

Bacteria from the *Lactobacillaceae* family in breast milk

Abstract

Breastfeeding is the gold standard in child nutrition, as confirmed by leading opinion-forming organisations such as the World Health Organization (WHO) and the American Academy of Pediatrics (AAP). Among the bioactive components, the microbiota of human milk can be mentioned. Potential probiotic microorganisms deserve special attention. The research undertaken in this study was aimed at characterizing, in the Polish population of breastfeeding women, the frequency of occurrence of *Lactobacillaceae* bacteria with potential probiotic activity in breast milk. Their properties, such as survival in low temperature conditions, resistance to antibiotics, possible impact on MCF-7 breast cancer cells and correlation with the content of macronutrients, were analyzed. Breast milk samples derived from 63 lactating women at various stages of lactation were tested. Simultaneously with the collection of milk samples, the women participating in the study were asked to complete a prepared questionnaire. The survey included questions relating to, among others: the circumstances of childbirth, state of health, medications and dietary supplements taken and the diet used. The questions were intended to characterise the study population and to indicate potential relationships between the microbiological composition of breast milk and the indicated factors. The study was approved by the local bioethics committee with the number KB921/2018. To identify bacteria belonging to the *Lactobacillaceae*, the method of culture inoculation on the MRS microbiological medium was used and the subsequent identification of the grown colonies using MALDI-TOF/MS and the polymerase chain reaction (PCR) technique. The research showed the presence of bacteria belonging to *Lactobacillaceae* in 22.2% of breast milk samples included in the study. Some of the identified strains were isolated and subjected to further research.

Antibiotic susceptibility testing was carried out using the disc diffusion method as well as antibiotic strips (E-TESTs). The study showed the sensitivity of bacterial isolates to ampicillin (10 µg), tetracycline (10 µg) and erythromycin (15 µg), as well as resistance to kanamycin (30 µg) and gentamicin (10 µg). In addition, the susceptibility to vancomycin (30 µg) among the tested strains varied. The obtained research results correlate with the results obtained by other authors.

Another research task undertaken in a doctoral dissertation was to characterise the survival rate of the tested strains in the environment of pasteurised human milk stored in refrigeration and freezing conditions. The pasteurisation process is intended to ensure microbiological safety for newborns using milk derived from milk banks. Unfortunately, the use of this technique adversely affects product quality. The high temperature (62.5 °C) causes the death of potential pathogens as well as microorganisms of a potentially probiotic nature. The conducted research indicated that the tested strains of bacteria belonging to *Lactobacillaceae* are characterised by good survival in the environment of pasteurized breast milk stored at a lowered temperature (which also takes place in milk banks), which makes it possible to use the discussed bacterial strains as supplementation of human milk subjected to high temperature.

In another study conducted as part of this doctoral thesis, the cytotoxic effect of metabolites of postbiotic strains of bacteria belonging to *Lactobacillaceae*, isolated from breast milk, against MCF-7 breast cancer cells was evaluated. The MTT test was used, in which supernatants containing post-biotic metabolites of the tested bacterial strains were used. The effect was checked after 24, 48 and 72 hours of incubation. The results showed that postbiotic metabolites derived from some bacterial strains isolated from breast milk could have a cytotoxic effect at the appropriate concentration.

The last issue undertaken in the doctoral thesis was characterising the profile of oligosaccharides in the tested samples. These determinations were made in cooperation with the Gdańsk University of Technology, where the analysis of HMOs contained in breast milk samples was performed. In addition, human milk samples were analysed, and the results were correlated with the content of microorganisms belonging to *Lactobacillaceae* and information obtained from completed questionnaires.

The conducted research indicates that breast milk originating from Polish breastfeeding women may be a source of bacteria belonging to *Lactobacillaceae*. Not all breast milk samples contain these bacteria, which does not mean that they are completely devoid of the probiotic microbiota, which includes many types of microorganisms. The conducted research is the basis for further research on using bacterial strains isolated from the breast milk environment in practice.