Abstract

According to the WHO, antibiotic resistance is a serious threat and one of the biggest public health problems. For many years, the problem of antibiotic resistance was associated only with the hospital environment. Over time, it has been shown that strains from other environments, including food can also be the source of antibiotic resistance. Many strains used industrially in food production have been recognized as its traditional ingredients, thanks to which they have obtained the status of safe and have been legally approved for use in food production in the European Union without the need to conduct tests confirming their safety. Since the analysis of the occurrence of resistance genes has been used relatively recently, there is no information about their occurrence in strains used in food production for many years.

The aim of the study was to analyze the occurrence and possibility of horizontal transfer of antibiotic resistance genes among strains from commercial starter and protective cultures. The impact of stress factors associated with food production and storage on changes in the expression of genes encoding resistance to antibiotics, as well as the frequency of gene transfer, was also analyzed.

The obtained results showed that the strains from the commercial starter and protective cultures may be carriers of acquired antibiotic resistance genes. The highest Minimum Inhibitory Concentrations (MICs) were reported for tetracycline (max. >256 µg/ml), kanamycin (max. 48 µg/ml) and chloramphenicol (max. 32 µg/ml). The most common resistance genes were *aph(3')-IIIa*, encoding resistance to kanamycin (n = 35; 60.3%) and chloramphenicol resistance *cat* (n = 32; 55.2%). The strains also demonstrated the ability to horizontally transfer resistance genes both *in vitro* and *in situ*. The *tet*M and *tet*K genes for resistance to tetracyclines were transferred most frequently.

After applying various variants of high-pressure treatment and after exposure to acid and osmotic stress, expression of genes encoding resistance to aminoglycosides (*aac(6')Ie-aph(2'')Ia* and *aph(3')-IIIa*), chloramphenicol (*cat*), ampicillin (*blaZ*) and/or tetracyclines (*tet*M, *tet*W) increased. Refrigerated storage resulted in a downregulation of *aac(6')Ie-aph(2'')Ia*, *aph(3')-IIIa* and *tet*M genes. The results obtained in the analysis of gene expression correlated with the results obtained in the phenotypic analysis. Changes in the frequency of transfer of the studied genes were also observed after exposure to stress factors.

The obtained results indicate that the strains included in the starter and protective cultures used in the food industry may be reservoirs of antibiotic resistance genes and participate in their spread. In addition, it has been shown that the conditions prevailing during the production, preservation or storage of food can contribute to changes in antibiotic resistance among strains that survived the process and to changes in the frequency of gene transfer, which leads to faster spread of resistance in the environment. It should be noted that while antibiotic resistance in starter and guard cultures is not an immediate threat to consumers, it is a cause for concern that cannot be ignored. This indicates the need to verify the strains used in the food industry in terms of their antibiotic resistance, so that they do not become an environmental reservoir of resistance genes.

Keywords: starter cultures, protective cultures, stress factors, antibiotic resistance, gene expression, horizontal gene transfer