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Enterococcus faecalis GFP biofilm susceptibility assay

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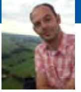

Aim

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.

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.

Materials and Methods

- Overnight culture of *E. faecalis* OG1RF with plasmid pMV158:GFP
- Correct for OD_{620} ≈ 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% CO_2 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
  - λ_{ex} 488 nm
  - λ_{em} 515 nm

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.

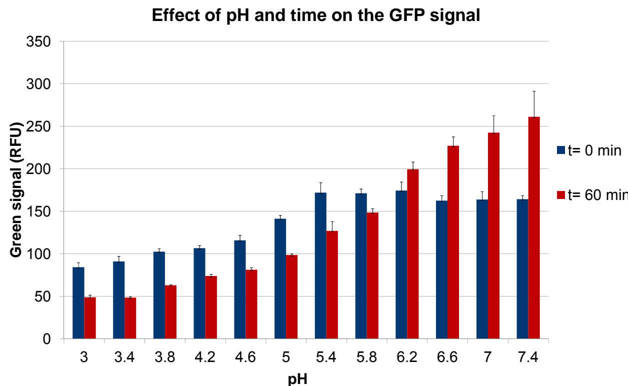

Fig 1

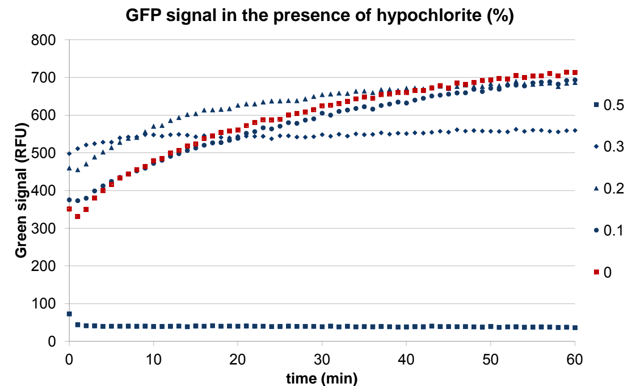

Fig 2

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.

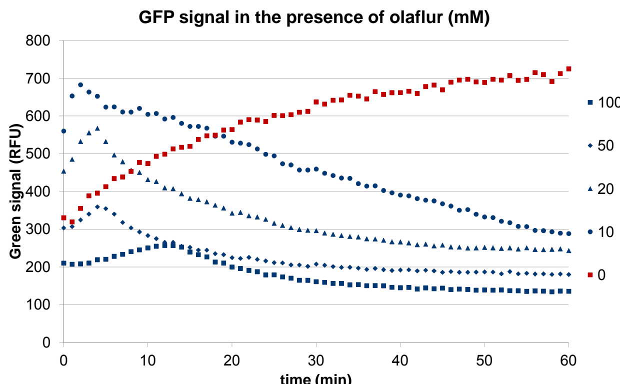

Fig 3a

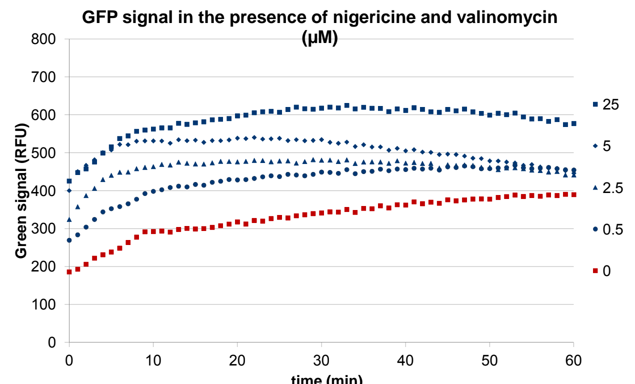

Fig 3b