RESEARCH ARTICLE OPEN ACCESS

Research Intensity Synthesis of Propionic Acid and Vitamin B12 Propionibacteria

Kuznetsova Tatyana[1], Halymbetova Aizhan[1], Kulnazarov Batyr[1], Saubenova Margarita[1]
[1]Institute of Microbiology and Virology Kazakhstan

Abstract
Conducted the selection and breeding of strains of *Propionibacterium shermanii* ability to synthesize vitamin B12 and propionic acid. Dedicated 9 propionibacteria strains synthesize vitamin B12 (180-1200 mcgr/ml) and propionic acid (0,63- 2,53 g/l).
Key words: Propionic acid bacteria, vitamin B12, propionic acid, strain, selection.

I. Introduction

In recent years, biotechnology in the food industry is focused on the development of functional foods and food additives of the new generation, as well as improvements in the safety and bioavailability of traditional food and feed [1]. Process action of microorganisms during this is due to their ability to synthesize specific active ingredients such as organic acids [2], bacteriocins, enzymes, vitamins, [3] and other that improve sanitary and microbiological and organoleptic characteristics of the finished product, and allowing to intensify the production process [4]. In this regard, along with lactic acid bacteria growing attention attracted less studied, but have already found practical application of decent (cheese-making, silage vegetable raw materials, production of probiotics) propionic acid bacteria [5-7]. Their physiological and biochemical features, simplicity to the conditions of existence, the activity of inhibiting the growth of fungi and other contaminating microorganisms are responsible for the high favor in the practical application and contributes to increased research in this direction.

Our work was aimed at finding strains of propionic acid bacteria which are promising for use in biotechnology.

II. Materials and Methods

A selection strains of propionic acid bacteria from different sources, such as rennet cheeses, silage, crop residues, animal rennet and agricultural al. Isolation of propionic acid bacteria produced from enriched cultures. For this test material (cheese, rennet and other farm animals). Was milled in sterile conditions and added to 3 grams in 250 ml flasks with dairy calcium broth further 3 day cultures were plated on corn- glucose medium in petri dishes, as well as corn - lactose into tubes containing higher layer protection, the method of tenfold dilutions. Cultured for 2 days at 30^0C .

The morphology of the propionic acid bacteria was studied by preparing preparations stained with methylene blue followed by microscopy system in an immersion lens ×100. Total allocated 9 strains of *Propionibacterium shermanii*.

To determine the synthesis of propionic acid bacteria are cultured on corn - lactose medium without agar cultivation mode 15 days at 30^0C. Control - corn - lactose medium. Number of propionic acid was determined by Mathieu.

Vitamin B12 content was determined spectrophotometrically by culture on corn - glucose medium supplemented with $CoCl_2 \times 6H_2O$ in an amount of 0,003 g/l. Control - corn - glucose medium. Mode of cultivation 3 days at 30^0C.

III. Environment

Wednesday to highlight the propionic acid bacteria

Milk and calcium soup (for Voitkevich): milk calcium salt - 2%, peptone - 1 %, sodium chloride – 0,5%.

Corn - glucose medium: corn extract - 2%, glucose - 1%, K_2HPO_4 – 0,2 %, $(NH_4)_2SO_4$ – 0,5 %, bacteriological agar - 20 g/l.

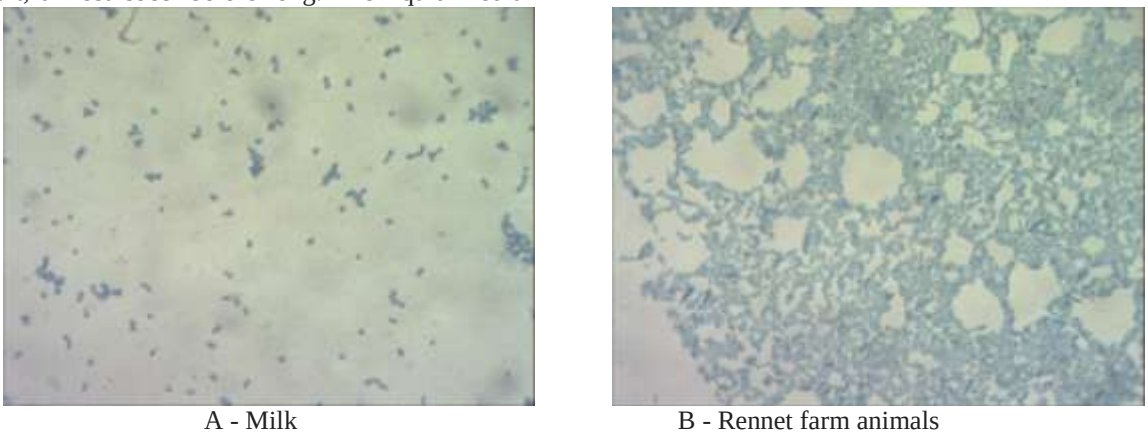
Corn - lactose medium: corn- milk mixture - 30 g/l, peptone - 30 g/l, lactose - 18 g/l, ascorbic acid -1 g/l, $Na_3C_6H_5O_7 \times 2H_2O$ - 12 g/l , MgSO4 - 0 24 g/l , KH_2PO_4 - 4 g/l, Na_2HPO_4- 2 g/l, bacteriological agar - 2 g/l.

In order to improve the synthesis of vitamin B12 using corn - glucose medium supplemented with $CoCl_2 \times 6H_2O$ in an amount of 0,003 g/l.

IV. Results and Discussion

Propionibacterium shermanii with growth of dense medium are round or in the form of buckwheat

grains. Colonies creamy, moist, shiny, oily. The test cultures of microorganisms grown on agar medium are the most sticks of various sizes, from the very short, almost cocci before long. The liquid medium cells have a coccoid form part of the collection in the chain, sometimes branching, and found sticks of short (Figure 1).



A - Milk B - Rennet farm animals

Figure 1 - The morphology of propionic acid bacteria isolated from different sources (×100)

Next was held propionic acid bacteria strains selection with high accumulation of propionic acid. As seen in Figure 2, the amount of propionic acid formed after 15 days of culture ranged from 0,63 g/l to 2,53 g/l.

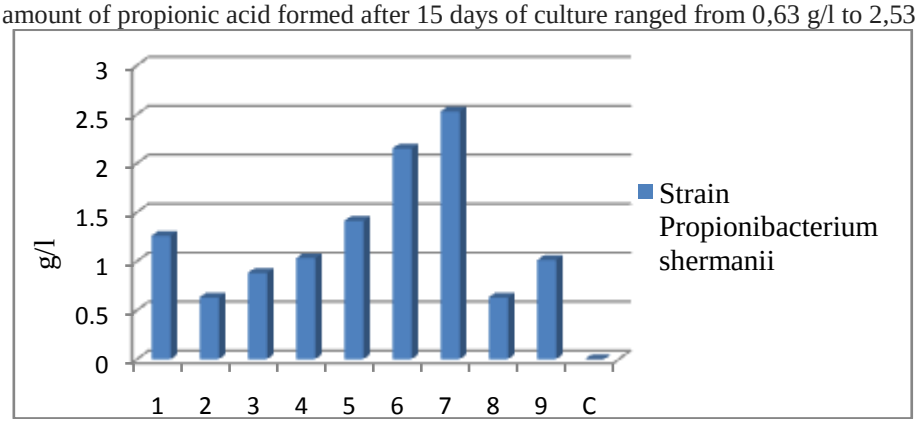


Figure 2 - Yield propionic acid after 15 days of culture

Maximum synthesis observed in embodiments №№ 6, 7 and is 2,15 g/l and 2,53 g/l respectively. Low acidifying strains possess the ability №№2, 3, 4, 8, 9 (0,63 – 1,03 g/l).

Also held propionic acid bacteria selected for the ability to synthesize vitamin B12. As seen in Figure 3 the best vitamin synthesizing capacity have strains №№1, 5, 6, 7. The number of synthesized vitamin is 1200-1070 mkg/ml.

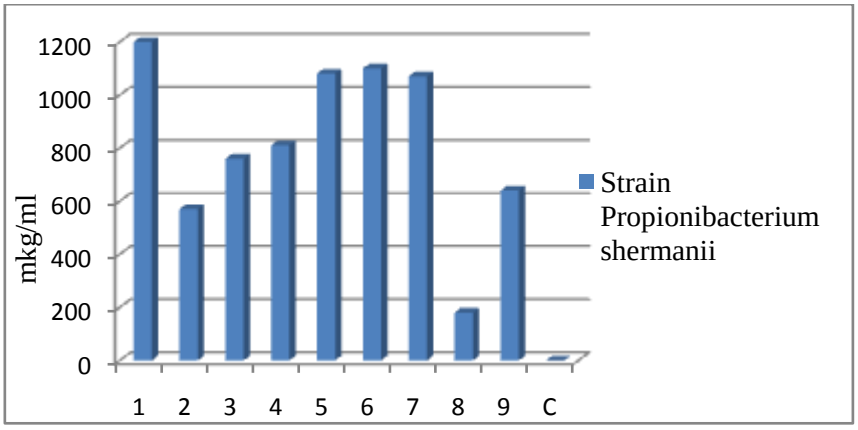


Figure 3 - Synthesis of vitamin B12 strains of propionic acid bacteria

Strain №8 synthesizes the least amount of vitamin B12 (180 mcgr/ml).

Thus, *Propionibacterium shermanii* isolates (№№ 1, 5, 6, 7), having the ability to synthesis of propionic acid and vitamin B12, are selected for further studies in order to improve safety, nutritional and biological value of food.

This study was conducted under the project "Development of the scientific basis for the use of new strains of propionic acid and lactic acid bacteria to improve safety, food and biological value of food" in the framework of the grant funding for research by the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan.

References

[1.] Ganin V.I. Stable leaven - quality and safe dairy products // Dairy industry. - 1999. - P. 25 - 26.

[2.] Bezborodov A.M. Biochemical basis of microbiological synthesis, M. - 1984. - 173 p.

[3.] Mountain O.N. A study of some basic factors that determine the production of dry preparations propionic acid bacteria // Engineering and technology of food production, 2011. - №4. - P.1-4.

[4.] Shenderov B.A. Medical microbial ecology and functional food. - M.: Grant, 2001 - Vol. 3. - 287 p.

[5.] Vorobova L.I. Propionic acid bacteria. M., 1999. - 300 p.

[6.] Khamagaeva I.S. Propionic acid bacteria ferments biotechnology. - Ulan-Ude, 2006. - 172 p.

[7.] Belozerova L.M. Development of the technology of fermented milk product using propionic acid bacteria // Abstract of diss ... candidate. tehn. sciences. Ulan-Ude. - 2000. - 36 p.