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Production of Single Cell Protein from Indigenous Fungi *Ashbya gosypii* and *Aspergillus fumigatus*

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Authors' contributions

This work was carried out in collaboration between both authors. Author SEAB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author ZMZ managed the literature searches, analyses of the study performed and authors SEAB and ZMZ managed the experimental process. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was producing single cell protein (SCP) by using *Ashbya gossypii* and *Aspergillus fumigatus* which are isolated from *Senna obtusifolia* (sickelpod "kawal") using Sabouraud's dextrose agar medium.
Study Design: This study was designated as an experimental study.
Place and Duration of Study: This study was conducted at the Department of Microbiology and Molecular Biology, Faculty of Science and Technology, Al-Neelain University, Khartoum – Sudan "1st March to 30th June 2015".
Methodology: The microbial biomass was obtained by inoculation of test microorganisms in different types of substrates including molasses substrate and mixture of banana peels plus wheat bran in equal amounts. The qualitative detection of protein in single cell protein biomass was done by ninhydrin method and agarose gel electrophoresis.
Results: The present study showed that *Aspergillus fumigatus* gave moderate (9.6 g) to significant (16 g) biomass while *Ashbya gossypii* gave none (0.0) or small (4.9 g) yield of biomass.

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Conclusion: According to these results, *Aspergillus fumigatus* are better than *Ashbya gossypii* in production of single cell protein which produced larger amount of dry weight in both substrates as 6.8 g when use molasses and 14 g when use the mixture of banana peels and wheat bran, compared with zero gram (0 g) and 4.6 g dry weight produced by *A. gossypii*. This indicates *A. fumigatus* can be used in large scale industrial production. This myco-protein is considered as generally regard as safe to use as alternative source of protein in food and animal feed unless more experiments are done.

Keywords: Ashbya gossypii; Aspergillus fumigatus; molasses; ninhydrin; Senna obtusifolia; single cell protein.

1. INTRODUCTION

Single Cell Protein (SCP) is a term coined in the 1960´s to embrace microbial biomass products which were produced by fermentation [1].

Microorganisms have been employed for many years in the production of high protein food content such as cheese and fermented soybean products [2].

Dried biomass of certain fungi is consumed as a protein source in human food and animal feed. Such fungal biomass is termed fungal protein or mycoprotein. A few yeasts and moulds are being consumed as SCPs. Fungi grow rapidly on a wide variety of cheap raw materials. They have lesser amount of nucleic acids than the bacterial SCPs. The digestibility and acceptability of fungal protein in human intestine is high. Therefore, dried fungal biomass is consumed as a SCP [3].

Since a large proportion of cell dry weight is accounted for protein, the nutritional value of a microbial derived food source is determined by the levels of protein produced [4].

Single-cell protein (SCP) production technologies arose as expecting way to solve the problem of worldwide protein shortage. Intensive research in fermentation science and technology for biomass production, as well as feeding, has resulted in a profound body of knowledge, the benefits of which now span far beyond the field of SCP production [4].

Intensive production (or extensive production) of protein from animal sources (meat) depends on protein from plants: grass for grazing or for production of animal feeds requires large areas of land, that may require nitrogenous fertilizers. This operation can causes a variety of problems if not used at the correct amounts; stored and prepared animal feeds also present a variety of economic and environmental problems. However, using of single cell protein depends mainly in certain benefits. Firstly, growth of microorganism is very much faster than of animals. Secondly, a broader range of materials may be considered as suitable substrates depending on the microorganism chosen [5].

Microorganisms have the ability to upgrade low protein organic material to high protein food, and this has been achieved 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 pre-war food input was replaced [6].

Bacteria and yeast are candidates for the synthesis of SCP. Bacteria grow more rapidly and efficiently than yeast on cheap substrates, and they provide a higher content of protein [7].

Single cell protein is gaining popularity day by day because they require limited land area for growth. Research on SCP has been stimulated by a concern over the eventual food crisis or food shortage that will occur if the world's population is not controlled.

In the 1960s, researchers at British Petroleum developed what they called "proteins-from-oil process": a technology for producing single cell protein by yeast fed by waxy *n*-paraffins, a product produced by oil refineries. Initial research work was done by Alfred Champagnat at BP's Lavera Oil Refinery in France; a small pilot plant there started operations in March in 1963, and the same construction of the second pilot plant, at Grangemouth Oil Refinery in Britain, was authorized [8].

The combination of sophisticated production with food processing technology yielded a new generation of SCP products which may be used

as meat substitutes, texture providing agents and flavour enhancers [1].

Future application of heterologous protein expression may further develop the potential of this food line, resulting in precisely tailored products which meet specific dietary requirements, or simulate high added value specialty products containing ammonium salts as the nitrogen source. This methodology is more efficient than brewing, but still resulted in some fermentation of the carbohydrate source, and sub-optimal yield of biomass obtained per unit of substrate; in feeding or fed batch process was thus born which is still successfully used in today's fermentations as part of a larger program for utilizing natural sources [9].

The post war period was characterized by the recognized need to tackle the problems of humanity on a global scale. A number of international organizations emerged for this task under the leadership of the United Nations. One such organization was The Food and Agriculture Organization of the United Nations (FAO), which brought forward the hunger and malnutrition problem of the world population in 1960, introducing the concept of the protein gap (25% of the world population had a deficiency of protein intake in their diet). The population growth predictions, moreover showed that the number of inhabitants would double between 1960 and 2000, from 2.5 billion to 5 billion (the actual figure reached 6 billion), and the greater part of this increase would take place in those countries suffering from malnutrition [10].

The Malthusian prospect of a limiting food supply was reinforced by fears that agricultural production would fail to meet the increasing food requirements of humanity. The resumption of peace had also procured a new atmosphere geared towards the academic study in civilian matters, and fermentation processes saw a very important period of progress in single cell protein production [11].

A greater involvement of private companies in the marketing of fermentation products had already begun. By the early 60´s a number of multinational companies decided to investigate the production of microbial biomass as a source of feed protein. The basic kinetic mechanisms ruling the growth pattern of microbes had been elucidated and were being established for yeasts and filamentous fungi. However, important technical challenges remained to be solved in industrial fermentations, and the field was boosting with activity. The relatively low market selling price set for this non-conventional towards low product cost and thus large scale production abundant substrates with low prices were sought [12].

The benefits of SCP production were thus extended from the production of food to the preservation of the environment. However, this same reasoning also conditioned the production volumes to match substrate consumption [13].

The impressive performance of the moulds on banana peel substrate may be attributed to the rich nutrient (particularly the crude protein 7.8% and crude fat 11.6% contents) composition of banana peels. The value of this agricultural waste can therefore be increased by its use not only in the manufacture of mycological medium but also in the production of valuable microfungal biomass which is rich in protein and fatty acids [14]. Also the wheat bran is multifarious food because is low in saturated fat, and very low in cholesterol and sodium. It is also a good source of protein, thiamin, riboflavin and potassium, and a very good source of dietary fiber, niacin, vitamin B_6, iron, magnesium, phosphorus, zinc, copper, manganese and selenium. It is also consider a perfect substrate for microbial growth [14].

The main objective of the present study was production of single cell protein by using *Ashbya gossypii* and *Aspergillus fumigatus*. The objective was specified as isolation of *Ashbya gossypii* and *Aspergillus fumigatus* from *Senna obtusifolia* (sicklepod "kawal"), production of single cell protein by using *Ashbya gossypii* and *Aspergillus fumigatus* and estimation and detection of total protein and amino acid in *A. gossypii* and *A. fumigatus* biomass.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Samples *Senna obtusifolia* (kawal), banana peels and wheat bran were collected from local markets in Khartoum city and as donation from students's home. Molasses sample was collected from the Distillery Unit of Kennana Sugars (D.U.K.S) Company located in White Nile State in the southern border of Sudan.

2.2 Preparation of Culture Media

2.2.1 Sabouraud dextrose agar (SDA)

An amount of 3.9 grams (SDA) were added to 100 ml distilled water in a conical flask, heated to boiling in water bath to obtain a homogeneous mixture then sterilized by autoclave at 121°C for 15 minutes before dispensed into pre-sterilized Petri dishes [15].

2.2.2 Molasses substrate

An amount of 225 ml molasses were added to 325 ml distilled water in conical flask, homogenized by shaking then sterilized by autoclave at 121°C for 10 minutes [15].

2.2.3 Banana peels + wheat bran substrate

Amount of 12.5 gram Banana peels powder plus 12.5 gram wheat bran powder, plus 1.5 gram KNO_3, plus 0.5 gram $MgSO_4$, plus 0.5 gram $ZnSO_3$, and 0.005 gram $FeCL_3$ were added to 500 ml distilled water in a conical flask, heated to boiling in water bath to obtain a homogeneous mixture then sterilized by autoclave at 121°C for 15 minutes [2].

2.3 Isolation of *Ashbya gossypii* and *Aspergillus fumigatus*

Senna obtusifolia Sicklepod (kawal) collected from market was ground and distributed randomly into Petri dishes by using forceps, then the plates were incubated in incubator at 34°C for three days. Subsequently, plates were examined for fungal growth, Lactophenol cotton blue stain technique was used to verify the results [16].

2.3.1 Lactophenol cotton blue stain technique

A needle was used to make thin smear, part of a colony was taken by a needle and put in a slide after adding Lactophenol cotton blue stain to labeled slide. The smear was then examined microscopically using 10X and 40X, to identify fungal cells [16].

2.3.2 Subculture purification of *Ashbya gossypii* and *Aspergillus fumigatus*

The plates were sub-cultured in other sterile plates containing SDA. Each susceptible colony was sub- cultured in a new plate. All the plates were incubated at 34°C for 72 hours. Then the grown microorganisms were identified microscopically by Lactophenol cotton blue stain [16].

2.4 Measurement of Microbial Biomass (SCP)

The two isolates were inoculated into molasses and banana peels plus wheat bran medium (500 ml), incubated at 34°C for five days. The biomass was collected and the total protein content was estimated by the Lowry method [2].

2.4.1 Protein and DNA extraction

2.4.1.1 Cell lyses

An amount of 0.1 g fresh frond material was ground with 500 µL extraction buffer and incubated for 1 hour in room temperature. An amount of 500 µL of 24:1 chloroform:isoamyl alcohol was added and vortexed on a low setting for 1 minute. Centrifuged for 10 minutes 15,000 x g. The aqueous layer transferred to a fresh tube. The volume of an aqueous phase was determined. Sequential addition was performed as 0.08 volumes of cold ammonium acetate and 0.54 volumes of cold isopropanol were added to precipitate the DNA. It was then mixed well and kept in freezer overnight, before centrifuged at 15,000 x g for 6 min to recover DNA. The pellet was saved and an aqueous layer was discarded. The rest volume was washed with 700 µL 70% cold ethanol mixed well and centrifuged at maximum speed for 3 minutes. The liquid was pipetted off. The excess ethanol was drought off in the vacuum dryer/speed for 20 minutes. Re-suspended in ddH20 (50 µL). The product was allowed to re-suspend for 1 hour at 55ºC or overnight in the freezer [17] and [18].

2.4.2 Ninhydrine test for protein estimation

The middle layer containing protein was separated to be used in protein analysis. The protein was dissolved by 2N NaOH, 5 drops of ninhydrin solution were added to 1 ml protein sample and then heated for few minutes [19].

2.4.3 NanoDrop to measure purity and concentration of DNA

The purity and concentration of DNA was measured by using NanoDrop ND-1000 spectrophotometer as follows:

The upper and lower optical surfaces of the spectrophotometer sample retention system was

cleaned by pipetting 2 µL of clean deionized water onto the lower optical surface. The lever arm was closed and taped a few times to clean the upper optical surface. Both optical surfaces were wiped with paper tissue. The NanoDrop was initialized by placing 1 µl clean water onto the lower optic surface and the nucleic acid module was selected. Then the lever arm was lowered and 'intialize' option in NanoDrop was selected. After completeness of initialization (~10 seconds); both optical surfaces were cleaned. After that the device was set as blank by adding 1 µl deionized water and the both optical surfaces were cleaned. The nucleic acid sample was measured by loading 1 µl of sample by selecting 'measure' option. Once the measurement was completed, both optical surfaces were cleaned using paper tissue [20].

3. RESULTS AND DISCUSSION

3.1 Cultural Features and Microscopic Identification of Test Microorganisms

3.1.1 *Ashbya gossypii*

The *Ashbya* can be seen from Fig. 1, were isolated from sickle pod samples using Sabouraud's dextrose agar medium. The isolated species were identified using the Lactophenol technique to determine their morphology. *Ashbya* species exhibited circular, flat to slightly raised, membranous, myceloid with advancing edge filamentous, moist, appraised hyphal growth with short, pointed, matted hair–like outgrowths over the surface. Growth appears which changes to a moist, translucent, dirty-white folded and wrinkled plechtenchyma. Swollen hyphal element, termed, bulb-forms are typical of the flavinogenic strains of *Ashbya gossypii*.

Microscopic appearance show the hyphae as hyaline, often vacuolated or contain a granular material and numerous hyaline droplets, at first the hyphae appeared non-septate. In flavinogenic strains of *A. gossypii*, riboflavin observed within certain cells as yellowish oily fluid, or needle – like orange crystals.

3.1.2 *Aspergullus fumigatus*

The fungus is capable of growth at 37°C or 99°F, and can grow at temperatures up to 50°C or 122°F, with conidia surviving. At 70°C or 158°F—conditions it is regularly encountered in self-heating compost heaps Its spores are ubiquitous in the atmosphere colony typically spread slowly but quickly assume a greenish-blue pigmentation due to abundant conidium formation. The pigmentation of mature conidia is at least partially due to melanin, Fig. 2.

Fig. 1.1. The morphological appearance of *Ashbya gossypii* using objective lens x40

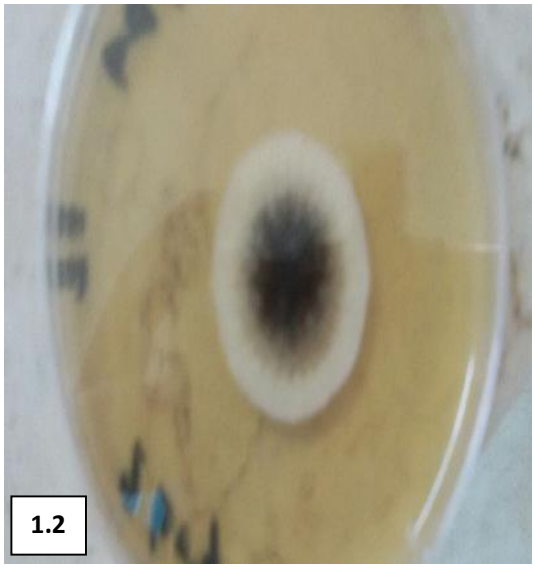

Fig. 1.2. The culture characteristics of *Ashbya gossypii*

3.2 Physical Properties of Microbial Biomass

As can be seen in Table 1, the wet weight of microbial biomass was varied due to the type of

substrate and test microorganism. *A. gossypii* exhibited non biomass when inoculated into molasses substrate and low biomass (4.9 g) when inoculated into mixed substrate consisting of banana peels + wheat bran in equal amounts. *A. fumigatus* gave significant biomass in both types of substrates, but in different amounts as 9.3 and 16 g when inoculated into molasses and mixed substrate respectively. The present study was slightly in agreement with Bozakouk [21] who reported that *A.fumigatus* gave 11.8 g of biomass when inoculated into glucose or maltose substrata, and it was in agreement with Jaganmohan et al. [2] who produced SCP using *Aspergullus terreus* inoculated banana peels+wheat bran as substrate and reported that it was gave 9.8 g of biomass when incubated for five day.

Fig. 2. Culture characteristics of *A. fumigatus* on Saboroud's dextrose agar

As can be seen in Table 2, the dry weight, moisture content, ash per gram and ash as percentage are varied due to type of substrate and test microorganism. *A. gossypii* exhibited non biomass when inoculated into molasses substrate that means it had zero dry weight, zero moisture content and zero ash. The low biomass of *A. gossypi* (4.9 g) was showed when inoculated into mixed substrate consisting of banana peels + wheat bran in equal amounst gave 4.6 g dry weight, 0.3 moisture content and 0.24 g ash which are 12% as percentage. *A. fumigatus* gave 6.8 g dry weight, 2.5 moisture content, 0.41 g ash and ≈ 20% ash when inoculated into molasses substrate, while it gave 14 g dry weight, 2 moisture content, 0.36 g ash which are 18% as percentage when inoculated into mixed substrate consist of banana peels + wheat bran in equal amounts. The present study was in agreement with Adedayo et al. [12] who reported that the ash content of most fungal biomasses ranged between 0.2-0.45 g. The present study was also in agreement Bozakouk [21] who reported that *A. fumigatus* moisture content ranged from 0.2 to 7.5 according to substrate used.

3.3 Electrophoresis

As can be seen in Fig. 3, *A. gosypii* from mixture substrate gave two bands when it is protein run on agarose gel electrophoresis. *A. fumigatus* from molasses and mixture substrate gave two sharp bands when it is protein run in agarose gel electrophorisis.

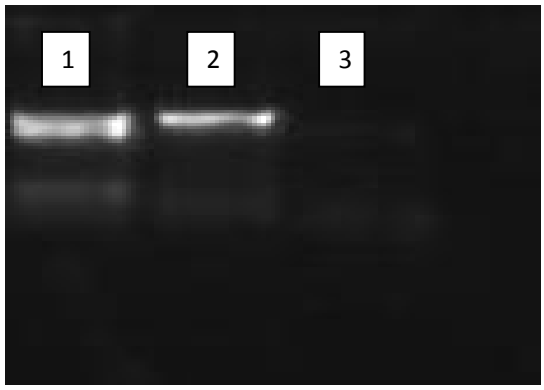

Fig. 3. (1) The protein bands of *A. fumigatus* inoculated into molasses; (2) The protein bands of *A. fumigatus* which inoculated in banana peels + wheat bran substrate; while (3) The protein bands of *A. gosypii* in banana peels + wheat bran substrate

3.4 Qualitative Protein Test

All three samples gave purple color which is known as Ruhemann's purple when react with ninhydrin (2,2-Dihydroxyindane-1,3-dione). Purple color indicates the presence of cyctine, tyrosine and glycin.

3.5 DNA Purity and Concentration

The purity of DNA was measured using NanoDrop ND-1000 with wavelength of 260/280 nm in different samples. The results showed that all the samples were in pure form as 0.995 and 0.997 which indicated the purity of DNA with references. The concentration of DNA varied in the range of 140 – 142 ng/l depending on the type of isolates and source it was obtained from (Table 3).

Table 1. Total biomass of test microorganisms in different substrate g/500 ml

Microorganisms	Wet weight/gram		Mean+STD
	Molasses substrate	Banana peels + wheat bran	
A. gosypii	0.0	4.9	2.45+4.18
A. fumigatus	9.3	16	12.65+10.37

Table 2. Physical properties of microbial biomass

Microorganism	Parameter							
	Dry weight		Moisture content		Ash/G		% Ash	
	*M	**B+B	*M	**B+B	*M	**B+B	*M	**B+B
A. gosypii	0.0	4.6	0.0	0.3	0.0	0.24	0.0	12%
A. fumigatus	6.8	14.0	2.5	2.0	0.41	0.36	≈ 20%	18%

**M≡ Molasses substrate while **B+B≡ Banan peels plus wheat bran*

Table 3. DNA purity (260/280) and concentration (ng/l)

Microorganism	Substrate			
			Banana Peels + Wheat Bran	
	DNA purity	DNA concentration	DNA purity	DNA concentration
A. gossypii	0.997	142	-	-
A. fumigatus	0.995	140	0.995	140

4. CONCLUSION

According to these results, *Aspergillus fumigatus* are better than *Ashbya gossypii* in production of single cell protein which produced larger amount of dry weight in both substrates as 6.8 g when use molasses and 14 g when use the mixture of banana peels and wheat bran, compared with zero gram (0 g) and 4.6 g dry weight produced by *A. gossypii*. This indicates *A. fumigatus* can be used in large scale industrial production. This myco-protein is considered as generally regard as safe to use as alternative source of protein in food and animal feed unless more experiments are done.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ugalde U, Castrillo J. Single cell proteins from fungi and yeasts. Appl. Myco Biotechnol. 2002;2:123.
2. Jaganmohan P, Purushottam D, Prasad S. Production of single cell protein (SCP) with *Aspergillus terreus* using solid state fermentation. Europ. J. Biol. Sci. 2013; 5(2):38-43.
3. Shivika B. Fungi as single cell protein. Biotich Articles; 2011.
4. Patel G. The DNA-protein cross: A method for detecting specific DNA-protein complex in crude mixture. Ind J Agr Res. 1995;3: 114-134.
5. Reed G, Nagodawithana T. Method for the preparation of a yeast extract, its use as a food flavor and a food composition comprising the yeast extract. Biotechnology enzymes, biomass, food and feed, Bibliographic Citation. 1995;9: 168-215.
6. Arora D, Mukerji K, Marth E. Single cell protein in hand book of applied mycology. India: Banaras Hind University. 1991;3: 499-539.
7. Oscar A, Jorgensen J, Jørgensen S. Systematic model analysis for Single Cell Protein (SCP) production in a U-Loop Reactor 20th European Symposium on Computer Aided Process Engineering – ESCAPE20; 2010.
8. Bamberg J. British petroleum and global oil, 1950-1975: The challenge of nationalism. Volume 3 of British petroleum and global oil 1950-1975: The Challenge of Nationalism, J. H. Bamberg British Petroleum serie. Cambridge University Press. 2000;426–428.
9. Stanbury P, Whitaker A, Hall S. Principles of fermentation technology. 2nd edition. Butterworth-Heinemann. Oxford; 2000.

10. Trinci A. Myco-protein: A twenty-year overnight success story. Mycol. Res. 1992; 96(1):1.
11. Trinci A. Evolution of the Quorn myco-protein fungus *Fusarium graminearum* A3/5. Microbiology. 1994;140:2181.
12. Adedayo M, Ajiboye E, Akintunde J, Odaibo A. Single cell proteins: As nutritional enhancer. Adv. Appl. Sci. Res. 2011;2(5):396-409.
13. Jhojaosadati S, Rasoul K, Abbas J, Hamid R. Bioconversion of molasses stillage to protein as an economic treatment of this effluent. Resources Conservation and Recycling. 1999;27(1-2): 125-138.
14. Essien J, Apkan E, Essien E. Studies on moulds growth and biomass production using waste banana peel. World J. Microbial. 2003;8:13-29.
15. Cheesbrough M. Medical laboratory manual for trobical countries, 3st edition, Cambridge University press. UK. 2008;2: 30-449
16. Aneja K. Experiments in microbiology, plant pathology and biotechnology, 4th edition, New Age International (P) Ltd; Publisher; Ansari Road, Daryaganj, New Delhi. 2005;438–441.
17. Sowell C, Thompson C, Batth R, Jernigan T, Toga A. Localizating age – related changes in brain structure between childhood and adolescence using statistical parametric mapping. Neuroimage. 1999;9:587-597.
18. Doyle JJ, Doyle JL. Barrington laboratory protocol DNA extraction -The polystichum homepage website for the hollyferns, members of the fern genus *Polystichum* and allies. This website is maintained for the use of hollyfern enthusiasts and scholars throughout the world by Dave Barrington at the University of Vermont in the United States; 1986.
19. Marsh K, Sims G, Mulvaney R. Availability of urea to autotrophic ammonia-oxidizing bacteria as related to the fate of 14C- and 15N-labeled urea added to soil. Biol Fert Soil. 2005;42:137–145.
20. Nanodrop.Com/Library/CPMB-1st.Pdf
21. Bozakouk A. Acid hydrolysis of *Phragmites austral*; is powder for production of single cell protein by *Candida utilis*. M.Sc. Thesis, Benghazi: Garyounis University; 2002.

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