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Effect of Lactoferrin and Iron on the Growth of Human Pathogenic *Candida* Species

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Abstract: Effect of lactoferrin and iron have been studied on the multiplication and pseudohyphae production by three pathogenic *Candida* species viz., *C. albicans*, *C. krusei* and *C. tropicalis*. Results showed that lactoferrin showed significant antifungal effect on the three species tested, while the addition of iron enhance the multiplication of *Candida* species.

Key words: Antifungal effects, lactoferrin, iron, *Candida* species

INTRODUCTION

Lactoferrin belongs to the transferrin family of iron binding protein and is a glycoprotein. Its not only found in the human milk but also in most epithelial surface secretions including tears, nasogastric, saliva and bronchial (Brock, 2002). Recently much attention has been given to lactoferrin because of following significant characteristics like, antibacterial, antifungal, anti-viral, antioxidant, immunomodulatory and to acts synergistally with lysozyme to potentiate the activity of proteins. Lactoferrin bind two molecules of iron with very high affinity thus making iron unavailable to pathogen which is an essential element for bacterial and fungal pathogen to survive and multiplication inside the host (Lupetil *et al*., 2000; Humphrey *et al*., 2002; Brissot *et al*., 2000; Di Mario *et al*., 2003; Weiss, 2002).

During infection, transferrin level increases and iron saturation decreases to allow increased availability of iron binding sites, a phenomenon known as hypoferremic response (Weiss, 2002).

Candida albicans and *C. glabrata* which causes yeast infections in vagina could be inhibited by activated lactoferrin and to blok yeast adhesion to the vaginal epithelial monolayer (Naidu *et al*., 2004). Iron chelators like gallic acid and salicylhydroxamate enhance the growth of *Candida albicans in vitro* (Weiss, 2002; Rehmani *et al*., 2004; Abe *et al*., 1990).

Fungicidal effects of lactoferrin have largely been done on *Candida albicans* because of its high pathogenicity (Viejo Diaz *et al*., 2004; Yamaguchi and Takakura, 2004; Anil and Samaranayke, 2002). There are very few reports about the antifungal effect of lactoferrin on other *Candida* species like *C. krusei* and *Candida tropicalis* (Nikawa *et al*., 1993; Xu *et al*., 1999).

In the presence of iron *Candida* species showed resistant to antifungal drugs and thus becoming a big challenge to control candidal infections (Bullen *et al*., 2006; Heyman *et al*., 2002; Howard, 1999).

Infections caused by *Candida albicans* remains considered as one of the most pathogenic human and animal *Candida* species but during recent years other species like *C. krusei* and *C. tropicalis* also emerged as a major pathogen of human and animals including all sites (Ruth *et al*., 2003; Pfaller *et al*., 2000; Rex *et al*., 2000).

The aim of present research was to study the effect of lactoferrin, lactoferrin free milk and added lactoferrin with iron on the growth of human pathogenic *Candida albicans, C. krusei* and *C. tropicalis*.

MATERIALS AND METHODS

This study was conducted at Department of Biology, College of Science, King Faisal University, Al-Hassa, Saudi Arabia during the period between June 2006-July 2008 and Candida species were collected from different hospitals in Riyadh and Al-Hassa as described latter.

Lactoferrin-free human milk was prepared by removing lactoferrin by treatment with heparin-sepharose. (Davidson *et al*., 1994). Lactoferrin purchased from Sigma-Aldrich (St. Louis, MO) was then added to the milk to obtain 3×10^3 μg mL^{-1} concentration. Sterile distilled water and lactoferrin free milk served as control. *Candida albicans. C. krusei* and *C. tropicalis* were isolated from different sources like blood, urine, vagina, sputum and Bronchoalveolar lavage (BAL) were cultured and maintained on Sabouraud dextrose agar. A total number of 100 isolates of each *Candida* species were collected for experiment.

Stock solution of *Candida* species were prepared from 24 h old colonies on Sabouraud dextrose agar to get a concentration of 10^3 cells mL^{-1} of yeasts into a 100 mL culture tubes. One milliliter from stock solution was added to 9 mL of test medium viz., whole milk, lactoferrin free

milk, added lactoferrin and added lactoferrin+iron medium, to get final concentration of yeast cells of 10^2 cells mL^{-1}. Yeast cells were counted in a hemacytometer at 24 h intervals. The number of CFU were also counted by culturing on SDA medium.

Five sets of each yeast in triplicate were prepared for each type of medium and incubated at 37°C for 24, 48 and 72 h. After each 24 h intervals cells were counted in the hemacytometer. The correlation between cell count and CFU was very high, results here reported are only for cell counts.

Added lactoferrin medium was prepared by adding 3000 μg of commercial lactoferrin per milliliter while 3×10^3 μg mL^{-1} of lactoferrin and 300 μg of ferrous sulphate were added to lactoferrin free milk to prepare lactoferrin + iron medium.

Candida species were isolated on Sabouraud dextrose agar and chromoagar medium (CHROMAGAR, Paris, France) and identified by Api Aux identification system (Biomerieux, France).

Candida species were collected from Riyadh Medical city, Central Hospital and various private hospitals in Riyadh and Al-Hassa region. These *Candida* species were isolated from Blood, Urine, Vagina, Sputum and Branchoalveolar Lavage (BAL). This project is not funded by any organization or a part of a research project.

RESULTS AND DISCUSSION

Almost similar type of increase in cell counts were shown by the *Candida albicans*, *C. krusei* and *C. tropicalis* (Table 1) after 24, 48 and 72 h of incubation. An insignificant decrease in cell counts was observed after 24, 48 and 72 h incubation period in lactoferrin free milk as compared to whole milk. The number of cell counts were significantly decreases in the case of added lactoferrin (3×10^3 μg mL^{-1}) but trends reversed in the medium with added iron and lactoferrin. Even the cell counts were more than the cell counts in the whole milk. There were gradual increase observed in all the cases after each 24 h of incubation period. In the medium containing added lactoferrin a significant reduction of cell count was seen even after 24 h of incubation for example. Only 650 cells were seen after 24 h of incubation in added lactoferrin as compared to 3350 cell in lactoferrin free milk and 3500 cells counts in the whole milk in the case of *Candida albicans*. A similar trends were also observed in other two species. In the case of lactoferrin + iron medium the cell counts were highest and even more than the cell counts observed in the whole milk medium. The results here clearly shows that lactoferrin does effect the growth of *Candida* species.

This is in coincide with earlier reports that lactoferrin has antifungal properties and affect the prevalence of pathogen in the host by binding iron molecules and making them unavailable to pathogen (Brissot and Guyadar, 2000; Di Mario *et al*., 2003). When iron added to the medium, even in the presence of lactoferrin increases the number of cell counts significantly. This is due to the availability of free iron in the medium which is an essential element for the multiplication of pathogen (Rehmani *et al*., 2004; Abe *et al*., 1990).

Results showed when lactoferrin added to the milk, no pseudohyphae were produced by any of the *Candida* species even after 72 h. of incubation but when iron added with lactoferrin, *Candida* species produced pseudohyphae although after 72 h of incubation as compared to whole milk where these *Candida* species produced pseudohyphae after 48 h of incubation (Table 2). Production of pseudohyphae of *Candida* species helps in faster spreading of *Candidal* infection

Table 1: Effect of whole milk, lactoferrin free milk, added lactoferrin and added lactoferrin with iron on the cell concentrations of *Candida* species (initial conc. 1000 cells mL^{-1})

		Whole milk			Lactoferrin free milk			Added lactoferrin (3×10^3 μg mL^{-1})			Added lactoferrin+iron (3000+300 μg)		
	Control	24	48	72	24	48	72	24	48	72	24	48	72
Candida species	sterile water	(h)			(h)			(h)			(h)		
C. albicans	Unchanged	3500±15	7680±20	19650±18	3350±10	7360±15	18400±20	650±10	965±10	1360±10	3630±20	8120±10	18650±15
C. krusei	Unchanged	2965±10	6790±15	16350±20	2275±12	6290±10	15450±15	593±12	936±12	1296±10	3110±15	7230±15	17250±20
C. tropicalis	Unchanged	3339±15	7346±20	18250±20	3260±10	6430±20	17330±15	636±10	969±10	1352±12	3562±15	7930±12	18930±20

±SD from the mean

Table 2: Production of pseudohyphae in the whole milk, lactoferrin free milk, added lactoferrin added lactoferrin with iron by *Candida* species

	Control sterile water			Whole milk			Lactoferrin free milk			Added lactoferrin (3×10^3 μg mL^{-1})			Added lactoferrin+iron (3000+300 μg)		
	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Candida species	(h)			(h)			(h)			(h)			(h)		
C. albicans	-	-	+	+	+	+	-	+	+	-	-	-	-	-	+
C. krusei	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+
C. tropicalis	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+

+: Present, -: Absent

Table 3: Source of isolation of *Candida* species

	No. of isolates					
Candida species	Blood	Urine	Vagina	Sputum	BAL	Total No. of isolates
C. albicans	30	20	20	20	10	100
C. krusei	15	30	25	15	15	100
C. tropicalis	20	20	20	25	15	100

in vivo, but lactoferrin not only checks the multiplication of yeast cells but also stopped the production of pseudohyphae by *Candida* species (Lupetil *et al*., 2000; Humprey *et al*., 2002; Brissot and Guyader, 2000).

A final total number of 100 isolated of each *Candida* species were collected from different types of specimen (Table 3). There were no significant differences were found as for as reaction to lactoferrin is concerned. All these isolates behaved similarly (Brock, 2002; Naidu *et al*., 2004; Nikawa *et al*., 1993; Xu *et al*., 1999).

In conclusion lactoferrin could be used safely as an antibiotics to checks bacterial and fungal infection (Lupetil *et al*., 2000; Humphrey *et al*., 2002; Di Mario *et al*., 2003).

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