INFLUENCE OF CULTURE MEDIUM ON THE ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA

Yu. M. Pohilko, N. O. Kravchenko

Institute of Agriculture Microbiology and Agroindustrial Manufacture, NAAS,
City of Chernihiv

The antagonistic activity of lactic acid bacteria isolated from the gastrointestinal tract of rabbits, and its dependence on the culture medium have been investigated. It was found that the antagonistic activity of lactic acid bacteria depends not only on strains, source of isolation, but also on their culture medium.

Key words: *antagonism, lactic acid bacteria, culture medium.*

Antagonistic properties of lactic acid bacteria attracted the attention of researchers and currently are widely described in the literature. Mechanisms of their antagonistic action are different. In some cases antimicrobial effect of these microorganisms is stipulated by the action of the main product of metabolism – lactic acid that lowers pH and plays the role of bactericidal factor in the enzymatic fermentation process upon obtaining food and feed [1; 2]. In other cases antagonistic effect of probiotic bacteria is stipulated by the neutral products that are released bacteria into the environment [3]. Separate substances produced by lactic acid bacteria are characterized by high antagonistic activity even at their low concentrations in the environment. This category includes antibiotic substances (lactocycl, lactobrevin, nisin, lactobacillin, etc.) [4; 5]. According to the literature data, lactic acid bacteria can produce hydrogen peroxide that inhibits *Staphylococcus aureus*, *Pseudomonas* or other pathogens and opportunistic pathogens. Antimicrobial effect of hydrogen peroxide is stipulated by denaturation of some enzymes, increased permeability of membranes and destruction of DNA of microorganisms by free radicals [6]. In addition, hydrogen peroxide activates lactoperoxidase system that forms oxidation products - inhibitors for a wide spectrum of gram-positive and gram-negative bacteria [7]. Another one mechanism of antibacterial

activity of lactic acid bacteria – the ability to produce lysozyme, which destroys bacterial cell walls, creating a nonspecific antibacterial barrier, has been established [3]. Antagonistic effect of some lactic acid bacteria is characterized by bactericidal properties of substances of protein nature (bacteriocins) [8]. The ability of lactic acid bacteria to inhibit the growth of a wide range of microorganisms (especially gram-negative) plays a major role in the formation of gastrointestinal microbiocenosis of humans and animals.

Lactic acid bacteria are the basis of many probiotic preparations both in human and veterinary medicine. They are widely used in starters of mono- and poly-component composition for making dairy products, in feed production - for preservation and improvement of feed nutrient value. [9]

Despite the abundance of the publications dedicated to the lactic acid bacteria and their antagonistic properties, search for the new antagonistic strains is up to date. Question on the selection of culture medium for culturing of lactic acid bacteria for examination of antagonistic properties is still unsolved.

Materials and methods. The study was conducted at the Laboratory of Probiotics of the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the NAAS.

Antagonist activity of bacterial strains of the genus *Lactobacillus*, isolated from the gastrointestinal tract of rabbits: *L. lactis* 4/1, *L. casei* 5/4, *L. helveticus* 13/2, *L. plantarum* 16/1, *L. plantarum* 16/3, *L. plantarum* 17/2, *L. plantarum* 17/3, *L. acidophilus* 31/2, *L. delbrueckii* 39/2, *L. acidophilus* 49/1 was studied.

As a typical representative of lactic acid bacteria (LAB), a strain of *L. acidophilus* CCM 4833, obtained from depository of D. K. Zabolotnyi Institute of Microbiology and Virology of the NAS of Ukraine. Eight strains of pathogenic and opportunistic microorganisms were used as a test strains: *Proteus mirabilis* No. 3177, *Proteus vulgaris* No. 13, *Staphylococcus aureus* No. 906, *Salmonella typhimurium* No. 89, *Shigella flexneri* 2a No. 160, *Klebsiella pneumoniae* No. 67, *Escherichia coli* 055K59 No. 3912, *Pseudomonas aeruginosa* ATCC-27853. The strains of opportunistic and pathogenic microorganisms were obtained from Chernihiv Regional Sanitary and Epidemiological Station. Strain of *Salmonella enteritidis* P1 var. *Issatschenko* was obtained from the collection of strains of microorganisms from the Laboratory of Probiotics.

Pathogenic microorganisms were cultured on meat-peptone agar (MPA) at 37 °C. The studied strains of lactic acid bacteria were cultured at 37 °C for 48 hours, on the following culture media: agarized sterile skimmed milk, agarized hydrolysed milk, MRS [10]. The study of antagonistic activity (AA) was performed *in vitro* by diffuse method of blocks [11].

Variants of culture media without blocks of antagonistic stains with the lack zones of growth retardation of pathogenic and opportunistic microorganisms were used as the control.

Consequently, as the result of conducted studies, antagonistic activity of 11 strains of lactic acid bacteria was established, of which 10 were isolated from the gastrointestinal tract of rabbits. Among the studied bacteria the following strains were the most active: *L. lactis* 4/1, *L. helveticus* 13/2, *L. plantarum* 17/2, *L.*

acidophilus 49/1, which formed the largest area of growth retardation of pathogenic and opportunistic microorganisms under cultivation on all studied media.

Obtained data suggest that lactic acid bacteria isolated from the digestive system of rabbits have a high bacteriostatic potential regarding pathogenic and opportunistic microorganisms, therefore, the use of the most promising ones in livestock breeding could significantly affect the reduction of the level of gastrointestinal diseases in animals.