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Mycota of distillery yeast sludge as source of single cell protein

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Abstract

This study focused on the isolation, identification, and utilization of the mycota present in distillery yeast sludge as source of single cell protein. Seven fungal isolates were described and identified. These include three species of yeasts (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen, *Candida parapsilosis* (Ashford) Langeron and Talice, and *Candida guilliermondii* (Castellani) Langeron et Guerra) and four species of molds (*Aspergillus flavus* Link, *Aspergillus niger* van Teigh, *Aspergillus japonicus* Saito var *japonicus*, and *Rhizopus* sp.).

The ability of the fungal isolates to produce single cell protein was evaluated by determining the crude protein content (CPC) of the distillery yeast sludge after 14 days of solid state fermentation. Results revealed that inoculation of the seven taxa produced significantly higher percentage CPC of the distillery yeast sludge. Apparently, *S. cerevisiae*-treated distillery yeast sludge had the highest percentage CPC of 33.7% and the highest percentage increase in CPC of 34.3%, while *Rhizopus* sp.-treated distillery yeast sludge had the lowest of 21.8%. Thus, the present study indicates the great potential of the seven taxa as source of single cell protein using the distillery sludge as substrate.

Keywords – crude protein content – distillery yeast sludge – fermentation – mycota – single cell protein

Introduction

In the Philippines, the sugar industry is one of the country's oldest and leading export earners. Based on reports, there are about 411,100 hectares of sugarcane plantation producing million metric tons of sugar and molasses. Molasses, a byproduct of sugar, provide a substantial supply of feed materials for the livestock industry, 67% of it is exported, 17% is used by distilleries, and 16 % is used for feeds (Rojas 1995).

Distillery yeast sludge (distiller's sludge) is produced as solid waste in the brewing industry. It contains a high amount of protein (21%) and is also rich in essential amino acids such as lysine, methionine, glycine, arginine, leucine, and histidine, which are essential for the growth and health of animals (Sudha Rameshwari & Karthikeyan 2005). Moreover, dried distillery grains are also sold as cattle feed and due to their abundant availability, it can also serve as an ideal substrate for microbial processes for the production of single cell protein.

Single cell proteins (SCP) are dried cell mass of microorganisms such as fungi, algae, and bacteria, which contain high concentration of nucleotides, inositol, and glutamic acid. It can also contain other biological molecules like lipids, carbohydrates, and vitamins. They can also be called

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as biomass, bioprotein, or microbial protein (Silva et al. 2009, Azam et al. 2014). Microorganisms are great sources of SCP due to its rapid growth rate, their ability to synthesize inexpensive substrates as carbon sources, and their high efficiency to convert carbon sources to protein (Glazer & Nikaido 2007).

Several studies have been reported to utilize different species of yeasts and molds using various agro-industrial wastes for single cell protein production. Hence, the study has been carried out to identify the mycota present in the distillery yeast sludge and to probe their potential in single cell protein production. The results would provide baseline information on the mycota thriving in distillery yeast sludge that would lead to further utilization of these taxa as source of microbial protein using the dried distillery yeast sludge as substrate which can then be used as supplement to costly livestock feeds. It can be of significance in the Philippines where there is a surplus of carbohydrates and inadequate supply of proteins.

Materials and Methods

Isolation of Mycota

Sugarcane distillery yeast sludge was collected directly from the discarding area of the distillery tank of Azucarera de Tarlac, Tarlac City, Tarlac, Philippines. The collected distillery yeast sludge were serially diluted up to 10^{-5} dilution and were pour plated into the Potato Dextrose Agar (PDA), Malt Extract Yeast Agar (MYA), and Yeast Agar (YA) plates. Cultures were then incubated for 4-7 days at 28°C in inverted position. After which, distinct colonies of yeasts and molds were isolated, purified, and maintained in MYA (yeasts) and PDA (molds) slants for further identification.

Identification of Mycota

Identification of mold isolates was based on their cultural and morphological characteristics and was referred to the taxonomic keys by Klich & Pitt (1988) and Lodder (1970). Meanwhile, API C AUX yeast identification system was employed for yeast isolates.

Preparation of the Inoculum

Inoculum was prepared by growing molds in PDA for seven days and yeasts in MYA for three days. Then, 20 ml of sterile water was added to the cultures and adjusted to 5.0×10^6 cells per ml with sterile distilled water. Cells were counted using a hemacytometer.

Preparation of the Substrate

Dried yeast sludge was collected from Central Azucarera de Tarlac. Dried yeast sludge was analyzed for its Crude Protein Content (CPC) using Kjeldahl method. This CPC served as the initial CPC of the substrate. One hundred fifty (150) grams of dried yeast sludge was placed in a clean culture bottle and 110 ml of water was added to the substrate to obtain 60-65% moisture content. These were covered with plastic and were sterilized at 121ºC, 15 psi for one hour.

Inoculation of Mycota in Sugarcane Distillery Yeast Sludge

Ten ml of the adjusted spore suspension of different mycota was aseptically transferred to the substrate. Cultures were covered with sterile cheesecloth and a sterile bamboo stick was inserted into the cheesecloth to facilitate the mixing of cultures every 72 hours to prevent the settling of the cells at the bottom of the cultures. The cultures were cultivated for 14 days at 28ºC.

Harvesting and Crude Protein Analysis

After 14 days of cultivation, the cultures were sterilized (121ºC, 15 psi for 1hour), air dried for 7 days, and were pulverized using mortar and pestle. Crude protein content (CPC) was analyzed using Kjeldahl method. The fungal-enriched sugarcane distillery yeast sludge CPC served as the final CPC. Whereas, the CPC of the uninoculated sugarcane distillery yeast sludge served as the

initial CPC.

Data were analyzed using Analysis of Variance (ANOVA) and Comparison among Means by Duncan's Multiple Range Test (DMRT) at 5% and 1% levels of significance.

Results and Discussion

Characterization and Description of Fungal Isolates

Three species of yeasts (Figs 1A- C) namely: *Saccharomyces cerevisiae* Meyen ex E. C. Hansen, *Candida parapsilosis* (Ashford) Langeron and Talice, and *Candida guilliermondii* (Castellani) Langeron et Guerra) and four species of molds (Figs 1D- F) such as *Aspergillus niger* van Teigh, *Aspergillus japonicus* Saito var *japonicus*, *Aspergillus flavus* Link, and *Rhizopus* species were isolated, described and identified based on the taxonomic keys by Klich & Pitt (1988) and Lodder (1970).

1. Saccharomyces cerevisiae Meyen ex E.C. Hansen

Colonies of *S. cerevisiae* as observed on MYA after three days of incubation were off-white in color, circular in shape, with dull and smooth texture, convex elevation, and entire margin (Fig 1 A). For the physiological characteristics, *S. cerevisiae* had a positive result in D-glucose, D-galactose, Methyl-α-D-glucopyranoside, D-maltose, D-saccharose, D-trehalose, D-melezitose, and D-rafinose. On the other hand, it had a negative result in glycerol, L- arabinose, calcium-2-keto-gluconate, L-arabinose, D-xylose, adonytol, xylitol, inositol, N-acetyl-glucosamine, D-cellobiose, and D-lactose.

2. *Candida guilliermondii* (Castellani) Langeron et Guerra

Colonies of *C. guilliermondii* grown on MYA were off-white in color and circular in shape. Colonies had a dull and smooth texture with convex elevation and margins were entire (Fig 1B). For its physiological characteristics, it had a positive result for D-glucose, glycerol, calcium-2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, D-sorbitol, Methyl-α-D-glucopyranoside, N-acetyl-glucosamine, D-cellobiose, D-maltose, D-saccharose, D-trehalose, D-melezitose, and D- raffinose. Meanwhile, it had a negative result for inositol and D-lactose substrate.

3. *Candida parapsilosis* (Ashford) Langeron and Talice

The colonies of *C. parapsilosis* grown on MYA were off-white in color, circular in shape, had dull and smooth texture and with convex elevation and with entire margin (Fig 1C). *C. parapsilosis* had a positive result for D-glucose, glycerol, calcium-2-keto-gluconate, L-arabinose, D-xylose, D-galactose, D-sorbitol, Methyl-α-D-glucopyranoside, N-acetyl-glucosamine, D-maltose, D-galactose, D-cellobiose, D-saccharose, D-melezitose and negative for adonitol, xylitol, inositol, D-trehalose, and D-raffinose.

4. *Aspergillus japonicus* Saito var *japonicus*

Aspergillus japonicus colonies on PDA were initially white, and became light to dark brown in color and the mycelium was floccose and sporulating densely (Fig 1D). The conidial head of *A. japonicus* was spherical to radiate in shape, splitting into well-defined divergent columns as it aged and was colored black. Its conidiophores were long, smooth-walled, hyaline, and slightly pigmented at the apex. From the conidiophores were the brown and subspherical vesicles. Then covering the vesicles was a single row of phialides where the conidia were borne. The conidia were hyaline to brown in color with subspherical to ellipsoidal shape. Hyphae were septated and hyaline.

5. *Aspergillus flavus* Link

Aspergillus flavus colonies on PDA were initially white, turning yellowish green to green in color as it matured (Fig 1E). Conidial heads of *A. flavus* were radiate to loosely columnar in

shape. The conidiophores were coarsely roughened and hyaline. Arising from the conidiophores were the globose to sub-globose vesicles. Then covering the entire vesicles was the metulae from where the phialides were borne. Conidia of *A. flavus* were globose to subglobose in shape with smooth to very finely roughened texture. The hyphae were septated and hyaline.

6. *Aspergillus niger* van Teigh

Aspergillus niger grown on PDA were initially white, quickly becoming black with the formation of its conidia. Conidial heads were radiate (Fig 1F) which split into columns during maturity. Their conidiophores were long, smooth in texture, and hyaline in color and became darker at the apex. From the apex of the conidiophores were the pale-brown and globose vesicles. Covering the entire vesicles of *A. niger* were the brown metulae which in turn gave rise to the phialides. Borne from the phialides were brown and globose conidia.

7. *Rhizopus* sp.

Rhizopus sp. on PDA were initially white turning grayish black and grew very rapidly (Fig 1G). Sporangiophores, rhizoids, sporangia, collumelae, and sporangiospores were visualized for the morphological characteristics of the *Rhizopus* sp. Its sporangiophores were long and brown in color. At the lower end part of the sporangiophores were the rhizoids and at the tip of the sporangiophores were the sporangia which were round in shape with flattened base. Collumelae with a hemispherical shape were also present. Borne from the sporangia and collapsed collumelae were the sporangiospores. Sporangiospores were unicellular, round to ovoid in shape, hyaline to brown in color, and smooth in texture. In addition, the hyphae of *Rhizopus* sp. were broad and non-septated.

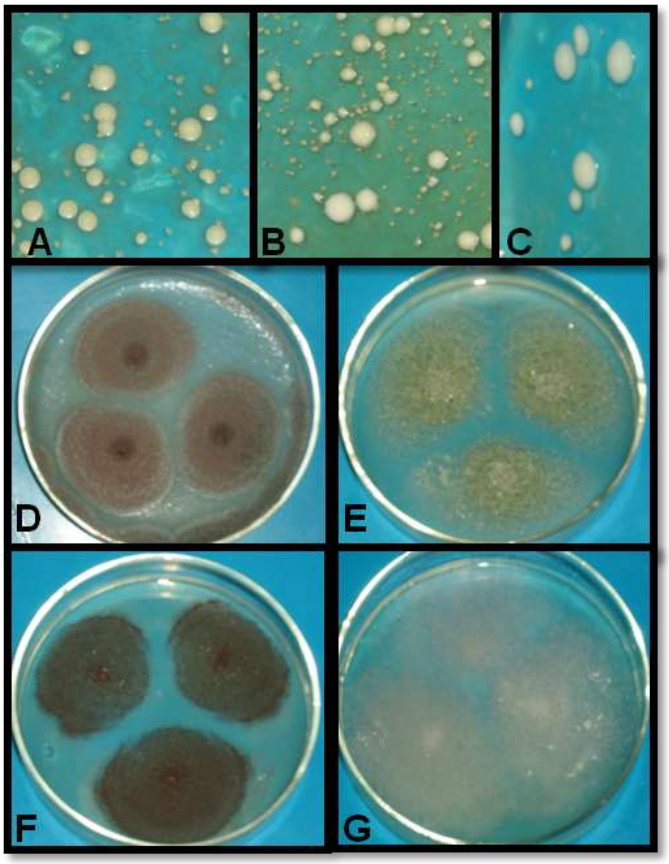

Fig. 1 – Cultural features of (A) *S. cerevisiae* (B) *C. guilliermondii* (C) *C. parapsilosis* on MYA after 3 days of incubation; (D) *A. japonicus* (E) *A. flavus* (F) *A. niger* (G) *Rhizopus* sp. on PDA after 7 days of incubation against blue background

Crude Protein Content (CPC) Profile of the Dried Distillery Yeast Sludge

Evaluation of the CPC of the fungal-enriched distillery yeast sludge was made through Kjeldahl method. The gross percentage CPC of the distillery yeast sludge are shown in Table 1. Interestingly, inoculation of fungal isolates increased the gross CPC of the distillery yeast sludge, which were significantly higher than the uninoculated sludge. *S. cerevisiae*-treated distillery yeast sludge registered the highest percentage mean of 33.7%, followed by *A. niger*-treated sludge and C. *guilliermondii*-treated sludge with means of 32.5% and 32.2%, respectively. Meanwhile, the uninoculated–distillery sludge had the least percentage CPC of 25.1%. Statistical analysis for the gross percentage CPC of distillery yeast sludge suggests that there is a highly significant difference among the treatment means. These indicate that the inoculation of the seven mycota affected the percentage CPC of the distillery yeast sludge thus increasing the CPC of the distillery yeast sludge and were significantly higher compared to the untreated yeast sludge. Similarly, Humdy (2013) reported the efficiency of *A. niger, R. oryzae*, and *S. cerevisiae* in SCP production wherein profiles of essential amino acids are comparable with FAO standards. While El-Deek et al. (2009) demonstrated the superior ability of different strains of yeast including *Candida utilis, C. tropicalis, Saccharomyces cerevisae, S. uvarum,* and *Rhodotorula rubra* to hydrolyze uric acid and produce protein when grown on dried poultry manure medium.

Results of the present study confirm the reports of Nasseri (2011) that several species of filamentous fungi such as *Aspergillus, Chaetomium, Paecilomyces, Penicillium, Trichoderma,* and yeasts including *Candida, Kluyveromyces,* and *Saccharomyces,* are SCP producers. This also coincides with Bacha et al. (2011), wherein *Saccharomyces cerevisiea* is considered the most vital source of single cell protein because of its easy harvesting, larger cell size with lower content of nucleic acids, and bioactive mixture of essential amino acids. Furthermore, Beningson (1992) & Pandey (1994) also revealed that most species of *Aspergillus* and *Rhizopus* produce carbohydrate-hydrolyzing enzymes in the likes of cellulases, xylanases, pectinases, α-amylase, glucoamylases, glucanases, lipases, hemicellulases, and proteases.

Table 1 Gross percentage CPC of distillery yeast sludge

Treatment	Mean percentage crude protein
A. niger -treated distillery sludge	32.5 [b]
A. japonicus-treated distillery yeast sludge	30.7[c]
A. flavus- treated distillery sludge	30.9[c]
Rhizopus- treated distillery sludge	30.5[c]
C. guillermondii- treated distillery sludge	32.2[b]
C. parapsilosis- treated distillery sludge	31.2[c]
S. cerevisiae- treated distillery sludge	33.7[a]
Control- uninoculated distillery sludge	25.1[d]

*Treatment means with the same letter are not significantly different at 0.01 level of significance

Percentage Increase in Crude Protein Content of the Distillery Yeast Sludge

Percentage increase in crude protein content of the distillery yeast sludge is presented in Table 2. *S. cerevisiae*-treated distillery yeast sludge registered the highest percentage increase in CPC of 34.3%, followed by *A. niger*-treated distillery yeast sludge, and *C. guilliermondii*-treated distillery yeast sludge with means of 29.6% and 28.6%, respectively. *Rhizopus* sp.-treated distillery yeast sludge obtained the lowest CPC of 21.8%. Among the seven isolated mycoflora, *S. cerevisiae* increased the percentage in CPC of the distillery yeast sludge which is significantly higher compared to the six fungal isolates. This implies greater ability of *S. cerevisiae* in the production of single cell protein. According to Davies (1994) *S. cerevisiae* can provide proteins, carbohydrates, fats, vitamins (mainly the B group), minerals, essential amino acids including lysine, which is generally higher than in bacteria, algae, and molds. Moreover, Ingram (2002) mentioned that *S. cerevisiae* also contains thiamine, tryptophan, riboflavin, biotin, niacin, pantothenic acid, pyridoxine, choline, streptogenin, glutathione, lysine, folic acid, and P-amino benzoic that contribute to its single cell protein production capability.

Table 2 Mean percentage increase in CPC of fungal-enriched distillery yeast sludge after 14 days of incubation

Treatment	Percentage increase in CPC
A. niger -treated distillery sludge	29.6[b]
A. japonicus-treated distillery yeast sludge	22.5[c]
A. flavus- treated distillery sludge	23.4[c]
Rhizopus- treated distillery sludge	21.8[c]
C. guillermondii- treated distillery sludge	28.6[b]
C. parapsilosis- treated distillery sludge	24.3[c]
S. cerevisiae- treated distillery sludge	34.3[a]

* Treatment means with the same letter are not significantly different at 0.01 level of significance

As indicated in this study among the species of molds, *A. niger* has the highest percentage increase in CPC. This result corresponds to that of Yigitoglu (1992), who found out that *A. niger* was superior to other species of *Aspergillus* and strains of fungi in biomass yield from agricultural waste. In addition, Ikenebomeh & Chikwendu (1997) observed high amylolytic activity of *A. niger* in biomass production while its ability to produce strong activity in beta glucosydase resulting to deglycosylation of the substrate was accounted by Rashid et al. (1997) and Suto & Tomita (2001). Moreover, single cell protein product obtained from *A. niger* contained 30.4% crude protein and had an essential amino acid profile featuring high lysine content and appreciable amounts of methionine and tryptophan (Singh et al. 1991). Thus, the superiority of *A. niger* among the species of molds was established.

In the present study, the great ability of the seven mycota present in the distillery yeast sludge to produce single cell protein was clearly demonstrated thus increasing the CPC of the yeast sludge. It also indicates the potentiality of the sugarcane distillery yeast sludge as carbon source for SCP production.

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To God be the glory. Thy will be done.

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