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Review article

BENEFITS AND RISKS OF USING *ENTEROCOCCUS* SPECIES IN FOOD OR AS PROBIOTICS

Rozalina Yordanova[1], Petya Hristova[2], Magdalena Platikanova[2]
1)Medical College, Trakia University, Stara Zagora, Bulgaria.
2)Department of Hygiene, Epidemiology, Microbiology, Parasitology, and Infectious Diseases, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria.

ABSTRACT:

Background: Enterococci are ubiquitous and can be found in water, soil, plants and several food products. They are also considered part of the normal beneficial gastrointestinal flora of both humans and animals. The aim of this review was to provide comprehensive information regarding the benefits and risks of *Enterococcus* species use in food or as probiotics.

Review Results: The presence of enterococci in foodstuffs has long been considered a sign of poor sanitation. Contrariwise, due to their enzymatic and proteolytic activities, they are used as starters in food fermentation and play an important role in the development of the organoleptic properties of fermented foods. Enterococci can spoil processed meats but, in contrast, are important for the maturation and flavor development of some traditional cheeses and sausages. Because of the ability to produce bacteriocins possessing antimicrobial action, enterococci are used as protective cultures in food biopreservation. Additionally, they showed probiotic properties with various beneficial characteristics. However, in the last few decades, enterococci have emerged as one of the most important bacteria worldwide, major opportunistic agents causing a wide range of nosocomial infections. They are resistant or tolerant to many antimicrobials, and multidrug resistant enterococci belong to the so-called "therapeutic problematic microorganisms". According to WHO, vancomycin-resistant representatives are high-priority pathogens on the list of microorganisms for the treatment of which the development of new antibiotics is urgently needed. In addition, they possess multiple virulence factors.

Conclusion: The role of enterococci in the pathogenesis of numerous and severe infections requires serious comments and discussion about the benefits and risks regarding their presence in food and their use as probiotics.

Keywords: *Enterococcus* spp., foodborne pathogens, food safety, probiotics,

BACKGROUND

Enterococcusus species are ubiquitous microorganisms associated with a wide variety of habitats- they could be naturally found in water, soil, plants, and several food products. They are considered part of the normal beneficial flora of the gastrointestinal tract of both humans and animals. They are also associated with food, most likely as a result of contamination from plant or animal sources, but also appear to play a role in a variety of fermented food [1].

Due to their ability to convert glucose into lactic acid as the main product of primary metabolism, enterococci are classified as lactic acid bacteria (LAB). Compared to other representatives of LAB, their wide distribution in nature is explained by their resistance to growth-inhibiting factors such as salt, acidity, heat, desiccation, chemical disinfecting agents [1].

Enterococci occur and grow in various fermented foods. Their presence in foodstuffs has long been considered a sign of poor sanitation during production and processing. Contrariwise, they can play an essential technological role in several fermented food products and are part of the microbiota of various commercial fermented products, such as dairy, meat, fish, sea food and vegetables. Due to their enzymatic and proteolytic activities, they are used as starters in food fermentation and play an important role in the development of the organoleptic properties of fermented foods [2]. Enterococci can spoil processed meats but, in contrast, are important for the maturation and flavor development of some traditional cheeses and sausages, especially those produced in the Mediterranean area [3]. Because of the ability to produce the so-called bacteriocins, possessing antimicrobial activity, enterococci are used as protective cultures in food biopreservation. They are also used as probiotics and in the production of feed additives with various beneficial characteristics - immunity stimulation, anti-inflammatory activity, diseases protection, etc. in humans and slaughter animals [4].

However, in the last few decades, enterococci have emerged as one of the most important bacterial species worldwide, major opportunistic agents causing a wide

range of nosocomial infections, especially in intensive care units. They are resistant or tolerant to many antimicrobials, and multidrug resistant enterococci belong to the so-called "therapeutic problematic microorganisms" group. Moreover, according to the World Health Organization (WHO), vancomycin-resistant members of the genus are high-priority pathogens on the list of microorganisms for the treatment of which development of new antibiotics is urgently needed. In addition, they possess multiple virulence factors that aid in their resistance to host defence mechanisms.

To date, the genus *Enterococcus* has not yet obtained the status generally recognized as safe (GRAS), and despite several safety issues, many members of the genus are well characterized and have served as starter cultures, co-cultures or protective cultures in the food industry and/or probiotics due to their positive features. They are also used in the production of feed additives to prevent diarrhea or to improve growth in animals [4].

Enterococcus spp. (at the same time, useful as participants in food fermentation and as a probiotic and dangerous as an opportunistic pathogen) is a critical topic that needs continuous debate. Therefore, the question of whether enterococci are safe for use in food and as probiotics is still difficult to answer.

The aim of this review was to provide comprehensive information regarding the benefits and risks of *Enterococcus* spp. use in food or as probiotics.

REVIEW RESULTS

It is essential to analyze food for the presence of pathogens ant their toxins to ensure a safe and reliable food supply and minimize foodborne pathogen diseases among consumers.

1. *Enterococcus* spp. applications

1.1. Enterococci as probiotics

According to the European Food Safety Authority (EFSA), in terms of safety, *Enterococcus* spp. do not meet the criteria for the "Qualified Presumption of Safety" and do not have Generally Recognized Safe Status due to the pathogenic nature of some of the members and the possessing of virulence factors [3, 5]. Therefore, due to safety concerns, lack of safety information, and legislation, only a limited number of enterococcal probiotics are commercialized and new recommendations regarding the probiotic legislative framework are needed to correctly distinguish between safe and potentially harmful *Enterococcus* strains.

Probiotics are "live microorganisms that provide health benefits to the host when administered in adequate amounts" [6]. In recent years, the consumption of probiotics has been increasing. A number of studies have shown that probiotics are known for their positive effects on human and animal health, such as modulating the immune system, reducing metabolic disorders, reducing hypersensitivity to allergens, enhancing intestinal barrier function, preventing intestinal inflammation, controlling diarrhea and improving feed digestibility [7]. In terms of feed regulation, the EFSA has authorized certain enterococcal probiotics for use as silage supplements and dietary supplements like intestinal ecosystem enhancers, antibiotic substitutes and feed additives to stabilize the microbial communities of the digestive tract in both monogastric and ruminant animals [8]. Enterococci have been used as probiotics for different purposes and in different fields- pharmaceutical industry, human and veterinary medicine and the food industry and a number of studies evaluating the probiotic characteristics of *Enterococcus* strains have reported their beneficial and significant health-promoting effects [9, 10]. It is really necessary for the various government institutions to make efforts to develop strategies and implement various practices to promote healthy eating among the general public [11].

1.2. Enterococci and their bacteriocins

A wide range of enterococci and the bioactive peptides (enterocins) they produce have been reported and approved as promising probiotics in the livestock industry in terms of growth promotion, health improvement, combating multiple drugs resistant pathogens, increasing metabolic efficiency and immunological parameters, alleviating antibiotic-induced diarrhea and maintaining animal integrity [12, 13]. Such beneficial effects are aimed at almost all types of animals - from farm animals to aquaculture and even pets. In recent years, purified and identified enterococcal bacteriocins have been added to foods in the form of concentrated preparations or produced in situ by bacteriocinogenic starter, supplemental or protective cultures [14]. The antimicrobial activity of enterococcal bacteriocins against food pathogens and spoilage bacteria has attracted considerable attention for their application in food preservation [15, 16]. In addition, the use of bacteriocins can help reduce the use of chemical preservatives and/or intensity of heat and other physical treatments [17].

1.3. Enterococci in dairy products

Enterococci are important to the dairy industry as well and can act as natural starters in raw milk. They are able to survive during milk refrigeration and pasteurization temperatures due to their psychrotrophic nature, heat resistance and adaptability to different substrates and growth conditions [18]. Enterococci are commonly present as non-starter microflora in cheese in Southern Europe and are important in the maturation of varieties of cheese due to their proteolytic or lipolytic activity, their ability to utilize citrate and to produce aromatic volatile compounds that contribute to the flavor and taste [2]. Some enterococci of dairy origin that produce bacteriocins are known to be effective in eliminating food spoilage or pathogenic bacteria such as *Listeria monocytogenes, Salmonella, Clostridium* spp., etc.

1.4. Enterococci in meat products

Enterococci are normal representatives of the natural microflora of many fermented meat products but can also be found in raw meat, fish and fermented seafood,

chicken and fermented meat products such as sausages [19, 20]. These microorganisms exhibit essential functional properties in meat, such as development of the sensory characteristics of fermented meat products, especially in sausages, metmyoglobin-reducing activity, and the ability to degrade antinutritional factors such and bile salt hydrolase production [19]. Bacteriocin-producing enterococcal strains are used as protective cultures in ready-to-eat meats [2]. The bacteriocins are heat stable, making it possible to add them to or on foods that can be heated or cooked [21]. Their use, combined with other barrier technologies, may represent a useful approach to increase antimicrobial efficacy [22]. However, because of the meat microbiota activity, harmful reactions, such as the formation of biogenic amines, are also possible. The presence of biogenic amines in food represents a potential risk to public health due to their physiological and toxicological effects. Because of their vasoactive properties, they could cause food poisoning in high concentrations [23]. These substances also affect the freshness and other organoleptic properties of meat and meat products [19]. *Enterococcus* spp. are among the microorganisms that can accumulate higher biogenic amines and are known to be the most efficient producers of one of the most dangerous biogenic amines (tyramine) in fermented foods [23]. Therefore, taking control measures (hydrostatic pressure, irradiation, controlled atmosphere packaging) to prevent the formation of biogenic amines in foods or to reduce their levels once they are formed is of utmost importance [24].

1.5. Other application

It is not clearly defined whether the source of *Enterococcus* spp. in plants is endogenous or a result of environmental contamination. Enterococci are found in fermented green olives and are probably involved in their fermentation process due to the good adaptation to the pH and salt concentration of the brine used in the process [25]. Some species are also found in the raw materials used in beer production [26]. In addition, they are associated with sorghum and soybean fermentations [27, 28]. Furthermore, enterococcal bacteriocins are used as bioprotective agents in many food matrices, such as fruit juices and ready-to-eat, fermented and unfermented vegetables [29].

2. Enterococcus spp. as nosocomial pathogens

Over the past few decades, *Enterococcus* spp. turn out to be one of the most important bacterial species worldwide, major opportunistic agents causing a wide range of nosocomial infections (urinary tract infections, including catheter-associated, surgical wound infections, endocarditis, bacteremia, meningitis, etc.), especially in intensive care units. The host or environmental factors, most notably exposure to antimicrobial agents, may favor an increase in the colonization density of enterococci in the gastrointestinal tract of hospitalized patients [30]. Antibiotic therapy that leads to the depletion of Gram-negative bacteria may promote the outgrowth of antimicrobials resistant enterococci [31]. On the other hand, high-density colonization of the patient gastrointestinal tract facilitates the transmission of antimicrobials resistant enterococci among hospital ward through fecal contamination [32].

Enterococci are resistant or tolerant to many antimicrobials. They possess the ability to exchange a wide variety of genetic elements for antibiotic resistance both within the genus and with other genera, for example, *Staphylococcus, Streptococcus* and *Bacillus* [33]. Multidrug resistant enterococci belong to the group of so-called "therapeutically problematic microorganisms". Moreover, according to the WHO, vancomycin-resistant members of the genus are high-priority pathogens on the list of microorganisms for the treatment of which the development of new antimicrobial agents is urgently needed. Vancomycin-resistant enterococci are a serious threat to hospitalized patients, especially catheterized ones, and to immunocompromised individuals. Their release can contaminate the environment or enter animal and human food chains, spreading vancomycin resistance.

Enterococci possess many genes encoding virulence factors that enable them to survive not only in harsh conditions but also in a hospital environment, which allows them to maintain infection in vulnerable hosts. The virulence factors that enterococci possess facilitate their adhesion to host cells, colonization, biofilm formation, invasion and support their resistance to host defence mechanisms. The substances that function in the adhesion process include the enterococcal aggregation substances and enterococcal surface proteins. Hyaluronidase, a proteolytic protein, is implicated in the degradation of mucopolysaccharides that connect tissue and cartilage and, therefore, in spreading bacteria [34]. Biofilm production plays an important role in the pathogenesis of enterococcal infections, provides a survival advantage to the microbial community, and favors the prolongation of the infection because of the limited penetration of antimicrobial agents [35].

CONCLUSION

Enterococcus species are part of the normal flora of humans and animals. Despite the characteristics that have led to their use in various fields as natural food preservatives, probiotics or feed additive, there is considerable concern regarding their application. Their increasing importance as nosocomial pathogens, the risk of transfer of antimicrobial resistance, the progressively frequent appearance of vancomycin-resistant strains, their flexible genome and the presence of virulence factors question their status as harmless bacteria in food. Therefore, it is necessary to pay special attention to the *Enterococcus* species to implement appropriate guidelines and relevant legislation regarding their application in food or as probiotics, and the benefit/risk ratio should be carefully assessed before using them.

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Address for correspondence:
Dr Rozalina Yordanova,
Medical College, Trakia University, Stara Zagora,
9 Armeiska Street, 6000 Stara Zagora, Bulgaria
Email: rozalina.yordanova@trakia-uni.bg