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Streptococcus gallolyticus subsp gallolyticus and *Enterococcus faecium* Gut Translocation: Virulence or Opportunism?

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Introduction and Purpose

Streptococcus gallolyticus subsp. *gallolyticus* (*S. gallolyticus*) and *Enterococcus faecium* (*E. faecium*) are implicated in endocarditis/bacteremia in colorectal cancer and immunocompromised patients, respectively.

The aim of this work was to investigate the differences of clinical relevant isolates in translocation across the intestinal epithelium, their cellular adhesion/invasion and their ability to form biofilm *in vitro.*

Methods

- Ten colonizing and invasive isolates, 6 *E. faecium and* 4 *S. gallolyticus* , were selected for the study (Table 1).
- For **translocation assay**, a differentiated monolayer of Caco-2 cells (TER 500-700Ω/cm^2) grown on polycarbonate transwell inserts (8µm pore size and 0.33cm^2) were inoculated at a MOI of 20 with of each isolate. Transepithelial electrical resistance (TEER) was monitored and translocated bacteria (CFU/ml in lower compartment) were determined by plating 10-fold dilutions onto agar plates at six time points along 8 hours. A *Lactobacillus reuteri* isolate was used as negative translocation control.
- To analyse the **adherence** of the isolates to the intestine epithelium, Caco-2 cells were grown until differentiation (10-14 days) and they were incubated with 10^7 CFU/ml of each isolate for 2 h at 37ºC 5% CO_2, then washed with PBS, treated with 0,1% triton X-100/PBS and 10-fold serial dilutions were plated on blood agar or m-Enterococcus agar for CFU counts. To study the **invasion ability,** after bacterial incubation and ulterior washing, extracellular adherent bacteria were killed during 1.5h incubation with 200 µg/ml streptomycin and 50 µg/ml ampicillin for *S. gallolyticus* and 10 µg/ml vancomycin and 100 µl/ml lysozyme for *E. faecium.* After PBS washing, monolayers were lysed with 1% Triton-X100/PBS. Lysates were plated onto blood agar or m-Enterococcus to count CFUs. Adherence was expressed as a percentage of the inoculum, and epithelial cell invasion as the percentage of adherence. Antibiotic killing efficacy in DMEM was tested previously for all strains employing (data not shown).
- **Biofilm formation** on polystyrene, collagen-I and collagen-IV covered microplates was quantified by the cristal violet method (Heikens *et al.*) (cutoff: OD_{600}>0.1). All experiments were performed in triplicate in at least three independent experiments and a multilevel linear regression statistical model was applied for translocation data analysis.

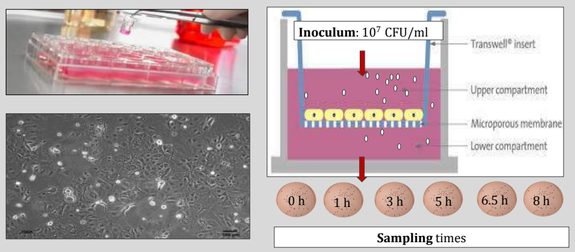


Figure 1. Translocation assay scheme.

Table 1. Characteristics of the isolates selected for the study.

Specie	Strain	Source	Sample	mlst (ST)	Antibiotic resistance	Other features	Reference
E. faecium	Efm106	OH patient	Feces	18	Amp, Hlr-Str, E	*esp, hyl, acm*	Sánchez-Díaz *et al.* 2015
E. faecium	Efm113	OH patient	Feces	117	Amp, Lvx, Hlr-Str, Hlr-G, E	*esp, hyl, acm*	Sánchez-Díaz *et al.* 2015
E. faecium	Efm121	OH patient	Blood	117	Amp, Lvx, Hlr-Str, E	*esp, acm*	Sánchez-Díaz *et al.* 2015
E. faecium	Efm197	OH patient	Feces	117	Amp, Lvx, Hlr-Str, Hlr-G,E, Lnz	*esp, hyl, acm*	Sánchez-Díaz *et al.* 2015
E. faecium	Efm217	outpatient	Feces	25	Amp, Lvx, Hlr-Str, Kan, Tet	*acm*	Tedim *et al.* 2015
E. faecium	Efm222	outpatient	Feces	699	--	--	Tedim *et al.* 2015
S. gallolyticus	Sg1	patient	Blood	NA	Min		Romero *et al.* 2015
S. gallolyticus	Sg6	patient	Blood	NA	Clin, Fos		Romero *et al.* 2015
S. gallolyticus	Sg74	Cow	Feces	NA	Hlr-Str, E Min, , Clin, Sxt,		Romero *et al.* 2015
S. gallolyticus	Sg78	Calf	Feces	NA	Clin, Sxt, Van, Q/D		Romero *et al.* 2015
E. faecalis	Ef29212	ATCC	--	NA			www.atcc.org
L. reuteri	Lr925	CECT 925T	--	NA			

Results

- **Translocation** ability across Caco-2 epithelium was demonstrated in 6/6 *E. faecium* and 3/4 *S. gallolyticus* isolates. Although non-significant differences in translocation speed (log CFUs/h) were found, *E. faecium* isolates Efm-106 (OH patient colonization) and Efm-121 (OH patient bacteraemia) translocated significantly more efficiently ($p<0.01$) than those from healthy volunteers colonization and *S. gallolyticus* strains.
- ***S. gallolyticus*** isolates (Sg1 Sg6, Sg74 and Sg78*) and L. reuteri* increased TEER values 10-20 % over the basal value.
- **TEER** values increments of **1%** were correlated with **translocation drops of 0. 04 logs**.
- ***E. faecium*** isolates from **OH patients** exhibited higher **adhesion ability** (1.5-3.5% of the inocumum) than *S. gallolyticus and E. faecium* from healthy volunteers (Efm-217, Efm-222).
- No significant biofilm formation was observed in *E. faecium* (6/6) and *S. gallolyticus* isolates (3/4) on polystyrene surfaces, but Sg78. Biofilm production was significantly higher on collagen IV coated surfaces.

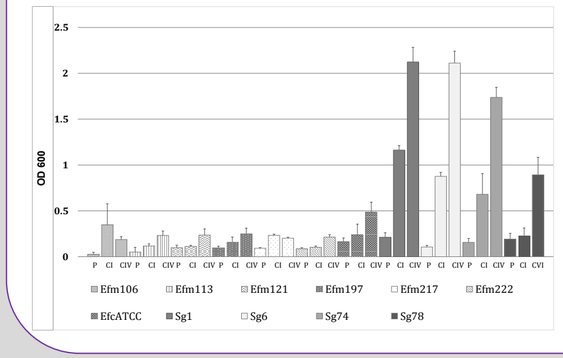
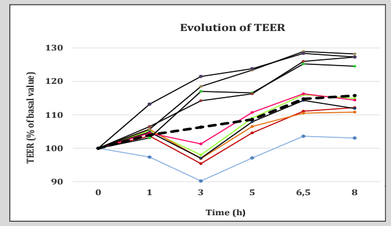


Figure 3. Biofilm production on polystyrene (P), collagen I (CI) and collagen IV (CIV) coated microplates determined by de crystal violet method.

Figure 1a) Mean evolution of TEER values during translocation experiments.
Figure 1b) translocation of Sg and Efm isolates across Caco-2 cells.



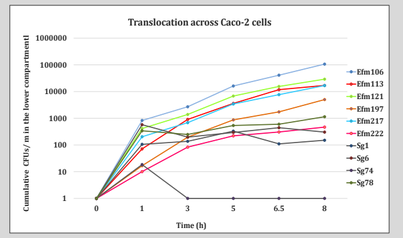
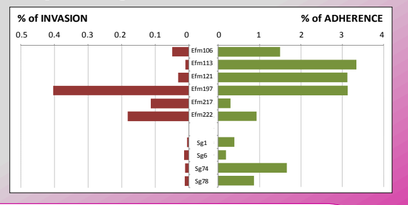


Figure 2. Adhesion and invasion percentages referred to the inoculum.



Conclusion

Both species form biofilms efficiently on collagen-rich surfaces, present good adhesiveness to intestinal epithelial cells and do not exhibited high invasion ability.

Under physiological conditions *E. faecium* isolates are able to efficiently translocate whereas intestinal epithelium alteration as it happens in colorectal cancer, seems to be required for *S. gallolyticus* translocation.