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# Risk and safety of Enterococci in probiotic use



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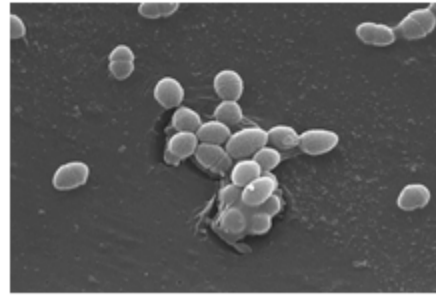
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## The genus *Enterococcus*

Gram-positive cocci initially described as streptococci (until 1980s)  
*Enterococcus* = 3rd-largest genus of lactic acid bacteria (LAB)  
after *Lactobacillus* and *Streptococcus*.

Most enterococcal species can grow in harsh conditions:  
(6.5% NaCl, 40% bile salts, pH 9.6, T°: 10 to 45°C)



*E. faecalis* and *E. faecium* = most important species of *Enterococcus*

in medical and food microbiology and studied as potential probiotics.

## Habitat of *Enterococcus*

Enterococci occur in many different habitats :  
Soil, surface water, sewage, plants, gastrointestinal tract of humans and animals  
Presence in foods of animal origin (fermented and cooked meats, cheese).

*E. faecalis* = predominant *Enterococcus* species  
of the human gastrointestinal tract

In faeces :  $10^5$ - $10^7$  CFU/g for *E. faecalis*  
 $10^4$ - $10^5$  CFU/g for *E. faecium*



Enterococci are also present in the intestine of animals.

This leads to contamination of milk and meat.

They can survive pasteurization due to their heat resistance.



## RISK

### Pathogenicity of *Enterococcus*

*Enterococcus* belongs to LAB,  
but there is controversy over considering them as GRAS  
(Generally Recognized As safe)



In the last decades, they have emerged as important nosocomial pathogens  
causing a variety of infections in immunosuppressed and intensive care unit  
patients : endocarditis, bacteremia, infections of the urinary tract...

*E. faecalis* predominates among enterococci isolated from human infections  
(more than 80%).

### Antibiotic resistance of *Enterococcus*

Resistance to a wide variety of antibiotics = contributing factor to pathogenesis

**Intrinsic resistance** : genes located on the chromosome  
( $\beta$ -lactams, cephalosporins, clindamycins, aminoglycosides)



**Acquired resistance** : genes on plasmids and transposons  
that can be transfer between strains.  
(chloramphenicol, erythromycin, clindamycins, aminoglycosides, tetracycline,  
 $\beta$ -lactams, fluoroquinolones and glycopeptides such as vancomycine),

Vancomycin : antibiotic considered a last resort for treatment of multiple  
resistant enterococcal infections.

The emergence of vancomycin-resistant enterococci (VRE) in hospitals  
has led to infections that cannot be treated with conventional antibiotic therapy.

### Virulence factors of *Enterococcus*

Virulence factors enhance the ability of a microorganism to cause disease.

Some virulence factors of *E. faecalis* are involved in :

- attachment to host cell and to extracellular matrix proteins (AS, Esp, EfaA..)
- resistance to macrophages (AS, HypR..),
- cell and tissue damage (Cyl, GelE, SprE..)
- immune system evasion (capsular polysaccharides).

Virulence factors are encoded by chromosomal regions  
Or plasmid genes : transmissible to other strains by gene transfer.  
*E. faecium* generally contains less virulence factors than *E. faecalis*.

## SAFETY

### *Enterococcus* in food technology

Use as dairy starter cultures,  
Role in improving flavour and quality of cheese.  
Contribution to the ripening and aroma  
due to proteolytic and esterolytic activities  
production of diacetyl and volatile compounds.



Production of bacteriocins, called enterocins  
(for example Enterocin A, Enterocin B and Enterocin P).  
Small peptides with antimicrobial activity  
towards closely related Gram-positive bacteria  
including spoilage or pathogenic bacteria, such as *Listeria*.



Use of bacteriocin-producing enterococci as starter cultures or protective  
cultures, or direct addition of the enterocins in food fermentation process  
may help to prevent the growth of pathogens.

### *Enterococcus* as probiotics : some examples

*E. faecium* SF68 (NCIMB10415), (Cerbios Pharma)  
found in Cylactin® and Bioflorin®  
Treatment of diarrhoea  
It is considered as an alternative to antibiotic treatment.



Symbioflor® 1 (Symbiopharm)  
contains *E. faecalis*  
for recurrent infections of the upper/lower respiratory tracts,  
inflammation of nasal sinuses (sinusitis) and bronchi (bronchitis).



SymbioPet : nutritional supplement for dogs and cats  
contains *E. faecium*  
stabilizes and reinforce the intestinal flora.



For safety assessment, antibiotic resistance and virulence factors must be checked  
in each new *Enterococcus* isolate before use as probiotic.

### Regulatory situation in Europa

The EU approach related to, for example, *E. faecium* is focused on  
demonstrating that the strain is safe and that safe strains can be  
differentiated from those that cause nosocomial infections in hospitals  
and possess antimicrobial resistance (AMR) genes.

The EU Commission, EFSA and the EU food and feed industries recognize  
that *E. faecium*, other lactic acid bacteria and *Bacillus* spp. are present in  
fermented foods, food supplements and pharmaceutical preparations. In  
the feed industry, they can also be used as feed additives, including  
fermented feeds such as silages.

In June 2017, EFSA published a draft version of a new guidance on the  
characterization of microorganisms, which contains useful information  
on how EFSA will potentially assess the safety of *E. faecium* : antibiotic  
resistance, markers of virulence... whole genome analysis.

[https://www.efsa.europa.eu/sites/default/files/engage/170615\\_0.pdf](https://www.efsa.europa.eu/sites/default/files/engage/170615_0.pdf)

