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SHORT COMMUNICATION

Antifungal Effects of *Lactobacillus* spp. Bacteria on *Candida* Yeast

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Summary

Thirty *Lactobacillus* were isolated from the stool of 5-15 year-old children using MRS agar and identified by API 50CHL. Also, 50 *Candida* were isolated from blood cultures using SD agar and identified by Vitek 2 instrument with YST kit. Antifungal effects of *Lactobacillus* bacteria on *Candida* were explored using well diffusion method in SD agar. *Lactobacillus* had the most prominent antifungal effect against on *C. albicans* (M29, M36), *C. parapsilosis* (M25, M26), *C. famata* (M28) and *C. guilliermondii* (M38) yeasts. This study shows the presence of antifungal effect of *Lactobacillus* on *Candida* isolated from blood cultures.

Keywords: *Antifungal effect, Lactobacillus, Candida, Blood culture*

Lactobacillus Bakterilerinin *Candida*'lar Üzerine Antifungal Etkileri

Özet

Otuz adet *Lactobacillus*, 5-15 yaş grubu çocuk gaitasından MRS agar'da izole edilerek API 50CHL cihazı tanımlanmaları yapılmıştır. Ayrıca 50 adet kan kültürü kaynaklı *Candida* ise SD agar'da izole edilerek Vitek 2 cihazında YST kiti ile tanımlanmaları yapılmıştır. *Lactobacillus*'ların *Candida*'lar üzerindeki antifungal etkileri SD agar'da kuyu difüzyon yöntemi ile tespit edilmiştir. *Lactobacillus*'lar en fazla *C. albicans* (M29 ve M36), *C. parapsilosis* (M25, M26). *C. famata* (M28) ve *C. guilliermondii* (M38) mayaları üzerinde antifungal etki göstermiştir. Bu çalışma *Lactobacillus*'ların, kan kültürlerinden elde edilen *Candida* izolatları üzerine antifungal etkisinin varlığını göstermektedir.

Anahtar sözcükler: *Antifungal etki, Lactobacillus, Candida, Kan kültürü*

INTRODUCTION

Yeasts are microorganisms commonly found in nature. They are present in the normal flora (in moist places like the intestinal system, mouth etc.) in a human body. Hospital-acquired fungal infections cause serious morbidity and mortality and it is clear that fungal diseases have emerged as important public health problems recently [1]. *Candida* constitutes 80% of hospital-acquired fungal infections. In recent years, there has been a significant increase in non-albicans *Candida* species, especially in blood cultures. *C. albicans* and non-albicans species pose a high risk of infection in the blood circulation system of children [2]. It has been reported that lactic acid bacteria (LAB) can produce antimicrobial substances with the capacity to inhibit the growth of pathogenic and spoilage microorganisms. Organic acids, hydrogen peroxide, diacetyl and bacteriocins are included among these compounds [3]. Recent years have witnessed an increase in the studies exploring the antifungal effects of *Lactobacillus* strains. These studies have primarily focused on protection against urogenital candidiasis and prevention of fungal contamination of food [4,5]. Atanasova et al.[6] established that *Lactobacillus paracasei* subsp *paracasei* M3 bacterium had an antifungal effect on *C. albicans*, *C. pseudointermedia* and *C. blankii*. Antifungal resistance of *Candida* species is an increasing problem [7]. Despite the wellfounded

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enthusiasm for new antifungal agents, it is very important to continue to assess the activity of newer agents [7].

Studies about the antifungal effect of *Lactobacillus* on *Candida* isolated from blood cultures are scarce. To our knowledge, this is the first report about the antifungal effect of *Lactobacillus* on *Candida* isolated from blood cultures. The aim of the present study is to examine the antifungal effect of *Lactobacillus* of human intestinal origin on *Candida* yeasts isolated from blood cultures. Besides, we have studied the pattern of *Candida* spp. to common usable antifungals in Turkey.

MATERIAL and METHODS

Microorganisms

The study used 30 *Lactobacillus* bacteria isolated from the stool of children in the 5-15 age group at the Microbiology Laboratory of Dr. Behçet Uz Children's Hospital. *Lactobacillus* was kept in bead tubes (Cryobank, Mast Diagnostics, France) at -20°C.

Bottles of blood cultures collected from the units were placed in BD Bactec 9240 (BD, USA) instrument. The bottles were incubated in the instrument for 7 days at 37°C and the bottles which were reproduction positive were transferred to SD agar (BD, USA) culture medium. Cultures that showed growth were microscopically examined (direct examination and gram staining) and those containing yeasts were separated. Each *Candida* strain was isolated from individual samples. Yeasts were sorted out using Vitek 2 (BioMérieux, France) kit. Isolates were stored in bead tubes (Cryobank, Mast Diagnostics, France) at -20°C [8].

Antifungal Assay

Antifungal effect of *Lactobacillus* on *Candida* was examined using well agar diffusion method [9]. *Lactobacillus* cultures were incubated in MRS liquid culture medium (BD, USA) under anaerobic conditions at 37°C for 24 h. The cultures were then centrifuged at 10.000 cfu for 10 min. The floating supernatant was recovered with an injector and filtered through 0.45 µ pore filter (Millipore, Molsheim, France) [9]. *Candida* were activated in SD agar and planted into 0.5 McF SDA (BD) culture media. In all media, 10 mm wells were opened and added 100 µl supernatant. The plaques were kept at room temperature for 2 h and left to incubate at 37°C for 24 h. Then, the zones around the wells were measured in mm [9].

RESULTS

Table 1 presents species of *Candida* yeasts isolated from blood cultures and their percentages. Of the 50 species isolated, 16 (32%) were *C. albicans* and 34 (68%) were non-albicans species. Yeasts isolated from blood cultures were 16 *C. albicans* (32%), 22 *C. parapsilosis* (44%), 4 *C. famata* (8%), 3 *C. guilliermondii* (6%), and 1 *C. lusitaniae* (2%). Our study shows that pathogenicity

Table 1. *Candida strains isolated from blood cultures and their distributions*
Tablo 1. *Kan kültürlerinden izole edilen Candida suşları ve onların dağılımları*

Services	***C. albicans***	***C. parapsilosis***	***C. famata***	***C. pelliculosa***	***C. guilliermondii***	***C. lusitaniae***
Emergency - ICU *	2	15	-	-	-	1
Infectious diseases	-	1	-	-	-	-
New-born unit	4	-	1	-	-	-
Oncology - Hematology	2	1	1	-	-	-
Burn	3	-	-	-	-	-
Surgical - ICU	3	2	2	2	2	-
Premature - ICU	1	2	-	2	-	-
Nursing infant	-	-	-	-	1	-
Cardiology	-	1	-	-	-	-
Big boy	1	-	-	-	-	-
Total (%)	**16 (%32)**	**22 (%44)**	**4 (%8)**	**4 (%8)**	**3 (%6)**	**1 (%2)**

** ICU : Intensive Care Unit*

of non-albicans species is much higher than that of *C. albicans*. Nearly similar findings have been reported by Levy et al.[2]. Various studies carried out in Turkey have revealed that non-albicans isolated from blood cultures are as common as or more common than *C. albicans* [10-12].

Table 2 shows the antifungal effects of *Lactobacillus* on *Candida* yeasts. *Lactobacillus* was seen to have antifungal effects against *C. albicans* (M29, M36), *C. parapsilosis* (M25, M26 and M44), *C. famata* (M28) and *C. guilliermondii* (M38) strains. However, the effect of *Lactobacillus* against *Candida* was observed as weak.

Table 2. *Antifungal effects of Lactobacillus on Candida yeasts*
Tablo 2. *Lactobacillus'ların Candida mayaları üzerine antifungal etkileri*

	Diameter of the Zones of Inhibition in mm								
***Lactobacillus* spp.**	***C. albicans* M29**	***C. albicans* M36**	***C. parapsilosis* M25**	***C. parapsilosis* M26**	***C. parapsilosis* M44**	***C. famata* M28**	***C. guilliermondii* M38**	***C. guilliermondii* M40**	***C. guilliermondii* M41**
L1	2	4	2		2	2			
L2	2	3.5	2		2	2			
L3	2	3		2	2	2			
L4		4			2		2		
L5		3	4						
L6							1.5		
L7	4		4		2	2	1.5		
L8		2	4		4				
L9		2				2			
L10						2.5			
L11		2				2			
L12					1.3				
L13			4		3.5	2	1.5		
L14									
L15			4						
L16		4							
L17	4	4	4	2			4	2	4
L18	4	3.5	4	2			3.5	2.5	
L19		1.5	5			2.5	4		
L20	2	1	5	2		2		2	
L21	2	6	4	2			3.4		
L22		3.5	5				3		
L23	4	4	4		3.5		2.5	2	8
L24	4	4	4				3.5		
L25	4	3.5	4				3		
L26		2.5	5	2	3.5		3		
L27	3.5	4	4				3		
L28	4	4	4				3		
L20	2	3	4						
L30	3.5	2	4		1,5		2		2

DISCUSSION

Various studies point out that some molecules with protein structure produced by bacteria have antifungal characteristics [6]. Özkaya et al.[13] studied the antifungal activity of *Lactobacillus* on various yeasts. This study, which used 19 *Lactobacillus* strains and 12 yeast strains, demonstrated that *Lactobacillus* bacteria had varying degrees of inhibitive effects against *C. krusei* 4B, *C. lipolytica* 5A, *C. lusitaniae* 7, *C. ciferrii* 10, *S. cerevisiae* 12, 13, *C. pseudotropicalis* 16 and *C. glabrata* 18 yeasts. An examination of *L. plantarum* and *C. albicans* mixed cultures under anaerobic conditions at 37°C for 48 h showed growth in *L. plantarum*, but not in *C. albicans* [14].

Studies about the strains isolated from the human intestinal system are scarce. It was found that *L. rhamnosus* and *L. paracasei* probiotic bacteria isolated from human stool had a high degree of antifungal effect against *C. albicans* ATCC 10291 [15]. In our study, however, *Lactobacillus* had a poor antifungal effect.

The literature does not contain studies about the antifungal effects of *Lactobacillus* in human intestinal system origin on yeasts (*Candida*) isolated from the human blood circulation system. Our study could be considered the first of its kind in this respect. Presence of yeasts in the blood circulation system in children poses a great risk, which needs to be eliminated. We know that over time resistance develops against antifungal drugs used against yeasts. *Lactobacillus* spp. of human origin, which has probiotic characteristics, may be used to reduce fungus through their antifungal effects or to eliminate yeasts, when combined with antifungal drugs. In conclusion, it is believed that outputs of this study will help to identify to find out new antifungal agents against resistance problem. Therefore, further and more detailed studies are needed in this field.

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