

EVALUATION OF THE SAFETY OF USING ENTEROCOCCI BASED ON THEIR COMPLETE GENOME ANALYSIS

Mora N.V.¹, Zelena L.B.¹, Tkachuk N.V.²

¹Kyiv National University of Technologies and Design, Kyiv, Ukraine;
zelenalyubov@gmail.com

²T.H. Shevchenko National University «Chernihiv Colehium», Chernihiv, Ukraine

Introduction. Enterococci are lactic acid bacteria (LAC) containing both pathogenic and commensal microorganisms that are widespread in the environment, even as intestinal symbionts. Due to their resistance to salts and acids, strains of *Enterococcus* spp. can adapt to various environments, they are also involved in the fermentation of cheeses and dry sausages, in which they are believed to contribute to the development of the organoleptic characteristics of these products [1]. Besides, some strains of *Enterococcus* have been shown to produce antimicrobial compounds, including bacteriocins. Bacteriocins are used as natural preservatives to preserve a wide range of food products; which is sometimes seen as a probiotic characteristic. Bacteriocins are considered as a promising alternative to combat antimicrobial resistance [2]. Although certain antibiotic-resistant infectious enterococci strains, including *E. faecium*, have been identified in hospital patients, they very rarely pose a risk of infection to people outside the healthcare setting.

Genomes of various microorganisms are also characterized for the presence of mobile genetic elements (MGEs). MGEs are represented DNA fragments that can move within bacterial genome or between different genomes, transferring in such way the various coding sequences, including virulence, toxin and antibiotic resistance genes. The last one is called horizontal gene transfer and brings either benefits or drawbacks [3].

Sequencing the complete genomes of microorganisms with further processing using bioinformatics analysis tools provides easy access and study of the information encoded in the genome using existing tools and databases [4]. Bioinformatic analysis of the genome allows obtaining detailed information about the potential metabolic capabilities of the organism and creating a map that reveals the potential for genetic engineering manipulations with the aim of improving the strain for use in industry. In the study of adaptation and evolution, bioinformatics analysis is used to identify new genes that allow microorganisms to adapt and survive in diverse environments [5]. In the process of finding and identifying microorganisms for further use in various industries, bioinformatics plays a key role in the initial analysis of the safety of these strains, in particular, it determines genes for virulence or the synthesis of toxic metabolites that cannot be detected using microbiological or biochemical methods [6].

The aim of the presented study was to analyze the complete genome of enterococci regarding the presence of antibiotic resistance genes and mobile elements sequences.

Materials and methods. The complete sequences of two *E. faecium* strains were downloaded from GenBank. The genome of *E. faecium* strains was analyzed in terms of antibiotic resistance genes and mobile genetic elements. The bioinformatic tools and

databases were used for this purpose. The Comprehensive Antibiotic Resistance Database (CARD), free available database on the Internet, was used to search for antibiotic resistance genes. Database web address is <https://card.mcmaster.ca/>. CARD includes tools for molecular sequence analysis, including BLAST and Resistance Gene Identifier software for resistome prediction based on homology and single nucleotide polymorphism patterns. The Alien_Hunter tool was used to search for mobile genetic elements that may facilitate horizontal gene transfer [7].

Results and discussion. Two strains of *E. faecium* with contrast isolation sources were chosen from the database: strain H7 isolated from the honey bee and strain UC7251 isolated from the fermented sausage. Both strains had the similar general genome characteristics presented in the table 1.

Table 1. Main characteristics of genome of *E. faecium* strains.

Strain	Genome size, Mbp	GC content, %	Protein coding genes	rRNA genes	tRNA genes
<i>E. faecium</i> H7	2,65	37,8	2574	18	70
<i>E. faecium</i> UC7251	2,61	38,1	2517	18	70

Results obtained in the study showed that genome of strain H7 and strain UC7251 contained genes encoding resistance to fluoroquinolone, macrolide and rifamycin antibiotics, besides genes of tetracycline and erythromycin resistance were defined in strain UC7251. Analysis of mobile genetic elements in enterococci genome revealed 28 and 51 such sequences in H7 and UC7251, respectively. Horizontal gene transfer carries many potential hazards: it can increase the adaptability of bacterial species or promote spreading of virulence and antibiotic resistance genes, for example, among microorganisms.

Conclusions. Thus, analysis performed in the presented research determined antibiotic resistance genes and high level of mobile genetic elements in *E. faecium* genome that maybe the potential hazardous factors of using these strains in any biotechnological processes. Therefore, it suggests that careful analysis of enterococci genomes should be carried out as an initial step of bacterial application in medical, agricultural, food, pharmaceutical and other fields.

References:

1. Moreno MF, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of enterococci in food and health. *International journal of food microbiology* 2006; 106(1):1-24.
2. Vieco-Saiz N, Belguesmia Y, Raspoet R, et al. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in microbiology* 2019; 10: 57.
3. Vogan AA, Higgs PG. The advantages and disadvantages of horizontal gene transfer and the emergence of the first species. *Biol Direct* 2011; 6 (1): 1-14.
4. Abdelhamid AG, El-Masry SS, El-DougDoug NK. Probiotic *Lactobacillus* and *Bifidobacterium* strains possess safety characteristics, antiviral activities and host adherence factors revealed by genome mining. *EPMA Journal* 2019; 10: 337-350.
5. Martino ME, Bayjanov JR, Cafrey BE. Nomadic lifestyle of *Lactobacillus plantarum* revealed by comparative genomics of 54 strains isolated from different habitats. *Environmental Microbiology* 2016; 18: 4974–4989.
6. Wang S, Yang B, Ross P, et al. Comparative genomics analysis of *Lactobacillus ruminis* from different niches. *Genes* 2020; 11: 1–17.
7. Horne T, Orr VT, Hall JP. How do interactions between mobile genetic elements affect horizontal gene transfer? *Current Opinion in Microbiology* 2023; 73: 102282–102289.