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Study of antimicrobial metabolites of lactic acid bacteria

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Abstract: In the past decades, detailed studies of SAB have revealed their ability to produce antimicrobial substances of various natures.Many SAB strains, in addition to lactic acid, produce a large number of non-specific low molecular weight compounds, such as organic acids, hydrogen peroxide, diacetyl, reuterin, etc., which determine the spectrum of their antimicrobial action.Many SAB strains, in addition to lactic acid, produce a large number of non-specific low molecular weight compounds, such as organic acids, hydrogen peroxide, diacetyl, reuterin, etc., which determine the spectrum of their antimicrobial action.As mentioned above, the main final metabolites produced by SAB during fermentation are lactic and acetic acids.Acetic acid has a broader antimicrobial activity than lactic acid. However, a synergistic effect is known for both acids:

A mixture of acetic and lactic acids inhibits the growth of pathogenic gram-negative enterobacteria Salmonella typhimurium.It is noted that L-lactate has a greater inhibitory effect than the D-isomer. Different microorganisms react differently.Depending on the acidity of the environment, for example, at a pH below 5.0, lactic acid inhibits the development of spore-forming bacteria, but does not affect the development of microscopic fungi and yeasts.

Keywords: Lactobacillus, strain, probiotic, SAB, isolation, antibacterial activity.

Introduction: The search for new promising probiotic strains, the identification of their probiotic properties, as well as the study of already known probiotic strains are important tasks of basic science.Research aimed at isolating and studying new strains of SABs is a widespread and urgent issue worldwide.One of the first and most important steps in the search and selection of a promising strain is to determine its taxonomy, which allows us to have a preliminary understanding of the safety, origin, habitat, and physiological characteristics of a microorganism, correctly isolating the strain at the species level.Lactobacteria are one of the most studied representatives of the SAB group, along with streptococci, lactococci, and enterococci.According to the modern phylogenetic classification of bacteria, the genus Lactobacillus has more than one hundred and fifty species and subspecies.Using 16S rRNA gene sequencing, the researchers identified 14 isolates of SABs as: Enterococcus mediterraneensis, Lactobacillus fermentum, and Streptococcus lutetiensis.

They studied the probiotic properties of the isolates.The authors' work investigated the antibacterial activity and probiotic properties of Lactobacillus spp., which strains were sensitive to ampicillin.

LITERATURE ANALYSIS AND METHODOLOGY

Dairy products were used to isolate SAB isolates.Enriched MRS-nutrient medium (Hi-Media, India) was prepared, homogenized in sterile saline (0.85%, pH 7).The homogenate was diluted to 10-5 and inoculated into 2 Petri dishes containing MRS-agar medium and incubated under anaerobic and aerobic conditions for 24-48 hours at 37-40°C.The isolated isolates were characterized according to their morphological, cultural, physiological, and biochemical properties according to the indicators specified in the "Bergji spectrometer".Samples with rod-shaped and blue cells under the microscope, Gram-negative and catalase-negative, were selected and cultured on MRS-broth medium (HiMedia, India).

DISCUSSION

The following composition was used to prepare the nutrient medium for the isolation of SAB isolates: MRS-broth nutrient medium composition (g\l);

peptone -10,0;
meat extract -10,0;
yeast extract -5,0;
twin 80-1,0 ml;
sodium salt of acetic acid -5,0;
ammonium citrate - 2,0;
glucose -20;
$MgSO_4$ x $7H_2O$ -0.2;
$MnSO_4$ x $4H_2O$ -0,05;
L-cysteine -0,2;
pH- 6,2-6,5., 15 for a minute 121 °C sterilized in an autoclave at a temperature of 1 atm.
MRS- agar medium composition (g\l);
peptone -10,0;
meat extract -10,0;
yeast extract -5,0;
twin 80-1,0 ml;
sodium salt of acetic acid -5,0;
ammonium citrate - 2,0;
glucose -20; $MgSO_4$ x $7H_2O$ -0.2;
$MnSO_4$ x $4H_2O$ -0,05;
L-cysteine -0,2;
agar-agar-18;
pH- 6,2-6,5., 15 for a minute 121 °C sterilized in an autoclave at a temperature of 1 atm.
Composition of MRS-1 medium without added carbohydrates, (100 ml);
peptone - 1 g;
meat extract - 0,5 g;
ammonium salt of citric acid - 0,2 g;
sodium salt of acetic acid - 0,5 g;
K_2HPO_4 - 0,2 g;
$MgSO_4$ -0,02 g;
$MnS0_4$ - 0,005 g;
Tvin 8 0 - 0 , 1 m l ;
pH 6,8-7,0.

Bromcresol purple (Reakhim, Russia) served as an indicator (1.4 ml of a 1.6% alcohol solution is added to 1 liter of medium). This indicator changes its initial purple color to yellow under the influence of acidic products formed when carbohydrates are broken down by lactobacteria. The prepared MRS-1 medium without added carbohydrates is placed in 5 ml test tubes and autoclaved for 15 minutes under 1 atm pressure.

A smear from a microbial culture is placed on a degreased glass slide, dried in a flame, and stained using the chemical Gram method. The Gram-stained sample is examined under a microscope to identify gram-negative and gram-positive bacteria; According to the Ziehl-Nielsen method - for staining acid-fast bacteria and detecting spores; By the Burry or Gins method - determination of the presence of capsules. The presence of capsules in the microorganisms under study is also determined by negative contrast using liquid black ink or a 10% aqueous solution of nigrosine. These dyes do not penetrate the capsule and are therefore clearly visible against the overall dark background of the drug. A drop of the studied suspension of microorganisms is placed in a drop of a diluted solution of fuchsin, mixed with a drop of ink, covered with a coverslip and viewed with an objective. (×40). To identify cultures, it is necessary to describe the type of spore formation, the location of the spores in the cell, and their size.

Study of the cultural characteristics of the strain

Cultural characteristics are characteristic of each type of bacteria, so they are an important distinguishing feature. The cultural properties of bacteria are determined by the nature of their growth in dense, liquid, and semi-liquid selective and industrial nutrient media. To obtain isolated colonies, the isolates are plated on dense nutrient media using the Drigalsky method.

The strain passport should describe the size, shape, nature of the edge contour of the colonies, the relief and surface of the colony, the color, structure and consistency of the colony. The characteristics of culture growth in liquid and semi-liquid media are described in detail. The growth of isolates should be studied at minimum, optimum, and maximum growth temperatures when grown under aerobic, anaerobic, or conditionally anaerobic conditions.

CONCLUSION

In this article, lactobacillus isolates were isolated from local sources. The culture (cells and colonies) should not have signs of differentiation, and the presence of cells and colonies that differed in morphological characteristics from the cells and colonies of the proposed strain in the studied cultures was not allowed. Preparation of nutrient media for isolation and cultivation of Lactobacillus isolates from local sources Dairy products were used to isolate SAB isolates. Identification of lactobacteria by carbohydrate digestion spectrum. To determine the enzymatic activity of each isolate against carbohydrates, the viability of lactobacillus isolates was restored by subculture 2-3 times in MRS broth nutrient medium and inoculated onto agar medium. The colonies that grew were selected. The isolated isolates were identified using MALDI-TOF Mass spectrophotometry EXS 2600 (Zybio) (Figure 3).

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