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Estimation of Esterase Activity, Adhesion Ability, in Various *Candida* species

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Abstract. Various species of *Candida* secrete lipolytic enzymes such as phospholipases and esterases. These yeasts' esterase operations have earlier been illustrated in a few research using the opacity test Tween-80. Systematic candidiasis is a severe infection in those who survive with a elevated mortality rate and morbidity. Candidate infections with mucocutaneous disease rarely lead to systemic candidiasis. Despite the morbidity induced by systemic candidiasis, little is known about the processes engaged in endothelial cells adhesion of *C. albicans* to or their subsequent transmigration. The present study was conducted with an aim to determine esterase activities and adherence abilities of some candida species: *C. albicans*, *C. glabra*, *C. krusei* and *C. kefyr*, isolated from oral cavities. The activity of esterase secretion between four species of candida was determined by Tween 80 opacity test medium. Two isolates; *Candida albicans* and *candida krusei* can hydrolyzed Tween 80 and the average of inhibition zone were (21.00-15.00 mm) respectively, otherwise *candida glabra* and *candida kefyr* non hydrolyzed Tween 80 after 1 week of incubation. Whereas the result of adherence ability had been recorded that all isolates of candida can adhere to Buccal Epithelial Cells (BECs) and conducted according to microscopic methods. The number of adhered *Candida krusei* cells recorded the highest value ranged between (10.00-43.00 cells) whereas, adherence of *C. kefyr* was showed the lowest value (20.00-22.00).

INTRODUCTION

The incidence of fungal infections is growing due to an growing amount of immunocompromised patients and the extensive use of antibiotics in the wide range (Aktas et al., 2002). Although *Candida albicans* survives the most frequently isolated pathogen, other *Candida* species such as *C. glabrata*, *C. tropicalis* and *C. krusei* emerge as opportunistic pathogens and are more resistant to antifungal agents than *C. albicans* (Baumgartner et al., 1996). Mycoses are therefore, an increasing medical issue that requires timely diagnosis and early-adaptation of antifungal treatment. Different *Candida* species secrete lipolytic enzymes like phospholipases and esterases. These yeasts' esterase activities have previously been demonstrated in a few studies using the opacity test Tween 80 (Ghannoun, 2000 and Slifkin, 2000).

identifying of virulence factors can currently play a main role in determining candidiasis pathogenesis and introducing new anti-candidate agents to support therapy strategies. Reviews showed that *Candida* spp. can secrete amount of exoenzymes such as phospholipase, esterase, hemolysin, and proteinase needed to colonize and attack host bodies (Maryam et al., 2017). The most prevalent human fungal infection, which manifests in a multitude of clinical forms, is regarded oral candidosis. One type of candidosis is *Candida* -induced denture stomatitis (CDS). *Candida* species' extra-cellular hydrolytic enzymes promote adherence and tissue penetration, thus invading the host (Arjuna et al., 2015 ; Atalay et al., 2015).

Candida albicans is a common fungus frequently found in the oral cavity, respiratory, intestinal and genital tracts and sometimes on the skin. The reported yeasts carrier frequency in the healthy oral cavity differs significantly (7–70%), possibly depending on the sampling technique, location and demographic research (Khaled, 2000).

Candida species' adherence to host cells is seen as an important early phase in disease development. *Candida* spp. can also adhere to the surfaces of medical devices and form biofilms, leading to an rise in candidemia and antifungal resistance associated with the introduction of catheters. The degree of virulence and the capacity to form biofilms are positively associated (Yun-Liang, 2003).

In this study, we aimed to estimate the production of esterase, and adherence ability of the *Candida* strains isolated from the oral cavity.

MATERIALS AND METHODS

Collection samples: The oral cavity samples were taken using the reported swabbing method (Ito-Kuwa et al., 1997). A sterile cotton swab was instantly inoculated into a 50 mg/ml chloramphenicol brain heart infusion broth. When streaking into Sabouraud dextrose agar (SDA) to get isolated colonies, tubes were incubated at 37°C for up to 48 h. At 35°C for 24-48 h, all plates were incubated, and yeast-like colonies were isolated. On SDA slants, all isolates were cultivated and stored at 4°C.

Identification of isolates: The yeast-like colonies recognized by the following experiments: germ tubes formation, micro-morphology study, fermentation and CHROMagar differential medium were used to verify the outcome by colony morphology and pigmentation (Odds and Bernaerts, 1994).

Esterase activity assay: Tween-80 opacity test medium was used to determine esterase activity. The test medium with a pH adjusted to 6.8 consisted of 1% peptone, 0.5% NaCl, 0.01% CaCl₂, and 1.5% agar. 0.5% of Tween-80 was added after cooling the medium to (50 °C). (10) microliters of previously prepared suspension from each isolate were carefully deposited on the opacity test medium Tween-80; in both aerobic and anaerobic conditions this was then incubated at 37 °C for 7 days. In the presence of a halo pervious to light around the inoculation site, esterase activity was regarded positive (Slifkin, 2000).

Candida adhesion assay: Adherence of yeast to epithelial cells differs significantly with the epithelium origin. Exfoliated samples such as BEC are the most common sources of epithelium. The BEC preparation technique was created by Kimura and Pearsall (1978), and is still used today by most of the researchers (McCarron et al. 2004) (see Table 2). BEC is thus acquired by softly rubbing the oral mucosa with sterile cotton swabs, followed by sterile PBS dispersion. After centrifugation and washing with PBS, cells are used immediately after the BECs screening under microscopy assay.

RESULT AND DISCUSSION

In this study, we aimed to estimate the production of esterase, and adherence ability of the *Candida* strains isolated from the oral cavity. **As illustrated in Table 1 and Table 2**

The present study was conducted with an aim to determine esterase activities and adherence abilities of some candida species: *C. albicans*, *C. glabra*, *C. krusei* and *C. kefyr*, isolated from oral cavities. Table 1 show result **esterase activities of (4) Candida strains on a Tween 80 opacity test medium** The activity of esterase secretion between four species of candida was determined by Tween 80 opacity test medium. Two isolates; *Candida albicans* and *candida krusei* can hydrolyzed Tween 80 and the average of inhibition zone were (21.00-15.00 mm) respectively, otherwise *candida glabra* and *candida kefyr* non hydrolyzed Tween 80 after 1 week of incubation (Mohammadi, 2013). Whereas the result of adherence ability had been recorded that all isolates of candida can adhere to Buccal Epithelial Cells (BECs) and conducted according to microscopic methods. The number of adhered *Candida krusei* cells recorded the highest value ranged between (10.00-43.00 cells) whereas, adherence of *C. kefyr* was showed the lowest value (20.00-22.00).

TABLE 1. esterase activities of (4) *Candida* strains on a Tween 80 opacity test medium

Candida strains	number of isolates	Zone of halo (mm)		
		St.Dev.	Minimum	Maximum
<i>C. albicans</i>	10	7.04	0.00	21.00
<i>C. glabra</i>	4	0.00	0.00	0.00
<i>C. krusei</i>	3	8.86	0.00	15.50
<i>C. kefyr</i>	3	0.00	0.00	0.00

Table 2 show result **Adhesion range for four Candida species** Loyalty of yeast to related to sacs that surround body organs cells differs significantly with the sacs that surround body organs origin. Exfoliated samples such as BEC are the most common sources of sacs that surround body organs. The BEC preparation way of doing things was created by Kimura and Pearsall (1978), and is still used today by most of the people who work to find information (Belazi, 2005) (see Table 2). BEC is this way owned received by softly rubbing the oral mucosa with (having no germs to have children cotton swabs, followed by having no germs to have children PBS breaking up out. After

centrifugation and washing with PBS, cells are used immediately after the BECs examining and testing so a decision can be made under microscopy test.

TABLE 2. Adhesion range for four *Candida* species

Candida species	number of isolates	St.Dev.	Minimum	Maximum
<i>C. albicans</i>	10	4.72	15.00	31.00
<i>C. glabra</i>	4	10.98	14.00	39.00
<i>C. krusei</i>	3	18.00	10.00	43.00
<i>C. kefyr</i>	3	1.00	20.00	22.00

The purpose of this research was to identify and evaluate different types of *Candida*

The aim of this study is to identify activities and adherence abilities of some candida species: *C. albicans*, *C. glabra*, *C. krusei* and *C. kefyr*, isolated from oral cavities. The activity of esterase secretion between four species of candida was determined by Tween 80 opacity test medium. Two isolates; *Candida albicans* and *candida krusei* can hydrolyzed Tween 80 and the average of inhibition zone were (21.00-15.00 mm) respectively, otherwise *candidaglabra* and *candida kefyr* non hydrolyzed Tween 80 after 1 week of incubation.

CONCLUSION

The present study was conducted with an aim to determine esterase activities and adherence abilities of some candida species: *C. albicans*, *C. glabra*, *C. krusei* and *C. kefyr*, isolated from oral cavities. The activity of esterase secretion between four species of candida was determined by Tween 80 .

Although *Candida albicans* survives the most frequently isolated pathogen, other *Candida* species such as *C. glabrata*, *C. tropicalis* and *C. krusei* emerge as opportunistic pathogens and are more resistant to antifungal agents than *C. albicans* (Schaller , 2005).Mycoses are therefore, an increasing medical issue that requires timely diagnosis and early-adaptation of antifungal treatment.

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