Enterococci: yin - yang microbes

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Summary

This review deals with the duality of enterococci, which can be illustrated by their yin - yang behaviour. The rough nature of this specific group of lactic acid bacteria promotes their dissemination in various environments where they significantly influence the outcome of a certain process. In the technological meaning, enterococci are leading microbes in fermentation processes of traditional foods, where their detrimental spoilage activities are equally significant. As therapeutics, enterococci manifest the probiotic properties through their positive effects on maintaining of the normal intestinal microflora, on stimulation of the immune system, on improved nutritional value of food and with the production of antimicrobial compounds (bacteriocins). At the same time, enterococci present an emerging pool of opportunistic pathogens for humans as they cause disease, possess agents for antibiotic resistance and their transfer mechanisms, and are frequently armed with potential virulence factors. Despite the yin - yang characteristics of enterococci, the long history of safe use of certain strains of enterococci in food/feed, and reliable identification and classification of enterococci with phenotypic methods supported with modern genetic tools, enables selection of promising enterococci, which could be safely used as starter cultures or food/feed additives.

Key words: enterococci, beneficial and detrimental role, identification, classification, safety

Introduction

Enterococci are notorious as ubiquitous microbes inhabiting soil, food, water and gastrointestinal (GI) tract of humans and animals. Genus *Enterococcus* currently comprises more than 26 species, with *E. faecalis* and *E. faecium* being two predominant species mostly found, especially in food (Giraffa, 2003; Klein, 2003).

Because of their abundance in different ecological niches, enterococci are significant in environmental, food and clinical microbiology. From the technological point of view they play an important role in the production of various traditional fermented food products in Europe (Gelsomino et al.,

2001; Andrighetto et al., 2001) where they are, on the other hand, also associated with spoilage of foods, especially meats (Franz et al., 1999). Another beneficial effect of enterococci is seen in their probiotic function, which is claimed to include maintenance of the normal intestinal microflora, stimulation of the immune system and improvement of foods nutritional value (Franz, 1999). Unlike their health promoting activities, certain enterococcal strains can cause human disease such as endocarditis, bacteraemia, urinary tract and neonatal infections. A specific cause for concern is their resistance to a wide variety of antibiotics. The intrinsic resistance of enterococci to a variety of antibiotics is mediated by genes chromosomally located while acquired resistance is mediated by genes residing on plasmids or transposons which can be transferred vertically and horizontally among bacteria (Kayser, 2003). Enterococci are also known to harbour useful biotechnological traits, such as production of bacteriocins, called enterocins. According to published reports, some enterocins have been described as antimicrobial peptides active against closely related and other lactic acid bacteria, listeria and clostridia, and therefore show potential in food control strategy (Giraffa, 1995).

The duality of enterococci rise doubts about their use as probiotics, as starter cultures in the food industry or as animal feed additives. Fortunately, the development of modern identification and classification methods together with clinical trials and long history of safe use of particular enterococci in production of traditional fermented foods classify enterococci as microbes with no particular risk for human health. However, the exact characterisation and safety assessment of a strain is crucial step needed prior any selection of strain in human or animal purposes. For instance, *Enterococcus faecium* SF68 has been studied and well documented over a period of more than 20 years for use as a human probiotic, especially in the treatment of diarrhoea (Franz, 1999).

Traditional fermented products offer a remarkable reservoir of "natural" microbes, including enterococci. With the use of proper phenotypic and molecular techniques, interesting and safe enterococci with potential use as starter cultures or as feed additives can be described and selected. Moreover, selection of natural isolates significantly contributes to the preservation and protection of the diversity and autochthonism of enterococci that are responsible for the deliciousness of traditional fermented products.

Irrespective of their yin-yang nature, enterococci remain one of the leading microbes concerning clinical, food and environmental future perspectives. Clearly, before application of enterococci in the dairy products, their thorough investigation, concerning clinical aspect, is demanded. This

review is focused on balanced evaluation of both, beneficial and detrimental nature of enterococci, because only clear understanding of their status may allow their safe use as a starter, or supportive cultures.

Enterococci - the ubiquitous microorganisms

It is well known that enterococci are natural inhabitants in soil, food, water and in GI tract of humans and animals, mostly mammals and birds. The main reason for their successful survival and persistence in such microbiologically diverse environments is in their rough nature. Enterococci are resistant to drying, heat and sanitazing agents. They grow from 10 to 45 °C, survive heating at 62.8 °C for 30 min, tolerate 6.5 % NaCl and 40 % bile, grow between pH 4.0 and 9.6 (Stiles, 2002) and can therefore efficiently compete for nutrients and space.

Enterococci were formerly classified as "faecal" or Lancefield group D streptococci which indicate that, despite enterococcal dissemination, the predominant habitat of enterococci still remains the gastrointestinal tract of humans and animals. Good adaptation on rigorous intestinal conditions gives enterococci excellent opportunity for their activities, whether beneficial or adverse. Moreover, faecal contamination seems to be one of the most important routes for their spreading, especially in various foods from animal origin. For a long time, the presence of enterococci in foods served as an indicator of poor hygiene, but nowadays enterococci are recognised as normal parts of the food microflora (Franz et al., 1999).

The environmental abundance and activities of enterococci have excited their high respect among scientists. Therefore, many researches have been lately focused on detailed identification and characterisation of this interesting group of microorganisms.

Beneficial activities of enterococci

From the positive side enterococci are closely involved with the production of traditional fermented foods, specially cheeses and meats. They colonise raw foods of animal origin (milk, meat) through intestinal and/or environmental contamination, where can survive and multiply during fermentation processes. The biochemical activities of enterococci in sausage matrix have not been studied thoroughly, but they might contribute to sausage aromatisation by their glycolytic, proteolytic and lipolytic activities (Hugas et al., 2003). Enterococci occur and grow in a variety of artisanal cheeses, produced from raw or pasteurised milk. Their historical bad reputation of faecal contaminants has recently turned into significant and important part of

the ripened cheeses bacterial flora. Enterococci show higher proteolytic activities than other lactic acid bacteria (LAB) which is important for cheese ripening. Their beneficial effect in cheese making has also been attributed to hydrolysis of milk fat by esterases. Additionally, enterococci produce typical flavour components such as acetaldehyde, acetoin and diacetyl. This beneficial role of enterococci in development of cheese aroma has led to inclusion of enterococcal strains in certain starter cultures (Franz et al., 1999). For example, two cheese isolates, *E. faecium* FAIR-E 198, a strain isolated from Greek Feta cheese, and *E. faecium* FAIR-E 243, a strain isolated from Sardinian cheese, were proposed to be applied as adjunct starter in Greek Feta cheese making for their positive impact during the ripening. Based on the overall evaluation of the results obtained from the microbiological, physicochemical and sensorial analyses, the most pronounced impact of both *E. faecium* strains, either as sole adjunct starter or in combination, has been observed on the growth of the starter culture, casein degradation and the organoleptic properties of the mature cheese (Sarantinopoulos et al., 2002a). Similarly, in Tetilla cheese production, an ideal starter should include *Lc. lactis* subsp. *lactis* and selected enterococci with the aim of achieving a high lipolysis and specific proteolysis over α_{S1}-casein (Menendez et al., 2004). Nevertheless, many reports describe the abundance of enterococci in traditional cheeses thus fortifying their position as normal part of cheese microflora, with *E. faecalis* being the predominant species (Andrighetto et al., 2001; Čanžek Majhenič et al., 2005).

Therapeutic properties of enterococci manifest through probiotic activities including maintenance or restoration of the normal intestinal microflora and thereby prevention or reduction of gastro-intestinal disorders, alleviation of lactose intolerance, reduction in serum cholesterol levels, anticarcinogenic activity, stimulation of the immune system and improved nutritional value of foods (Franz et al., 1999). Most probiotic LAB strains derive from healthy humans and are therefore naturally associated with the mucosal surfaces of the mouth, GI and genitourinary tract, where they are present as typical commensals. Enterococci are minority (up to 1%), but important members of the bacterial community inhabiting large bowel of adult humans with *E. faecalis, E. faecium and E. durans* as prevailing species (Tannock and Cook, 2002). Products for human consumption containing probiotic organisms are most often sold as infant foods, cultured milks and pharmaceutical preparations/animal feed additives where few strains of *E. faecium* and *E. faecalis* find application. *E. faecium* SF68 is probably the best studied in terms of probiotic activity. When administered to both human and animals, strain

was shown to be effective in the treatment of intestinal disorders. This strain is also resistant to low pH, insensitive to bile salts and individuals show a high tolerance to it, with no side effects (Franz et al., 1999). Another controlled studies revealed that *E. faecium* SF68 significantly reduced the incidence of diarrhoea in chronic pulmonary tuberculosis patients receiving long-term treatment. It was also effective as lactulose in lowering blood ammonia and for improving mental state and psychometric performance of patients with hepatic encephalopathy. At present, there are no unified regulations available concerning correct labelling, safety and functionality of the commercially available probiotic preparations. Labelling issue and antibiotic susceptibility of bacterial isolates (including *E. faecium*) of 55 probiotic products has been recently reviewed by Temmerman et al. (2003), where they conclude that quite a number of products are incorrectly or inadequately labelled, as well as certain strains showed antibiotic resistance. It is important that probiotic products designed especially for their health promoting purposes are safe and well-documented in order to provide consumers with the full benefits of the probiotic aspects.

Another common and beneficial trait among enterococci seems to be bacteriocin production. These, so called enterocins, are mainly produced by *E. faecalis* and *E. faecium* strains and generally belong to class II bacteriocins which are regarded as small, heat stable and membrane active non-lantibiotics (van Belkum and Stiles, 2000). From the very beginning of enterococcal bacteriocins researches, enterocins have been frequently reported as bacteriocins with antilisterial effect (Giraffa, 1995). Concerning the latter, inhibition of *L. monocytogenes* and other *Listeria* spp. by enterococci gives the essential information on their interest as food-grade preservatives, to be used in dairy technology. Two main applications concerning bacteriocins are possible: the use of either antimicrobial proteins as additives or bacteriocinogenic strains directly in food system. Many reports consider possible use of enterocins/bacteriocinogenic enterococci as starters in different food models, where can efficiently serve as a barrier in a hurdle technology (Laukova et al., 1999a; Laukova et al., 1999b; Sarantinopoulos et al., 2002b; Foulquie Moreno et al., 2003; Sabia et al., 2003). Moreover, enterococcal isolates, selected from slovenian traditional Karst ewe's cheese, exerted strong antilisterial effect which could be a result of bacteriocinogenic activity (Mohar et al., 2005). Well-characterised *Enterococcus* bacteriocins are enterocin A, enterocin B, enterocin 50, bacteriocin 31 and enterocin AS-48 (Franz et al., 1999). In their research, Rodriguez et al. (2000) confirmed high incidence of bacteriocin-producing LAB in raw milk samples, with

inhibitory activity against both spoilage and pathogenic microorganisms. Moreover, AS-48 producing enterococci have been found at high proportion especially in goats' and ewes' milk. Enterocins with broad inhibitory spectra undoubtly indicate their use as biopreservative agents in dairy systems. As proposed by Giraffa (1995), careful identification followed by thorough testing based upon certain criteria must be accomplished before putting any of enterocins (or enterocin producing strains) to practical use in the dairy industry.

Detrimental activities of enterococci

Unlike their positive technological traits, enterococci can become a spoilage problem in traditionally fermented meat products and also in cooked, processed meats, especially if initially present in high numbers (Giraffa, 2002). The persistence of enterococci in fermented meat products can be attributed to their wide range of growth temperatures and their high tolerance to salt (Hugas et al., 2003). According to some authors, formation of biogenic amines seems to be unfavourable activity of enterococci in fermented meat products, where microbial growth, acidification and proteolysis offer propitious conditions for biogenic amines production. All non-starter enterococci, isolated from fermented pork sausages, were reported to produce tyramine and phenylethylamine, while no tryptamine, putrescine or cadaverine was detected (Bover-Cid et al., 2001). The prolific growth of enterococci of a dairy origin in milk and milk products leading to formation of significant levels of biogenic amines has been observed as well (Martuscelli et al., 2005).

According to many authors, enterococci numbers vary from 10^4 to 10^6 CFU/g in cheese curd and from 10^5 to 10^7 CFU/g in the fully ripened cheese (Franz et al., 1999; Giraffa, 2003). Similar observations were recently reported for Tolminc, an artisanal Slovenian cheese, where in fully ripened cheese enterococci reached numbers of up to 10^6 per g (Čanžek Majhenič et al., 2005). Varying levels in different cheeses result from the cheese type, the production season, the extent of milk contamination, and survival in the dairy environment (dependent on seasonal temperature), as well as survival and growth under the particular conditions of cheese manufacture and ripening (Foulquie Moreno et al., 2006). High levels of contaminating enterococci, most often caused by poor hygienic practices during cheese manufacture, lead to deterioration of sensory properties in some cheeses. It is known that casein degradation performed by enterococci play an important role in the development of texture in cheese. This statement could be true to the certain

level as some peptides contribute to the formation of flavour, while others, undesirable bitter-tasting peptides can lead to off-flavours. Concerning the lipolytic activity of enterococci in cheese, limited and even contradictory data appears in the literature. Lipids in cheese contribute to cheese flavour as being source of fatty acids, which may be further converted to methyl ketones and lactones, being a cause of oxidation of fatty acids, leading to the formation of unsaturated and flavoured aldehydes causing a flavour defect referred to as oxidative rancidity, and being solvents for aromatic compounds of fatty acids (Foulquie Moreno et al., 2006).

More seriously, a number of nosocomial infections such as endocarditis, bacteraemia, urinary tract and neonatal infections have been associated with an increasing incidence of enterococci, predominantly strains of *E. faecalis* and, to a lesser extent, *E. faecium*. Over the last two decades, enterococci, formerly viewed as organisms of minimal clinical impact, have emerged as opportunistic pathogens of humans. Major risk factors for acquiring nosocomial enterococcal infections are serious underlying disease, a long hospital stay, renal insufficiency, neutropenia, liver or bone marrow transplantation, presence of urinary or vascular catheters, treatment in an intensive care facility, and a preceding antibiotic therapy for other infectious diseases with antibiotics against which enterococci possess a natural resistance. In this connection, superinfections with enterococci can occur (Kayser, 2003).

The virulence gene expression

Enterococci are intrinsically (naturally) resistant to a number of antibiotics, which are mediated by genes located on the chromosome. This is distinct from acquired resistance that is mediated by genes residing on plasmids or transposons. Acquired resistance can occur if two prerequisites are present: the genetic potential by the microorganisms (accumulation of mutations in the "own" DNA that finally leads to resistance or acquisition of transferable resistance genes from donor cells) and the antibiotic selective pressure (therapeutic use of antibiotics) (Klare et al., 2003). Examples of intrinsic antibiotic resistance include resistance to cephalosporins, β-lactams, sulphonamides and low levels of clindamycin and aminoglycosides, while examples of acquired resistance include resistance to chloramphenicol, erythromycin, high levels of β-lactams, fluoroquinolones and glycopeptides, such as vancomycin (Franz et al., 2003). Vancomycin resistance is of special concern because this antibiotic was considered a last resort for treatment of multiple resistant enterococcal infections. The emergence of vancomycin-

resistant enterococci (VRE) in hospitals has led to serious infections that cannot be treated with conventional antibiotic therapy. Today, 6 different gene clusters mediating glycopeptide resistance have been described in enterococci: *vanA*, *vanB*, *vanD*, *vanE* and *vanG* which are known to be acquired traits, while *vanC* is an intrinsic property of motile enterococci. The *vanA* cluster mediates high-level inducible resistance to both glycopeptides (vancomycin and teicoplanin), while *vanB* cluster causes inducible low- to high-level resistance to vancomycin only. *vanA* and *vanB* resistance are usually acquired through the Tn*1546* transposon. *vanD*, *vanE* and *vanG* clusters do not appear to be transferable (Kak and Chow, 2002). The *van*A type of enterococcal glycopeptide resistance is the most important one and its main reservoir is *E. faecium* (Klare et al., 2003). Many debates concerning the source of VRE have been opened, but it seems that farm animals, due to the avoparcin use (glycopeptide antibiotic used as a growth promoter in animal feeds), constitute an important reservoir of VRE that could be transmitted to the hospital environment via contaminated meat (Franz et al., 1999). Besides VRE, isolated from a wide variety of farm animals, there are some reports on traditional European cheeses dealing with detection of multiple drug resistant enterococci. The later indicates strong correlation between the use of antibiotics in human medicine and animal husbandry, and opens the question on their entering to the food chain. Moreover, some authors isolated VRE from healthy subjects living in the community and with no history of hospitalisation or antibiotic treatment. This suggests that development of strains resistant to antibiotics is not a phenomenon restricted to hospitals and that the food chain may play a role in spreading the glycopeptide-resistant enterococci (Mannu et al., 2003) Therefore, enterococci can be naturally transmitted from food animals or foods to the human intestinal tract.

Antibiotic resistance alone cannot explain the virulence of enterococci. In order to become pathogenic, they need to express virulence traits associated with adhesion, translocation and evasion of immune response and cause pathological changes either directly by toxin production or indirectly by inflammation (Franz et al., 2003). Known virulence traits in enterococci include adherence to host tissues where aggregation substance (*agg*) and extracellular surface protein (*esp*) play a mayor role, production of adhesin-like *E. faecalis* and *E. faecium* endocarditis antigens (*efaAfs*, *efaAfm*), secretion of cytolysin (*cyl*) and other toxic products (*gelE* protease) and production of plasmid-encoded sex pheromones (*cpd*, *cob*, *ccf*, *cad*). Cytolysin is responsible for lysing a broad range of eukaryotic and gram-positive cells, while gelatinase (*gelE*) acts on collagenous material in tissues. Sex

pheromones facilitate conjugation (Eaton and Gasson, 2001). Although mechanisms of acquisition of antibiotic resistance and spread have been well studied and enterococcal virulence and pathogenic mechanisms are still largely unknown, some facts concerning virulence determinants are ascertained. When Eaton and Gasson (2001) investigated the incidence of known virulence determinants in starter, food, and medical strains of *E. faecalis* and *E. faecium*, they have found that medical *E. faecalis* strains had more virulence determinants than food strains did, which in turn, had more than starter strains did. *E. faecium* strains were generally free of virulence determinants, with notable exceptions. *gelE* and *esp* determinants were found in *E. faecium* strains from all three origins, although in the past, these were found exclusively in *E. faecalis* strains. This phenomenon could be related to the increasing occurrence of pathogenic *E. faecium* strains. *efaA* determinant was found at similar frequencies in the *E. faecalis* and *E. faecium* strains and appears in all three groups. Similar distribution of virulence determinants was determined for *E. faecalis* strains isolated from Tolminc traditional Slovene cheese (Čanžek Majhenič et al., 2005). On the other hand, low incidence of virulence determinants in *E. faecium* from dairy, ovine and medical strains was reported by Mannu et al. (2003) as no strain carried more than one virulence determinant. Moreover, among enterococci isolated from food, Franz et al. (2001) observed, that 10.4 % of *E. faecium* strains were positive for one or more virulence determinant, compared to 78.7 % of *E. faecalis* strains.

Regarding to all written down, the question whether enterococci are safe for use as starter cultures remains difficult to answer. The principal concern for enterococci in the food supply is their pathogenic potential based on horizontal transfer of genes for factors associated with virulence and antibiotic resistance. With *in vitro* studies, Eaton and Gasson (2001) were able to show that virulence genes on a pheromone-responsive plasmid could be transferred to strains of *E. faecalis* used as started cultures in food, but they were not able to transfer virulence genes into strains of *E. faecium* starter cultures. However, present evidence does not suggest enterococci as foodborne pathogens, but the food chain has clearly been established as an important source of enterococci in human environment, where some strains may bear antibiotic resistance and virulence traits. The control and safety of foods that contain enterococci is a special challenge to food industry because of their robust nature, their wide distribution and their stability in the environment.

How to distinguish the good guys from the bad guys?

Fortunately, this question is not doubtful anymore because of available identification and classification methods, including modern phenotypic and powerful molecular tools (for review see Domig et al., 2003).

Besides fundamental differentiation of enterococci, well known phenotypic typing methods are used as follows: biotyping, meaning the detection of carbohydrate fermentation and enzyme pattern, usually includes a battery of bacteriological tubes containing different carbohydrate sources and indicator dyes. Most of the commercially available kits are indicator-based determination of sugar utilisation or show reactions based on their specific microbial enzymes, and are known as: *api 20 Strep* (Bio-Merieux, Mracy l'Etoile, France), *api 50 CH* (Bio-Merieux), *Rapid ID32 Strep* (Bio-Merieux) and *api zym* (Bio-Merieux). Recently, *PhenePlate*™ PhP plate system (PhPlate Microplate Techniques, Stockholm, Sweden) proved successful for environmental (Čanžek Majhenič et al., 2005) and epidemiological studies of enterococcal isolates, yielding results similar to those obtained by pulsed-field gel electrophoresis (PFGE) (Kuhn et al., 1995). Another phenotypic method is sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) which compares the whole-cell protein patterns of unknown isolates on the species and/or subspecies level (Descheemaeker et al., 1994). Among other typing methods there are multilocus enzyme electrophoresis (MLEE), antimicrobial susceptibility testing, serotyping, long-chain fatty acid analysis, fatty acid methyl esters (FAME) analysis, enterocin typing, pyrolysis mass spectrometry (pyMS), vibrational spectroscopic methods and proton magnetic resonance spectroscopy (1^{H}MRS) (Domig et al., 2003).

More rapid and reliable identification and differentiation of strains is conducted by application of molecular biology techniques. The first generation of genotypic typing methods based on the analysis of plasmid content and plasmid DNA restriction digests. Next step of typing methods included total chromosomal restriction digests, known as DNA fingerprint or profile, and analysis of restriction fragment length polymorphism (RPFL) by hybridisation with probes (eg. ribotyping). The development of pulsed-filed gel electrophoresis (PFGE), which allows global chromosomal comparisons, in the mid-1980s ushered in the third generation of genetic methods together with the discovery of polymerase chain reaction (PCR) and consecutively most PCR-based amplification methods: randomly amplified polymorphic DNA (RAPD), specific and random amplification (SARA-PCR), amplified fragment length polymorphism (AFLP), Rep-PCR, PCR-ribotyping, amplification of intergenic rRNA spacer regions (ITS-PCR), amplified ribosomal DNA

restriction analysis (ARDRA), RFLP of PCR-amplified 16 S rDNA, broad range PCR-restriction fragment length polymorphism (PCR-RFLP), temporal temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE). The most sophisticated, fourth generation approaches base on multilocus sequence typing (MLTS) and DNA sequencing (Domig et al., 2003).

From this brief overview it can concluded that the importance and applicability of each of the methods may vary from one species to another, and also according to the precise geographical location in which they are being used. For some species, combined use of several different phenotypic methods may offer a reasonable approach, but such an approach may not be possible, or may take a long time to develop, for organisms which have not been well-studied previously. In contrast, genotypic methods have potential to be used for studying diversity in any microbial species, with some genotypic methods also offering the possibility of providing the universal approach to microbial typing in which the same basic methodology can be used to study isolates of any microorganism. Namely, PCR identification of enterococci with genus-specific primers, designed by Deasy et al. (2000) from published 16 S rRNA, enables specific discrimination of *Enterococcus* genus from other bacteria. These primers were also used to characterise the enterococcal diversity in an Irish cheddar-type cheese-making factory (Gelsomino et al., 2001). To characterise clinically relevant enterococci, Dutka-Malen et al. (1995) developed primers at the species level (*E. faecalis* and *E. faecium*), targeting the *ddl* gene. *E. durans* and *E. hirane* species specific primers, targeting *ddl* gene as well, were developed and validated by Knijff et al. (2001). Many publications report PCR detection of *van* genes in enterococci with variations of *vanA*, *vanB*, *vanC1*, *vanC2* and *vanC3* primers that originally were all derived from the sequences designed by Dutka-Malen et al. (1995). A molecular screening of *Enterococcus* virulence determinants using PCR technique was performed by Eaton and Gasson (2001). Moreover, Hufnagel et al. (2003) propose an additional immunological test to assess the safety of enterococci by testing the susceptibility to opsonophagocytic killing. In recent study of Cocolin et al. (2004), DGGE method seems to efficiently differentiate and identify *E. faecium* and *E. faecalis*.

New knowledge, new methods and techniques, development and improvement of analysing procedures, which are gained on daily bases, widen our perspectives in detection, characterisation and identification of enterococci in the area of food microbiology and in epidemiological researches. Only well-conceived plan on combining different fundamental and advanced methods

can provide reliable identification of a certain strain of enterococci that could be used as a starter culture or as probiotic preparation.

Conclusions

It appears that there is a very thin line separating enterococci-contaminants from enterococci-pathogens. The emergence of many enterococci resistant to glycopeptides and other antibiotics, production of biogenic amines into the food, and finding of virulence traits within both clinical and foodborne isolates pose the presence of enterococci in foods questionable (Giraffa, 2003). Moreover, highly efficient ability of enterococci to transfer different genes, e.g. antibiotic resistance genes, to pathogenic bacteria, even more decrease the beneficial credit of enterococci. As such, they could constitute a definite health risk and special care should be taken to ensure that enterococci used as probiotics or starter cultures do not posses potential virulence factors or acquire antibiotic resistance. Therefore the question of whether enterococci are safe for use in food/feed could only be answered after thorough and explicit setting up of selection criteria defining which enterococci are not pathogenic. Growing range of techniques with varying taxonomical resolution is used to identify, characterise and type enterococci. Genotypic methods, in particular, have become a powerful tool in food microbiology and in epidemiological search of enterococci, and can scientifically support discrimination between beneficial and detrimental enterococci regarding the testing schemes, which so far are not mandatory in the food legislation of the EU.

Despite their detrimental nature, enterococci definitely find application as technological microorganisms, especially in cheese and meat fermentation processes. It has to be emphasized that in the most traditional food fermentation studies so far, enterococci are proposed to be a part of defined microbial population, where they appear to play at least some positive role. Low milk acidifying and proteolytic activities make enterococci of minor importance as primary starter cultures in cheese manufacture, but they are significant as starter adjuncts. In cheeses made with added enterococci, taste, aroma, colour, structure, as well as the overall sensory profile of ripened cheese seem to be positively affected (Giraffa, 2003). Moreover, some strains with a long history of safe use as probiotics and their large-scale commercial application are also known. For example, *E. faecium* SF68 has more than 20-year-long history of safe association in probiotic therapy. Numerous strains of enterococci associated with food systems produce enterocins with activity against *Listeria monocytogenes*, *Staphylococcus*

aureus, *Clostridium* spp. (Giraffa, 1995). Therefore enterocins or enterocin producers show a potential for dairy application as biopreservatives or protective cultures.

However, based on a long history of safe association of particular enterococci with some traditional food fermentations, the use of such strains appears to bear no particular risk for human health. Abundance of experiences as well as progress in molecular techniques enables exact characterisation and safety assessment of a strain. Therefore, the yin-yang duality of enterococci should not be our stoic dark future but challenging and promising present as we posses tools and knowledge to command enterococci.

ENTEROKOKI: YIN - YANG MIKROBI

Sažetak

Ovaj revijalni prikaz opisuje dualizam enterokoka kojega možemo ilustrirati njihovim yin-yang ponašanjem. Otporna narav ove veoma specifične skupine bakterija mliječne kiseline omogućava njihovu proširenost u različitim sredinama gdje signifikantno utječu na ishod pojedinih procesa. Tako su u tehnološkom smislu enterokoki vodeći mikrobi u fermentacijskim procesima a istovremeno su i dobro poznati uzročnici kvarenja hrane. Kao terapeutici, enterokoki manifestiraju svoja probiotička svojstva pozitivnim djelovanjem na održavanje normalne intestinalne mikroflore simulirajući imunološki sustav, te poboljšavaju nutritivnu vrijednost hrane proizvodnjom antimikrobnih supstanci (bakteriocina). Pored toga enterokoki predstavljaju rastući rezervoar oportunističkih patogena za ljude jer izazivaju bolesti, sadrže činitelje za antibiotičnu rezistenciju i mehanizme njihova transfera, te često nose i potencijalne virulentne faktore. Ali usprkos yin-yang naravi enterokoka, duga povijest bezopasne upotrebe pojedinih enterokoka u hrani i krmi, te pouzdana identifikacija i klasifikacija enterokoka fenotipskim metodama poduprtim modernim genetskim tehnikama, omogućava selekciju obećavajućih enterokoka, koje bi mogli, bez posebnog rizika po zdravlje čovjeka, upotrijebiti kao starterske kulture ili dodatke hrani i krmi.

Ključne riječi: enterokoki, korisno i štetno djelovanje, identifikacija, klasifikacija, bezopasnost enterokoka

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