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Extraction and Purification of the Potential Allergen Proteins from Candida Albicans

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Abstract: A commercial strain of *Candida albicans*10231 was grown and isolated using Sabouraud's Dextrose Agar Media. The samples were then subjected to lyophilization before protein extraction. TCA/acetone/methanol/phenol method has been used for protein extraction. For estimation of the percentage of extracted protein, BCA Protein Macro Assay Kit was applied. The samples were then transferred to the spectrophotometer for reading at wave length of 562 nm. The results show that the profile of the quantity of protein concentrations obtained upon supplementation of the following chemicals: PBS, ammonium bicarbonate, and 50mM of SDS buffer, were 0.133mg/mL, 0.080mg/mL, and 0.068mg/mL respectively.

Keywords: Allergy, Fungal allergy, *Candida albicans*, protein extraction

Introduction

Allergy can be described as an abnormal response of the immune system. The immune system of individuals with allergies develops an extreme reaction to substances (allergens) that are usually harmless, such as surrounding pollen granis, molds, animal hairs. Allergic skin diseases are more common in all age, ranges from children to the elderly, due to excessive urbanization today, rapid increase in negative environmental factors, increasingly moving away from natural nutrition (Günenç, 2020). About 20-30% of the world's population is affected by different allergic problems, and about 30% of them are caused by fungal spores (Grinn, 2011). More than 80 species of fungi allergic more than 100 species with respiratory symptoms have been associated with serious infections in humans and animals, while other species cause serious diseases in plants. Allergic effects on atopic individuals are important because of their opportunistic pathogenic effects in immunocompromised/suppressed individuals (Fang et al., 2005; Chakrabarti et al., 2012). When air as a medium is examined, it appears to contain viruses, bacteria, protozoans, algae, fungal spores and pollen (Schillinger et al., 1999). As a result of mycological research, which has a history of about 150 years, it has been found that so far there are more than 100,000 yeast and mold fungi, and only about 100 of them cause diseases in humans and animals (Töre, 1996). Meterological factors such as wind, humidity, temperature, precipitation, geographical location, air pollution, vegetation and human studies influence the concentration and type of fungal spores in the atmosphere (Bezerra et. al., 2014; Aydoğdu & Asan, 2008). Fungi and fungal spores may remain suspended in the air for long time , among these fungi are many genus of family *Saccharomycetaceae*, for example *Candida albicans*.

Habitat of Candida Albicans:

Mcosal membranes of human and other warm blooded animals. Also found in the gut, in the vagina and also on the surface of the skin. Found in the digestive tract of birds. Isolated from soil, animal, hospitals, in-animate objects and food. Small, oval, measuring 2-4 μm in diameter. Yeast form, unicellular, reproduce by budding. Single budding of the cells may be seen. Both yeasts and pseudo-hyphae are gram positive. Encapsulated and diploid, also form true hyphae. Dimorphic fungai (yeast and pseudohyphal form) Can form biofilms. Normal condition: Yeast. Special condition (pH, Temperature): Pseudohyphae 80-90% of cell wall is carbohydrate (Carlile et. al., 2001).

The cell wall is formed of an inner chitin layer, a β-1,3-glucan layer, a β-1,6-glucan layer, and an outer mannan layer (Kapteyn et. al., 2000). In *C. albicans*, there are two Pir proteins: Pir1 and Pir32. Pir1 was found to be an essential protein, required for the stability and rigidity of the cell wall (Martı´nez et. al., 2004). Pir32 is a 422 amino acid long protein (Bahnan et. al., 2012). In laboratory, *C. albicans* usually grown on Sabouraud's Dextrose Agar Media at incubation temperature between 26-37°C and incubation time between 24-48 hrs.

The present work deals with the application of new method for the isolation and purification of *C. albicans* proteins in an attempt to obtain a high yield of protein from this fungus. Such a protein may serve as a drug for the treatment of many diseases, in addition to suggest recommendations that may be helpful in the reconsidering or changing of prescription strategy and dosing protocol.

Method

Preparation of C. albicans 10231 culture

C. albicans 10231 strain was purchased from local market. It was cultured on cooled sterilized SDA agar medium in petri dishes and kept in in incubator, previously adjusted at 37°C, for 48 hrs incubation.

Extraction of total proteins

Total protein extraction procedure based on the method described by. Pure culture of *C. albicans* was then transferred to eppendrof tubes, followed by the addition of chloroform and 70% of methanol (2:1 v/v). The mixture in, eppendrof tubes, was well mixed by vortex, and then kept in the shaker at 4°C for 24 hrs. After 24 hrs the mixtures in the tubes were subjected to mixing by vortex. The samples in eppendrof were then subjected co centrifugation at 15000rpm for 15 min. The samples kept in the incubator for 48 hrs at 37°C. 40mL of PBS was added and the samples then mixed well by vortex and followed by addition of phenol to the samples and kept in the incubator for 3 days at 4°C. The samples were then freezed at -20°C, and subjected to lyopholization for 24hrs. 10% of TCA was then added, and the samples left at room temperature for 15 min and the centrifuged at 13000rpm for 3min. The supernatant was discarded. To the precipitate, ammonium acetate solution (7.7mg ammonium acetate in 1 liter methanol) was added. The sample mixed well by vortex, then centrifuged at 13000rpm for 3min.The supernatant was discarded and 80% of cold acetone was added to the precipitate, then centrifuged The supernatant was discarded. The precipitate kept in the incubator for 24 hrs. at 37°C, then 0.8 ml phenol and 0.4 ml of SDS buffer (5% at pH 8) were added, mixed by vortex, then centrifuged at 15000rpm for 3min. The supernatant was discarded; while10% methanol and 1 M ammonium acetate were added to the precipitate. The sample kept at -20°C for 24hrs. Then centrifuged at 13000rpm for 3min. The supernatant was discarded; the precipitate was washed by 8% acetone (for seven times) by centrifugation at 13000rpm for 20min. and incubated at 37°C for 2 days, then stored at -20°C for 2 days. The sample is divided in to three aliquots, each aliquot contained 0.025mg of the sample, and to each aliquot the following chemicals were added: 50mM SDS, PBS, and ammonium bicarbonate. These 3 samples were then put in an orbital shaker previously adjusted at 250rpm and 4°C for 72hrs.

Detection of total protein

Determination of Total Protein Concentration Total protein concentration of mushroom extracts Smith et al. (1985) by using the bicinchoninic acid (BCA) method. Commercially purchased BCA Macro Assay Kit (Serva Electrophoresis GmbH) was used to determine protein concentration. BCA analysis was performed in

accordance with the protocol suggested by the manufacturer. All above experiments were done in triplicate and the average value of the data was considered.

Results and Discussion

The table below represents the results of the current study (Table 1). It shows that the profile of the quantity of protein concentrations obtained upon supplementation of the following chemicals: PBS, ammonium bicarbonate, and 50mM of SDS buffer, were 0.133 mg/mL, 0.080mg / mL, and 0.068 mg/mL respectively. This means that the highest yield of total protein obtained is 0.133 mg/mL developed upon supplementation of PBS. However, Kustrzeba-Wojcicka, et.al.(2009) have shown that the highest concentration of the total protein extracted from *C. albicans* was 0.095mg / mL. This means that the increment in the amount of total protein of the present work is 0.098 mg/mL (Kustzeba, 2009), compared to Kustrzeba-Wojcicka, et al study. Since it become well known that *C.albicans* protein is considered as an allergen and source of allergic reaction, therefore finding of new technique for improvement of the production of a such protein (as immunogen) and other drugs may help to improve the current medication status especially in those patients who suffering from severe allergic response and reactions to the common allergen. Therefore, the future work should be concentrated on these aspects since little or no work concern this field.

Tablo 1. Total protein concentration values of *C. albicans* extracts measured by BCA assay

Alerjen adı	Absorbance Measurements	Absorbance Average	Protein concentration (mg/mL)
Candida albicans (Ammonium bicarbonate)	0,812/0,787/0,834	0,811	0,080 mg/mL
Candida albicans (PBS)	0,978/0,893/0,876	0,916	0,133 mg/mL
Candida albicans (50mM SDS Buffer)	0,808/0,738/0,706	0,750	0,068 mg/mL

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