Probiotic enterococci: current status

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Enterococci

- typical lactic acid bacteria (LAB)
- of importance in food and clinical microbiology
- ubiquitous microorganisms, but have predominant habitat in GIT of human and animals (Giraffa, 2002)

Introduction

- former representative Group of the genus *Streptococcus* → fecal streptococci or Lancefield‘s group D streptococci
- later: separated from the genus Streptococcus on the basis of modern classification techniques and serological studies in the 1980s.
- the large conglomeration of streptococci was thus subdivided into three separate genera: *Streptococcus, Lactococcus* and *Enterococcus*

Introduction

- the typical pathogenic species (except *S. thermophilus*) remained in the genus *Streptococcus* and separated from the non-pathogenic and technically important species of the new genus *Lactococcus* (Devriese and Pot, 1995)
- the faecal ‚streptococci' associated with the GIT of human and animals, with some fermented foods and a range of other habitats, constituted the new genus *Enterococcus.*

Introduction

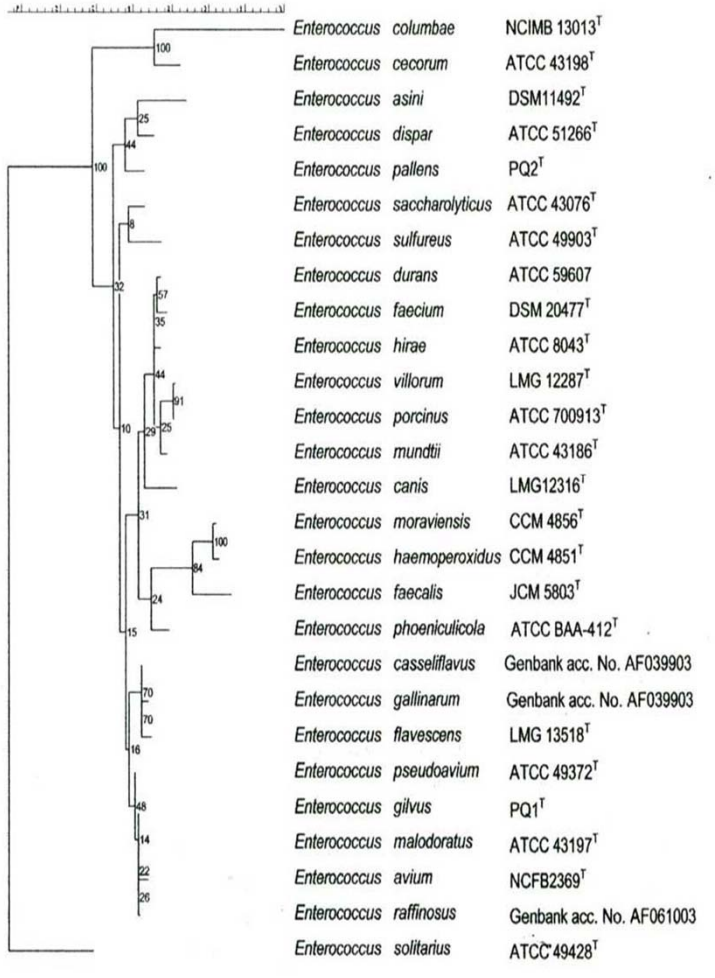
- Two most prominent representatives of the genus *Entrerococcus*:

  Enterococcus faecalis

  Enterococcus faecium
- *E. faecalis* and *E. faecium* play the most important roles both in human disease and in fermented foods and probiotics (Franz et al., 1999)

Taxonomy and identification

- Thirty *Enterococcus* species are currently recognized based on sequence analysis of 16S rRNA genes.



Taxonomy and identification

- Genus *Enterococcus* can be distinguished into 7 Groups:

***E. faecium*-group:** *E. faecium, E. durans, E. hirae, E. mundtii, E. villorum, E. canis*
***E. avium*-group:** *E. avium, E. malodoratus, E. pseudoavium, E. raffinosus, E. gilvus*
***E. gallinarum*-group:** *E. gallinarum E. casseliflavus, E. flavescens*
***E. dispar*-group:** *E. asini, E. dispar, E. pallens, E. hermanniensis, E. canintestini*
***E. saccharolyticus*-group:** *E. saccharolyticus, E. sulfureus, E. saccharominimus, E. italicus, E. aquimarinus*
***E. cecorum*-group:** *E. cecorum, E. columbae*
***E. faecalis*-group:** *E. faecalis, E. haemoperoxidus, E. moraviensis, E. ratti*

Physiological properties

Table 1 Characteristic Physiological Properties of Validly Described *Enterococcus* Species

Species	Growth at		Growth in the presence of					
	10°C	45°C	pH 9.6	6.5% NaCl	40% Bile	0.04% Sodium azide	Esculin hydrolysis	Group D antigen
E. asini	(+)	(+)	n.d.	−	+	n.d.	+	+
E. avium	V	+	+	V	V/+	n.d.	+	+
E. casseliflavus	+	+	+	V/+	+	+	+	+
E. cecorum	−	+	(+)	−	(+)	−	+	−
E. columbae	−	n.d.	n.d.	−	(+)	−	+	−
E. dispar	+	−	n.d.	+/−	+	−	+	−
E. durans	+	+	+	+	+	+	+	(+)
E. faecalis	+	+	+	+	+	+	+	+
E. faecium	+	+	+	+	+	+	+	V
E. flavescens	V/−	V/+	n.d.	+	+	+	+	+
E. gallinarum	+	+	+	+	+	+	+	+
E. gilvus	+	+	n.d.	+	+	n.d.	+	+
E. haemoperoxidus	+	−	n.d.	+	+	+	+	+
E. hirae	+	+	+	+	+	+	+	V
E, malodoratus	+	−	+	+	+	n.d.	+	+
E. moraviensis	+	−	n.d.	+	+	+	+	+
E. mundtii	+	+	+	+	+	+	+	+
E. pallens	+	+	n.d.	+	+	n.d.	+	+
E. phoeniculicola	n.d	n.d	n.d.	−	−	n.d.	n.d.	n.d.
E. porcinus	+	+	n.d.	+	n.d.	n.d.	+	+
E. pseudoavium	+	+	+	+/−	V/+	n.d.	+	−
E. raffinosus	(+)	+	+	+	V/+	n.d.	+	n.d.
E. ratti	+	+	n.d.	+	n.d.	n.d.	+	(+)
E. saccharolyticus	+	+	n.d.	(+)	+	n.d.	+	−
E. solitarius	+	+	n.d.	+	+	n.d.	+	+
E. sulfureus	+	−	n.d.	+	+	n.d.	+	−
E. villorum	n.d.	n.d.	n.d.	+	+	+	+	n.d.

n.d., not determined, (+), weak positive; V, variable; +/−, differing reports in literature.

Habitat

- Environment

  soil, surface waters, waste water, on plants,
- Gastrointestinal tract
- Foods

  - cheese

  - fermented vegetables

Habitat

Table 20.1 Numbers and predominant isolates of *Enterococcus* spp. in cheeses from Mediterranean countries

Cheese	Country of origin	Milk source	Enterococci in curd (log CFU/g)	Enterococci at end of ripening (CFU/g)	Predominant bacteria in end product (% of isolates)	Reference
White-brined cheese	Greece	Raw goat milk or mixed goat and ewes' milk	4.0	6.7	*L. plantarum* (47%) [b] *E. faecium* (12%) *L. paracasei* subsp. *paracasei* (10%) *E. faecalis* (9%)	Litopoulou-Tzanetaki and Tzanetakis (1992)
Kefalotyri cheese	Greece	Ewes' milk, cow milk or mixed ewes' and goat milk	4.9	5.8	*E. faecium* (35.6%) *L. plantarum* (18.4%) *L. casei* subsp. *casei* (15.8%) *E. durans* (9.2%) pediococci (9.2 %)	Litopoulou-Tzanetaki (1990)
Teleme cheese	Greece	Pasteurised ewes' milk	n.r.[a]	n.r.	Lactobacilli Leuconostocs Enterococci	Tzanetakis and Litopoulou-Tzanetaki (1992)
Orinotyri cheese	Greece	Raw ewes' milk	n.r.	6.8	lactococci, enterococci, leuconostocs	Prodromou *et al.* (2001)
La Serena ewes' milk cheese	Spain	Raw ewes' milk	6.2	7.2	Lactobacilli Leuconostocs Enterococci	Del Pozo *et al.* (1988)
Manchego cheese	Spain	Raw ewes' milk	n.r.	n.r.	Enterococci	Ordoñez *et al.* (1978)
Cebreiro	Spain	Raw cow milk	n.r.	6.5	*E. faecalis* (30.1%) *E. faecalis* (var *liquifaciens*) (11.9%) *Lact. lactis* (19.0%) *W. (Leuc.) paramesenteroides* (7.9%) *Leuc. mesenteroides* subsp. *mesenteroides* (6.3%) *E. faecium* (4.8%)	Centeno *et al.* (1996)

Habitat

Table 20.1 Continued

Cheese	Country of origin	Milk source	Enterococci in curd (log CFU/g)	Enterococci at end of ripening (CFU/g)	Predominant bacteria in end product (% of isolates)	Reference
San Símon cheese	Spain	Raw cow milk	5–6	6–7	*E. faecalis, E. faecium, E. durans, Staph.* spp. *Micrococcus* spp	García *et al.* (2002)
Tetilla cheese	Spain	Raw cow milk	n.r.	7.3	*E. faecalis, L. casei* subsp. *casei,* Leuconostoc *mesenteroides* subsp. *mesenteroides*	Menéndez *et al.* (2001)
Caprino d' Aspromonte	Italy	Raw or heated goat milk	4–6	5–7	enterococci, lactobacilli, mesophilic and thermophilic cocci	Caridi *et al.* (2003)
Montasio	Italy	Raw or heated cow milk	4	ca. 5–7	*S. thermophilus, E. durans, E. faecalis, E. faecium*	Marino *et al.* (2003)
Serra cheese	Portugal	Raw ewes' milk	n.r.	n.r.	*Leuc. lactis, Lact. lactis, Leuc. mesenteroides* subsp. *mesenteroides/ dextranicum E. faecium*	Macedo *et al.* (1995)
Picante da Beira Baixa cheese	Portugal	Mixture of raw goat and ewes' milk	n.r.	n.r.	*E. faecium, E. faecalis, E. durans, L. plantarum, L. paracasei*	Freitas *et al.* (1995)

[a] n.r. = not reported; [b] : *L. = Lactobacillus; E. = Enterococcus; Lact. = Lactococcus; Leuc. = Leuconostc; W. = Weissella, Staph. = Staphylococcus*

Use of enterococci as probiotics

- *E.faecium* SF68

  diarrhoea treatment
- Causido® culture

  - consists of *S. thermophilus* and *E. faecium*

  - hypocholsterolaemic effect

Virulence factors

Table 20.2 Virulence factors which may be present in some *Enterococcus* strains, and (suggested) association with stage of virulence

Virulence determinant	(Suggested) association with stage of virulence
Aggregation substance (AS)	Adhesion to eukaryotic cells (adhesin)/ promotes colonisation Invasion of eukaryotic cells (invasin) Adhesion to extracellular matrix proteins (may promote translocation) Increases survival in immune cells (evasion of host immune response)
Cytolysin (Cyl)	Eukaryotic cell toxin Lyses immune cells (evasion of host immune response)
Gelatinase (Gel)	Can hydrolyse various biological peptides, e.g. collagens and fibrin (role in translocation?) Can hydrolyse antibacterial peptides (evasion of host innate immune response)
Enterococcal surface protein (Esp_{fs} and Esp_{fm})	Adhesin, promotes colonisation Exhibits characteristics of MSCRAMMs – role in evasion of immune response?
Adhesin to collagen of *E. faecalis* (Ace) or *E. faecium* (Acm)	Adhesion to extracellular matrix proteins (may promote translocation) Exhibits MSCRAMM characteristics: role in evasion of immune response?
Endocarditis antigen from *E. faecalis* or *E. faecium* ($EfaA_{fs}$ and $EfaA_{fm}$)	Adhesin: role in endocarditis
Hyaluronidase	Degrades hyaluronic acid, a major extracellular matrix constituent: role in translocation?
Pheromones	Cause inflammation, induce superoxide production
E. faecium secreted antigen (Sag)	Adhesion to extracellular matrix proteins
Superoxide and hydrogen peroxide	May cause cell/DNA damage, improves colonisation
Capsule	Evasion of host immune response

Incidence of virulence factors

Table 20.3 Reported incidences of virulence factors among *E. faecalis* and *E. faecium* strains from foods

	% incidence of virulence factor of enterococci from foods				
	Fermented sausages[1]	Fermented sorghum[2]	Cheese/other foods[3]	Cheese[4]	Foods[5]
For *E. faecium* strains:	(n=55)	(n=22)	(n=48)	na	(11)
EfaA	100	90.9	nd	na	82
GelE	5.5	0	0	na	0
AS[a]	5.5	0	0	na	0
Esp	5.5	9.1	2.1	na	0
Haemolysin or cytolysin[b]	nd	0	8.3	na	0
For *E. faecalis* strains:	(n=5)	na	(n=47)	(n=10))	(9)
EfaA	100	na	nd	100	89
GelE	100	na	48	70	78
AS	100	na	48.9	100	67
Esp	99.3	na	36.2	50	33
Haemolysin or cytolysin	nd	na	21.3	50	44

nd: not determined; na: not applicable as no strains of the indicated species were investigated
a: aggregation substance: data pertaining to information on either the clumping phenotype or presence of the *asa1* gene
b: haemolysin or cytolysin: data pertaining to information on either the presence of cytolysin genes or lysis on blood agar
1 data adapted from Martin *et al.* (2005)
2 data adapted from Yousif *et al.* (2005)
3 data adapted from Franz *et al.* (2001)
4 data adapted from Majhenic *et al.* (2005)
5 data adapted from Eaton and Gasson (2001)

Incidence of antibiotic resistance among food enterococci

Table 20.4 Reported incidences of antibiotic resistances among *E. faecium* and *E. faecalis* strains isolated from foods

Resistance to antibiotic	% incidence of resistance of enterococci from foods						
	Fermented sausages[1]	Fermented sorghum[2]	Cheese/other foods[3]	Retail meats (turkey/chicken)[4]	Retail meats (pork/beef)[4]	Produce[5]	Ready to eat foods[6]
For *E. faecium* strains:	(n=55)	(n=22)	(n=48)	(n=213/245)	(n=114/245)	(n=97)	(n=47)
Ampicillin	30.9	0	0	54/23	4.4/2.8	nd	6.4
Penicillin	58.2	9.1	45.8	53/20	9.6/8.7	7	12.8
Erythromycin	56.4	31.8	27.1	87/43	60/39	10	29.8
Tetracycline	29.1	0	6.3	0.9/0.4	0.9/0.4	29	17
Chloramphenicol	20	0	10.4	41/22	7/19	5	2.1
Ciprofloxacin	54.5	13.6	56.3	nd	nd	28	25.5
Gentamicin	0	0	2.1	nd	nd	0	nd
Streptomycin	nd	0	4.2	nd	nd	3	nd
Vancomycin	1.8	13.6	2.1	nd	nd	0	0
Rifampin	69.1	nd	nd	nd	nd	nd	19.1
For *E. faecalis* strains:	(n=5)	na	(n=47)	(n=110/51)	(n=161/66)	(n=38)	(n=52)
Ampicillin		na	2.1	nd	nd	nd	0
Penicillin		na	12.8	0	0/0	0.6/0	0
Erythromycin	93.3	na	63.8	3	42/33	8.1/4.5	26.4
Tetracycline	86.7	na	44.7	0	94/67	89/39	41.5
Chloramphenicol	93.3	na	31.9	3	0/0	3.1/0	24.5
Ciprofloxacin	46.7	na	27.7	5	0/0	0.6/0	0
Gentamicin	0	na	25.5	0	nd	nd	nd
Streptomycin	nd	na	46.8	0	nd	nd	nd
Vancomycin	0	na	0	0	nd	nd	0
Rifampin	100	na	nd	nd	nd	nd	11.3

nd: not determined; na: not applicable as no *E. faecalis* strains were investigated

1 data adapted from Martin *et al.* (2005)
2 data adapted from Yousif *et al.* (2005)
3 data adapted from Franz *et al.* (2001)
4 data adapted from Hayes *et al.* (2003)
5 data adapted from Johnston and Jaykus (2004)
6 data adapted from Baumgartner *et al.* (2001

Conclusions

- it is still hard to make decision whether enterococci are risk for consumer or not.
- many enterococci occur in large numbers in foods; however there many strains can harbour (multiple) virulence determinants and/or antibiotis resistance genes.
- The ‚host factor‘ appears to play a key or determining role in the establishment of an infection with enterococci.

- Thank you!