Enterococcus faecalis GFP biofilm susceptibility assay

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Aim
To develop a green fluorescent protein (GFP)-based screening assay to monitor the effect of antimicrobials on Enterococcus faecalis biofilms using the intrinsic fluorescence as a susceptibility marker.

Materials and Methods
- Overnight culture of E. faecalis OG1RF with plasmid pMV158:GFP
- Correct for OD620 ≈ 0.7
- 1:50 dilution in SDMY medium with 0.2% glucose and 10 µg/ml tetracycline
- Inoculation of 96-well microtiter plate
- Incubation under 5% CO2 at 37°C for 24 hr, with medium refreshment after 8 hrs.
- Removal of supernatant
- Add 100 µl test compound dissolved in 50 mM PIPES and 0.9% saline (pH 7.2)
- Follow GFP fluorescence kinetically
  λex 488 nm
  λem 515 nm

Conclusion
This GFP-based assay can be used, under strictly controlled environmental conditions, to monitor the antimicrobial susceptibility of E. faecalis. In addition, this GFP-based assay can be used as a pH sensor to monitor localized pH changes in biofilm. By following the GFP response in time it can be used to predict the mode of action of novel antimicrobials.

Results
Figure 1 shows the biofilm GFP-signal which is pH sensitive (reduced at lower pH) and time dependent.

Oxidizing agents such as sodium hypochlorite decreased the cell viability in a dose dependent manner and reduced GFP fluorescence. See fig. 2 and the video for real-time GFP reduction determined using a BioFlux system.

Compounds, that affect cell membrane integrity (Olaflur) or proton motive force (nigericin and valinomycin), see fig 3a and 3b, showed increased GFP fluorescence in a dose-dependent manner, likely due to leakage of hydrogen ions over the cell membrane.