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Optimization of Conditions for Culturing Probiotic Bacteria-Antagonists of Agents Involved in Hospital-Acquired Infections

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ABSTRACT : *Thereby, it was established that the proposed association against the agents of hospital-acquired infections has a broad spectrum of activity when grown on the tested nutrient media. The best result in antagonist activity was achieved when it was grown in the media MRS and those based on yeast water and molasses, as well as on the milk whey supplemented with 3 g/L yeast extract. No significant differences in the titer of microorganisms were observed when grown in these nutrient media. Culturing should be carried out at a temperature of 300C for 24 hours.*

Keywords: *probiotics, culturing, antagonism, hospital-acquired infections*

I. INTRODUCTION

Modern scientific evidence cited in the works of foreign and domestic researchers suggest that hospital-acquired infections (HAI) occur in at least 5-12% of the patients admitted to hospitals. Thus, up to 2 million illnesses are recorded each year in hospitals in USA, 500-700 thousand in Germany, which is about 1% of the population of these countries. In USA, about 25% out of 120 thousand and more patients infected with HAI die and, according to expert findings, HAI are a major cause of fatal outcomes. The data obtained in recent years indicate that HAI significantly extend the hospital stay for patients, and the damage they cause every year is 5 to 10 billion dollars in the United States [1-2].

These data show the relevance of the research aimed at the prevention and treatment of diseases caused by HAI. We have developed the complex probiotic Polylactobac representing an association of lactic and propionic acid bacteria inhibiting growth of broad-spectrum pathogens causing intestinal infections and inflammatory and septic processes. According to the results of trials carried out in the Scientific Center of Urology named after B.U. Dzharbusynov (Almaty), the probiotic was recommended for the comprehensive treatment of urinary tract infections [3, 4]. Due to its effectiveness and broad-spectrum activity, the drug Polylactobac is very promising for the medical practice. In connection with the above, we have carried out research to improve the probiotic effectiveness against HAI due to optimization of conditions for culturing bacteria.

II. MATERIAL AND METHODS

The association for the prevention and treatment of hospital-acquired infections, comprising strains of lactic acid bacteria Lactobacillus cellobiosus 20, Lactobacillus brevis 139, Lactodacillusplantarum 14d and propionic acid bacteria Propionibacteriumshermanii 2/10 in equal proportions (probiotic Polylactobac), served as object of the study. To prepare the association, the bacterial cultures were grown together in the tested nutrient media at a temperature of 32^0C for 24 hours. Experiments were run in three replicates. The average values are presented in the tables.

Accumulation of bacterial cells was established by plating cultures of appropriate dilutions in Petri dishes with the MRS solid nutrient medium, antagonist activity was estimated using agar diffusion technique against the following test cultures: Escherichia coli, Salmonella gallinarum, MRSA Staphylococcus aureus 9, MRSA Staphylococcus aureus 3316, Klebsiellapneumoniae 444, Pseudomonas aeruginosa 342, Pseudomonas aeruginosa835, Proteus sp.,Acinetobacter sp. 1182, Acinetobacter sp. 1522, Candida albicans, MycobacterimB_5 [5].

III. RESULTS AND DISCUSSION

1.1 To select the best nutrient medium, an experiment was conducted to grow the association in the following medium variants: 1 – MRS; 2 - combined [6]; 3 - yeast extract -5.0; molasses -20.0; $CoCl_2$ -0.01; 4 - molasses - 20.0; $CoCl_2$ -0.01, yeast water - up to 1 L.

The experimental results are presented in Table 1.

Table 1 - Growth inhibition zones of test cultures by Polylactobac association when grown in various nutrient media, mm

option No.	*E.coli*	*S.gallinarum*	*S.aureus*9	*K.pneumoniae*444	*C. albicans*	*P.multocida*	*P.aeruginosa*342	*P.aeruginosa*835	*Acinetobacter*1182	*Acinetobacter*1522	*Proteus sp.*
1	12,5	14,0	18,0	16,0	10,0	15,0	17,0	15,5	13,0	19,0	18,0
2	9,0	18,0	12,0	11,5	10,0	11,5	11,5	11,5	9,0	10,0	9,5
3	12,5	16,5	16,0	12,0	9,0	17,5	16,5	13,0	0,0	21,0	13,0
4	17,0	14,0	16,0	15,0	12,0	18,0	18,0	17,0	10,5	18,0	14,0

As can be seen from the table, the association against hospital-acquired infection has a broad spectrum of activity. The best result in the antagonist activity against the examined test-cultures was achieved when it was grown in the media MRS and No. 4 on the basis of yeast water and molasses. A good result was also provided by using a nutrient medium No.3 based on yeast extract and molasses, but in this variant, antagonism against *Acinetobacter*1182 was not revealed. In the combined medium, the higher antagonist activity as compared with the other variants was established against *S. gallinarum*. The association, grown in this medium, exhibited the lower activity against the rest of test cultures.

No significant differences were observed in titer of microorganisms when grown in various nutrient media, but the variant with the combined medium differs from the others by the higher pH value.

1.2 Further studies were carried out to select the milk whey-based nutrient medium. To this end, the following variants of media were tested:

1) Milk whey without additives
2) Milk whey + 10 g/L corn extract + 10 g/L of molasses +0.2 g/L yeast extract
3) Milk whey + 10 g/L molasses
4) Milk whey + 3 g/L yeast extract
5) Milk whey + 10 g/L of molasses + 0.2 g/L yeast extract
6) Milk whey + 15 g/L oat flour
7) Control – MRS medium
8) Combined medium

The pH values for all media were 6.8-6.9.

The experimental results are presented in Table 2.

Table 2 – Antagonist activity and bacterial titer of probiotic Polylactobac when growing cultures in various milk whey-based nutrient media

option No.	Growth inhibition zone, ⌀ mm									Titer, CFU/ml
	E.coli	*S.gallinarum*	*S.aureus*9	*K.pneumoniae*444	*P.multocida*	*P.aeruginosa*342	*P. aeruginosa*835	*Acinetobacter*1522	*Proteus sp.*	
1	10,0	18,0	13,0	15,0	16,0	12,0	13,0	12,0	14,0	$8,0x10^8$
2	10,0	16,0	16,0	14,0	17,0	9,0	15,0	13,0	12,0	$3,0x10^9$
3	12,0	13,0	19,5	15,0	16,0	10,0	17,0	14,5	14,5	$2,3x10^9$
4	13,0	15,0	19,5	15,0	17,0	12,0	15,5	15,0	15,0	$5,1x10^9$

5	9,0	10,0	11,0	10.0	11,0	0,0	14,0	0,0	12,0	$1,2x10^9$
6	10,0	12,0	12,5	12.0	11,0	0,0	13.0	0,0	11,0	$1,2x10^9$
7	11,5	15,5	14,0	15,0	16,0	11,0	16,0	12,0	16,0	$1,6x10^9$
8	11,0	12,0	12,0	16,5	12,0	0,0	17,0	0,0	13,5	$2,5x10^9$

As seen from the table, the association grown in the MRS medium as well as in the milk whey-based variants: No. 1 (milk whey without additives), No.2 (milk whey + 10 g/L corn extract + 10 g/L of molasses + 0.2 g/L yeast extract), No.3 (milk whey + 10 g/L molasses) and No. 4 (milk whey + 3 g/L yeast extract), has the broadest spectrum of antimicrobial activity. It was active against all examined test cultures in these nutrient media. The association showed the minimal activity against all test cultures in nutrient media No. 5 (milk whey + 10 g/L of molasses + 0.2 g/L yeast extract), and No. 6 (milk whey + 10 g/L oat flour). In these nutrient media, antagonism against *P. aeruginosa* 342 and *Acinetobacter*1522 was not established. The association grown in the combined nutrient medium did not inhibit these test cultures, but revealed high activity against *K. pneumoniae* 444 and *P. aeruginosa*835, while against the rest of the test cultures it was at the level of variants Nos. 5 and 6.
The minimal accumulation of bacterial cells in the association occurred in the milk whey without additives ($8.0x10^8$ CFU/ml), in the rest of nutrient media it made up $1.6\text{-}5.1x10^9$ CFU/ml. Acidification of the culture media by the association after a day of culturing was 40 to 127^0T. The lesser acidity was found in the combined medium (40^0T) and milk whey without additives (97^0T), the higher one - in variant No. 4 (127^0T), in the other variants - 100 to 124^0T.

By antagonist activity and content of viable cells the nutrient medium based on the milk whey with addition of 3 g/L yeast extract is the most appropriate. The probiotic activity when using this nutrient medium is similar to that in MRS medium.

1.3 To carry out the final selection of the nutrient medium and optimization of culture conditions (temperature and process duration), the bacterial association against hospital-acquired infection was grown in four variants of nutrient media: 1 – MRS medium; 2- milk whey + 3 g/L yeast extract; 3 - milk whey + 10.0 g/L molasses; 4 - water + yeast 20.0 g/L molasses. Culturing was carried out at 30 and 37^0C for 24 and 48 hours.
The data on culturing the association in the listed nutrient media for 24 hours at temperatures of 30 and 37^0C are shown in Table 3.

Таблица 3 – Antagonist activity and bacterial titer of probiotic Polylactobac when grown for 24 hours in various nutrient media

option No.	Growth inhibition zone, ⌀ mm											Titer, CFU/ml
	E.coli	*S.gallinarum*	*S.aureus*3316	*S.aureus*9	*K.pneumoniae*444	*C.albicans*	*P.multocida*	*P.aeruginosa*342	*P.aeruginosa*835	*Acinetobacter*1522	*Proteus sp.*	
The cultivation temperature 30^0C												
1	18,0	20,0	12,5	21,0	18,0	9,0	16,0	13,0	17,5	15,0	18,0	$3,5x10^9$
2	13,0	14,0	10,0	15,0	16,0	9,0	12,0	0	13,0	0	14,0	$1,8x10^9$
3	12,0	15,5	10,0	14,0	15,0	9,0	14,0	9,0	13,0	0	13,0	$9,0x10^8$
4	18,0	16,0	12,0	20,0	15,0	9,0	13,0	13,0	14,5	0	14,5	$8,0x10^8$
The cultivation temperature 37^0C												
1	14,0	18,0	14,0	20,0	19.5	9,0	16,0	14,0	20.0	11,0	16,0	$8,0x10^8$

2	11,0	14,0	9,0	18,0	15,5	9,0	11,0	9,0	14,0	0	15,0	$9,0x10^8$
3	13,0	15,0	9,0	15,0	15,0	0,0	12,0	0,0	13,0	0	15,0	$6,0x10^8$
4	16,0	17,0	12,5	17.0	15,0	10,0	13,0	11,0	15,0	9,0	17,0	$6,0x10^8$

It is seen from the table that a greater accumulation of bacterial cells in the association occurs when grown at a temperature of 30^0C in MRS medium and medium No. 2 based on milk whey with yeast extract. When culturing the bacteria at 37^0C, their content after a day of culturing in all variants of media is somewhat lower than at 30^0C. The higher antagonism in the association was revealed when culturing in the media MRS and the one based on the yeast water and molasses (No. 4). Antagonist activity against the test cultures at different temperatures of culturing in the main differs slightly. This indicates that both temperatures are suitable for culturing the association, but the temperature of 30^0C is more preferable.

When culturing the association on above-mentioned nutrient media for 48 hours at temperatures of 30 and 37^0C, the decrease in the antagonistic activity of association against the examined test cultures was recorded in all variants. Increase in the number of viable cells after 48 hours of culturing was not observed in any of the variants compared to the 24-hour culture.

IV. CONCLUSIONANDFUTUREWORK

Thereby, the conditions for culturing probiotic bacteria forming the drug Polylactobac have been selected. The optimal culture media include MRS and a medium based on the yeast water and molasses. Culturing should be carried out at a temperature of 30^0C for 24 hours.

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