Candida utilis* was kept for mass scale culture with the optimized growth parameters such as pH-5, 30°C 0.5% NaCl, 54 hrs incubation, glucose as carbon source (4%), and 0.5% yeast extract as nitrogen source. After incubation harvest was done and cells were separated by centrifugation at 10, 000 rpm for 30 min. The weight of biomass was gravimetrically done and expressed as on dry weight basis g/L.

Preparation of cell extract

The cell extract was prepared by the sonication protocol adapted from Ruiz *et al.*, 1999. Cell pellet was washed twice with normal saline (0.1% NaCl) and suspended in phosphate buffer (0.1 M, pH 7.0) in 1:1 ratio. Washed cell pellet was sonicated at 0°C for 2 min at an interval of 30sec. with LABSONIC U sonicator (133 V, 0.5 repeating cycles per sec.) Unbroken cells were removed by centrifugation at 4°C for 20 min at 10,000 rpm. The crude preparations were used for further analysis.

Estimation of protein, carbohydrate and nucleic acids

Protein

The method of Lowry *et al.*, 1951 was adapted for the estimation of total protein. 10 mg of dried yeast cells

were thoroughly homogenized with 1 ml of deproteinising agent (10% TCA) by keeping the tubes in an ice bucket and samples were centrifuged for 20 min. at 3000 rpm. The precipitate thus obtained was used for protein estimation. The precipitate was dissolved in 2 ml in NaOH and to 1 ml of this solution, freshly prepared 5 ml of alkaline reagent was added. This was kept at room temperature for 10 min., after which 0.5ml of 1N Folin-ciocalteu reagent was added and mixed rapidly.

The standard stock solution was prepared using BSA (Bovine Serum Albumin) at a concentration of 25 mg/5ml 1N NaOH. Different dilutions in the range of 0.25-2.5 mg/ml were prepared from this stock solution, the alkaline reagent and Folin-phenol reagent were added as in the case of yeast dried samples. A blank was prepared with 1 ml 1N NaOH and treated the same way as above.

All the test tubes were kept for 30 min. at room temperature and the optical density (OD) of the blue colour developed was measured against the blank at 660 nm.

$$\text{Protein \%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \text{ x } 100$$

Carbohydrate

The total carbohydrate was estimated by phenol-sulphuric acid method of Dubois *et al.*, (1956). About 5 mg of dried yeast cells were taken for carbohydrates analysis. The dried yeast cells were taken in a test tube and 1 ml of phenol (5%) and 5 ml concentrated H_2SO_4 were added in quick succession. The tubes were kept for 30 min. at 30°C and the optical density (OD) of the colour developed was measured at 490 nm against the blank. D-Glucose was used as a standard and carbohydrate content was calculated by using the following formula.

$$\text{Carbohydrate \%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \text{ x } 100$$

Nucleic acids

Estimation of DNA

The colorimetric method of Sadasivam and Manikam (1996) was adapted for the estimation of DNA on basis of quantitative reaction of deoxy sugar with diphenylamine reagent. 0.5 mg of dried yeast cells were thoroughly mixed with 1ml of saline citrate and made up to 3 ml with distilled water, to which 6 ml of diphenyalamine reagent was added. After mixing, the tubes were kept for 10 min. in boiling water bath and then cooled. The optical density (OD) of the colour developed was measured at 600nm against the blank. Known DNA was used the standard and DNA in samples was calculated by using this formula.

$$\text{DNA\%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \text{ x } 100$$

Estimation of RNA

The colorimetric method of Sadasivam and Manikam (1996) was adapted for the estimation of RNA on the basis of pentose determination as well as orcinol, phloroglucinol, aniline etc. 50 µg of dried yeast cells were throughly mixed with 1 ml of distilled water and kept in ice chilled 10mM Tris-acetate, 1mM EDTA buffer (pH 7.2), and made up to 3 ml with distilled water. To this 6 ml of orcinol acid reagent, and 0.4 ml of 6% alcoholic orcinol were added. After mixing, the tubes were kept for 20 min. in boiling water bath and then cooled. The optical density (OD) of the colour developed was measured at 660 nm against the blank. Known RNA was used the standard % of RNA of unknown samples were calculated as per the formula given below.

$$\text{RNA\%} = \frac{\text{Standard value x OD of the sample}}{\text{weight of the sample}} \text{ x } 100$$

Amino acid profile of protein

Bruckner (1995) method was used for estimating amino acids in the yeast dried cells. The samples were hydrolyzed with 6N HCl at 110°C for 22 hrs. The amino acids were determined by an automatic amino acid analyser (Lachrom E Merck). 5 µl eluded amino acids were quantitatively detected by a fluorescent detector (SPD-10A VP) using acetonitrile which was read at 254 nm. The amino acids were then identified by comparing their retention time (Rt) with the standard amino acids run at identical conditions.

Results and Discussion

Food grade yeasts can provide proteins, carbohydrates, fats, vitamins (mainly the B group), minerals and essential amino acids. Generally, the lysine content in yeasts is higher than that of bacteria and algae. Moreover, yeasts contain low amounts of nucleic acids. The acceptability of a particular microorganism as food or feed depends on its nutritional value and safety issues including nucleic acid content, presence of toxins and residual undesirable compounds such as heavy metals.

SCP is normally considered as a source of protein. However, like any other biological material, it also contains nucleic acids, carbohydrate cell wall material, lipids, minerals and vitamins. Nevertheless, these contributions are given little importance by nutritionists, who generally value SCP in terms of Kjeldhal nitrogen x 6.25 (standard factor relating amino nitrogen to protein content). However, about 10-15 % of the total nitrogen in fungi and yeasts is in the form of nucleic acids. These are not metabolized in the same way as proteins but follow a different route. Amino N, therefore represents approximately 80% of total microbial nitrogen, and is composed of all essential amino acids required for human growth and nutrition.

C. utilis is frequently used in single cell protein (SCP) production because of its ability to utilize a variety of carbon sources and to support high protein yield (Nigam, 2000). It has been used for production of several industrial products for both human and animal consumption (Zayed and Mostafa, 1992 and Kondo *et al.*, 1997).

Microorganisms have the ability to upgrade low protein organic material to high protein food, and this has exploited on by industry. This phenomenon was employed in Germany during the First World War when the growth of *Saccharomyces cerevisiae* was exploited for human consumption. Moreover, *Candida arborea* and *C.utilis* were used during the Second World War and about 60%of the country prewar food input was replaced (Litchfield, 1983).

In the present study totally 65 morphologically distinct yeast strains were isolated from the serially diluted water and sediment samples of Vellar estuary. From water 30yeast strains and from sediment 23 strains were obtained. Based on growth at 600nm in Sabouraud glucose broth and protein content isolate VEW12 was selected for further study.

The selected yeast strain with higher protein content was subjected to morphological, cultural and various sugar assimilation tests to identify the species. It was identified as *C. utilis* based on the biochemical tests. The sugar assimilation results obtained are shown in Table 1.

In this study, pH ranging from 4 to 9 was studied for the detection of optimum pH for the growth of *C. utilis* in the SG broth. The maximum growth was noted at pH-5 (Fig - 1). *Candida* sp. was capable of growth over a wide pH range of 3.0 to 6.2 (Adoki, 2007). Rosma and Ooi (2006) obtained maximum yield of *Candida utilis* pH 4.5. In another study, Rajoka *et al.*, 2005 and Munawar *et al.*, 2010 also produced maximum biomass of *Candida utilis* at pH 6.0.

The temperature ranging from 25 to 40^{o}C were studied for the detection of optimum temperature for the growth of *C. utilis* in the medium. The maximum growth was noted at 30^{o}C and minimum growth was observed at 25^{o}C (Fig-2). Similar observation was noted by Adoki (2007) with *Candida* sp. at 30^{o}C. Yalemtesfa *et al.*, 2010 found maximum protein content with *Chaetomium* sp and *A.niger* at 25°C.

The salinity ranging from 0 to 4% were studied for the detection of optimum salinity for the growth of *C. utilis* in the medium. The maximum growth was noted at 0.5% with an OD value of 1.89 (Fig-3).

The different incubation period from 0 to 60 hrs were studied for the detection of optimum incubation period for the growth of *C. utilis* in the medium. The maximum growth was noted at 54 hrs. Irrespective of parameters tested, after 18 hrs, growth rate increased and at 54 hrs maximum biomass was observed (Fig-4). Munawar *et al.*, 2010 reported 96 hrs whereas Li *et al.*, 2009 optimized fermentation period of 69h was best maximum cell biomass production of *C. utilis*.

When glucose, maltose, fructose, sucrose and starch at 5% concentration were tested, maximum growth was observed with glucose whereas minimum was observed with starch (Fig-5). When the concentration of ideal carbon source (i.e) glucose was tested from 1 to 5%, 4 % resulted in maximum growth (Fig-6).

When 0.5% yeast extract, beef extract, peptone, ammonium nitrate, ammonium sulphate and potassium nitrate were examined, yeast extract as a nitrogen source exhibited the maximum growth (Fig-7). When yeast extract was tested at varying concentration (i.e) 0.1 – 1.0%, 0.5% resulted in the maximum growth (Fig-8). Dunlap (1975) observed production of SCP is enhanced with nitrogen sources of urea and peptone.

The potential strain identified as *Candida utilis* was kept for mass scale culture with the optimized growth parameters such as pH-5, 30^{o}C 0.5% NaCl, 54 hrs incubation, glucose as carbon source (4%), and 0.5% yeast extract as nitrogen source. After incubation harvest was done and cells were separated by centrifugation at 10, 000 rpm for 30 min. The weight of biomass was gravimetrically done and expressed as on dry weight basis g/L. The biochemical composition

of *Candida utilis* biomass is presented in Table 2. The total biomass yield was 4.32g/L. Crude protein, total carbohydrate and total nucleic acid content of biomass were respectively 39%, 32%, and 6.5% before nucleic acid reduction whereas after nucleic acid reduction they were found be 43.0%, 32.0% and 1.7% respectively (Table 2).

Table 1: Biochemical identification of *C. utilis*

Test	Result
Glucose	+
Galactose	-
Sorbose	-
Sucrose	+
Maltose	+
Cellobiose	+
Trehalose	Weak/Latent
Lactose	-
Melibiose	-
Raffinose	-
Melizitose	+
Inulin	-
Soluble Starch	-
Xylose	+
L Arabinose	-
D Arabinose	-
Ribose	-
Rhamnose	-
Ethanol	+
Glycerol	+
Erythritol	-
Ribitol	-
Galactitol	-
Mannitol	+
Sorbitol	-
A M D Glucoside	+
Salicin	+
Lactic Acid	+
Succinic Acid	+
Citric Acid	Unknown
Inositol	-
Gluconolactone	Unknown
Glucosamine	Unknown
Methanol	Unknown
Xylitol	Unknown
NH4 2SO4	+
KNO3	+
Ethylamine	+
Cadaverine	Unknown
Lysine	Unknown

Chandra and Chakrabarti (1996) reported SCP production by yeast using plant liquid waste. Totally 3 yeast strains such as *Saccharomyces cerevisiae, Torula utilis, Candida lipolytica* were characterized in their studies. The crude protein content of those yeasts were respectively 45.6%, 54.3% and 50.5%.

Table 2: The composition of lyophilized *C. utilis* biomass grown on optimized medium

Composition	Before nucleic acid reduction (%)	After nucleic acid reduction (%)	Standard values of Food Grade Yeast
Crude protein	39	43.0	35.7
Total Carbohydrate	32	32.0	46.3
Total Nucleic acid	6.5	1.1	Below 3
RNA	4.0	0.8	
DNA	2.5	0.1	

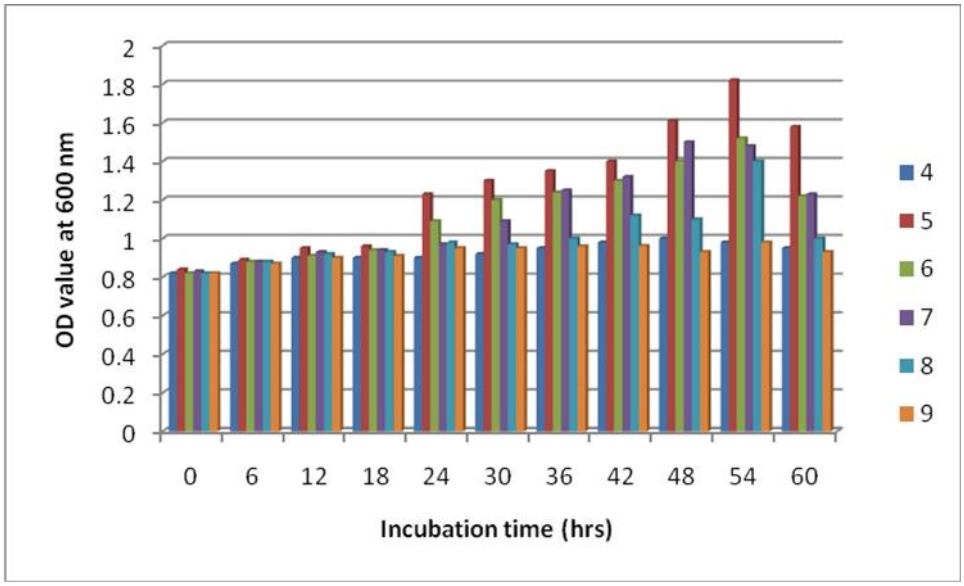

Fig. 1: Effect of pH on growth of *C.utilis*

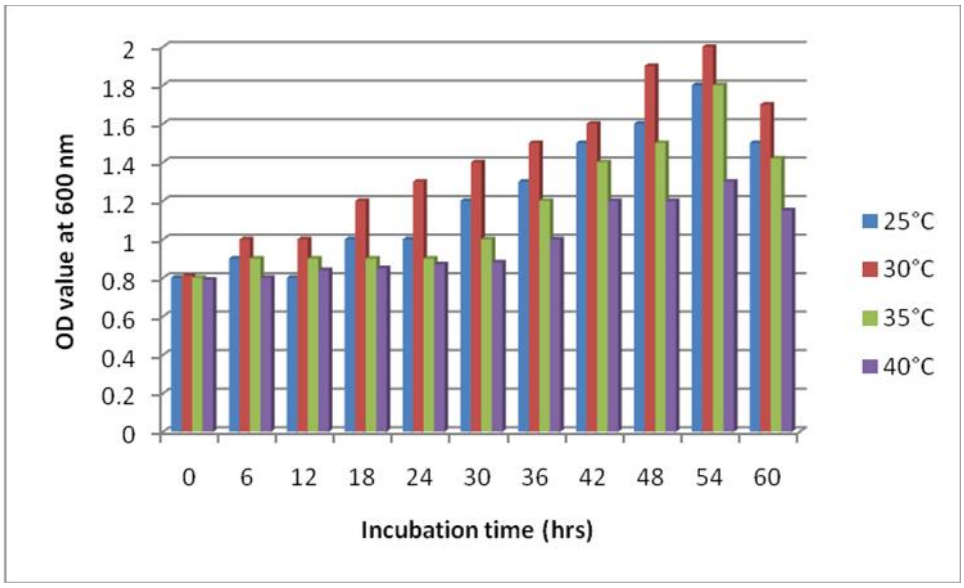

Fig. 2: Effect of temperature on growth of *C. utilis*

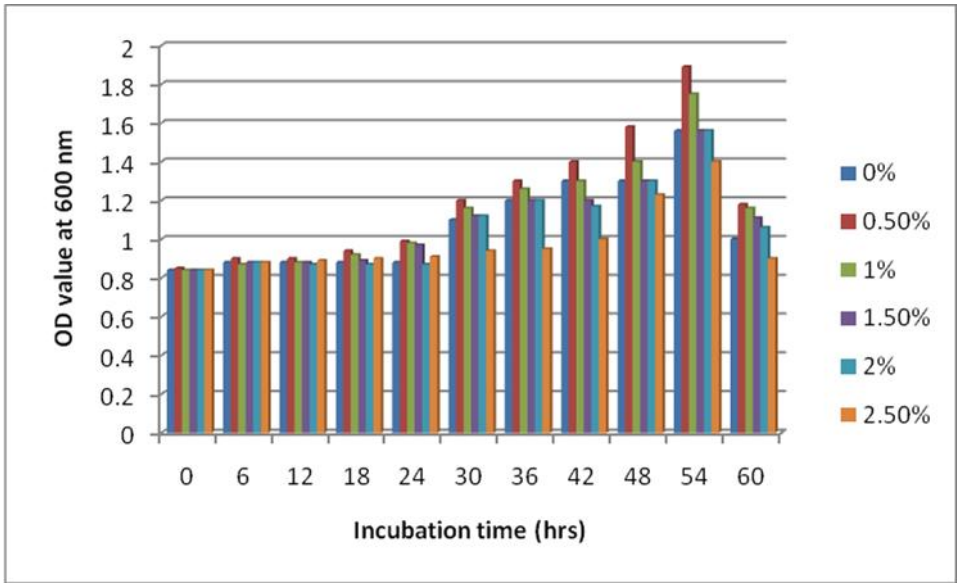

Fig. 3: Effect of salinity on growth *C.utilis*

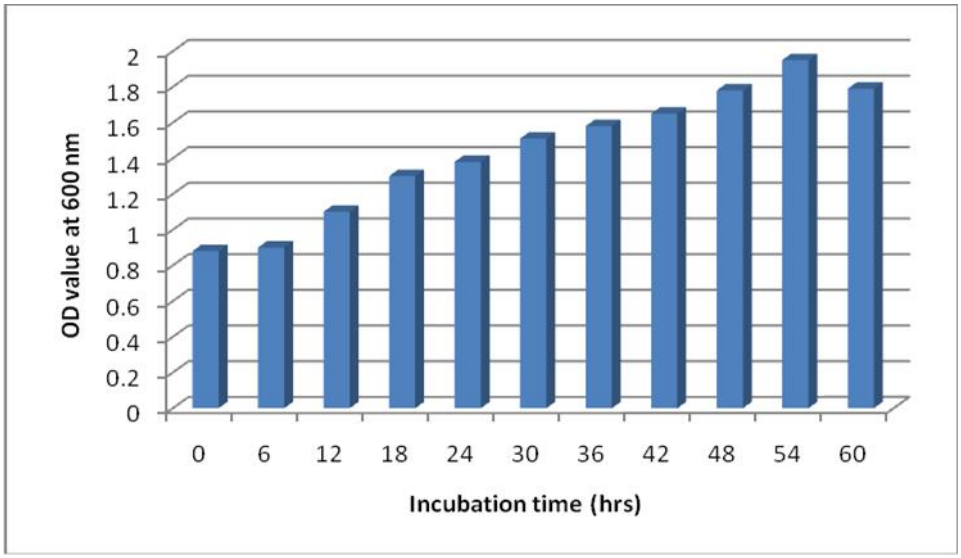

Fig.4: Effect of incubation period on growth of *C.utilis*

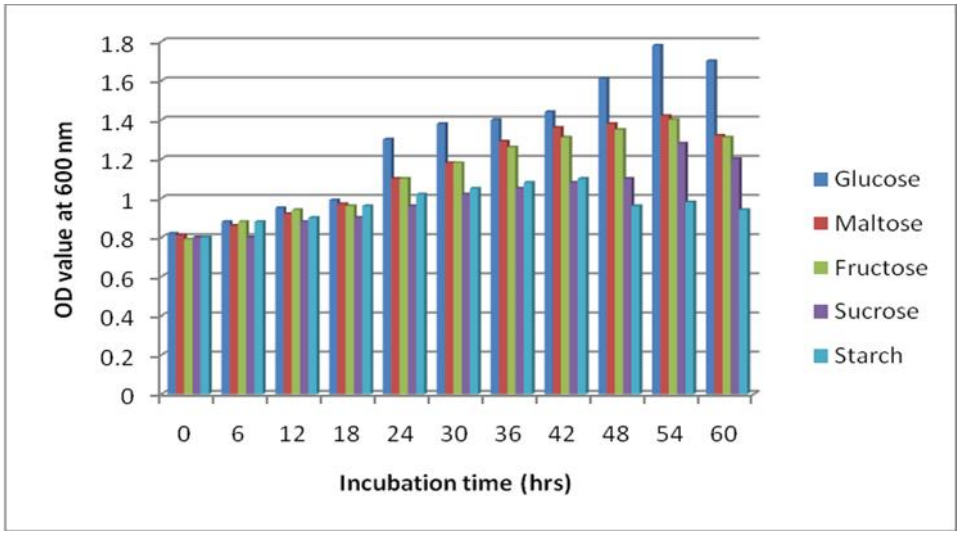

Fig. 5: Effect of carbon source on growth of *C.utilis*

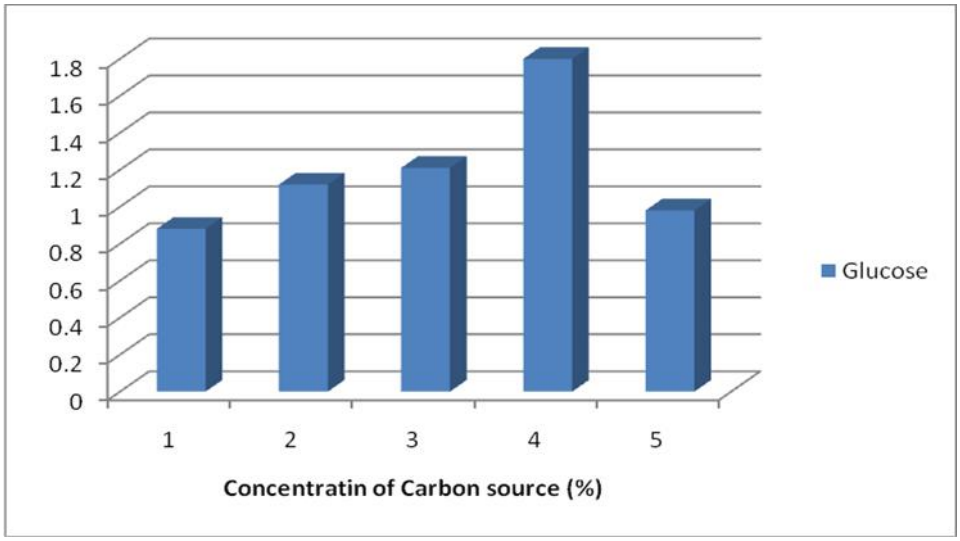

Fig. 6: Concentration of ideal carbon source on growth of *C. utilis* at 54hrs of incubation

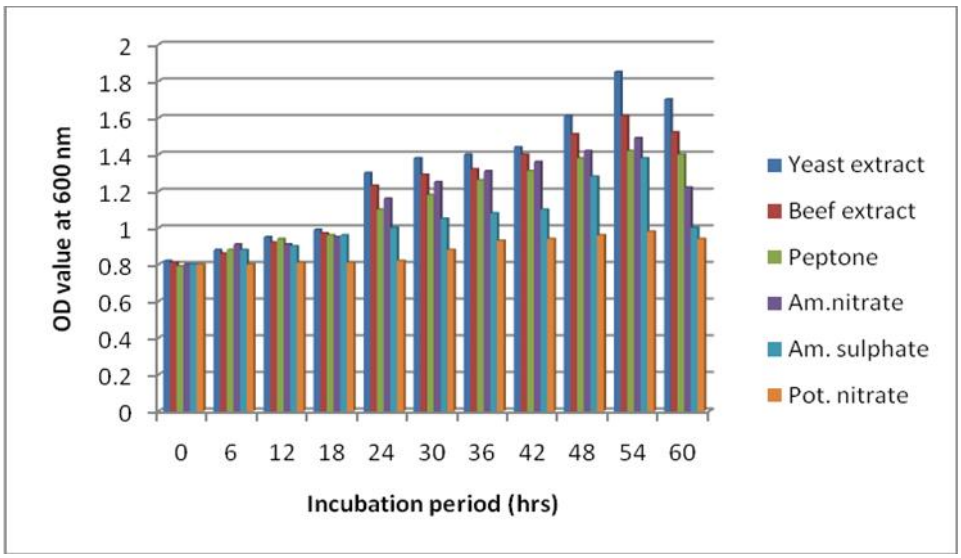

Fig. 7: Effect of nitrogen source on growth of *C.utilis*

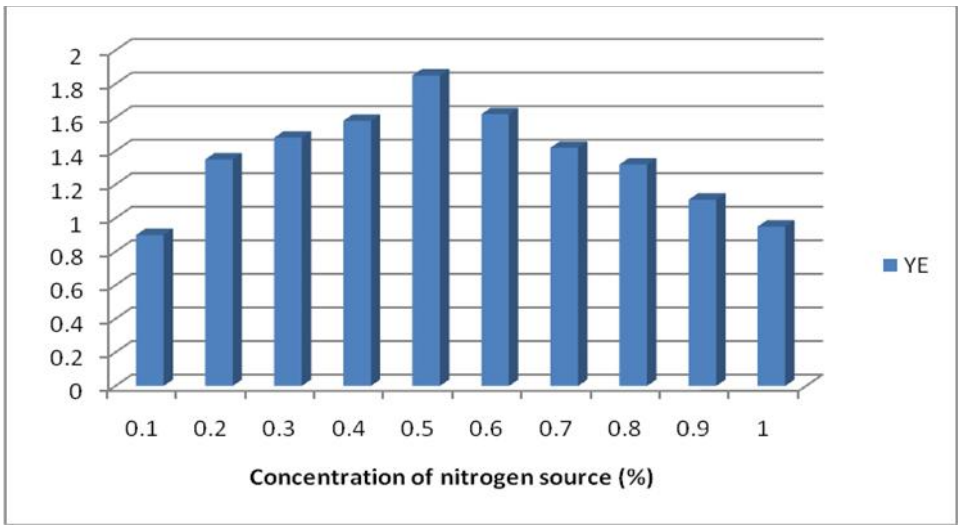

Fig. 8: Concentration of ideal nitrogen source on growth of *C.utilis* at 54hrs of incubation

There has been an increasing interest in single cell protein production by yeast because of the increased level of protein, optimal growth rate, low nucleic acid content and good amino acid profile (Gilland, 2002).

The amino acid composition of *C. utilis* and FAO reference protein is given in Table 3. In the present study totally 18 amino acids were present in this strain. Among these, 4 amino acids such as Lysine, Phenyl alanine, Leucine and Cystine were found to be high comparable to FAO (Food and Agricultural Organization) reference protein (Table 3). Nelson *et al.*, 1960 reported the average lysing content (as % of total protein) of 34 strains of *Candida* was 7.4% (range 5.0±9.8) whereas methionine and tryptophan were 1.2 and 1.3% respectively.

Table 3: Amino acid composition of SCP from *C. utilis*

Amino Acids	Mg g-1	FAO (mg g-1)
Aspartic acid	56	-
Glutamic acid	84	-
Serine	30.5	-
Glycine	37	-
Threonine	35.5	40
Arginine	14	-
Alanine	46.5	-
Cystine	26	20
Tyrosine	33.5	-
Histidine	10	-
Valine	41	42
Iso-leucine	38	42
Phenyl alanine	44	28
Leucine	44	28
Lysine	56.5	42
Proline	38	-
Tryptophan	6	-
Methionine	17.5	22

References

Adoki, A., 2007. Factors affecting the yeast growth and protein yield production from orange, plantain and banana wastes processing residues using *Candida* sp. African Journal of Biotechnology, 7: 290-295.

Barnett, M.J., N.L., Morgan, J.S. Rockey, and S. Higton, (Edts.) 2001. Industrial Microbiology: An Introduction. BlackWell Science Ltd. London: 219-226.

Bruckner, H., 1995. Estimation of Amino acids. *J. Chrom.*, 697:295-307.

Chanda, S. and Chakrabarti, S., 1996. Plant origin liquid waste: a resource for single cell protein production by yeast. *Bioresour. Technol.*, 57:51-54.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28:305-356.

Dunlap, C.E., 1975. Production of single cell protein from insoluble agricultural wastes by mesophiles in SCP II: Cambridge, Massachusetts, MIT Press, 244-268.

Gilland, B., 2002. World population and food supply: Can food production keep pace with population growth in the next half-century. Food Policy, 27:47-63.

Herbert, D., P.J. Phipps, and R.E. Strange, 1971. Chemical analysis of microbial cells. In:*Methods in Microbiology*, Vol. 5B, ed. J.R.Norris and D.W.Ribbons. Academic Press, London. 244-249.

Kondo, K., Y. Miura, H. Sone, K. Kobayashi and H. Iijima, 1997. High level expression of a sweet protein, monellin, in the food yeast *Candida utilis*. *Nature Biotechnol*., 15: 453-457.

Kurtzman, C.P. and J.W. Fell (eds), 1998. *The yeasts, A Taxonomic Study*, 4th edn.

Li, X., O. Xu, M. Chen, X. Song, Q. Yong and S. Yu, 2009. Optimization of culture conditions for production of yeast biomass using bamboo wastewater by response surface methodology. *Bioresour. Technol.*, 100: 3613-3617.

Litchfield, J.H., 1983. Single cell Protein. *Sci*., 219: 740-746.

Lowry, O.H., N.J. Farr, A.L. Rosebrough and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. chem*., 193: 265-275.

Munawar, RA, M. Irfan, M. Nadeem, Q.A. Syed and Z.H. Siddique, 2010. Biosynthesis of single cell biomass of *Candida uilis* by submerged fermentation. *Pak. J. Sci.*, 62(1): 1-5.

Najafpur, B. D., 2007. Single cell Protein. *Biochem. Eng. Biotechnol.*, 332-341.

Nigam, J.N., 2000. Cultivation of *Candida langeronii* in sugarcane bagasse hemicellulosic hydrolyzate for the production of single cell protein. *World J. Microbiol. Biotechnol.*, 16: 367-372.

Rajoka, M.I., S.H. Khan, M.A. Jabba., M.S. Awan and A.S. Hashmi, 2005. Kinetics of batch single cell protein production from rice polishing with *Candida utilis* in continuously aerated tank reactors. *Bioresour. Technol.*, 97: 1934-1941.

Rosma, A. and K.I. Ooi, 2006. Production of *Candida utilis* biomass and intracellular protein content: effect of agitation speed and aeration rate. *Malaysian J. Microbiol.*, 2: 15-18.

Ruiz, C., V.J. Cid, M. Lussier, M. Molina and C. Nombela, 1999. A large-scale sonication assay for cell wall mutant analysis in yeast. Yeast. 15:1001–1008.

Sadasivam, S. and A. Manickam (Eds) 1996. Estimation of DNA. In:Biochemical method. New age international (p) limited. p159.

Singh, B.D., 1998. Biotechnology. New Delhi: Kalyani Publishers, pp. 498–510

Zayed, G. and N. Mostafa, 1992. Studies on the production and kinetic aspects of single cell protein from sugar-cane bagasse saccharified by *Aspergillus niger*. *Biomass Bioener.*, 3: 363-367.

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