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CHAPTER 1.2.5

The Genus Enterococcus: Taxonomy

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Introduction

The genus *Enterococcus* contains bacterial species associated with animals and plants. Only species from humans and domestic animals have been studied in some detail. Limited information is available on plant-associated species and this has been mainly derived from the study of strains transiently associated with humans or animals.

The enterococci are most often considered as components of the intestinal flora of humans and animals acting as opportunistic pathogens in different extra-intestinal compartments of the body. They have received considerable attention in medical bacteriology because of their increasing role in hospital-acquired (nosocomial) infections. An important factor contributing to this phenomenon undoubtedly has been their natural (intrinsic) and acquired resistance to frequently used antibiotics. Numerous studies have been devoted in recent years to this topic. Genetic studies (not treated in the present contribution), except those undertaken for taxonomic purposes, have largely concerned plasmids and transposons in connection with antibiotic resistance, and two genetic systems that have been described in *Enterococcus faecalis*: conjugative plasmids and sex pheromone plasmids.

Phylogeny

Relation to Other Genera

The enterococci have been separated from the streptococci, first based on DNA-DNA and DNA-rRNA hybridization studies (Schleifer and Kilpper-Bälz, 1984; Schleifer et al., 1985; Schleifer and Kilpper-Bälz, 1987). This separation was confirmed by 16S rRNA sequence analysis (Ludwig et al., 1985) which showed that the enterococci also differed from the lactococci and certain other Gram-positive cocci. The enterococci belong to the Firmicutes with low G+C content, the so-called clostridial branch. Phylogenetically the closest relative of the enterococci, but well separated from the latter, is the genus *Vagococcus* and next *Carnobacterium*, *Tetragenococcus*, *Aerococcus*, *Alloiococcus*, *Dolosigranulum*, *Facklamia*, *Globicatella* and *Abiotrophia* (Collins et al., 1997). The streptococci and the lactococci to which the enterococci have been linked in the past, are more distantly related, as are the lactobacilli.

Species Groups

16S rRNA Reverse Transcriptase Sequence Analysis.

Within the genus certain groups of species (Table 1) have been shown by 16S rRNA reverse trancriptase sequence analysis to be more closely related to each other than to others (Williams et al., 1991). *Enterococcus faecalis* forms a distinct lineage, as do *E. saccharolyticus*, *E. sulfureus* and *E. dispar*. The intraspecies group distances between *E. cecorum* and *E. columbae* are larger than the distances seen within other species groups. Patel et al. (1998) produced by the same technique a distance matrix tree that was nearly identical except for the fact that *E. sulfureus* and *E. saccharolyticus* appeared to form still another group with its two distantly related members.

Relation to Phenotypic Characteristics

Most interestingly, these "species groups" show a fairly large number of phenotypic characteristics which are typically shared by all members of a given group (Devriese et al., 1993b). These phylogenetic groups are therefore useful natural groups whose common characteristics can be used for identification. It is far easier to differentiate the various species groups from each other than the species within the groups. Moreover, a number of important characteristics are common and unique to certain species groups. For example the *E. cecorum* group is carboxyphilic and does not exhibit the unusual resistance to drying commonly attributed to the enterococci. All strains of the *E. gallinarum* group possess the *vanC*-gene cluster conferring low-level resistance to glycopeptide antibiotics such as vancomycin.

Table 1. Phylogenetic enterococcal species groups as determined by reverse transcriptase DNA sequence analysis of 16S rRNA.

Species group	Species
E. faecium group	*E. faecium*
	E. durans
	E. hirae
	E. mundtii
E. avium group	*E. avium*
	E. malodoratus
	E. raffinosus
	E. pseudoavium
E. gallinarum group	*E. gallinarum*
	E. casseliflavus
E. cecorum group	*E. cercorum*
	E. columbae

From Williams et al. (1991).

Table 2. *Enterococcus* species.

Species	Described by
E. faecalis	Schleifer and Kilpper-Bälz, 1984
E. faecium	Schleifer and Kilpper-Bälz, 1984
E. durans	Collins et al., 1984
E. gallinarum	Collins et al., 1984
E. casseliflavus	Collins et al., 1984
E. avium	Collins et al., 1984
E. malodoratus	Collins et al., 1984
E. hirae	Collins et al., 1986
E. mundtii	Collins et al., 1986
E. pseudoavium	Collins et al., 1989
E. raffinosus	Collins et al., 1989
E. cecorum	Williams et al., 1989
E. columbae	Devriese et al., 1990
E. saccharolyticus	Rodrigues and Collins, 1990
E. dispar	Collins et al., 1991
E. sulfureus	Martinez and Collins, 1991
E. asini	de Vaux et al., 1998

Formal infraspecies divisions have not been made in the genus, though some ecovar-related variability has become apparent in *E. faecium*. These ecovars pertain to biochemical reaction types (biotypes), and more convincingly, genotypes associated with certain animal host species (Devriese et al., 1987; Quednau et al., 1999).

Other Techniques

A phylogenetic tree derived from sequences of internal fragments of structural D-alanine: d-alanine ligase genes and alignments of deduced amino acid sequences was found to be largely superposable on that derived from 16S rRNA sequences (Evers et al., 1996). Similar attempts to determine subdivisions with other techniques proved less satisfactory. Polymerase chain reaction (PCR) amplification of the intergenic spacer (ITS-PCR) between the 16S and 23S rRNA, as determined by Tyrrell et al. (1997), recognized the *E. avium* group as well as *E. hirae* with *E. durans*, but failed to separate *E. faecalis* from *E. faecium* or *E. gallinarum*. Broad-range PCR (BR-PCR) amplification (Monstein et al., 1998) of 16S rDNA fragments including variable regions V3, V4 and V9 resulted in 12 different species groups, which partially corresponded to the 16S rRNA species groups determined by reverse transcriptase sequencing of the nearly complete 16S rRNA genome by Williams et al. (1991). Randomly amplified polymorphic DNA (RAPD) analysis using the unweighted pair group method of association (UPGMA) clustering showed good agreement with the 16S rDNA groups and individual species (Monstein et al., 1998).

Taxonomy

Classification Errors and Problems

About twenty species have been allocated to date (August 1999) to the genus *Enterococcus*. However, certain taxonomic and nomenclatural difficulties are apparent. *Enterococcus solitarius* (Collins et al., 1989) has been shown to be more closely related to the genus *Tetragenococcus* (Collins et al., 1990; Williams et al., 1991). *Enterococcus seriolicida* (Kusuda et al., 1991) is identical to *Lactococcus garvieae* and has to be reclassified as such (Teixeira et al., 1996).

Another problem concerns *Enterococcus flavescens* (Pompei et al., 1991). This species appears to be identical to *Enterococcus casseliflavus*, which has nomenclatural priority. Neither protein analysis nor PCR-based typing was able to differentiate between strains allocated to either one of the two species (Descheemaeker et al., 1997). Their ligase genes showed high levels of similarity (Navarro and Courvalin, 1994; Dutka-Malen et al., 1995).

Melissococcus pluton (Bailey and Collins, 1982), the etiological agent of European foulbrood disease of honey bees, is phylogenetically closely related to the genus *Enterococcus* (Cai and Collins, 1994; de Vaux et al., 1998). Despite some doubts, the genus *Melissococcus* has been retained as a separate genus for nomenclatural convenience and because of its branch point at the periphery of the the *Enterococcus* cluster.

Current Species

These observations taken into consideration, 17 *Enterococcus* species are to be retained (Table 2).

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types (biotypes), and more convincingly, genotypes associated with certain animal host species (Devriese et al., 1987; Quednau et al., 1999).

Habitat

Intestinal

The best known, though not the only habitat of the enterococci, is the gut of mammals and birds. They may be significant components of other animal groups as well. Apart from some sporadic observations, few data are available on this topic (see, for example, Benno et al., 1992 on *E. faecalis* in the Japanese tree frog, Hlya japonica). Most enterococcal species known to date are typically associated with the intestines of humans and domestic animals, and when found outside the gut, they are interpreted as indicators of fecal pollution. Others, notably the plant-associated yellow-pigmented *E. casseliflavus* and *E. mundtii* (Collins et al., 1986; Vaughn et al., 1979) may occur transiently in the intestines.

Host Species Variation

Certain host-specific variations in the occurrence of different species in different animal hosts are known to exist. In humans as well as in many other animal species, *E. faecalis* and *E. faecium* are most frequently found. The first is more common and usually occurs in larger numbers than the second (see review in Murray, 1990). *E. cecorum* is a prominent member of the enterococcal flora of poultry and pigs (Devriese et al., 1991a; Devriese et al., 1994), whereas *E. columbae* is the dominant component of the gut flora of pigeons. *E. hirae* is a frequent inhabitant of the porcine gut and may occur in poultry, cattle, dogs and cats (Devriese et al., 1987). *E. durans* has been isolated from humans, chickens and calves. Despite its name, *E. avium* is rarely isolated from the intestines of birds, and *E. gallinarum*, similarly, is not an important member of the flora of chickens. The habitat of the members of the *E. avium* species group (*E. avium*, *E. malodoratus*, *E. raffinosus* and *E. pseudoavium*) is largely unknown. *E. malodoratus* is often found in the tonsils of cats (Devriese et al., 1992a; author's correction in this reference: *E. raffinosus* is to replaced by *E. malodoratus*).

Age Variation

In certain hosts, variations in the enterococcal flora according to age have been documented. Enterococci are among the dominant flora of the intestine in the very first days of life in many animals, but they decline to markedly lower levels at 2 to 3 weeks of life (Smith and Crabb, 1965). In ruminants they are frequent in the preruminating period but they decline to very low levels later on (Devriese et al., 1992). Age-dependent variations in species distribution have been observed in the enterococcal flora of chickens: *E. faecalis* and *E. faecium* prevail during the first days of life. Later on *E. faecalis* starts to decrease first, followed by *E. faecium*, to be replaced by *E. cecorum* (Devriese et al., 1991b). Also in humans, *E. faecalis* largely outnumbers the other species in infants less than 1 week of age (Noble, 1999).

Variation in Different Compartments

The enterococcal flora may differ in different compartments of the intestine as has been documented in chickens (Devriese et al., 1991a): *E. durans* and *E. hirae* were part of the small intestinal flora of 3- to 4-week-old chicks but were not detected in the crop and the caeca of the same animals.

Variation Due to Feeding

A well-known though somewhat special example of the influence food ingestion may have is the low enterococcal content of feces from breast-fed infants (mean count per gram in 4–7 week-olds: 6.3 $\log_{10}$) compared with formula-fed infants (9.6 $\log_{10}$; Stark and Lee, 1982). Other food-dependent variations are much less clear.

Other Body Sites

Enterococci may also occur in the throat and in the vagina of humans, usually less than 20% of individuals being positive. Most isolates are *E. faecalis*, though in certain hospital settings *E. faecium* may outnumber *E. faecalis* (McGowan and MacFarlane, 1983).

Association with Plants and Invertebrates

Certain species are known to be typically plant-associated. This is notably the case with the yellow-pigmented *E. mundtii* and *E. casseliflavus* (Martin and Mundt, 1972). However, *E. faecium* and *E. faecalis* are also frequently isolated from this source (Ulrich and Muller, 1998). *E. casseliflavus* was the predominating species in forest industry wastewater in Finland. Enterococcal-like strains from pristine waters could not be identified except for some rare *E. faecalis* isolates (Niemi et al., 1993). In moderate climates, the enterococci disappear from the plant world during the winter, reappearing during the spring and becoming more and more frequent as the plants grow and flourish. It has been speculated

that insects play an important role in this seasonal variation (Martin and Mundt, 1972). Undetermined *Enterococcus* strains have been shown to play a role in the gut metabolism of wood-feeding termites (Tholen et al., 1997).

Isolation

Enterococci are usually isolated from pathological specimens on nonselective blood agars or on blood agar supplemented with colistin (polymyxin) and nalidixic acid or oxolonic acid active against mainly Gram-negative bacteria. These are most useful additions. Experienced people may be able to recognize *E. faecalis* colonies on blood agar relying on their relatively large nonhemolytic or β-hemolytic colonies. Most *E. faecium* group strains produce α-hemolytic colonies. This approach yields only presumptive identifications, of course.

Many different selective media have been devised for the enterococci but none has proved specific (Reuter, 1992). Sodium azide is the main selective component in most of them. In certain formulations bile salts or antibiotics (such as neomycin or gentamicin) with poor anti-enterococcal activity are added, and aesculin or tri-tetrazolium figure as indicator substances. Higher temperatures (42 to 45C°) may be applied as well to improve enterococcal selectivity (Niemi and Ahtiainen, 1995). One of the most used media is Slanetz and Bartley agar (also named M-*Enterococcus* agar). The visual quality and selective capacity of this medium may vary depending on the heat exposure of tetrazolium during preparation, though this affects the yield of intestinal streptococci such as *S. bovis* and *S. gallolyticus* much more than the enterococci. Usually, *E. faecalis* colonies can be recognized on this medium, which confers to this medium an advantage over others.

It should be known that not all enterococcal species grow on enterococcal selective media (Devriese et al., 1993b).

Identification

Genotypic Methods

Several genotypic methods have been evaluated for their ability to identify enterococcal strains to species level. Full sequencing of the 16S-rRNA gene was done by Williams et al. (1991) and by Patel et al. (1998). On the basis of these sequences, a phylogenetic tree was constructed from which species groups can be distinguished. Homology values within the genus *Enterococcus* ranged from 93.7% to 99.8% for a 1,452-nucleotide region (Williams et al., 1991). Because this is a costly and time-consuming method, several other tools have been studied for the ability of identification.

A species-specific PCR assay has been developed (Dutka et al., 1995). This multiplex PCR assay makes use of four primer pairs. Two primer pairs are directed to the genes encoding D-alanine:D-alanine ligases (*ddl* genes), one pair being complementary to *ddlE. faecium*, another to *ddlE. faecalis*. The *vanC-1* and *vanC-2* genes are specific for *E. gallinarum* and *E. casseliflavus*, respectively. These genes encode for intrinsic vancomycin resistance.

Tyrrell et al. (1997) used intergenic ribosomal PCR, which amplifies the noncoding region between the 16S and 23S rRNA genes, to discriminate enterococcal strains to species level. Profiles of several species, such as *E. avium*, *E. raffinosus*, *E. malodoratus* and *E. pseudoavium* and also *E. faecalis* and some *E. hirae* strains, were highly similar. Differentiation was made possible by digestion of the amplification products with *Sau3A*, except between *E. avium* and *E. pseudoavium*.

Descheemaeker et al. (1997) and Quednau et al. (1998) investigated the usefulness of RAPD in the identification of enterococci. The use of primer D11344 resulted in different amplification patterns for the species *E. faecalis*, *E. faecium*, *E. hirae*, *E. durans*, *E. gallinarum* and *E. casseliflavus* (Descheemaeker et al., 1997). Quednau et al. (1998) could visually distinguish all clinically relevant species on the basis of their fingerprint without the need of computer-based analysis. In both studies, *E. flavescens* strains showed the same pattern as *E. casseliflavus*, thus confirming that the former name is to be disregarded.

In the multiplex PCR of Dutka-Malen et al. (1995), speciation is limited to *E. faecium*, *E. faecalis*, *E. gallinarum* and *E. casseliflavus*. The RAPD and other assays may be made applicable to any desired species, provided that the discriminatory capacity and interlaboratory reproducibility of the method is satisfactory.

Phenotypic Methods

Genus Identification The classical species *E. faecalis* and *E. faecium*, as well as the species forming a species group with the latter, have a number of characteristics in common which separate them to some extent from the other Gram-positive, catalase-negative, facultatively anaerobic cocci: ability to grow in 6.5% NaCl broth, at pH 9.6, at 10°C and at 45°C; presence of group D antigen. These characteristics which traditionally are attributed to the enterococci are far from common to the other enterococcal species. Espe-

Table 3. Characteristics common to all or nearly all enterococci.

Characteristic	Result
VP	+ (negative in *E. saccharolyticus*)
β-Glucosidase	+
β-Glucuronidase	– (positive in most *E. cecorum* strains)
Urease	–
Resistance to 40% (v/v) bile	+
Aesculin hydrolysis	+
Acid from	
N-acetylglucosamine	+
Amygdalin	+
D-Arabinose	–
Arbutin	+
Cellobiose	+
Erythritol	–
D-Fructose	+
Galactose	+
β-Gentiobiose	+
Glucose	+
Glycogen	– (positive in some *E. gallinarum*, *E. cecorum* and *E. columbae* strains)
Inositol	– (delayed positive in *E. raffinosus*)
D-Fucose	–
L-Fucose	–
Lactose	+ (negative in certain *E. faecalis* strains)
Maltose	+
D-Mannose	+
Methyl-β-D-glucopyranoside	+ (not reported in some of the newer species)
α-Methyl-D-xyloside	–
Pullulan	– (not reported in some of the newer species)
Ribose	+ (negative in *E. asini* and in some *E. casseliflavus* strains)
Salicin	+
Trehalose	+ (negative in some *E. faecalis* strains)
L-Xylose	–

VP = Voges Proskauer test.

cially the newer species are often negative in one or more of these tests.

A fairly large number of other characteristics are found in nearly all enterococci (Table 3) but, with certain useful though not absolute exceptions, they are not specific for the genus. The VP (Voges-Proskauer or acetoin reaction) and acid production from ribose have a high differential value especially with regard to the streptococci. Only *S. agalactiae*, *S. uberis* and the β-hemolytic *S. porcinus* react positive in both tests as do all enterococci, except *E. saccharolyticus* (VP–) and *E. asini* and some *E. casseliflavus* strains (ribose–).

Although no single phenotypic test or combination of tests is able to characterize the genus *Enterococcus* adequately, certain practical approaches can be used. Enterococcal-like colonies growing to "normal" colony size on media containing 0.04% sodium azide selective for enterococci and able to grow in 6.5% NaCl broth are most probably enterococci. In case of doubt, VP and/or ribose testing can be added. Typically, only streptococci of the *Streptococcus bovis* species group show colony characteristics similar to those of the enterococci on these media. These streptococci are always ribose-negative and they do not grow in 6.5% NaCl broths.

This procedure is only valid when the "classical" enterococci are looked for exclusively, and when the newer species can be disregarded.

Species Groups The enterococcal species groups differ from each other in a number of characters which are useful to confirm identifications (Table 3). A more extensive version of this table has been published by Devriese et al. (Devriese et al., 1993b).

Species Identifications of species within a species group are more difficult to make, and errors are more likely to occur within groups than between groups. Further details are provided by Devriese et al. (1993b). In routine diagnostic bacteriology, usually presumptive identifications based on growth characteristics will be confirmed, and phenotypic identifications will be made by using identification galleries or applying

Table 4. Identification of species groups.

Test	*E. faecalis*	*Faecium* group	*Avium* group	*Gallinarum* group	*Cecorum* group
Motility	–	–	–	+	D
Capnophilic growth	–	–	–	–	+
Group D antigen	+	D+	D	+	–
APPA	–	–	+	–	–
PYRA	+	+	+	+	–
Alkaline phosphatase	–	–	–	–	D+
α-Galactosidase	–	D	D	+	+
Arginine dihydrolase	+	+	–	+	–
Acid from					
Adonitol	–	–	D+	–	–
L-Arabinose	–	D	D+	+	D
D-Arabitol	–	–	+	–	D
α-Methyl-D-glucopyranoside	–	–	+	+	D
D-Raffinose	–	D	D+	D	+
L-Sorbose	–	–	+	–	–

short identification schemes such as the one produced by Facklam and Collins (1989). These procedures are reliable with nearly all *E. faecalis* and most *E. faecium* strains, but less trustworthy with the others.

Certain of the tests differentiating between groups indicated in Table 3 are particularly useful to confirm identifications of the less well-known species. An example of such use is the acidification of methyl-α-D-glucopyranoside, which differentiates *E. gallinarum* and *E. casseliflavus* (the *E. gallinarum* group) from *E. faecalis* as well as from *E. faecium* and related organisms (Devriese et al., 1996).

Preservation

Enterococcus strains are notoriously resistant to adverse environmental conditions such as drying, which makes preservation easy. This is to be nuanced, however, in that certain species, notably *E. cecorum*, are not as resistant as others. The classical enterococci can be preserved for many years at –20°C in cryopreservative media (lyophylization media). Caution is warranted when the less well-known species are involved. Preservation at –70°C or lyophilization is recommended for such strains as well as for strains whose characteristics are to remain as intact as possible for certain applications and research purposes.

Epidemiology

The epidemiology of enterococci has mainly been focused on the species *E. faecium* and *E. faecalis*. The epidemiology of glycopeptide-resistant *E. faecium* has been investigated most intensely. These studies concerned hospital epidemiology and the possible spread of resistant strains from animals to humans.

Tools

The epidemiology of enterococci has been investigated with different molecular techniques, and depending on the method used, different and frequently opposite conclusions have been drawn. The highest discriminative power was obtained using PFGE (pulsed-field gel electrophoresis). This technique can clearly divide the enterococcal species into diverse clones. Ribotyping has been shown to be less suitable in typing *E. faecalis* strains (Gordillo et al., 1993). Restriction endonuclease analysis (REA) is discriminative in *E. faecium*, and may yield valuable results comparable to those obtained with PFGE. No single ideal method can be used without clinical epidemiological investigation, but any of these techniques is helpful (Savor et al., 1998). Biochemical characteristics do not allow distinction of enterococcal strains from different host species. However, raffinose-positive *E. faecium* strains are found solely among poultry strains (Devriese et al., 1987).

Transfer Between Host Species

Studies on the transfer of enterococci between host species have mainly focused on the transfer of vancomycin-resistant *E. faecium* from animals to humans. Ribotyping did not differentiate between vancomycin-resistant *E. faecium* strains from different host species (Bates et al., 1994). By using PFGE, a high genetic variability was found among the strains (Klare et al., 1995) and transfer between hosts appeared to be uncommon (Descheemaeker et al., 1999; Klare

et al., 1995; vandenBraak et al., 1998). However, on some occasions similar strains were found in both animals and humans (Descheemaeker et al., 1999; Jensen, 1998; Stobberingh et al., 1999).

Using REA of total DNA on *E. faecium* strains, a clear division could be made between strains from different animal hosts and humans (Quednau et al., 1999). This was the first firm indication of the host specificity of enterococci.

Nevertheless, transient in vivo colonization of the human gut by animal strains is possible when high doses are administered. Enterococci do not seem to survive in a different host for a prolonged period (Berchieri, 1999).

Hospital Epidemiology

Traditionally, enterococcal infections have been considered to be endogenous, arising from the patient's own flora. More recently, however, they have been shown to spread from patient to patient and from hospital to hospital. Health care personnel and inanimate objects may be responsible for transmission (Korten and Murray, 1993). In hospitals, the clonal spread of resistant strains, mainly vancomycin-resistant *E. faecium* strains, has been investigated most often by using PFGE. It has been demonstrated that some multiple resistant strains could spread within a hospital and between hospitals, even over a period of 6 years. However, equally as many outbreaks were polyclonal (McDonald et al., 1997).

Disease

Enterococci used to be known only as causes of endocarditis and rare cases of meningitis. This picture has changed considerably in the last decade: these bacteria have become one of the leading causes of nosocomial (hospital-acquired) bacteremia, and of surgical and urinary tract infections.

Infections Caused by Enterococci

In Humans The most frequent form of enterococcal disease, urinary tract infection, is most often caused by instrumentation or structural abnormalities of the urinary tract. Prior antibiotic therapy is another risk factor. Use of antibiotics especially those lacking effective anti-enterococcal activity such as cephalosporins, fluoroquinolones, polymyxins, macrolides, lincosamides and potentiated sulfonamides, is an important predisposing factor. Acquired resistance against other agents may aggravate the situation (Gray and Pedler, 1992).

Intra-abdominal and pelvic infections are often polymicrobial, but the enterococci are important components of the infecting flora. They are involved in peritonitis associated with chronic ambulatory peritoneal dialysis, spontaneous peritonitis in cirrhotic or nephrotic patients, as well as in acute salpingitis, pelvic abscesses and other forms of peripartum pathology.

Enterococcal bacteremia is associated with endocarditis in a minority of cases, other conditions such as urinary tract infection being much more frequent. Intraurinary or intravascular catheters are often involved. Polymicrobic bacteremia is very common, and mortality is generally high, most probably because of severe underlying disease and complicating factors. In countries such as the United States, enterococci rank among the most common causes of bacteremia, but in others they may be much less frequent (Pfaller et al., 1999). Endocarditis due to enterococci is seen most frequently in elderly males, often suffering urinary tract infection or undergoing invasive tests involving instrumentation. Rarer disease conditions include neonatal infections and infection of the central nervous system. Most cases represent complications of underlying disease.

In Animals Animals are not hospitalized except for some pets, and even these are much less frequently hospitalized than humans. Debilitated and aged animal patients are less often treated. For this reason the typical enterococcal pathology seen in humans is virtually unknown in animals.

Nevertheless, certain pathological conditions associated with enterococci have been documented. Most often, birds appear to be involved and *E. hirae* strains have been implicated as causes of septicemia and focal brain necrosis (Devriese et al., 1991b). Other *E. hirae*-like or *E. durans*-like strains have been shown to adhere to the intestinal villi of sucklings of several mammalian species, and may cause relatively mild enteritis (Cheon and Chae, 1996; Dooley, 1998).

Pathogenicity Among the enterococci *E. faecalis* is the species most frequently associated with disease in humans. Although *E. faecium* strains have become resistant to antibiotics more often than *E. faecalis* strains, the relative importance of these species does not appear to change dramatically (Huycke et al., 1998). This suggests that *E. faecalis* is more virulent or that *E. faecalis* strains more often possess virulence factors. These include cytolysin, pheromone-responsive plasmid transfer with production of aggregation substance, extracellular superoxide production, and a surface protein (designated as Esp). Their

pathogenic role is still unclear for the greater part.

Cytolysin Cytolysin production has been shown to be clearly associated with *E. faecalis* strains isolated from pathological conditions (Ike et al., 1987). Enterococcal cytolysin causes lysis of different target membranes including those of erythrocytes, resulting in hemolysis on some types of blood agar. It has been shown to contribute to the severity of several experimental infections and is associated with increased risk of sudden death from nosocomial bacteremia reviewed in (Huycke et al., 1998). The cytolysin is expressed and processed through a complex maturation pathway (Booth et al., 1996).

Sex Pheromone and Aggregation Substance Adherence is important in the pathogenesis of *E. faecalis* urinary tract infection and endocarditis (Guzman et al., 1989). One possible mechanism underlying adherence is mediated by small peptides seven to eight amino acids in length called pheromones. Sex-pheromone plasmid-carrying *E. faecalis* donor cells are stimulated by the excretion of pheromones by plasmid-free potential recipient cells, to synthesize a corresponding adhesive protein, called aggregation substance. This results in a tight aggregation of both types of cells, thus making the conjugative transfer of sex-pheromone plasmid possible. This unique system is remarkably regulated and versatile: inhibitor peptides are excreted by donor cells which neutralize the effects of the corresponding sex pheromones, and donor cells may produce sex pheromones not related to the sex-pheromone plasmid they harbor. The prototype of this class of genetic elements is Tn*916*.

The N-terminal part of the adhesin is responsible for the clumping effects, with a region between amino acid 525 and amino acid 617 playing a dominant role (Muscholl, 1998). This aggregation substance mediates also adhesion to eukaryotic cells such as cultured renal tubular cells (Kreft et al., 1992), and it has been shown to enhance pathogenicity in animal models of *E. faecalis* endocarditis (Schlievert et al., 1998), though in other infection models it appears to play a less determinative role (Dupont et al., 1998). The role of plasmid-encoded aggregation substance in the transition from bacteremia to endocarditis is still a matter of debate (Berti et al., 1998).

Superoxide Production Nearly all *E. faecalis* and very few *E. faecium* strains produce substantial amounts of extracellular superoxide, and production is higher in strains from septicemia than in strains from carriers. The role of this substance in the pathogenicity of *E. faecalis* is still unknown (Huycke et al., 1998).

Esp Esp is a cell wall-associated protein of unusual repeating structure (Shankar et al., 1999) which bears global organizational similarity to the Rib and C-α- proteins of group B streptococci. It has been detected in infection-derived *E. faecalis* strains but not in other enterococcal species. Its role in disease is as yet unsure.

Antibiotic Treatment Most enterococcal infections are treated with single-drug therapy. Ampicillin, penicillin, vancomycin have been used most often. Classically, β-lactams and aminoglycoside antibiotics are combined to treat endocarditis. However, these regimens are inadequate when strains with high-level aminoglycoside resistance are involved, and facing resistance situations single-drug policies may have to be changed as well.

Antibiotic Resistance Enterococci are intrinsically resistant to many antibiotics. Certain β-lactams such as penicillin, ampicillin, piperacillin and imipenem show good bacteriostatic activity (Huycke et al., 1998). Combinations of these β-lactam antibiotics with aminoglycosides have been most widely used to achieve bactericidal effects. The glycopeptides vancomycin and teicoplanin have been valuable alternatives.

Therapeutic possibilities are hampered by increasing numbers of strains with acquired resistance, especially among *E. faecium*. Resistance determinants against all useful antibiotics in the treatment of enterococcal infections have been described, even against the newest agents, which as yet have not been used extensively. Important regional differences in resistance prevalence have been noted (Pfaller et al., 1999).

Resistance against β-lactam antibiotics is mostly due to alterations in the penicillin-binding proteins of the strains. However, it is not clear whether the prevalence of strains with higher resistance levels is increasing or is simply due to selection or identification of resistant strains that were already present naturally (Moellering, 1991). β-lactamase-mediated resistance has been reported only in some *E. faecalis* strains isolated in the United States. This resistance gene resembles a staphylococcal gene and supposedly the gene has recently been transferred from this genus to the enterococci (Rice et al., 1991).

Only high level resistance against aminoglycosides is of importance because it affects the β-lactam-aminoglycoside synergy. This type of resistance is mediated by aminoglycoside-modifying enzymes, inactivating these antibiotics. The resistance genes are mostly located on

plasmids. Also these genes are identical to the staphylococcal aminoglycoside resistance determinants (Ounissi et al., 1990).

Resistance against glycopeptides including vancomycin has been reported for the first time in 1989 in Europe. Ever since, the number of reports on resistant strains has increased. Especially United States hospitals are coping with increased resistance, in contrast to Europe where infections with GRE (glycopeptide-resistant enterococci) are still uncommon (Schouten et al., 1999).

Animal strains investigated in the United States showed few resistances to therapeutic antibiotics (Thal et al., 1995). Resistance against most antibacterial growth promoters used in animal feed has been demonstrated in enterococci, especially *Enterococcus faecium* isolated from farm animals, pets and foods (Butaye et al., 1999b). Certain of these are potentially important because of their cross-resistance with therapeutically used drugs. The use of avoparcin (a glycopeptide antibiotic) in animal nutrition as a growth-promoting antibiotic has been incriminated as a source of the GRE in Europe. This is not the case in the United States where avoparcin has not been in use, but where high hospital use of vancomycin has resulted in high resistance frequencies. There is still discussion on the impact of glycopeptide resistance among enterococci from animal origin on the resistance of enterococci in humans (Butaye et al., 1999a).

Applications

Enterococci may play a beneficial or a detrimental role in foods. They may cause spoilage or they may contribute to ripening and flavoring processes of certain foods. A special application concerns their use as indicator strains to detect fecal contamination of water, and certain strains are used as additives in feeds or even as therapeutics meant to improve certain intestinal conditions.

Role in Foods

Food Contamination Enterococci are among the most thermotolerant of the nonsporulating bacteria, and the classic species are surprisingly resistant to drying, which makes them prone to cause spoilage in cooked or heated processed meats (Franz et al., 1999). Because of these properties, they can be considered as indicators of sanitary quality of foods. *E. faecalis* is the dominant species in most of these foods.

In marked contrast to this, their presence is highly desirable in a variety of cheeses to achieve certain aromas or other sensory properties. *E. faecium* and *E. faecalis* usually dominate and their numbers are often as high as 10^6 or 10^7 colony forming units/g. In other types of cheese, different lactic acid bacteria predominate, but also in these varieties enterococci represent an important part of the flora of the ripened products (data summarized in Franz et al., 1999). Their proteolytic activity, production of acetaldehyde, acetoin and diacetyl, and possibly also esterase activity on milk fat are considered important for cheese ripening (Centeno et al., 1996; Tsakalidou et al., 1998).

Indicators of Fecal Pollution Enterococci are able to survive for long periods on inanimate surfaces even in direct sunlight (Bale et al., 1993). The presence of enterococci in drinking water supplies is monitored mainly because *E. faecalis* and *E. faecium* strains survive much longer than other enteral bacteria in water. Their presence in the absence of *E. coli*, indicates a more distant contamination. Another application concerns the assessment of surface- and recreational water quality (Godfree et al., 1997). The existence of plant-associated species and strains is to be taken into account on certain occasions when fecal pollution is to be verified (Niemi et al., 1993). The detection and enumeration of enterococci in water is carried out by membrane filtration or by enrichment in liquid media (Leclerc et al., 1996).

Additives The favorable properties of enterococci have led to the inclusion of certain *E. faecalis*, *E. faecium* or *E. durans* in starter cultures of certain types of cheese. They make it possible to achieve desirable properties on a constant basis (Franz et al., 1999).

Another possibly useful characteristic of enterococci concerns their production of bacteriocins active against other enterococci, "*Listeria*" and some "*Clostridium*" species. Bacteriocins produced by *E. faecium* and *E. faecalis* generally belong to class II (Abee et al., 1994; Cintas et al., 1998).

Certain strains of enterococci are in use as "probiotics." They are applied to prevent or to treat enteric disease in humans and animals (O'Sullivan et al., 1999).

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