DOI:10.31557/APJEC.2021.4.S1.5

RESEARCH ARTICLE

Characteristics of the Karakalpak Strain of *B.Bifidum Kk*, Promising for Use as a Probiotic

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Abstract

The article presents the results of a study on the physiological, biochemical and technological properties of the local (karakalpak) strain of Bifidobacterium bifidum KK. Antagonistic activity of the strain in relation to Escherichia coli, Staphylococcus aureus, Proteus vulgaris, relation to intestinal metabolism products and sensitivity to drugs was studied. It has been established that the karakalpak strain of Bifidobacterium bifidum KK has high technological properties and can be recommended as a probiotic to supplement the actual nutrition of children and adults.

Keywords: lactic acid bacteria- microbiota- Aral Sea region- bifidobacteria- technological properties- probiotic

Asian Pac Environ Cancer, **4 (Suppl 1)**, 5-7

Submission Date: 10/05/2021 Acceptance Date: 11/02/2021

Introduction

One of the urgent problems of modern medicine is the influence of microbiota on human health. A connection has been established between microbiome disorders and the development of diseases of the gastrointestinal tract, skin, respiratory system, etc. The study of the microbiota of the body makes it possible to establish safe and effective strains of probiotic bacteria and create a new generation of multiprobiotics based on them [1].

Currently, products and preparations created using lactic acid bacteria are the basis of functional nutrition. Products of local production based on local microflora adapted to extreme environmental conditions gear special value in healthy nutrition. It is proved that the effect of products made on the basis of local train is many times more effective than imported ones [2].

Bifidobacteria and lactobacteria are the main representatives of the normal microflora of the gastrointestinal tract of the human body. Bifidobacteria play a leading role in maintaining and normalizing intestinal microbiocenosis, ensuring nonspecific resistance of the body; and improving protein-vitamin and mineral metabolism [2-5]. It has been proved that bifidobacteria are a "supplier" of a number of essential amino acids, including tryptophan, vitamins; their anticarcinogenic and antimutagenic activity, the ability to reduce cholesterol levels in the blood, etc. have been established [1,2][6,7]. All these positive properties of bifidobacteria allow us to consider them as the basis of functional nutrition.

The aim of this work is to study the physiological, biochemical and technological properties of the local (karakalpak) strain of Bifidobacterium bifidum KK and to determine the possibility of its use as probiotics.

Materials and Methods

The material for the study is a local strain culture of Bifidobacterium bifidum KK, isolated from the body of an infant. Microbiological and biochemical research methods were used in this study.

For the cultivation of bacteria strain of Bifidobacterium bifidum. KK and culture preservation were used modified medium MRS and semi-liquid medium Blaurocca with lactose [6].

The study of the physiological and biochemical properties of the culture was carried out according to the generally accepted microbiological method. The activity of acid formation was determined by titrometric method after 24 hours of cultivation at 28 and 37° C. The results were expressed in Turner degrees. The sensitivity of the culture to antibiotics was determined by the method of "paper

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disks". Sensitivity to 15 antibiotics was tested.

The antagonistic activity of the strain in relation to conditionally pathogenic indicator bacteria was determined by the method of delayed antagonism. To do this, 0.1 ml of a culture of bifidobacteria of 10^{-7} dilution was applied to the surface of the liquid nutrient medium of the MRS and cultured for 72 hours at 37° under anaerobic conditions.

The antagonistic activity of the strain was studied in relation to Escherichia coli, Staphylococcus aureus and Proteus vulgaris.

Results and Discussion

At the study, the morphological and cultural, physiological and biochemical, antagonistic, technological properties of the Bifidobacterium bifidum KK strain were studied in detail.

Morphological-cultural and physiological-biochemical properties of the strain:

On the semi-liquid medium of Blaurocca, the cells of a two-day culture have a straight or slightly branched shape with clavate thickenings; the cell sizes are (0.4-0.7) x (3,2-3,8) microns. In the smear, they are arranged singly, in pairs, rarely in the form of chains. When stained with methylene blue, rounded granules are visible at the edges of the cell, are gram-positive, not acid-resistant, do not form spores, and are motionless. Typical obligate anaerobes grow in the thickness of the medium with the zone of anaerobiosis. On dense media, colonies in the form of thickened carnations of 1-2, 2-4 mm in size are formed. The color of the colonies varies from white and gray to dark brown. On liquid media in test tubes, they grow in the form of a "column" of turbidity.

Physiological and biochemical propertie

Chemoorganotrophs, the strain actively assimilates glucose, lactose, maltose, galactose, fructose, mannitol, xylose and weakly ferments sucrose and trehalose. It has no proteolytic activity. The optimal growth temperature is 37-39° C, and grows at 42-45° C.

It is established that the culture has a high growth rate. It is seen from the data in Table 1 that when cultured on various nutrient media, a high cell titer was achieved and long-term strain activity was observed for 48 hours, while the cell titer was 10^8-10^9 (CFU/ml).

Determining the susceptibility of a bacterial strain to antimicrobial drugs is an important prerequisite for its approval as a probiotic. Some authors state that in cases of concomitant prescription with antibiotics for the prevention and treatment of intestinal disorders, probiotics must be resistant to certain antibiotics in order to survive in the gastrointestinal tract [3, 8].

Table 1. The activity of the Strain of B.bifidum pcs. KK on Various Media

Nutrient medium	Cultivation time, hours	Cell titer (CFU in ml)
Hepatic environment	24	10^7
	48	10^8
Corn-lactose	24	10^7
	48	10^8
Milk with growth activators	24	10^7
	48	10^9

Table 2. Attitude of Bifidobacterium Bifidum KK SLE-134 to Antibiotics

Antibiotic	Stability (no suppression zone)	Small suppression zone (less than 10mm)	Stunted growth, i.e. sensitivity (more than 15mm)
Ristomycin	-	+	+
Amikacin	-	+	-
Oxacillin	+	-	-
Ampicillin	+	-	-
Tetracycline	+	-	-
Oleandomycin	-	+	-
Metacyclin	-	+	-
Kanamycin	-	+	-
Streptomycin	+	-	-
Carbenicillin	+	-	-
Benzylpenicillin	+	-	-
Erythromycin	+	-	-
Gentamicin	-	-	+
Levomycetin	+	-	-
Neomycin	-	-	+

The test for the sensitivity of the Bifidobacterium bifidum KK strain to antimicrobial drugs was performed by the disc diffusion method. It has been studied the ratio of the strain to 15 antibiotics. At the same time, benzylpenicillin G, ampicillin, chloramphenicol, tetracycline and erythromycin were chosen to test the most common mechanisms of acquired resistance for gram-positive anaerobes.

Table 2 shows the ratio of strain of B. bifidum KK culture to antibiotics. It was found that the culture showed a high degree of resistance to oxacillin, ampicillin, tetracycline, streptomycin, carbenicillin, benzylpenicillin, erythromycin and levomycetin. High sensitivity was observed in relation to ristomycin, gentamicin and neomycin.

Antagonistic activity

The antagonistic activity of bifidobacteria is associated with the production of organic acids (acetate and lactate). This strain has antagonistic activity against pathogenic and conditionally-pathogenic microorganisms. In co-cultivation, the strain suppressed the growth of the "test culture" after 48 hours. It was found that this strain has antagonistic activity in relation to Eshcherichea coli - 100%, Staphylococcus aureus - 100%, Proteus vulgaris - 75%, Proteus mirabilis - 60%.

It relations to intestinal metabolic products: resistant in an environment with 40% bile, 0.5% phenol, 3% salt, resistant to low pH.

The strain has high technological properties

It accumulates biomass well, when added to milk in an amount of 5-6%, fermentation occurs after 12-14 hours of growing, the maximum acidity is 90-110° T (does not rise above up to 5 days), the titer reaches $5x10^{10}$, which is 1-2 orders of magnitude higher than that of other strains. The strain retains its activity even after multiple passages [7-10]. Reseeding was carried out every 30 days. Storage conditions: on a medium with milk and on Blaurokka's medium. The culture withstands lyophilization well.

In conclusions, thus, our research shows that the Karakalpak strain of Bifidobacterium bifidum has high technological properties and can be recommended as a probiotic for supplementing the actual nutrition of children and adults. This preparation can be used as a means that increases the body's nonspecific resistance to adverse environmental factors and contributes to the overall health of the human body. All these positive effects allowed us to consider bifidobacteria as one of the main categories of functional nutrition.

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