

The Study Bio film Forming Activity, Survival in the Gastrointestinal Tract and Antibiotic Resistance of Propionic Acid Bacteria

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ABSTRACT

The study biofilm forming activity and survival in the gastrointestinal tract of propionic acid bacteria *Propionibacterium freudenreichii* subsp. *shermanii* №№ 1, 10, 12, 13, 14, isolated from various fermented milk products. It has been established that strains of propionic acid bacteria, isolated from sour-milk products, have an increased propensity to biofilm formation, resistance to gastrointestinal tract conditions, and antibiotic resistance, which is beneficial in their practical application.

Keywords: propionic acid bacteria, biofilms, resistance to bile, acid resistance, antibiotic resistance.

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I. INTRODUCTION

Various chemical ingredients, that are widely used to reduce the risk of contamination and damage of products, have adverse effects on consumers health [1]. An alternative to chemicals are microorganisms - producers of organic acids, namely lactobacilli, which are representatives of normal microflora of gastrointestinal tract [2-4]. Propionic acid bacteria have also found application in food and feed industry. These organisms, due to their antifungal activity along with lactic bacteria become the object of attention as bio-preservatives in food and feed [5]. In order to be used as bioconservants, propionic acid bacteria must possess a set of properties that allow them to compete with pathogenic and opportunistic microorganisms. Such properties include antagonistic, bacteriocin-producing and antioxidant activity, adhesive ability, resistance to bile, acid resistance, resistance to antibiotics, biofilm forming activity [6].

Thus, the study of microorganisms that have the ability to form biofilms, antibiotic resistance and resistance to aggressive conditions of the gastrointestinal tract is a topical area of research in the field of biotechnology of microorganisms. The purpose of this work was to study biofilm forming activity, acid resistance, resistance to bile, antibiotic resistance of propionic acid bacteria used for the preservation of fermented milk products.

II. MATERIALS AND METHODS

The objects of the study were cultures of propionic acid bacteria *Propionibacterium freudenreichii* subsp. *shermanii* №№ 1, 10, 12, 13, 14, selected from various fermented milk products. To determine the biofilm-forming activity, direct colony count of propionic acid bacteria in the biofilm was done by serial dilution. The coverslips were placed on the "edge" in sterilizing drum and filled with 90% ethyl alcohol for 45 minutes, then the alcohol was drained and dried at 37°C for 30-40 minutes, then 4 ml of corn-glucose medium and 0.1 ml of a daily culture of propionic acid bacteria (108 cfu / ml) were added. Incubation was at 37°C for 24 hours. The culture liquid was drained, the coverslips were removed with tweezers and pipetted in 10 ml of sterile water, followed by dilution and seeding on corn-glucose agar. As a control, the microorganisms placed in the suspension were washed off the surface of the cover glasses for 2 seconds. The inoculations were incubated at 37°C for 24 hours, followed by counting of grown colonies in different dilutions and expressed in CFU / ml.

In the framework of the experiment, the state of the gastrointestinal tract was simulated. The cells of propionic acid bacteria were grown on a corn-glucose medium (72 hours), precipitated by centrifugation, resuspended in an equal volume of gastric juice with the addition of 17% by weight of oat flakes and incubated for 3 hours at 36.6°C and pH 3.0. The mixture was then neutralized, 5% by

weight of bile acid mixture was added and incubated for 5 hours at 36.6°C and pH 8.0. The number of viable cells at each stage, simulating the state of the gastrointestinal tract, was expressed in CFU / ml; colonies were obtained by incubation on corn-glucose agar at 30°C for 3 days.

Sensitivity to antibiotics was studied by the method of disks. Plates with discs were incubated for 24 hours at 37°C. Evaluation of the results was carried out according to the diameter of the growth inhibition zones of microorganisms, taking into account the disc diameter (5 mm). The studies used the following antibiotics: doxycycline (Doc), benzylpenicillin (Pen), rifampicin (Rif), lincomycin (Lin), neomycin (Neo). These are broad-spectrum

antibiotics that are active against both gram-positive and gram-negative bacteria.

The data was subjected to statistical processing on a computer program Microsoft Office Excel 2003 by calculating arithmetic mean and standard error of the mean. Probability of difference values (p). The difference between the compared values was considered reliable at a value of p <0.05.

III. RESULTS AND DISCUSSION

The result of the experiments showed that propionic acid bacteria possesses biofilm-forming activity. The number of studied bacteria on the surface of the cover glass varies within $1,9 \times 10^9$ - $7,1 \times 10^{10}$ CFU / ml (table 1).

Table 1 - Biofilm formation of propionic acid bacteria selected from fermented milk products.

Propionic acid bacteria	The number of bacteria from surface of the cover slips, CFU / ml	
	Control (0 h)	Experiment (24 h)
<i>Propionibacterium shermanii 1</i>	$1,4 \pm 0,3 \times 10^3$	$7,1 \pm 0,2 \times 10^{10}$
<i>Propionibacterium shermanii 10</i>	$2,6 \pm 0,1 \times 10^3$	$6,4 \pm 0,1 \times 10^{10}$
<i>Propionibacterium shermanii 12</i>	$4,8 \pm 0,1 \times 10^3$	$1,9 \pm 0,1 \times 10^9$
<i>Propionibacterium shermanii 13</i>	$3,2 \pm 0,2 \times 10^3$	$8,7 \pm 0,3 \times 10^9$
<i>Propionibacterium shermanii 14</i>	$2,9 \pm 0,4 \times 10^3$	$2,1 \pm 0,2 \times 10^{10}$

Colonization of the surface of the cover glass is considerably higher in *Propionibacterium shermanii 1*, *Pr. shermanii 10*, *Pr. shermanii 14* ($2,1$ - $7,1 \times 10^{10}$), the lowest is in *Pr. shermanii 12* ($1,9 \times 10^9$). When the glass was immersed for a few seconds into the suspension of microorganisms, the

residual amount of biomass on the glass was $1,4$ - $4,8 \times 10^3$.

It was found that after the transferred stress by sequential exposure of gastric juice and bile acids, the number of propionic acid bacteria decreased by 1-3 orders of magnitude (Table 2).

Table 2 - Number of propionic acid bacteria in the model GIT, CFU / ml

Propionic acid bacteria	Number of living cells, CFU / ml				
	Initial quantity (0 h)	Experiment (3 h)	Control (3 h)	Experiment (8 h)	Control (8 h)
<i>Propionibacterium shermanii 1</i>	$4,7 \pm 0,1 \times 10^8$	$1,4 \pm 0,1 \times 10^7$	$7,3 \pm 0,3 \times 10^8$	$2,9 \pm 0,1 \times 10^5$	$3,1 \pm 0,1 \times 10^9$
<i>Propionibacterium shermanii 10</i>	$1,9 \pm 0,1 \times 10^8$	$4,6 \pm 0,2 \times 10^7$	$4,8 \pm 0,1 \times 10^8$	$1,4 \pm 0,1 \times 10^5$	$1,3 \pm 0,3 \times 10^9$
<i>Propionibacterium shermanii 12</i>	$2,1 \pm 0,1 \times 10^8$	$3,8 \pm 0,1 \times 10^7$	$5,3 \pm 0,1 \times 10^8$	$1,9 \pm 0,1 \times 10^5$	$1,6 \pm 0,4 \times 10^9$
<i>Propionibacterium shermanii 13</i>	$4,3 \pm 0,2 \times 10^8$	$6,2 \pm 0,2 \times 10^7$	$7,9 \pm 0,2 \times 10^8$	$2,3 \pm 0,3 \times 10^5$	$3,5 \pm 0,3 \times 10^9$
<i>Propionibacterium shermanii 14</i>	$5,2 \pm 0,1 \times 10^8$	$3,9 \pm 0,1 \times 10^7$	$8,4 \pm 0,3 \times 10^8$	$4,4 \pm 0,2 \times 10^5$	$4,2 \pm 0,2 \times 10^9$

In control samples, the number of microorganisms increased by 8 hours in one order. The study of the dynamics of growth of propionic acid bacteria that underwent aggressive factors of the gastrointestinal tract showed that their growth slowed down. The biomass density of experimental variants in the stationary phase is 2 times less than in the control. Gastric juice acts on cells stronger

than bile acids, but the number of viable cells remains at a fairly high level.

Antibiotics have a big influence on the development of propionic acid bacteria. The negative effect of antibiotics in case of getting them into food raw materials, and in particular to lactic acid products, affects the inhibition of the microflora of starter cultures in the technological process of food production, which creates the

danger for the development of an extraneous antibiotic-resistant microflora. With this in mind, the important role in the selection of tertiary cultures of microorganisms in the composition of food products is given special attention to such properties as resistance to antibiotics. In this regard, the study

of the antibiotic resistance of strains of propionic acid bacteria is topical.

The results of the studies of the antibiotic susceptibility of propionic acid bacteria are presented in Table 3.

Table 3 - Antibiotic resistance of propionic acid bacteria

Propionic acid bacteria	Area of growth inhibition, mm				
	Doc	Pen	Rif	Lin	Neo
<i>Propionibacterium shermanii 1</i>	6,0±0,1	7,1±0,2	6,7±0,2	0	7,4±0,2
<i>Propionibacterium shermanii 10</i>	6,4±0,1	7,5±0,1	7,2±0,1	0	7,1±0,1
<i>Propionibacterium shermanii 12</i>	7,1±0,2	7,3±0,3	6,9±0,1	0	6,7±0,2
<i>Propionibacterium shermanii 13</i>	8,2±0,1	9,0±0,1	6,7±0,2	0	6,9±0,1
<i>Propionibacterium shermanii 14</i>	7,4±0,1	7,1±0,2	7,5±0,3	0	6,8±0,1

It should be noted that all strains have little sensitivity to doxycycline, benzylpenicillin, rifampicin, neomycin, and full resistance to lincomycin.

IV. CONCLUSION

Thus, the strains of propionic acid bacteria isolated from sour-milk products have an increased tendency to biofilm formation, resistance to the conditions of the gastrointestinal tract, and also antibiotic resistance, causing a high benefit in their practical application.

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