

Isolation and Selection of Propionic Acid Bacteria Promising for Biotechnological Production

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Abstract—Bacteria of the genus *Propionibacterium* are the microorganisms with great biotechnological potential. The article deals with studies of biological properties of new propionic acid bacteria strains isolated from raw milk in different regions of Republic of North Ossetia-Alania.

According to morphological characteristics, the cells of the isolated strains are motionless sticks with rounded ends that do not form spores and are gram-positive. The ability of strains to ferment carbohydrates in biochemical tests was determined. The research results showed that the studied bacterial strains were referred to *Propionibacterium freudenreichii*. Resistance of microorganism strains to low pH values and gastrointestinal tract secretions was studied.

Keywords—propionic acid bacteria; genus *Propionibacterium*; *Propionibacterium freudenreichii*; identification; acid resistance; bile resistance

I. INTRODUCTION

It is known that the change in the intestinal microflora caused by pathogens involves various violations of vital body functions, a complication of chronicity. Currently, one of the promising methods of human and animal diseases prevention is the use of new generation fermented dairy products and medioprophyllactic preparations-probiotics [1]. Live bacteria that are part of probiotics are antagonists of pathogenic and opportunistic microorganisms, and their use normalizes the microflora in the gastrointestinal tract. The technological effect of microorganisms is associated with their ability to synthesize specific biologically active components, such as organic acids, bacteriocins, enzymes, vitamins and others, contributing to the improvement of sanitary-microbiological and organoleptic indicators of the finished product, as well as allowing to intensify the production process [2].

Increased attention of researchers, along with lactic acid bacteria to make probiotic preparations, is attracted by less studied, but already found good practical use (in cheese-making, silage of plant raw materials, probiotics production) - propionic acid bacteria [2-5]. Probiotic properties of propionic acid bacteria are due to the synthesis of a great amount of vitamin B12 [3], as well as the formation of propionic acid, minor organic acids, amino acids, enzymes and bacteriocins in the fermentation process.

Bacteria of the genus *Propionibacterium* are the microorganisms with great biotechnological potential. Classical propionic acid bacteria have antibacterial [4], antioxidant [3], immunostimulatory [3] and antimutagenic effect [5], capable of being well – fixed on the intestinal walls, due to high adhesive properties, creating a barrier to protect against the action of some chemicals, pathogenic bacteria and UV rays. Therefore, the search for new strains of microorganisms with the necessary biological properties and technological characteristics is a promising trend in the biotechnological research.

The research aim was to isolate and study the properties of new propionic acid bacteria strains that possess biotechnological potential.

II. EXPERIMENTAL

The research objects were 10 propionic acid bacteria isolates that were isolated from raw milk in different regions of RNO-Alania. Isolation of pure cultures was conducted in liquid media – corn-lactose and milk-calcium broth (by Voytkovich) and solid-corn-lactose medium containing 1,5% agar. The time of cells reaching the middle of the exponential phase for each strain of the microorganism was experimentally determined. The cultures were maintained by replanting to fresh nutrient media and stored at 4°C.

The dry weight of bacterial cells in suspension was determined after the sample drying in the drying cabinet at 105 °C to the constant weight [4].

Resistance to the body secretions simulating transit through the gastrointestinal tract (GIT) was investigated using apothecary bile and pH changes [5].

Identification of propionic acid bacteria species was carried out according to generally accepted methods in microbiological practice [6] and the Bergey determinant [7].

Statistical processing of the obtained results was carried out by calculating the relative reproducibility variance and the confidence interval for the mean value using the program Statistica 6.0.

III. RESULTS AND DISCUSSION

As a result, 10 isolates propionic acid bacteria with good acid formation activity (53,5-102 To) were isolated.

In the process of preliminary studies 4 strains of rod-shaped microorganisms (IL-1, IL-2, IL-7, IL-10) were selected from 10 isolated strains. The resulting group of strains was analyzed by the parameter of cell growth rate, that is, the strain ability to accumulate maximum biomass in a short period of time. Dry weight of cellular biomass was determined after incubating strains of microorganisms in liquid corn-lactose medium for 24 hours at 36,5°C (table I).

According to the results of the experiment, the maximum biomass accumulation was recorded for IL-1, IL-2 strains, which were selected for further studies.

According to morphological characteristics, the cells of the isolated strains are motionless sticks with rounded ends that do not form spores and are gram-positive. The cell wall thickness varied slightly with the age of the culture, being in the range of 21,0-25,0 nm. When staining with lead acetate solution, it is possible to distinguish two layers of the cell wall. A large number of membrane cells were found in micropreparations prepared by negative contrast method.

When growing in a nutrient-dense medium – colonies are rounded or in the shape of buckwheat grain, moist, shiny white or cream colour. When growing in a liquid medium, the strains of isolated microorganisms form heavy viscous sediment.

Comparison of biochemical characteristics showed that the isolated strains are catalase-positive, able to ferment lactose, glucose, galactose, fructose, rhamnose, mannose and restore nitrates. It was found that the studied strains are not able to break up gelatin and assimilate maltose, sucrose; mannite, sorbite, arabinose, raffinose, xylose, dulcete.

Basing on the analysis of the materials for studying cultural, morphological and biochemical properties and by means of diagnostic aid in Bergey bacteria determinant [7], the isolated local strains were referred to the genus *Propionibacterium*. Strains IL-1, IL-2 we identify with the species *Propionibacterium freudenreichii*.

According to the requirements imposing on microorganisms that are a part of probiotics, they must be resistant to low pH, bile. This is because the probiotic must survive its passage through the stomach before reaching the large intestine [5]. The resistance of isolated propionic acid bacteria strains to hydrochloric acid in a liquid nutrient corn-lactose medium inoculated by the tested bacteria, setting by the hydrochloric acid the pH values from 2,0 to 4,0 was studied. The studied strains show not only good resistance to low pH, but also the ability to further growth and reproduction (Fig.1).

TABLE I. BIOMASS ACCUMULATION BY STUDIED STRAINS ($\bar{X} \pm SE, N = 8$)

Strain index	Dry weight biomass, g/l
Control	0,47 ± 0,011
IL - 1	1,20 ± 0,036
IL - 2	1,38 ± 0,012
IL - 7	0,36±0,012
IL - 10	0,81±0,014

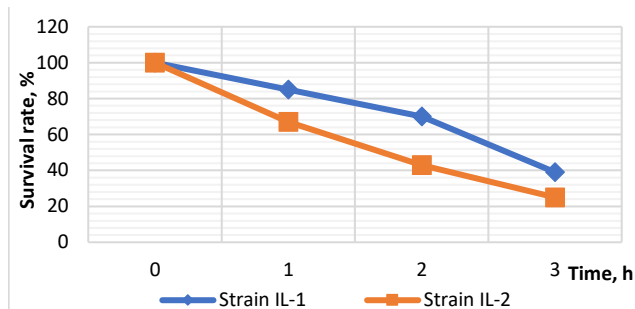


Fig. 1. Survival rate of IL-1, IL-2 strains of propionic acid bacteria at pH 2.

Fig.1 shows that a marked decrease in the number of viable cells occurs with increasing incubation time. The survival rate of IL-1 strain of propionic acid bacteria at pH 2 in an hour of incubation is 81%, and for IL-2 strain 67% of the viable cells number is preserved.

To study the resistance of the isolated strains the bile action was supplemented with the introduction of a daily culture of microorganisms into the corn-lactose medium containing a bile concentrate of 0,3% and 0,5%. Bacterial growth was analyzed by viable colonies in 0, 2 and 4 hours of cultivation and when further inoculating in agaric medium at 37°C during 48 hours. 0,1 ml of a solution that contained a specific, previously isolated strain was added to 10 ml of corn-lactose medium, then it was inoculated 24 hours at 37°C.

Strains of the studied cultures of propionic acid bacteria showed significant resistance to the bile action. It was found that the increase in incubation time and bile concentrate in the nutrient medium to 0,5%, leads to a decrease in the viable cells number, which can be caused by the death or inactivating some part of cells in the aggressive medium of the “stomach” or “small intestine”.

Thus, the studied strains showed good resistance to low pH and bile action, which is an indirect indicator of cells viability that may ensure the implementation of probiotic properties in the studied strains.

IV. CONCLUSION

The isolated bacterial strains were referred to the genus *Propionibacterium* in accordance with Bergey bacteria determinant [7]. The results of studying cultural-morphological and physiological-biochemical properties allow to previously refer the isolated and studied strains to *Propionibacterium freudenreichii*. The obtained data indicate that the isolated strains of propionic acid bacteria are physiologically active and, in the future, can be used as a basis for making probiotic preparations

REFERENCES

- [1] R. Saarela, G. Mogensen, R. Fonden, J. Matto, S. Matilla, “Probiotic bacteria: safety, functional and technological properties”, Journal of Biotechnology, № 3 (84), pp. 197–215, 2000.
- [2] I.S. Khamagaeva, I.V. Boyarineva, N.Yu Potapchuk, “Study of probiotic properties of the combined ferment”, Technique and technology of food production, № 1, pp. 1 – 5, 2013.
- [3] L.I. Vorobyova, Propionic acid bacteria. Publ. of the Moscow State University, 1995.

- [4] T.V. Kuznetsova, A.E. Khalymbetova, M.G. Saubenova, "Isolation and selection of propionic acid bacteria with antagonistic activity", Actualscience, № 2 (1), pp. 19 – 20, 2015.
- [5] O.E. Khaeva, L.P. Ikoeva, "Isolation and study of propionic acid bacteria to acid stress", Proceedings of Gorsky SAU, № 2 (55), pp. 152 – 156, 2018.
- [6] O.V. Prunova, O.N. Sakhno, Laboratory course in General Microbiology. Publ. of the Vladimir State University, 2005, 76 p.
- [7] Hoult J., Krieg N., Snita P., Staley J., Williams S., Bergey's manual of determinative bacteriology. Publ. of the Moscow: Mir, 1997.