

**Influence of algae *Chlorella vulgaris* on the growth and probiotic features of lactic acid bacteria**

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## Abstract

Algae are great, although currently underestimated, source of biologically active compounds, and may be used for the development of functional food. Despite the availability of extensive literature regarding the antimicrobial properties of algae, only a few studies have focused so far on the practical use of fermented food. The addition of algae improves the quality of food products and limits the use of chemical preservatives. The combination of fermented products containing lactic acid bacteria with algae allows not only to create products rich in all nutrients necessary for the proper functioning of the organism but also to create a new segment of fermented foods.

The scientific aim of the study was to determine the influence of algae on the growth and selected probiotic properties of lactic acid bacteria, such as survival in adverse environmental conditions (low pH, bile salts, phenol, and sodium chloride) and enzymatic activity. Moreover, the application aim of the research was to develop an innovative fermented product with the addition of algae *Chlorella vulgaris* and probiotic lactic acid bacteria, fixed by lactic fermentation, without the addition of chemical preservatives.

The effect of *Chlorella vulgaris* on the growth and acidifying activity of the lactic acid bacteria was determined. Tested strains of *Levilactobacillus brevis* reached a stationary phase after approximately 24 hours, while the addition of algae into the bacteria growth medium shortened their logarithmic phase (the stationary phase after about 18 hours). Another desired effect that was achieved by introducing algae into the bacteria culture medium was reduced production of D-lactic acid and increased production of L-lactic acid. The study of enzymatic activity showed that algae stimulate the synthesis of enzymes responsible for the breakdown of proteins and enzymes responsible for the synthesis of flavor-forming compounds in fermented foods.

In the next stage of the research, the protective effect of algae on the survival of lactic acid bacteria under adverse environmental conditions was demonstrated. One of the main requirements for selecting probiotics is their survival in bile salts. The obtained results revealed that the negative influence of bile salts was minimized due to the protective effect of algae. Moreover, apart from the ability of potential probiotics to tolerate bile salts, it is also expected that the probiotics will tolerate an acidic environment, therefore the survival of lactic acid bacteria at low pH was investigated. However, the protective effect of algae at low

pH is a strain-dependent feature. The addition of algae to the growth environment of *Levilactobacillus brevis* ŁOCK 0980 and MG451814 increased their survival at pH 2.5.

Therefore, on the basis of the obtained results, it was found that the tested strains of *Levilactobacillus brevis* cultivated in the presence of *Chlorella vulgaris* are characterized by faster growth, increased production of L-lactic acid, as well as high survival in adverse environmental conditions, which allows the use of algae in fermented products, and thus to create a new product on the health food market.

At the last stage of the work, an innovative lactose-free probiotic soya drink supplemented with *Chlorella vulgaris* was developed. Based on the previous research, the *Levilactobacillus brevis* ŁOCK 0944 strain with proven probiotic properties was selected for soya drink fermentation. Fermentation conditions and the appropriate amount of lactic acid bacteria were selected for the soybean drink. Moreover, the level of pH and total acidity were monitored. The finished product was obtained after 24 hours (4 hours of fermentation at 30°C and 20 hours of maturation at 20°C). The addition of algae accelerated the growth of bacteria in the soya drink and increased the amount of L-lactic acid production. Therefore, *Chlorella vulgaris* can be used as a natural growth stimulator of starter bacteria in the production of fermented beverages. The bacteria introduced into the gastrointestinal tract simulator together with a soya drink after the 30 days of refrigerated storage exhibited a considerably higher survival rate. Due to the number of bacteria and the survival rate during storage (8.4 - 8.7 log CFU/mL), the developed fermented soybean drink with algae *Chlorella vulgaris* (and also with the addition of xylitol) meets the criteria for a fermented probiotic product.

The results revealed that the combination of microalgae *Chlorella vulgaris* and *Levilactobacillus brevis* shows great potential for creating innovative, functional products that can provide a significant amount of lactic acid bacteria to the consumer, in addition to the nutritional properties of algae.

Key word: Algae, *Chlorella vulgaris*, *Levilactobacillus brevis*, fermented food, probiotic functional drink