

THE SPECTRUM OF ANTIMICROBIAL ACTION OF STRAINS OF LACTOBACILLI TO STRAINS OF ENTEROCOCCI ENTEROCOCCUS FAECIUM, ENTEROCOCCUS FAECALIS

Ogay Daria Kisenovna, Miralimova Shikhlo Mirdzhamalovna, Kutlieva Guzal Jumaniyazovna
Elova Nilufar Arashovna, Sahibnazarova Honsuluw Abduvahabovna
Institute of Microbiology of AS RUz, Tashkent, Uzbekistan
E-mail: aziz0761@gmail.com

ABSTRACT

The spectrum of antimicrobial and bacteriocinogenic action of 5 strains of lactobacilli to 14 strains of enterococci was studied. It was found that the antimicrobial activity of lactobacilli against enterococci *E. faecium* and *E. faecalis* depends on the species and strain belonging of both lactobacilli and enterococci. Comparison of two species of enterococci showed that enterococci *E. faecalis* are more sensitive than *E. faecium*. Isolated strain *L. plantarum* 42 from highly salted cabbage, showing antimicrobial and bacteriocinogenic effect on enterococci.

Keywords: enterococcus, colonization, bacteriocin, plantaricin, peptides, bacteriocinogenic activity

XULOSA

Энтерококкларнинг 14 та штаммига қарши лактобактерияларнинг 5 та штаммининг антимикроб ва бактериоциноген таъсир спектри ўрганилган. Лактобациллаларнинг *E. faecium* ва *E. faecalis* энтерококкларига нисбатан антимикроб таъсири лактобациллалар каби энтерококкларда ҳам тур ва штамм тегишлигига боғлиқ эканлиги аниқланган. Энтерококкларнинг 2 та турини ўзаро таққослаш шуни кўрсатганки, *E. faecalis* энтерококклари *E. faecium* га нисбатан лактобактериялар таъсирига сезgirроқ. Ўта шўр тузланган карамдан антимикробларга қарши антимикроб ва бактериоциноген фаолликка эга *L. plantarum* 42 штамми ажратиб олинган.

Kalit so'zlar: enterokok, kolonizatsiya, bakteriosin, plantaritsin, peptidlar, bakteriosinogen ta'sir.

РЕЗЮМЕ

Изучен спектр антимикробного и бактериоциногенного действия 5 штаммов лактобактерий к 14 штаммам энтерококков. Выявлено, что антимикробная активность лактобацилл к энтерококкам *E. faecium* и *E. faecalis* зависит от видовой и штаммовой принадлежности как лактобацилл, так и энтерококков. Сравнение двух видов энтерококков показало, что энтерококки *E. faecalis* более чувствительны, чем *E. faecium*. Выделен штамм *L. plantarum* 42 из сильно засоленной капусты, проявляющий антимикробное и бактериоциногенное действие на энтерококки.

Ключевые слова: энтерококк, колонизация, бактериоцин, плантарицин, пептиды, бактериоциногенная активность

INTRODUCTION

Enterococcus faecalis involved in the initiation of human's inflammatory diseases due to genetic sensitivity and dysregulation of the immune response. *Lactobacillus plantarum* 42 is locally distinguished strain, which has antimicrobial and bacteriocinogenic activity with *Enterococcus faecium*, *Enterococcus faecalis*.

The one of the relevant problems in the food industry and medicine is the search for microorganisms with antimicrobial activity to the agents of food spoilage and human infectious diseases in the food industry and medicine. Lactic acid bacteria, especially lactobacilli due to the synthesis of lactic, acetic, short-chain fatty acids aldehydes, ketones, possess these valuable properties.

In nature it is extremely rare meets lactobacilli - producers special bacteriocine-like substances, which actively inhibit the growth and development of pathogenic and conditionally pathogenic microorganisms. However, it is known as the *Lactobacillus plantarum* forms such as bacteriocins Plantaricin S [1], Plantaricin C11 [2], Y21 [3], TF711 [4].

These plantaricins inhibit the growth of gram-positive bacteria (*Bacillus cereus*, *Clostridium sporogenes*, *Staphylococcus aureus*, *Listeria monocytogenes*) and gram-negative *Salmonella typhi*, *Vibrio cholera*, *E. coli*, *Shigella dysenteriae*. From existing indicator cultures studied poorly antimicrobial activity of lactobacilli on the growth and development of enterococci. Only in the past decade to focus on enterococci *Enterococcus faecium* and *Enterococci faecalis*, especially fecal enterococcus, which belongs to commensal bacteria (normal flora of the intestine) and does not exhibit pathogenic properties in the normal functioning of the human immune system.

However, when the genetic sensitivity of the human body and dysregulation of the immune system is involved in the initiation of inflammatory bowel disease. Thus, in mice with deficient in Interlace 10 and non-association with *enterococcus faecalis* is shown in 10-12 weeks after colonization by bacteria develops distal colitis of the colon, which then progresses to inflammation of the duodenum up to its obstruction, for a longer time than distal colitis, more than 30 weeks.

MATERIALS AND METHODS

OBJECTS: Cultures used for testing – *Lactobacillus plantarum* 8PA-3 (typical), local strains of *L. plantarum* 42,44, *L.rhamnsus* 41, *L.casei* subsp *paracasei* 48. Indicator culture: American strain *Enterococcus faecalis* OGIFR1, FI 2-2, 4610, *E. faecium* 2897, 50K, M76, 4605, 1026, 2714, from the collection of the Museum of all-Russian Institute of genetics of industrial microorganisms and local strains of *E.faecium* 364, 302; *E. faecalis* 422, 345 from the collection of the laboratory of the genetics of lactic acid bacteria, Institute of Microbiology of the Academy of Sciences. The recovery of the lyophilized in vial of tested strains of lactobacilli was performed in MRS-broth (HiMedia) and indicating cultures of enterococci in common hydrolysate-milk-broth (HM), according to the standard technique. Received hydrolyzed soft agar by adding 0,75% agar in HM-broth, solid MRS- agar by adding 1,8% of dry agar in MRS-broth. To study the antimicrobial activity of lactobacilli were grown in MRS-broth at (37±1)°C in the anaerobic jar in a nitrogen atmosphere for 48 hours, enterococci grown in GM-broth for 24 hours at the same temperature in the thermostat. Liquid culture of lactobacilli in the amount of 1-3 µl was dropped on the surface of agar media were grown in anaerobic jar at (37±1)°C for 12 hours, conducted the induction of bacteriocines by methodical induction 4.2.2602-10.4.2 (Moscow, 2010), after which the lactobacilli were grown in anaerobic jar for 48 hours. Then close with the resulting spot-grown culture was dripping a solution of the enzyme pepsin 5 µl (50 µg), was maintained at room temperature for 1 hour. Then the top was covered with a second layer of hydrolyzed soft agar 7 ml, seeded with enterococci at a concentration of 10⁶ CFU/ml then were incubated for 18-20 hours. Checking the presence or absence of zone of inhibition of indicating culture as well as the growth of enterococci at the application site of the enzyme. Isolation and purification of bacteriocins-like substances conducted by L.Saavedre, F. Sesma [6]. Only proteins of the culture fluid was precipitated not 40 % solution of ammonium sulphate, but with 98,55%, then were dialyzed in a dialysis bag with a pore size of 1000 Da, the content substance of the dialyzed bag was lyophilized dried. Dry extract of proteins at a concentration of 2,5% (0,0025 g dry extract soluble in 0,1 ml sterile distilled water) was used to test for the presence of the bacteriocins-like substance.

To study bacteriocinogenic to the crude extract proteins sterile paper discs of 5 mm is soaked in the solution of extract of proteins impose on MRS agar, which is then covered with a second layer of soft agar with hydrolyzed milk concentration of enterococci 10⁶CFU/ml and then noted the presence of bactericidal action of crude extract proteins into the culture of the indicator enterococci.

RESULTS AND DISCUSSION

Antibacterial activity of lactobacilli to enterococci depends on the titer of the indicator culture of enterococci in the upper soft layer of hydrolyzed agar. Thus, when the titer of cells of the indicator strain is equal to 10⁷CFU/ml no inhibition of growth of enterococci (expert strain 2714), while at lower titer to 10⁶/ml all sensitive enterococci. The lactobacilli in different ways exhibit antimicrobial activity to *E. faecium* (table 1). Typical strain of *Lactobacillus plantarum* 8 PA-3 inhibits the growth of all eight strains of faeciums. Strain *L. plantarum* 42 inhibits the growth of all strains of enterococci area with bactericidal action from 20 to 28 mm, *L. plantarum* 44 does not inhibit the growth of two strains – M 74 and M 76, to the remaining six strains culture is active, area of the bactericidal effect of *L. rhamnosus* is between 18 and 30 ml. Lactobacilli *L. rhamnosus* 41 and *L. casei* subsp *paracasei* 48 in a lesser extent inhibit the growth of enterococci. Indicating cultures most sensitive to the inhibitory action of the lactobacilli strain *E. faecium* 2897 and 4605.

Enterococci *E. faecalis* both standard and local strains were susceptible to the antimicrobial activity of lactobacilli (table 2). Studied five strains expert strain 4610, insensitive to 41 and *L. rhamnosus* 41, *L.casei* subsp *paracasei* 48 and sensitive to the two *L. plantarum* strains, all 5 strains sensitive to all kinds of lactobacilli. Area of bactericidal action of the lactobacilli to *E. faecalis* significantly larger than *E. faecium* strains. From the studied strains of lactobacilli is the most active *L. plantarum* 42, which effectively inhibits the growth of cultures of *E. faecium* and *E. faecalis*.

Identifying bacteriocinogenic properties by the presence of the growth indicator of the cultures of enterococci in the area of bactericidal action of lactobacilli after destruction bacteriocinogenic substances by the enzyme pepsin also showed that bacteriocinogenic is a strain of *L. plantarum* 42. From cultures of *E.faecium* sensitive model strains 2897, M 76, 1026 and local strain 364, from 6 cultures of *E. faecalis* sensitive 5 strains. 3 model – 2714, OGJFR, FI 2-2 and two local 422 and 345 (table 1), are not sensitive strain 4610.

Table 1 Antimicrobial and bacteriocinogenic activity of the lactobacilli to enterococci (zone of inhibition in diameter, mm)

№	Indicator culture of enterococci	Tested lactobacilli				
		L. pl-m 8 RA-3	L. pl-m 42	L. pl-m 44	L.rhamnosus 41	L. casei 48
1.	E.faecium 2897	-	28,0*	28,0	25,0	18,0
2.	K 50	-	24,0	30,0	-	-
3.	M 76	-	26,0*	-	16,0	-
4.	364	-	24,0*	26,0	10,0	10,0
5.	302	-	20,0	18,0	-	-
6.	4605	-	21,0	22,5	22,5	12,5
7.	1026	-	26,2*	27,2	-	-
8.	M 74	-	25,0	-	17,5	-
9.	E. faecalis 2714	22,3	23,3*	21,0	18,0	23,0
10.	422	23,4	19,3*	25,0	22,5	18,0
11.	FI 2-2	10,0	25,4*	19,0	17,5	16,7

12.	345	15,0	25,0*	23,5	25,0	25,0
13.	4610	-	27,4	27,0	-	-
14.	OGIFR 1	10,0	15,0*	25,0	22,5	25,0
Note: *Bacteriocinogenic activity to enterococci						

The test strain OGIFR1 the sensitivity by the disco diffusion method, when depositing in the hole 5 µl of extract zone of inhibition of growth is 22 mm. the Comparison of the spectrum of sensitivity of the indicator strains of enterococci established by microbiological method, their sensitivity to the crude extract proteins showed a coincidence of the data obtained from *E. faecium* strains 2897, M76, 364, 1026; from *E. faecalis* 2714, 422, OGIFR1, 2-2 PI, 345.

CONCLUSIONS

In recent years, there has been an increase in the number of people with immunodeficiency states, which leads to an increase in the incidence of infectious processes caused by opportunistic microorganisms, including enterococci. Particular attention is paid to two types of enterococci - *E. faecalis* and *E. faecium* - as nosocomial pathogens.

In this regard, particular interest is caused by microorganisms that inhibit the growth and development of fecal enterococci. Such is the local strain *L. plantarum* 42, isolated from highly salted cabbage, which shows antimicrobial and bacteriocinogenic effect on all types of enterococci studied. Studies on the antimicrobial activity of lactobacilli in relation to enterococci have shown a dependence on species and strain belonging. Comparison of two types of enterococci showed that enterococci *E. faecalis* are more sensitive than *E. faecium*.

Thus, the local strain *L. pl* 42 obtained by us can be suitable for use as a probiotic strain for the production of therapeutic biologics, dietary supplements to food, as well as food products for functional purposes.

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