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

Wioleta Chajęcka-Wierzchowska*, Anna Zadernowska, Łucja Łaniewska-Trokenheim

Chair of Industrial and Food Microbiology, Faculty of Food Science, University of Warmia and Mazury, Plac Cieszyński 1, 10-726 Olsztyn, Poland

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

* Corresponding author.
E-mail address: wioleta.chajecka@uwm.edu.pl (W. Chajęcka-Wierzchowska).

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