Isolation and biochemical characterization of Bacillus subtillis Nattō

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Introduction

Bacillus subtilis natto belongs to the *Bacillus subtilis* species and it is the basis for the production of traditional Japanese food. Nattō is a traditional Japanese dish made from fermented soybeans and is usually combined with soy sauce. It's very rich in vitamins. Nattō part of 100 g provides 29% of the recommended daily intake of vitamin K, 22% of vitamin C, 76% of manganese and 48% of daily requirements for iron and dietary fibre. It contains vitamins, amino acids, proteins, sugars, fats, minerals and dietary fibres, and polypeptides consisting of 275 amino acid residues with anticoagulant, fibrinolytic, blood pressure lowering effects and antioxidant activity[1]. Enzymes and proteins of this strain also show antithrombin effects similar to heparin, as well as antitumor activity. It has also been shown that *Bacillus subtilis natto* contains a nattokinase, which exhibits a strong fibrinolytic activity and activates other fibrinolytic enzymes[2].

Methods

The microorganism is isolated from the Japanese speciality: 1 g of Nattō is added to 9 mL of saline, resuspended and incubated in an aqueous bath at 80 °C. A dilution series (10^{-1}-10^{-9}) is made from which 1 mL of culture is taken and seeded on Petri mug with nutrient agar (peptone 1, 15 g, meat extract, 3 g, sodium chloride, 5 g, dicalium hydrogen phosphate, 0.3 g, agar, 18 g, distilled water, 1 L) and incubated at 28 °C. Pure, individual colonies (K1 and K2) were isolated by the serial dilution method.

The isolated microorganism was characterized by API 50 CHB/E tests.

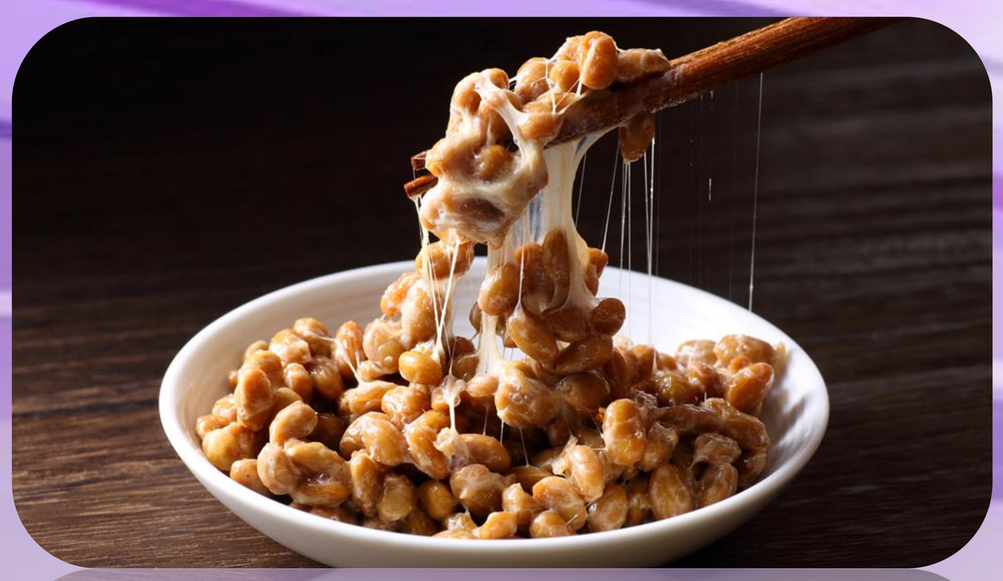

Figure 2. Japanese food- Nattō

The results

The results of the API 50 CHB/E test showed that the resulting microorganism belongs to the genus *Bacillus subtilis* with a percentage of agreement of 99.9%, with the literature data.

Figure 1. The results of the API 50CHB / E test for two colonies K1 (left) and K2 (right).

REFERENCES

1. Ho, H., Nguyen, H. Optimization of Bacillus subtilis Natto Imobilization Process on Alginate – Chitosan Complex and Its Application for Nattokinase Fermentation. Int. J. Pharm 2016;5:25-30.
2. V. Mani, L. C. Ming, Tempeh and Other Fermented Soybean Products Rich in Isoflavones. In: J. Frias, C. Martinez-Villaluenga, E. Peñas, Fermented Foods in Health and Disease Prevention. Academic Press 2017: 453-474.

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