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Single Cell Protein Production from *Torula* Yeast (*Cyberlindnera* sp.) Using Banana Peel Hydrolysate

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Single cell protein is a source of protein derived from one-celled organisms (algae, yeasts, fungi or bacteria) grown on different carbon sources and is used as a substitute for protein-rich supplement for human and animal feeds.
Methods: In this study, ten yeast isolates from saw dust samples that were obtained from small scale wood processing enterprises from Gondar town, Ethiopia were screened and identified through morphological, cultural and physiological tests.
Results: From the different yeast isolates, an isolate SDY6 was found to be *Torula* yeast (*Cyberlindnera* sp.). The biomass production capacity of this yeast isolate was evaluated using banana peel hydrolysate under batch and aerobic conditions in which pH and temperature were adjusted to 4.5 and 30°C, respectively. $(NH_4)_2SO_4$ was added to the banana peel hydrolysate to increase biomass yield. The produced biomass was measured at 0, 24, 48, 72 and 96 h. Maximum biomass of 8.82±1.21 g/L was produced at 48 h incubation. On the other hand, crude protein content of the product was analysed using Kjeldahl apparatus. About

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10.25±2.73% of maximum crude protein content was obtained by this yeast.
Conclusion: In conclusion, a higher biomass yield and crude protein content can be obtained from *Torula* yeast (*Cyberlindnera* sp.) using banana peel hydrolysate as a substrate.

Keywords: Banana peel hydrolysate; single cell protein; Torula yeast (Cyberlindnera sp.) SDY6.

1. INTRODUCTION

People especially those living in the developing world are facing the increasing protein deficiency from time to time. Since 1950s, continuous and intense efforts have been made to explore new, alternative and unconventional protein [1]. For this reason, in 1996, new sources mainly bacteria, yeast, fungi and algae named single cell protein as coined to describe the protein production from biomass, originating from different microbial sources [1,2].

Single cell protein has been considered an alternative to conventional protein source as human food or animal feed. The most attractive features of microorganisms for large scale processes for single cell protein production include [1,3,4]:

- The wide variety of methodologies, feedstocks (cheap agro-industrial wastes) and microorganisms that can be used for this purpose.
- Superior efficiency in substrate conversion.
- High productivity, derived from their fast growth rate.
- Independence of seasonal factors (not influenced by external factors such as their origin, and season or climatic changes).
- There is no need for light energy to produce biomass in heterotrophic microorganisms.

Yeast was the first and foremost microorganism whose importance as animal feed supplement was recognised almost a century ago. During Second World War, Germany replaced half of the imported protein sources by yeast. Pruteen was the first commercial single cell protein used as animal feed additive [1]. Microorganisms including bacteria (*Cellulomonas*, *Alcaligens*, *Methylomonas*, etc.), yeast (*Candida*, *Saccharomyces*, *Torula*, *Pichia*,*Geotrichum* etc.), algae (*Spirulina*, *Scenedesmus*, *Chlorella*, *Dunaliella*, etc.) and molds (*Trichoderma*, *Fusarium*, *Rhizopus*, *Aspergillus*, *Sclerotium*, *Poliporus*, etc.) are used in the production of single cell protein [1,5]. Superior nutritional quality, efficiency to use low cost raw materials, the ease to harvest because of its large size, its ability to grow at acidic, lower nucleic acid content, high malic acid and lysine content make yeast as a suitable candidate for single cell protein production [1,5].

Torula yeast (*Cyberlindnera* sp.) commonly *Cyberlindnera jadinii* (Acc.No: SAMN02616005) is widely used for the production of single cell protein that is used as food for humans and feed for dogs, cat and fish [1]. The yeast is also used for flavoring in processed foods. It is often grown on wood liquor, a byproduct of paper production, which is rich in wood sugar, xylose [6]. *Cyberlindnera jadinii* (synonym, *Candida utilis*) can also be used, in a blend of various other yeasts, as secondary cheese starter culture to inoculate pasteurised milk to improve cheese flavour [7]. It is also used in neutralisation a curd [7].

Microorganisms can utilize a variety of cheap substrates like agricultural wastes (rice straw, rice hulls, manure and starchy residues etc.), effluents, industrial wastes (molasses, wheat bran, etc.), petroleum byproducts, natural gases (like methane), ethanol, methanol, that also help in decomposing pollutants [2,8].

For the production of single cell protein, selected stains of microorganisms can be cultured on suitable cheap raw materials in technical cultivation process. In the current study, the selected yeast species, Torula yeast was isolated, characterised and optimised. The optimised Torula yeast was fermented with banana peel hydrolysate as a substrate and supplemented as biomass increment. Banana (*Musa paradisiaca*) fruit peel is an organic waste that is highly rich in carbohydrate content and other basic nutrients that could support the microbial growth. In tropical climates, the banana trees continue bearing fruit throughout the year. Sugar represents that part of the fruits which is used by microorganisms for single cell protein for food and feed applications [9,10]. The different parts of the banana tree including the fruit peel are disposed as waste. It is time to use such fruit peel as a substrate for the production of single cell protein from microorganisms, especially of

the yeasts. The use of such a cheap and readily available substrate lowers the cost of production, reduces waste disposal and management problems, conserves natural resources and provides feed for livestock purposes. Therefore, the use of banana peel hydrolysate as a fermentation medium was taken into consideration as an alternative raw material for the production of Torula yeast biomass in this investigation.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of Yeast Strains

Saw dust samples were obtained from four small-scale wood products processing enterprises from Gondar town, Ethiopia. All samples were put into sterile polythene bags, transported to the laboratory, and processed within a short period of time. The saw dust was milled. About 1 g of each of sample was transferred to 9 mL of sterile distilled water and mixed thoroughly. Serial dilutions (10^{-1}− 10^{-6}) were done. At each successive step of the series, the saw dust samples were maintained in increasing dilute aqueous suspensions. Aliquots of 0.1 mL from appropriate dilutions were spread plated on yeast extract malt extract (YM) media containing (g/L): yeast extract 3, malt extract 3, peptone 5, glucose 10 and agar 15. Chloramphenicol (0.01 g/L) was added to each media to inhibit bacterial growth and 0.01 mL propionic acid was added to inhibit mold growth. The pH of the medium was kept at 4.5. The samples were incubated at a temperature of 30°C for 48 h. Colonies were enumerated, characterised and recorded. Those containing isolated colonies with the morphology typical of yeasts were transferred to slant YM agar slants and preserved at 4°C for further study.

2.2 Yeast Identification

2.2.1 Asexual propagation

From young culture of yeasts grown in YM broth with temperature 30°Cand pH 4.5, a drop of culture on a glass slide showed cells budding.

2.2.2 Observation of ascospores

Yeast colony was suspended in a drop of water on a glass slide. To observe types of ascospores, spore staining method of Yarrow [11] was used. Heat fixed preparations were flooded with 5% aqueous malachite green for 30 − 60 sec. After flooding, the heat fixed preparations with 5% aqueous malachite green, were heated to steaming 3 to 4 times. The excess stain was run off under running tap water for half a min. The preparations were then counterstained with 0.5% safranin red for about 30 sec. Again the excess stain was run off under running tap water for half a min. The preparations were observed both under high power and oil immersion objectives.

2.2.3 Fermentation of carbohydrates (fermentation tests)

The sugars used for fermentation by yeasts as a test used were glucose, galactose, sucrose, maltose, lactose, raffinose, trehalose, inulin, soluble starch, melibiose, cellobiose, and xylose [11,12]. Before inoculating yeasts, fermentation basal medium was prepared as follows. Powdered yeast extract with 4.5 g and peptone 7.5 g were dissolved in 1 L of distilled water. A basal medium of 2 mL was then put into Durham tubes. Then, the ingredients were sterilised at 121°C for 15 min. The pH of the medium was kept at 4.5. A 1 mL concentrated, filter- sterilized sugar solution was added into the tubes to give a final sugar concentration of 2%, w/v (4% w/v for raffinose). The tubes were inoculated with cells from a 24−48 h old culture and incubated at 30°C for 28 days. The tubes were shaken and inspected for an accumulation of gas in the insert. The results were recorded and scored.

2.2.4 Assimilation tests of carbon and nitrogen compounds

Assimilation tests of yeasts were done using auxanographic method following Yarrow [11] and Barnett et al. [12]. For assimilation test of carbon compounds, ingredients containing (g/L): $(NH_4)_2SO_4$ 5, KH_2PO_4 1, $MgSO_4.4H_2O$ 0.05 and agar 20 were prepared. Then 2 g of yeast extract was added. The pH of the medium was kept at 4.5. The medium was dispensed into tubes and sterilized at 121°C for 15 min. The basal agar medium was melted and cooled to between 40 and 45°C. A suspension of young cells in water was either added to a tube of the medium, mixed and then poured into petri dishes. The petri dishes were then left on a level surface for the agar to set and then allowed to stand for a few h to allow the surface of the agar to dry. Petri dishes of basal agar medium in which yeast cells suspended were seeded with carbon sources (glucose, galactose, sucrose, maltose, lactose,

raffinose, trehalose, inulin, soluble starch, melibiose, cellobiose, and xylose) at various points around the periphery. The results were then read by inspecting the plates for an opaque zone of growth around the point where a carbon source were applied. The bottom of the petri dish was marked around the periphery to locate and identify the carbon sources and a small amount of each compound was deposited as aseptically as possible on the surface of the agar. The plates were examined every two days for up to a week. Similarly, in testing the ability of a yeast to assimilate nitrogen sources, the basal medium was prepared without nitrogen source, instead it contains glucose as carbon source. Auxanographic method was used. In such method, yeast carbon base medium was seeded with a yeast suspension similar to the above. A yeast suspension from a 24 – 48 h old colony was prepared in sterile distilled water. The basal medium was prepared with ingredients containing (g/L): glucose 20, KH_2PO_4 1, $MgSO_4$ 0.5 and agar 20. The ingredients were sterilised by autoclaving at 121°C for 15 min. The medium was then allowed to cool to 50°C. Then 1.0 mL of the yeast inoculum was added to the medium and mixed. The medium was poured into sterile petri dishes and allowed the agar to harden at room temperature. Aseptically, discs containing KNO_3, L-lysine, creatine, and glucoseamine were placed on the medium surface. The plates were labeled. After the nitrogen sources were introduced, the plates were incubated at 30°C and inspected after 2 and 4 days for zones of growth around the sites of the nitrogen sources.

2.3 Inoculum Preparation

The selected and identified yeast strain was grown in a broth culture medium containing yeast extract 3 g/L, peptone 5 g/L, glucose 2 g/L, sucrose 15 g/L, $Na_2HPO_4.12H_2O$ 2.4 g/L, $MgSO_4.7H_2O$ 0.075 g/L and $(NH4)_2SO_4$ 5.1 mg/L. The pH of the medium was kept at 4.5.The medium was sterilized at 121°C for 15 min. A 0.9% v/v inoculum size was used in each case [13,14].

2.4 Yeast Cultivation

The prepared inocula were grown on fermentation media (banana peel hydrolysate media) at different incubation periods (0, 24, 48, 72 and 96 h). The banana peels were obtained from local fruit juice sellers, Gondar, Ethiopia. The wastes were washed, dried and milled before the addition of distilled water at a ratio of 1:1 (w: v). The particle size of the powdered banana peel was approximately 80 mesh. The extract was heat-treated (160°C for 30 min) and filtered using centrifugation. $(NH_4)_2SO_4$ as a nitrogen source was added to hydrolysate to increase the biomass yield and pH was fixed at 4.5 using 1M NaOH and 1M HCl. The extract was autoclaved at 121°C for 15 min. The experiments were done in triplicate.

2.5 Cell Harvest and Biomass Yield Determination and Crude Protein Determination

In series of the propagation processes, cell harvest and biomass determination was done according to Somaye et al. [14]. The Erlenmeyer flasks containing the inoculated banana peel hydrolysate with $(NH_4)_2SO_4$ as nitrogen supplement were kept at pH 4.5 and temperature of 30°C and different incubation periods (0, 24, 48, 72 and 96 h). After incubation periods, the suspensions in each flask were centrifuged using tubes. The sedimented yeast cells (pellets) up on 4000×*g* for 10 min each were washed with cold distilled water and re-centrifuged. The supernatant was discarded. The biomass was then dried in vacuum oven at 60°C for 8 h and weighed.

Total nitrogen content of the harvested biomass was determined using Kjeldahl apparatus according to the instructions published by Krishna and Ranjhan [15]. Crude protein content per percent was also determined following the methods of AOAC [16].

3. RESULTS

3.1 Characterization of Yeast for Identification

In this study, ten yeast isolates from saw dust samples that were obtained from small scale wood processing enterprises from Gondar town, Ethiopia were screened and identified through morphological, cultural and physiological tests. Identification of the isolated yeasts was based on the already known standards (colony characteristics, budding patterns, physiological and biochemical features and formation of ascospore formation or sexual reproduction). From these ten isolates, an isolate SDY6 was found to be Torula yeast (*Cyberlindnera* sp.). The colonies of this isolate were circular and shiny in their appearance with creamy color on the solid media, having a smooth texture on agar surface.

The yeast showed colony diameters ranging from 1–2 mm up on incubation for 3–5 days. Shape of the yeast was oval (Fig. 1). The isolated yeast did not display ascospore formation, however, it displayed similar pattern of multipolar budding. The ability of the yeast to ferment carbon sources as well as its ability to assimilate carbon and nitrogen compounds is presented (Table 1).

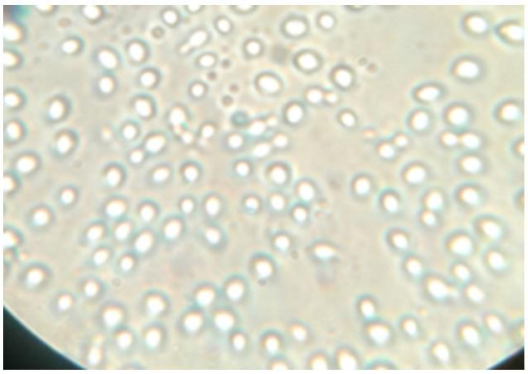

Fig. 1. Microscopic appearance of Torula yeast (*Cyberlindnera* sp.) SDY6

3.2 Biomass Yield and Crude Protein Determination

The biomass yield of yeast that is obtained from this work was measured at different times during the batch culture operation. After fermentation for 48 h where the medium was kept at temperature of 30°C and pH 4.5, the yeast produced a maximum biomass of 8.82±1.21 g/L (Table 2). On the other hand, crude protein content of the product was analyzed using Kjeldahl apparatus. About 10.25±2.73% of maximum crude protein content of the biomass was obtained by this yeast (Table 2).

4. DISCUSSION

From the 10 yeast isolates, an isolate SDY6 was found to be Torula yeast (*Cyberlindnera* sp.) SDY6.This isolate was identified using the following features. These were microscopic observation (oval shape and also the isolate showed multipolar budding features), its colony characteristics (smooth and circular in appearance, creamy texture (color) on agar media). In addition, in ascospore analysis there were no asci formed. This is a negative result indicating that, the yeast was Torula (*Cyberlindnera* sp.) SDY6 because most yeasts possess aci within their ascospore but Torula (*Cyberlindnera* sp.) SDY6 does not possess this feature. Furthermore, fermentation of carbohydrates and assimilation of both selected carbon and nitrogen sources was undertaken (Table 1). These results are in line with the standards set by Yarrow [11] and Barnett et al. [12].

Table 1. Fermentation and assimilation tests of Torula yeast (*Cyberlindnera* sp.) SDY6

Test compound	Glucose	Galactose	Sucrose	Maltose	Lactose	Cellobiose	Melibiose	Trehalose	Soluble starch	Inulin	Xylose	Raffinsoe	Glucoseamine	Nitrate	L-lysine	Creatine
Fermentation	+	-	+	-	-	-	-	-	-	w	-	-	-	NA	NA	NA
Assimilation	+	-	+	+	-	+	-	+	-	+	+	+	-	+	+	-

(+) positive result, (-) negative result, (w) weak result, (NA) not applicable

Table 2. Yield dry weight for Torula yeast (*Cyberlindnera* sp.) SDY6 grown on banana peel hydrolysate

Time (h)	Biomass yield dry weight (g/L)	Crude protein content (%)
0	0.44±0.05	4.25±0.75
24	0.62±0.08	8.27±0.88
48	8.82±1.21	10.25±2.73
72	3.11±0.72	7.59±1.09
96	2.4±0.22	6.22± 0.56

Substrate is a determinant factor for high yield of yeast biomass. Banana peel is available in excess in Ethiopia and it is disposed as waste. So that, its hydrolysate was chosen as a substrate for Torula yeast (*Cyberlindnera* sp.) SDY6 in the current study. It was confirmed that, banana peel hydrolysate was a suitable substrate for the production of single cell protein by the Torula yeast (*Cyberlindnera* sp.) SDY6.

Here it can be inferred that, it is highly rich in carbohydrate content and helps the growth of the yeast, desirable to lower the cost of production and reduce waste disposal and management problems.

The biomass yield of yeast that was obtained from this work was measured at different times during the batch culture operation. After fermentation for 48°h where the medium was kept at temperature of 30°C and pH 4.5, the yeast produced a maximum biomass of 8.82±1.21 g/L (Table 2). It is known that different microorganisms have different growth kinetics. Different yeast species also have different growth phases. Torula yeast (*Cyberlindnera* sp.) SDY6 in this study gave high biomass yield at 48 h of fermentation. This indicates that maximum exponential growth phase of Torula yeast (*Cyberlindnera* sp.) SDY6 is after 48 h of incubation. On the other hand, crude protein content of the product was analyzed using Kjeldahl apparatus. About 10.25±2.73% of maximum crude protein content of the biomass was obtained by this yeast (Table 2). These results are in concurrent with findings for single cell protein produced by *Penicillium expansum* on orange peel which gives 9.89% protein content [17]. *Arachniotus* species when grown on corn cob waste for single cell protein production gave 11.20% content [18]. Microorganisms in general have a high rate of multiplication and high protein content (30 – 80%) in terms of dry weight.

The application of microbial biomass is broadly used for fermentation starter cultures for food and beverage industries, waste treatment processes, and agricultural inoculants; as a source of protein for human food because it is odorless and tasteless, as animal fodder and as functional foods, because it has flavor, fat and water binding property, dispersing action, whipping and foaming action and extraction properties.

5. CONCLUSION

In conclusion, a higher yield of biomass from Torula yeast (*Cyberlindnera* sp.) SDY6 was possible using banana peel hydrolysate as a substrate. The supplementation of banana peel hydrolysate with nitrogen nutrients improved the Torula yeast (*Cyberlindnera* sp.) SDY6 growth, i.e., the biomass yield was best with banana peel hydrolysate medium with $(NH_4)_2SO_4$ as nitrogen source. Banana peel hydrolysate offers a good option, if researches on the possibilities of augmenting its nutritional status are carried out. With regard to the results obtained from this work, fermented banana peel hydrolysate is a proper substrate for single cell protein production under conditions provided in this study, however, for profitable production, interruption of the process after 48 h fermentation could be useful.

After these findings, the following recommendations can be forwarded: Further studies should be done to investigate the nucleic acid content; cost effective single cell protein process should be performed in a pilot and in an industrial scale and genetic improvements of microbial cell is required for high yield of biomass especially Torula yeast (*Cyberlindnera* sp.) SDY6 gives high protein efficiency and protein content when it is treated than natural one.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nasseri AT, Rasoul-Amini S, Morowvat MH, Ghasemi Y. Single cell protein: Production process. Am. J. Food Technol. 2010;6(2):103–116.
2. Ukaegbu-Obi KM. Single cell protein: A resort to global protein challenge and waste management. J. Microbiol. Microb. Technol. 2016;1(1):1–5.
3. Roth FX. Microorganisms as a source of protein for animal nutrition. Anim. Res. Dev. 1980;12:7−19.
4. Parajo JC, Santos V, Dominguez H, Vazquez M. NH_4OH-based pretreatment for improving the nutritional quality of single cell protein. Appl. Biochem. Biotechnol. 1995;55:133−149.
5. Sharma I, Sharma K. Banana peel extracts for the production of single cell protein by

using *Saccharomyces cerevisiae.* Int. J. Adv. Res. 2017;5(3):531−535.
6. Yosef BZ. Essays in Kashrus and Food Science. – Kosher Food Production. 2nd Edition. Iowa: Wiley-Blackwell. John Wiley and Sons; 2008.
7. Law BA, Tamime A. Technology of Cheese making. 2nd Edition, Society of Dairy Technology Series. Chichester, West Sussex: Wiley-Blackwell. John Wiley and Sons, Ltd; 2010.
8. Huang YT, Kinsella JE. Functional properties of phosphorylated yeast protein: Solubility, water- holding capacity and viscosity. J. Agri. Food Chem. 1986;34(4): 670−674.
9. Enwefa C. Biomass production from banana skins. Appl. Microbial. Biotechnol. 1991;36(2):283−284.
10. Yabaya A, Ado SA. Mycelial protein production by *Aspergillus niger* using banana peels. Sci. World J. 2008;3(4):9−12.
11. Yarrow D. Methods for the isolation, maintenance and identification of yeasts. In: The Yeasts: A Taxonomic Study. Kurtzman CP, Fell JW. Editors. Elsevier Science, BV. 1998;77–100.
12. Barnett JA, Payne RW, Yarrow D. Yeast-characteristics and identification. 3rd Ed. Cambridge: Cambridge University Press; 2000.
13. O-Neto AJ, Infanti P, Vitolo M. Influence of pH, temperature and dissolved oxygen concentration on the production of glucose 6-phosphate dehydrogenase and invertase by *Saccharomyces cervisiae*. Braz. J. Chem. Eng. 1997;14.
14. Somaye F, Moosavi-Nasab M, Lale N. Single cell protein production from UF cheese whey by *Kluyveromyces marxianus*. 18th National Congress Food Technology, 15-16, October, Mshnod, Iran; 2008.
15. Krishna G, Ranjhan SK. Laboratory manual for nutrition research. New Delhi: Vicas Publishing House; 1980.
16. AOAC. Official methods of analysis. 17th Edition, Association of Official Analytical Chemists, Washington, DC; 2000.
17. Yakoub KM, Umar DM. Effects of various agricultural waste and pure sugars on the production of single cell protein by *Penicillium expansum*. World Appl. Sci. J. 2010;8:80–84.
18. Javed AM, Asghar M, Yaqub M, Khurram S. Production of single cell protein from delignified corncob by *Arachniotus* species. Pak. J. Agri. Sci. Tot. 2000;37: 130–132.

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