LWT - Food Science and Technology 75 (2017) 670–676

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Virulence factors of *Enterococcus* spp. presented in food

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ARTICLE INFO

Article history:
Received 24 June 2016
Received in revised form
15 September 2016
Accepted 8 October 2016
Available online 11 October 2016

Keywords:
Enterococcus spp.
Virulence factors
Food safety

ABSTRACT

Enterococci have emerged as important nosocomial pathogens and have been found to possess many virulence factors, some of which are considered very important in the pathogenesis of diseases caused by them. While clinical *Enterococcus* strains have been extensively described in the literature, the knowledge of the virulence factors and the genetic structure of enterococci found in food is limited. In addition, enterococci are intrinsically resistant to several antimicrobial agents and they can easily acquire resistance to antimicrobials. High-level resistance to a wide range of antibiotics together with the presence of virulence factors reinforces the potential role of enterococci as effective opportunists in nosocomial infections. Although foodborne enterococcal infections have never been reported, the consumption of food carrying virulence enterococci is a possible route of transfer. This review was carried out to characterise of some virulence factors which most often occur in *Enterococcus* strains isolated from food including ready-to-eat food.

1. Introduction

Enterococci are commonly found in the digestive tract of humans and farmed animals and are an integral part of commensal bacteria included in the physiological microbiota (Hammerum, 2012; Lebreton, Willems, Gilmore, 2014). Studies on the ecology and epidemiology of *Enterococcus* spp. indicate that along with feces these bacteria are entered into the environment which they easily colonise due to its high adaptability. Hence their widespread occurrence in soil, water, sewage, on plants and fruits. This way they subsequently enter raw materials of animal and plant origin, such as milk, meat and vegetables (Giraffa, 2002). The prevalence of *Enterococcus* spp. in foods, including ready-to-eat type of foods, results mainly from their resistance to adverse environmental conditions related to the production technology as well as food storage conditions. The ability of growth in the presence of NaCl in a concentration of 5–10%, bile salt in a concentration of 40% and pH range from 4.6 to 9.9, the ability of growth in aerobic and anaerobic conditions and the ability to survive in a temperature of 63,5 °C for 30 min means that they often constitute the residual microflora in food (Domig, Mayer, & Kneifel, 2003; Van den Berghe, De Winter, & De Vuyst, 2006).

In hospital environment, enterococci are considered to be potentially opportunistic pathogens that can cause infections in immunocompromised patients. A continuous growth in the number of strains resistant to antibiotics have been reported in recent years (Carlet et al., 2012). Vancomycin-resistant enterococci are currently one of the main opportunistic pathogens in hospital environment (O'Driscoll & Crank, 2015). Therefore, an increasing number of reports concerning these bacteria's mechanisms of resistance to antibiotics have emerged. Presence of resistance genes alone does not indicate pathogenicity of a strain, however, combined with the presence of virulence factors it may cause the strain to become dangerous (Heidari, Emaneini, Dabiri, & Jabalameli, 2016). In particular, it occurs because genes conferring/expressing resistance to antibiotics and virulence factors are often placed on the same mobile genetic elements. As well as transmissible antibiotic resistance plasmids, virulence factors are known to be transmissible by highly efficient gene transfer mechanisms (Eaton & Gasson, 2001).

Pathogenesis of most infections involves a sequence of events which include colonisation, adhesion to the host's cells, invading tissues and resistance to non-specific defensive mechanisms (Upadhyaya, Ravikumar, & Umapathy, 2009). Studies have shown that strains of enterococci that have virulence factors cause more severe infections than those without them. The increasing role of *Enterococcus* species in the aetiology of infections in patients with impaired immunity has encouraged researchers to attempt to characterise the factors which allow bacteria to effectively colonise the host's organism by passing the immune barriers and causing

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http://dx.doi.org/10.1016/j.lwt.2016.10.026
0023-6438/

pathological changes.

Considerable progress has been made in recent years in the detection of virulence factors in enterococci of clinical origin. This has made it possible to detect virulence factors in strains isolated from food and to determine any differences in the virulence potential in enterococci from ~~both of these~~ two sources. Two virulence factors have been isolated and characterised: i) surface factors that affect colonisation of host cells, and ii) agents secreted by enterococci, which damage the tissues (Sava, Heikens, & Huebner, 2010) (Table 1).

2. Virulence factors that promote colonisation

Enterococci are capable of adhering to their host's tissues (Tomita & Ike, 2004). This and their resistance to low pH and high concentrations of bile salts (Foulquié Moreno, Sarantinopoulos, Tsakalidou, & De Vuyst, 2006) contribute to enterococci being among the most common of the bacteria colonising the colon. Their adhesins enable them to bind to receptors on the mucous membrane or to proteins of the extracellular matrix, which favour colonisation of the epithelium (Franz, Stiles, Schleifer, & Holzapfel, 2003). If they could not bind, they would be removed by peristalsis of the intestines. Obviously, colonisation itself is not proof of pathogenicity, but combined with other factors of virulence and with the presence of a number of resistance genes, potentially harmful. Virulence factors that promote colonisation include: aggregation substance (AS), collagen-binding protein (Ace), cell wall adhesin (Efa A), enterococcal surface protein (Esp). (Hollenbeck & Rice, 2012; Strzelecki, Sadowy, & Hryniewicz, 2011).

2.1. Aggregation substance

The aggregation substance is the first enterococcal surface protein to be described. As it often acts as a virulence factor and it transfers antibiotic resistance genes, it is ~~still~~ a subject of current intensive studies. This protein has a molecular weight of 137 kDa and a hairpin-like structure. The strongly conservative ~~motive~~ motif of LPXTG is an important part of its molecule and its distinctive sequence is regarded as the site of recognition and cleavage by sortases which bind them by a covalent bond to the cell wall (Dramsi, Trieu-Cuot, & Bierne, 2005). The aggregation substance includes a range of highly homologous adhesins, encoded on large conjugative plasmids transferred in a so-called facilitated conjugation system, mediated by sex pheromones (Strzelecki et al., 2011). Sex pheromones are short, hydrophobic peptides, which enter AS and interact with a specific conjugative plasmid (Clewell, An, Flannagan, Antiporta, & Dunny, 2000). The process is of special importance in the conjugative transfer of genes between cells. In the presence of pheromones secreted by the recipient's cells, the donor's cells synthesise AS which binds to a related EBS ligand on the recipient cell surface (Dunny, Leonard & Hedberg, 1995). The process results in the formation of large conjugative aggregates consisting of bacterial cells, which facilitates their exchange of genetic material between cells. In the presence of a specific ligand, with a structure similar to that of teichoic acid, the AS protein can have the features of a superantigen (Kozlowicz, Dworkin, & Dunny, 2006; Wardal, Sadowy, & Hryniewicz, 2010). Moreover, it plays a role in propagation within a species of plasmids, on which other factors of enterococci virulence are encoded, such as cytolysin (described below) and determinants of antibiotic resistance. Finally, the aggregation substance and cytolysin can act synergistically, thereby increasing the strain's virulence by switching on cytolysin regulation in the *quorum-sensing* system, making it possible to damage deeper tissues (Gilmore, Coburn, Nallapareddy, & Murray, 2002; Foulquié Moreno et al., 2006).

Currently, there are 20 pheromone-dependent plasmids found in enterococci, on which genes encoding antibiotic resistance were found together with those that encode AS. The following are the conjugative plasmids with genes responsible for production of AS proteins: pAD1 (Asa1 protein), pPD1 (Asp1 protein) and pCF10 (Asc10 protein) (Clewell, 2007; Dunny, 2007). The genes responsible for production of aggregation substance proteins are strongly conserved and are 90% mutually homologous. This is with the exception of the *asa373* gene, which is situated on the pAM373 plasmid and which encodes the Asa373 protein; the gene sequence is considerably different from those mentioned above (Hendrickx, Willems, Bonten, & Van Schaik, 2009). It has been observed that only Asa337 is capable of binding to the recipient's cells that are devoid of the active binding substance (Suk-Kyung, Koichi, Haruyoshi, & Yasuyoshi, 2006)

2.2. Collagen binding protein - Ace

Ace (*Accessory colonisation factor*) is another surface protein with adhesive properties, with a molecular weight of about 74 kDa. It is encoded by the *ace* gene (Rich, Favre-Bonte, Sapena, Joly, & Forestier, 1999). The protein was isolated from *E. faecalis* strains both from healthy carriers and from people with enterococcal infections, which suggested that this feature can be used to identify the species (Duh, Singh, Malathum, & Murray, 2001). Like the AS protein, Ace also plays an important role in colonisation by binding to proteins of the extracellular matrix (ECM); it also participates in binding type I and IV collagen (Nallapareddy, Qin, Weinstock, Hook, & Murray, 2000). Ace is a member of the family of surface proteins described by the acronym MSCRAMM (microbial surface component recognizing adhesive matrix molecules), with LPXTG (L - leucine, P - proline, X – any amino acids, T – threonine, G – glycine), with high affinity and specificity of binding a ligand which

Table 1
Enterococcal virulence factors.

Enterococcal virulence factors	Gene	Function/biological effect
Virulence factors that promote colonisation:		
- aggregation substance (AS),	*agg*	- binding to host cells, enables cell-to-cell contact between donor and recipient strains for conjugation
- collagen-binding protein (Ace),	*ace*	- colonisation by binding to proteins of the extracellular matrix (ECM); it also participates in binding type I and IV collagen
- cell wall adhesin (Efa A),	*efa*A	- virulence factors associated with infective endocarditis
- enterococcal surface protein (Esp).	*esp*	- associated with biofilm production
Virulence factors with affect tissues:		
- cytolysin (Cyl),	*ace*	- bactericidal properties towards Gram-negative bacteria and toxic properties (β-haemolysis) towards erythrocytes, leukocytes, macrophages
- gelatinase (GelE)	*efa*A_{fs}, *efa*A_{fm}	- hydrolyses gelatine, elastin, collagen, haemoglobin, as well as other bioactive peptides, e.g. proteins bound to pheromones
- hyaluronidase (Hyl)	*esp*	- plays a role in destroying mucopolysaccharides of the connective tissue and cartilage

is among the components of ECM (Hendrickx et al., 2009).

Considering the functionality and structure of the Ace protein, it has been noted that it is similar to one of the first MSCRAMM's discovered in Gram-positive bacteria: the Cna protein which occurs in *S. aureus*. The structure of an Ace molecule contains a short signal sequence consisting of 31 amino acids at the N-terminal end; the A domain consisting of 335 amino acids, which is responsible for binding a ligand in ECM proteins; the B domain, consisting of a variable number of tandem replications and the C region consisting of LPXTG (Hendrickx et al., 2009; Nallapareddy et al., 2000). A protein whose structure is similar to Ace in *E. faecalis* has been found in *E. faecium* – Acm. The Acm protein is encoded by the acm gene, which is homologous to *ace*. Like Ace, it is responsible for binding collagen (Nallapareddy, Singh, Okhuysen, & Murray, 2008). Since Ace in *E. faecalis* shows some similarity to the Acm protein in *E. faecium* only within the A domain, whereas similarity to the Cna protein in *S. aureus* can be observed within the A and B domains, it has been suggested that it is more similar to *S. aureus* (Nallapareddy, Weinstock, & Murray, 2003). Moreover, both Acm and Cna bind to collagen, whereas Ace also binds to lamin; they also show greater affinity to collagen I than IV, while Ace shows identical affinity to both (Nallapareddy et al., 2000; 2003).

2.3. Endocarditis specific antigen - EfaA

EfaA (*endocardis antigen*) is a protein with a molecular weight of about 34 kDa encoded by the efAfs gene in *E. faecalis* strains, and by efArm in *E. faecium* (Eaton & Gasson, 2001; Sava, Heikens, & Huebner, 2010). The *efa*A gene is part of the *afaCBA* operon which encodes the ABC transporter (permease), regulated by magnesium ions (Abrantes, Kok, & Lopes Mde, 2013). The EfaA protein is homologous to the adhesins present in cell walls of streptococci, e.g. to the FimA protein, produced by *Streptococcus parasanguis*, ScaA in *S. gorgonii*, PsaA in *S. pneumoniae* and SsaB in *S. sanguis* (Archimbaud et al., 2002). It has been shown by genetic methods that homologous genes to *efa*A are present in strains of *E. avium, E. asini, E. durans* and *E. solitarius* (Jiménez et al., 2013; Semedo et al., 2003b).

2.4. Surface protein - Esp

Entercococcal surface protein (Esp) with a molecular weight of about 200 kDa is the largest identified enterococcal protein. The *esp* gene encoding this protein is located on the pathogenicity island (PAI), which also contains proteins responsible for the active outflow of antibiotics (Leavis et al., 2004). This location is probably a result of horizontal gene transfer between *E. faecalis* and *E. faecium*. The Esp protein shows some structural similarity to other proteins present in Gram-positive bacteria: C-α in β-haemolytic *Streptococcus agalactiaceae* encoded by the *bca* gene, R28 in *Streptococcus pyogenes* and Bap in *Staphylococcus aureus* - a protein associated with formation of biofilm (Donlan & Costerton, 2002; Hendrickx et al., 2009; Toledo-Arana et al., 2001). Studies of the Esp protein have confirmed its participation in the formation of biofilm which can play an important role in the exchange of genetic material between cells and increase their resistance to antibiotics (Donlan & Costerton, 2002; Foulquié Moreno et al., 2006; Latasa, Solano, Penadé, & Lasa, 2006). It has been shown that the occurrence of the *esp* gene in *E. faecium* is correlated with resistance to ampicillin, ciprofloxacin and imipenem (Billström, Lund, Sullivan, & Nord, 2008). The latest reports suggest a correlation of the presence of a surface protein and resistance to vancomycin. Studies of clinical strains have shown that 83.3% of vancomycin-resistant strains of *E. faecium* (VREF) had the *esp* gene (Ochoa et al., 2013). Moreover, most of those genes showed multi-drug resistance, which has been confirmed by Billström et al. (2008). It has also been shown that the *esp* gene can be transferred between strains of *E. faecium* by way of plasmid conjugation, and also between strains of *E. faecalis* by the chromosome-chromosome transposition (Oancea, Klare, Witte, & Werner, 2004).

3. Virulence factors which affect tissues

After the colonisation process, pathogenic strains of *Enterococcus* spp. secrete toxic substances which have a destructive effect on the host's tissues. Virulence factors secreted by enterococci include: cytolysin (Cyl), gelatinase (GelE) and hyaluronidase (Hyl).

3.1. Cytolysin - Cyl

Cytolysin is one of the best characterised enterococci virulence factors. It is a bacteriocin-type exotoxin, which shows bacteriocidal properties towards Gram-negative bacteria and toxic properties (β-haemolysis) towards erythrocytes, leukocytes and macrophages (De Vuyst et al., 2003). Production of cytosine is the responsibility of the operon containing eight genes: *cylR$_1$, cylR$_2$, cylL$_l$, cylLs, cylM, cylB, cylA and cylI.* The operon can be located on strongly conservated pheromone-dependent conjugative plasmids (e.g. pAD1) or within the island of pathogenicity in the bacterial chromosome near other determinants of virulence: surface protein Esp and the aggregation substance AS (Eaton & Gasson, 2001; Shankar, Coburn, Pillar, Haas, & Gilmore, 2004). Expression of cytolysin requires at least five proteins encoded by the *cylL$_l$* and *cylLs* genes, which encode structural subunits, which undergo post-translation modification by a protein encoded by the *cylM* gene. Cytolysin produced in a cell is secreted outside with the help of a protein encoded by the *cylM* gene. Outside the cell, cytolysin is activated by serine protease encoded by the *cylA* gene, and the cell itself is protected by a surface protein encoded by the *cylI* gene, which affects its resistance to cytolysin. Expression of cytolysin is regulated by a regulatory system which comprises the *cylR$_1$* gene, encoding the transport protein CylR1 and the *cylR$_2$* gene, which encodes the DNA binding protein – CylR2. The regulatory system is activated by the *quorum-sensing* mechanism, when the concentration of the smaller sub-unit of cytolysin CylLs outside the cell is sufficiently high (Hällgren et al., 2008; Semedo et al., 2003a). Cytolysin-encoding genes have been found both in *Enterococcus* strains isolated from infections and from those which make up commensal microbiota. There have been a number of reports which indicate their frequent occurrence in strains isolated from animals and food products, both of animal and plant origin (Eaton & Gasson, 2001; Ben Omar et al., 2004; Franz et al., 2001; Trivedi, Cupakova, & Karpiskova, 2011). Cytolysin-encoding genes have been found in the following species: *E. faecalis*, *E. faecium, E. avium, E. casseliflavus, E. cecorum, E. durans, E. gallinarum, E. malodoratus* and *E. raffinosus* (Semedo et al., 2003a).

3.2. Gelatinase – GelE

Gelatinase is an extracellular, zinc-dependent metalloendopeptidase, with a molecular weight of about 30 kDa. This enzyme is capable of hydrolysing gelatine, elastin, collagen, haemoglobin, as well as other bioactive peptides, e.g. proteins bound to pheromones (Archimbaud et al., 2002). Gelatinase is encoded by the *gel*E gen situated on the chromosome. The gene is controlled by the transmembrane protein FsrB, which is regulated by locus fsr consisting of three genes: *fsrA, fsrB, fsrC* (Hancock & Perego, 2004; Pillai et al., 2004). The set of *fsrABC* genes encodes the regulatory protein FsrA, FsrB – pheromone transporter GBAP and histidine kinase FsrC (Podbielski & Kreikemeyer, 2004). Deletions within

locus *fsr* produce mutants which - despite having the *gel*E gene - do not produce gelatinase, which decreases their virulence. Mutations within the *fsrA*, *fsrB* or *fsrC* genes have been shown to reduce the biofilm synthesis by 28–32% (Mohamed & Huang, 2007).

The *gel*E gene is regulated in the *quorum-sensing* system and this depends on the proper level of the gelatinase biosynthesis activation pheromone (GBAB) (Pinkston et al., 2011; Teixeira et al., 2012). Locus *fsr* in enterococci is functionally similar to locus *agr* in *S. aureus* (Dunman et al., 2001). The presence of the *gel*E gene is one of those determinants of virulence assayed in enterococci, which is found both in clinical strains and in those isolated from food. It usually occurs in *E. faecalis* and in individual strains of *E. faecium* (Eaton & Gasson, 2001).

3.3. Hyaluronidase – Hyl

Hyaluronidase, an *E. faecium* genome protein with molecular weight ~~is~~ close to 45 kDa and encoded by the *hyl* gene, *E. faecium* has not been found in the chromosomal DNA of strains of *E. faecalis* (Archimbaud et al., 2002). It is homologous with hyaluronidases of other cocci including: *Streptococcus pyogenes, Staphylococcus aureus* and *Streptococcus pneumoniae.* This enzyme plays a role in destroying mucopolysaccharides of the connective tissue and cartilage and, consequently, in spreading bacteria. The *hyl* gene in clinical strains has been usually found in *E. faecium* and it occurs extremely rarely in *E. faecalis* (Vankerckhoven et al., 2004). It has also been found in other strains of species isolated from food including: *E. casseliflavus*, *E. mundtii* and *E. durans* (Trivedi et al., 2011).

Strains of a clinical origin usually contain more factors of virulence than those isolated from other sources, including food, whereas the latter - although not an immediate source of infection themselves - can promote the spreading of genes of virulence (Comerlato, Resende, Caierão, & D'azevedo, 2013; Eaton & Gasson, 2001; Jiménez et al., 2013). Bacteria of genus *Enterococcus* are capable of exchanging genetic material by conjugation and the process frequently occurs in the gastrointestinal tract (Huddleston, 2014). A number of enterococcal factors of virulence, such as haemolysin-cytolysin, adhesive substances or antibiotic resistance, can be transferred by the mechanism of gene exchange. It is often the case that one plasmid contains genes that encode pheromones, antibiotic resistance and factors of virulence (Franz et al., 2001; Giraffa, 2002). Therefore, considering consumer safety, it seems reasonable to monitor the presence of virulence factors in strains of genus *Enterococcus* isolated from food.

4. Sex pheromones

Enterococci have a mechanism of plasmid accumulation based on the production of chromosomally-encoded genes of sex pheromones - *cpd, cob, ccf, cad* (Clewell, Francia, Flannagan, & An, 2002). Pheromones are small peptides of 7–8 amino acids, which facilitate conjugative transfer of plasmids between cells (Chandler & Dunny, 2004). Pheromones secreted by recipients are donor-specific and induce expression of conjugative operons of its plasmid. A strain usually secretes several different pheromones. Apart from pheromones themselves, each pheromone-dependent plasmid encodes the secretion of peptides which act like inhibitors competitive to the corresponding pheromone. When pheromones bind to receptors on the surface of the donor's cells, this signal is transduced and induces the gene of the aggregation substance (Clewell, 1993; Dunny et al., 1995). Expression of the *asa*1 gene results in the formation of cell aggregates, which enables effective plasmid transfer (Clewell et al., 2002). However, it is not the only role of pheromones; they can also be chemically attractive to human neutrophils and the induce production of superoxides (mutagenic substances) and initiate inflammatory conditions (Bhardwaj, Malik, & Chauan, 2008).

5. Virulence potential of *Enterococcus* strains isolated from food

Knowledge of the virulence characteristics of circulating *Enterococcus* strains may help to understand the complex pathogenic process of these opportunistic microorganisms (Sharifi et al., 2012). Data ~~about~~ on the virulence of *Enterococcus* isolated from food are still scarce. Researchers have shown that the virulence of *Enterococcus* seems to depend on the species rather than on the source from which they were isolated. Many more virulence factors have been found in *E. faecalis* than in isolates of other species. Franz et al. (2001) found that 10.4% of *E. faecium* strains and as much as 78.7% of *E. faecalis* strains isolated from food had at least one virulence factor. Cariolato, Andrighetto, and Lombardi (2008) examined strains isolated from dairy products and showed isolates of *E. faecalis* to have in their genome from 1 to 6 factors of virulence, whereas other species were practically free of them. Furthermore, Jiménez et al. (2013) examined strains of *Enterococcus* spp. isolated from animals and healthy humans and they showed isolates of *E. faecalis* to contain in their genome several factors of virulence each (*cad, ccf, cob, cpd, efa*A_{fs}, *agg, gel*E, *cyl*A, *esp*), whereas *E. faecium* contained only the *efa*A_{fm} gene.

Medeiros et al. (2014) compared strains isolated from material of clinical origin and from food (raw vegetables, raw meat, pasteurized milk, soft cheese) and showed that a number of strains isolated from food have in their genomes the same factors of virulence as those found in strains of clinical origin. Their research on *E. faecalis* isolates (57 of clinical isolates, 55 isolates from food) showed that isolates of clinical origin contained genes of cytolysin (*cyl*A), the aggregation substance (*agg*) and the enterococcal surface protein (*esp*) more frequently. On the other hand, the presence of genes of gelatinase (*gel*E) and the collagen binding protein (*ace*) were present at the same level, which suggests their widespread occurrence in both environments.

Since the presence of sex pheromones is regarded as one of the factors that facilitate conjugation, their high incidence in isolates from ready-to-eat food should be considered a food safety risk. The presence of ~~those~~ these factors in the genomes may be indicative of their ability to exchange genetic material at every stage of their production, and when they reach the human gastrointestinal tract. The occurrence of sex pheromones is frequently associated with the presence of the aggregation substance. The isolates that have the *asa*1 gene respond to the recipient's cells by producing pheromones to accept the pheromone-dependent plasmids. This is an important relationship in the process of exchange of ~~the~~ resistance genes in the conjugation process. Among sex pheromones, the *ccf* gene is responsible for activating the conjugation of the pCF10 plasmid, which transfers resistance genes to tetracyclines (Akhtar, Hirt, & Zurek, 2009). Moreover, numerous studies have shown that pheromones mediating in the system of conjugation are also responsible for acquiring resistance to glycopeptides, including vancomycin. Transfer of the *van*A gene during the conjugation by the method of membrane filters is mediated by plasmid pCF10 (Paoletti et al., 2007; Werner et al., 2011). Sex pheromones seem to be a very important factor in to the virulence of enterococci and participate~~s~~ in the spreading of antibiotic resistance. Epidemiological studies have shown that they are isolated more frequently from patients with bacteremia and wound infections than from feces samples from healthy volunteers and hospitalised patients (Wardal et al., 2010). The incidence of pheromones in food isolates is worrying in light of their ability to facilitate the process of gene

exchange. In studies performed on enterococci (n = 35) isolated from retail raw (20 samples), cooked (20 samples), and ready-to-eat shrimps (20 samples), genes encoding sex pheromones *cpd* (100%), *cob* (94.3%) and *ccf* (94.3%) were found with high frequency. All of the tested sex pheromone determinants (*cpd, cob, ccf*) were present in 31 out of 35 isolates (Chajęcka-Wierzchowska, Zadernowska, & Łaniewska-Trokenheim, 2016). Also, in enterococci isolated from retail beef and chicken meat samples in Turkey, the most prevalent virulence determinants were *cpd* (100% and 92.4%) and *ccf* (98% and 99%) (Yılmaz, Aslantaş, Pehlivanlar, Türkyılmaz, & Kürekci, 2016).

Gelatinase is the most frequently occurring factor of virulence (Ali, Hasan, Bin Asif, & Abbasi, 2014; Jiménez et al., 2013; Ribeiro et al., 2011). The gene which encodes this metalloprotease has been found in the majority (>70%) of isolates from food (cassava, beetroot, potato, sweet potato, parsley, cabbage, raw meat, pasteurized milk and dairy products, such colonial cheese type and soft cheese) in Brazil (Medeiros et al., 2014). Similarly, high level occurrence of *gel*E was detected in enterococci isolated from Turkish white cheese (İspirli, Demirbaş, & Dertli, 2017). Frequent occurrence of gelatinase in isolates from meat and dairy products is attributed by the authors to the presence in these products of gelatine, collagen and casein, which are hydrolysed by gelatinase. It is important that gelatinase is often a factor of virulence found in the genome of strains of clinical origin, including vancomycin-resistant alarm pathogens. Sharifi et al. (2012) found the presence of the *gel*E in all vancomycin-resistant strains of clinical origin of *E. faecalis*. The *gel*E gene were detected in 82.9% of chicken isolates, and 75% of beef isolates in the study performed by Yılmaz et al. (2016).

Phenotypic expression of the *gel*E gene is usually determined to be around the 50% level (Brtkova, Revallova, & Bujdakova, 2011; Medeiros et al., 2014). The lack of expression of the gene in laboratory conditions is attributed to the loss of one or more of the genes that are parts of the *fsr* operon which participates in the regulation of expression of the *gel*E (Cariolato et al., 2008), or mutation of the genes (Comerlato et al., 2013). These observations confirm the complexity of the processes which accompany the virulence of enterococci.

The presence of *ace* was detected in isolates of *E. faecalis* from food ~~than in~~ as well as in isolates of clinical origin (Cariolato et al., 2008; Medeiros et al., 2014). Abriouel et al. (2008) showed the gene to occur more frequently in isolates of clinical origin (>80%) than in isolates from vegetables, water and soil. Owing to the ability to bind collagen, the Ace protein plays an important role in the early stages of colonisation. Singh, Nallapareddy, Sillanpää, and Murray (2010) found deletion of the *ace* gene greatly suppressed the colonising potential of *E. faecalis*.

Another alarming factor is the ability of enterococci isolated from food to form a biofilm. Biofilm increases the survival rate and propagation of the genes of resistance in different environmental conditions. Strains which live in the biofilm have been shown to be more resistant to antibiotics (Holmberg & Rasmussen, 2016). Bacteria which live in mature biofilm can tolerate antibiotics at concentrations of about 10–1000 times higher than outside them (Simões, Simões, & Vieira, 2010). The presence of factors that mediate adhesion to cells and the formation of biofilm are the main stages in the process of infection or colonisation of a host.

Some authors reported an association between the ability to form biofilm and the presence of *esp* (Chuang-Smith, Wells, Henry-Stanley, & Dunny, 2010; Tendolkar, Baghdayan, Gilmore, & Shankar, 2004) while others suggested that mechanisms for biofilm formation are independent from *esp* (Hufnagel, Koch, Creti, Baldassarri, & Huebner, 2004; Kristich, Li, Cvitkovitch, & Dunny, 2004; Mohamed, Huang, Nallapareddy, Teng, & Murray, 2004). Gelatinase has also been suspected of being involved in the process of biofilm formation, by mediating signals arriving through the quorum-sensing *fsr* system (Hancock & Perego, 2004). However, Mohamed and Murray (2005) found a lack of a correlation between gelatinase and biofilm formation in a large collection of *E. faecalis* isolates. The results obtained by Mohamed et al. (2004) indicated that a more important factor than gelatinase in biofilm production may be serine protease. A recent report on enterococci strains isolated from food indicated that there was no association between the presence of *esp* and *gel*E and the ability to form biofilm (Chajęcka-Wierzchowska et al., 2016).

The least frequently occurring virulence factor of enterococci isolated from food is haemolytic activity. The *cyl*B gene related to cytolysin metabolism were detected in enterococci from Turkish white cheese (İspirli et al., 2017) while *cyl*A gene were detected in isolates from Egyptian fresh raw milk cheese (Hammad, Hassan, & Shimamoto, 2015). Some authors reported discrepancies resulting from the presence of genes and their expression. The presence of cytolysin is not always associated with haemolysis on blood agar. According to literature data, lack of haemolysis may result from ~~too~~ a weak expression of the gene, which does not allow the detection of phenotypic changes, or from the presence of an inactive gene product (Gaspar, Crespo, & Lopes, 2009; Upadhyaya et al., 2009). In the study ~~of~~ by Trivedi et al. (2011), β-haemolytic activity was higher in *E. faecalis* (29%) compared to *E. faecium* (10%) isolated from milk and dairy products, ready-to-eat meat products and fruits and vegetables. Moreover, although the majority of β-haemolytic strains belong to *E. faecalis* and *E. faecium* species, the feature seems to be genus-dependent rather than species-dependent, because it has been observed in *E. mundtii* and *E. durans* of dairy origin and two *E. casseliflavus* strains of dairy and meat origin (Trivedi et al., 2011). Different researchers reported that *Enterococcus* strains isolated from a variety of fermented food products exhibited no β-haemolytic activity (Yoon et al., 2008; Özmen Toğay et al., 2010).

It is interesting to point out that the virulence factors have been also found in the genome of strains of *E. casseliflavus, E. durans, E. hirae E. avium, E. cecorum, E. gallinarum, E. malodoratus and E. faffinosus* and *E. mundtii* (Jackson et al., 2015; Poeta et al., 2005; Semedo et al., 2003a; Semedo et al., 2003b; İspirli et al., 2017) This is important, considering the fact that researchers usually focus on assessing the virulence of *E. faecalis* and *E. faecium*, which are the most common in food. Meanwhile, it turns out that the other species also contain invasiveness factors, which make them more virulent.

6. Conclusions

Strains isolated from food including ready-to-eat food, might have properties which are typical of strains isolated from cases of bacteraemia and hospital infections. The presence of virulence factors does not necessarily mean that the strains isolated from the foods cause diseases in humans, but may have pathogenic potential as these factors have been found to contribute to the severity of infection. Although factors of virulence are less frequent in isolates from food than in isolates of clinical origin, they can be a potential reservoir of virulence factors and they can make it possible to transfer them to human microbiota in the food chain. There is no data showing a direct relationship with the consumption of foods containing virulent enterococci and illness but enterococcal strains with virulence traits and antibiotic resistances exogenously transferred into the human gut via food products can represent risk for immunocompetent individuals. It is very important issue in immunocompromised patients, a risk for enterococcal disease by such strains cannot completely be excluded.

Acknowledgments

This review was was financed by the National Science Centre allocated on the basis of a decision number DEC-2013/09/N/NZ9/01630, supported by a grant from the Ministry of Science and Higher Education, Poland, no. N N312 236138 and University of Warmia and Mazury (523-0713-0882, 523-0713-0883)

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