Research Article

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Medical Mycology: Open Access

2015
Vol.1 No.1:2

In vitro Antagonistic Activity of *Candida albicans* against Filamentous Fungi

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Received date: October 17, 2015; **Accepted date:** October 27, 2015; **Published date:** November 26, 2015

Abstract

Background: *Candida albicans* is a special type of yeast that is naturally found on the surface of various mucosal layers of the human body. Biofilms forming is well known synergistic relationship between *C. albicans* and various organisms with no or little aggressive acted toward each other.

Methods and findings: *C. albicans* that isolated from patient with cutaneous candidiasis was tested for antifungal activity against six species of filamentous fungi by measuring of percentage inhibition. The effects of incubation periods and different concentrations of glucose on the antifungal activity of *C. albicans* were also tested. The percentage inhibition of growing filamentous fungi had been observed at high values when they cultivated on media that previously cultured with *C. albicans* than when both of fungi and *C. albicans* cultured at the same time, especially at low concentrations of glucose. *Mucor* spp. and *C. sitophila* had been affected by *C. albicans* only when they cultivated on media that previously cultured with *C. albicans*.

Conclusion: *C. albicans* had been shown the ability to inhibit various species of filamentous fungi. Incubation periods and glucose concentrations had been effected on the inhibitory action of *C. albicans* against other fungi.

Keywords: *C. albicans*; Filamentous fungi; Antifungal

Introduction

Candida albicans considers one of the most important fungi that is found in commensal with other normal microflora on mucosal surface of the mouth, esophagus, gastrointestinal tract, urogenital tract and on the skin of healthy individuals [1-3]. It is rarely found in the environment outside human body [4]. Under special conditions, *C. albicans* can be turned from unharmful yeast to pathogenic fungi causing many clinical forms of diseases that are called Candidiasis [1,5]. Some of these diseases located on superficial layers such as Cutaneous and vulvovaginial Candidiasis, while other invaded with dissemination into other tissues such as candidemia and candidial myocarditis [2,6]. Morphological alteration from yeast to pseudohyphae or hyphae and production of various virulence factors facilitate *C. albicans* to penetrate the mucosal layer and to cause systemic infections [1,6-7].

One of very important types of relationship between *C. albicans* and other normal microflora in the human body, especially fungi and bacteria, is the ability of *C. albicans* to form biofilms with the same specie or with different types of organisms that are found on the tissue surfaces through the production of tyrosol molecules [8]. These biofilms are more likely to hold *C. albicans* with heterogeneous organisms such as bacteria and non-*candida albicans* candidia species (NCAC) than with other strains of *C. albicans* [9-10].

Although *C. albicans* living in a synergistic form with different organism, it is also has the ability to eliminate other organism. The in vitro antagonistic ability of *C. albicans* toward other groups of fungi was investigated as the main aim in this study.

Materials and Methods

Fungal isolation

C. albicans was isolated from the skin of patient with Cutaneous Candidiasis who attended at AL-Hussein general teaching hospital of Karbala province in July 2014. In addition to germ tube and morphological characters, API 20C sugar assimilation system was performed to diagnosis of *C. albicans* [11-12]. *Chrysonilia sitophila* and four species of Aspergillus

including *A. flavus*, *A. fumigates*, *A. niger*, and *A. oryzae* were isolated from air by exposure a plate of Sabouraud's dextrose agar (Himedia, India) to air for 10 min. *Mucor* spp. was isolated from contaminated bread. The isolated species of filamentous fungi were diagnosed based on morphological and microscopically characters [11,13], in addition to molecular characters that had been described by Gautam and Bhadauria [13]. All isolated fungi had been maintained on Sabouraud's dextrose agar as stock cultures.

Fungal and media preparation

The cell number of *C. albicans* and other isolated fungi had been standardized at 3 × 10^8 cells/ml by using McFarland Nephelometer barium sulfate standards [14]. Sabouraud's glucose agar was prepared by mixing peptone 10 g, agar 15 g, 1 L of distilled water and different concentrations of glucose (10 g, 15 g, 20 g, 25 g and 30 g). Those media were prepared in order to study the effect of glucose levels on the culturing of *C. albicans* with filamentous fungi.

Antifungal assay

The antagonistic relationship between *C. albicans* and other filamentous fungi was evaluated according to the method that mentioned by Randhawa et al. [15]. Briefly, *C. albicans* was cultivated like arc or half of the circle by swab around the center of culture plate that inoculated with 0.01 ml of filamentous fungi. The protocol was performed in two ways that differ in the time of inoculation of filamentous fungi with *C. albicans*. Firstly, *C. albicans* and other fungi were cultivated at the same time and incubation at 37°C for 48 h. Secondly, *C. albicans* was first incubated alone for 24 h at 37°C before inoculation of filamentous fungi, then incubated together at 37°C for 48 h. Meanwhile, each of *C. albicans* and other fungal species were separately cultured on prepared media as a control. Perpendicular colony diameters (mm) of growing species were measured and percentage inhibition was calculated according to the formula that mentioned by Kumar et al. [16] as follow:

$$Percentage\ inhibition = \frac{[(C-T)\times 100]}{C}$$

Where, C=colony diameter (mm) of control

T=colony diameter (mm) of tested fungi

Results

The ability of *C. albicans* to inhibit the growth of other fungi had been investigated against six species of filamentous fungi. After 48 h incubation of testing fungi with *C. albicans*, the growth of *A. niger*, followed by *A. oryzae* and *A. fumigatus* had been ceased with high percentage inhibition, especially at low concentrations of glucose (10 g). However, the inhibition rate of those three species was decreased in concomitant with increasing of glucose concentrations. For *A. flavus*, the result was reflected when fungal growth decreased on media containing low concentrations of glucose (**Figure 3**). On the other hand, the growth of *Mucor spp.* and *C. sitophila* had not been effected in the presence of *C. albicans* even glucose concentration was affected (**Figure 1**).

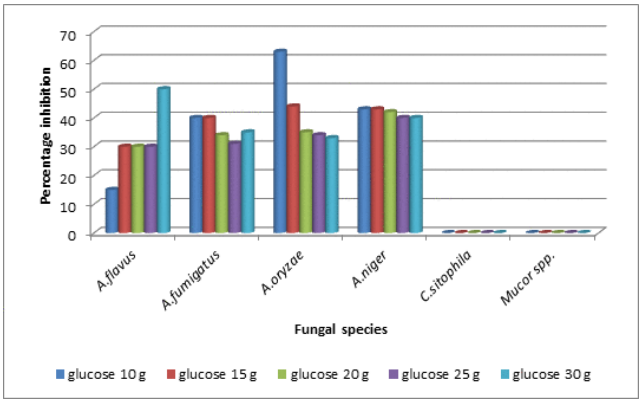

Figure 1: Percentage inhibition of filamentous fungi cultured together with *C. albicans* on media containing different concentrations of glucose.

The growth of filamentous fungi that had been inoculated after 24 h cultivation of *C. albicans* was clearly decreased with high percentage inhibition than when fungi and *C. albicans* cultured together at the same time. *A. oryzae* followed by *A. fumigatus* and *A. niger* had been shown more sensitivity toward the growth of *C. albicans* with decreasing in inhibition values in concomitant with decreasing of glucose concentrations (**Figure 2**).

In the second method, the growth of *Mucor species* and *C. sitophila* was differed from that in the first method. Both of them had been affected by the culturing of *C. albicans*, especially at low concentrations of glucose for *C. sitophila* and high concentrations for *Mucor species* (**Figure 4**).

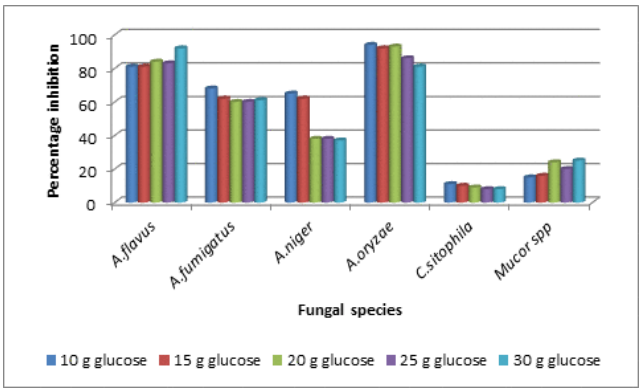

Figure 2: Percentage inhibition of filamentous fungi cultivated after 24 h of *C. albicans* grown on media containing different concentrations of glucose.

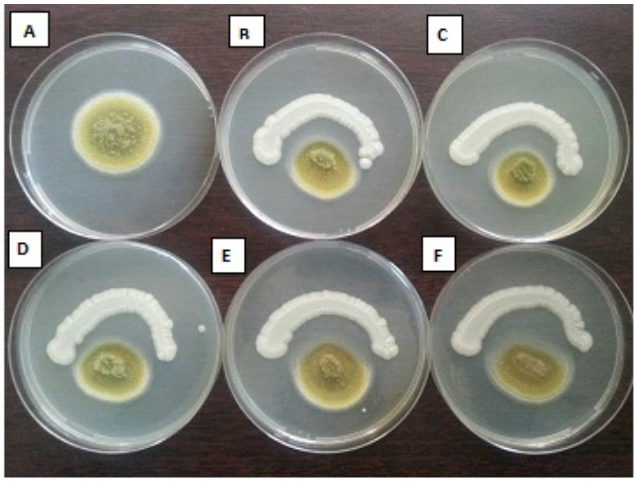

Figure 3: *A. flavus* cultured together with *C. albicans* on media containing different concentrations of glucose. **A**: Control; **B**: glucose (10 g); **C**: glucose (15 g); **D**: glucose (20 g); **E**: glucose (25 g); **F**: glucose 30 (g).

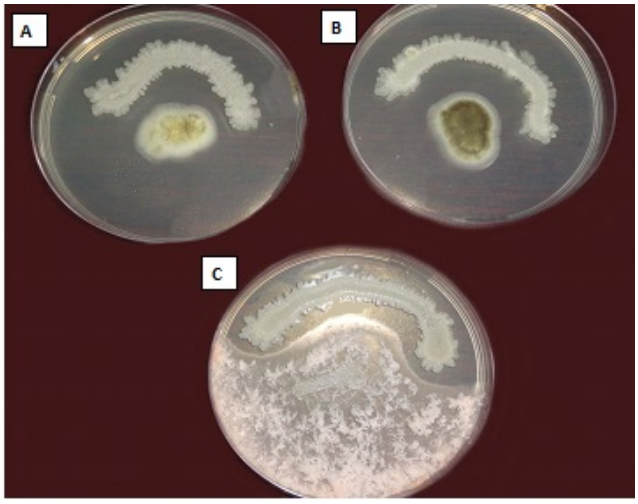

Figure 4: Filamentous fungi cultivated after 24 h of *C. albicans* grown on media containing different concentrations of glucose. **A**: *A. oryzae*; **B**: *A. niger*; **C**: *C. sitophila*.

Discussion

C. albicans that is found on mucosal surface of different organs of the human body is usually cannot live alone. It represented one of various groups of organisms that are living together in the form of microflora. Although *C. albicans* can not cause any harmful effects on the healthy individuals body, but in special conditions such as a deficiency in the immune system, it can become pathogenic fungi and have the ability to cause various forms of Candidiasis [1,5].

The relationship between *C. albicans* and other fungi does not consider in peace all of time as in forming of biofilms. It can be aggressive relationship when *C. albicans* tends to eliminate other fungi as noted for *A. fumigatus* [15]. This inhibitory activity of *C. albicans* may be related to its ability to produce suppressive materials that are effecting on the growth of other fungi. In human body, *C. albicans* usually secreted various hydrolytic enzymes such as aspartyl proteases, phospholipase A to D, serine peptidase and at least nine lipases [2-3,17-20]. It is also producing gliotoxin [21-24] which is an epipolythiodioxoperazine with a molecular mass of 326 Da that mainly produced by *A. fumigatus* [25-28]. However, hydrolytic enzymes and gliotoxin consider an important virulence factors for the pathogenicity of *C. albicans* [1,5].

C. albicans that was cultivated with filamentous fungi on media containing different concentrations of glucose had been revealed the ability to inhibit the growth of other tested fungi. Although we didn't identify the types or nature of the substances that had secreted by *C. albicans* into culture media and had showed antifungal effects, gliotoxin that is encoded by the gli gene cluster as detected in *A. fumigatus* [29] is assumed to be one of them due to its known toxicity against various groups of fungi [30]. In mammalian cells, the toxicity of gliotoxin is related to the presence of disulphide bridge in its structure [28,31]. Whereas, in fungal cells, gliotoxin has often interfered with the activity of redox homeostasis or protein modification leading to a disorder of the cell function and growth [29]. Otherwise, gliotoxin have the ability to inhibit the function of acetolactate synthase that is important to produce acetaldehyde in fungal cells [26,32].

Filamentous fungi had been shown a variable degree of sensitivity toward the presence of *C. albicans* and that may be depended on the type and amounts of secreted materials from growing yeast. The colony of *A. niger* had been clearly inhibited in the presence of *C. albicans* when they were cultured at the same time, while it inhibited less than *A. oryzae* and *A. fumigatus* in plated that previously cultured with *C. albicans*. The synthesis of gliotoxin in fungal cells is usually needed a long period of incubation time due to its produced in the late stage of growth as secondary metabolites [28]. Thus, delay incubation of *C. albicans* for 24 h may give the yeast enough time to increase its secretion of gliotoxin in the media and that would be elevated the inhibitory action of it against tested filamentous fungi. This explanation could be confirmed by the results of Carberry et al. who found that A niger was inhibited at low concentration of gliotoxin (5 µg/ml) than *A. flavus* and *A. oryzae* which they need a high concentration of gliotoxin (10 µg/ml) to inhibit [30]. This observation was also cleared with *Mucor spp.* and *C. sitophila* in our study. They hadn't affected when they cultured together with *C. albicans*, while their growth had been inhibited on media that previously cultured with *C. albicans*.

In addition to incubation periods, temperature could be considered another factor that effects on the secretion of gliotoxin. Incubation at 37°C found to be increased the production of gliotoxin by *A. fumigatus* than at 25°C due to the role of high temperature in inducing fast growing of fungal hyphae [28].

A. fumigatus is one of important gliotoxin producing fungi. It has a specific self-protection system against the effect of gliotoxin [29], unless it loses one of gliotoxin synthesis genes that rendered the fungus more sensitive to exogenous gliotoxin [33-34]. Thus, to explain the sensitivity of *A. fumigatus* toward the cultured of *C. albicans* in our study, there must be other substances produced by *C. albicans* in addition to gliotoxin. Glucose is the main source of energy for all living organisms including fungi. In *C. albicans*, glucose is usually catalytic through various metabolic pathways to get energy and to produce many useful chemical compounds for its survival, such as ethanol [35]. Ethanol that considers germistatic fungal agent has the ability to inhibit the growth of a wide range of fungal species [36]. Its production by *C. albicans* had been found to be increased at 37°C than at low temperatures [37] and because we was incubated *C. albicans* with other fungi at 37°C , so we were assuming that ethanol was produced in amounts enough to inhibit the growth of filamentous fungi. Moreover, *C. albicans* can also produce CO_2 from the metabolic pathway of glucose. In the presence of 25% CO_2, the growth of many fungi had been reduced [38], while *A. niger* had been inhibited at 3% CO_2 [39,40].

For most of tested fungi, the percentage inhibition had been affected by glucose concentrations. At low concentrations of glucose, the growth rate was very clearly decreased than at high concentrations. Thus, if we assume that *C. albicans* needs a short time to convert glucose to ethanol when it is in small amounts, then greater quantity of glucose often takes a long time to break down. However, the high percentage inhibition that had been shown in plates with previously cultured of *C. albicans* may result from the presence of many secondary metabolites such as gliotoxin and ethanol.

The confirmation of the inhibitory action of *C. albicans* against other fungi is not just made *C. albicans* more competent fungi among other normal flora, but it also gives the human body, another line of defense against opportunistic fungi. The main route of entering of fungi into the human body is usually through the respiratory system. *C albicans* is usually found on the mucosal surface of the nose and mouth [1-2]. Thus, entering of fungal cells into human body by inhalation and deposit most of them on the mucosal surface will follow by the destruction of these cells with no chance to grow due to the presence of *C. albicans* and its inhibitory secretions. Thus, *in vivo* study is recommended to confirm the capacity of *C. albicans* to eliminate filamentous fungi from the human body.

Conclusion

C. albicans had been shown the ability to inhibit various species of filamentous fungi. Incubation period and glucose concentration had been effected on the inhibitory action of *C. albicans* against other fungi. The human body will get another advantage from the presence of *C. albicans* as normal flora through its ability to eliminate other opportunistic fungi.

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