In vitro detection of porphyrin-producing wound pathogens with real-time bacterial fluorescence imaging

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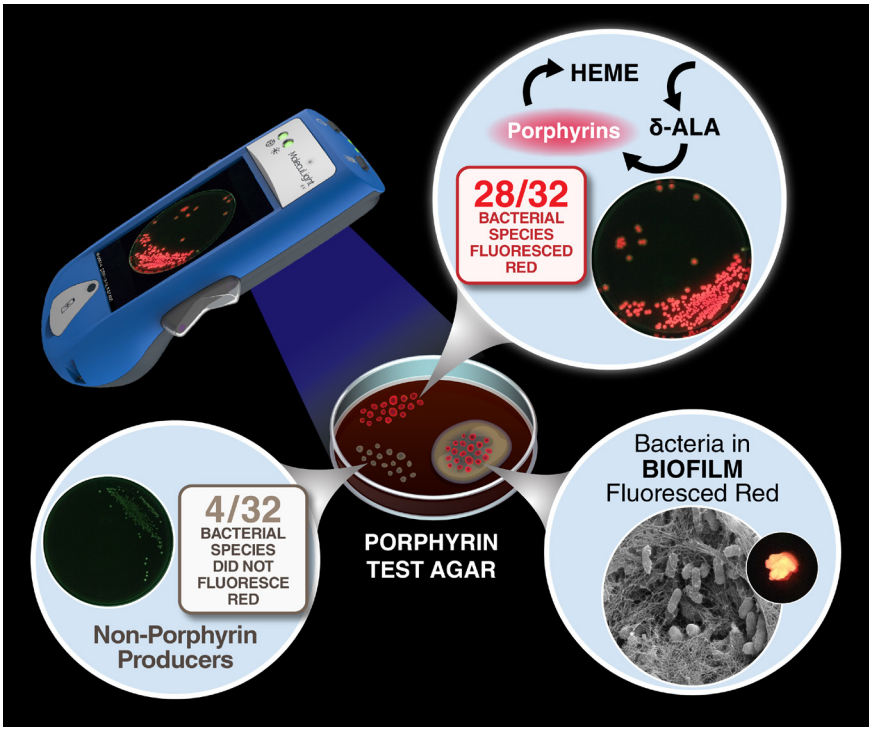
In this study, the porphyrin production and red fluorescing capabilities of **32 common wound pathogens** were investigated, grown in both planktonic and biofilm forms, were investigated using the MolecuLight ***i:X***® fluorescence imaging device. This is the first published study to report detection of red fluorescence from bacteria in biofilm under violet light illumination.

Background

- The handheld MolecuLight ***i:X*** fluorescence imaging device is used by wound care practitioners around the world to visualize the presence of bacteria in wounds. Across multi-site trials, the presence of red fluorescence has a positive predictive value of >95% for moderate-to-heavy (>10^4 CFU/g) bacterial loads.[1,2]
- Unique fluorescence from bacteria is attributed to intrinsic production of red-fluorescent **porphyrins**, intermediates of the heme biosynthesis pathway.[3,4]
- In the present study, 32 bacterial (26 aerobic, 6 anaerobic) and 4 yeast species were plated in triplicate on Remel Porphyrin Test Agar plates and examined for red fluorescence under 405 nm violet light illumination using the MolecuLight ***i:X*** device.



- **28/32 porphyrin-producing bacterial species produced red fluorescence** when illuminated with the MolecuLight ***i:X*** after 40 or 120 hours of incubation while 4 non-porphyrin producing bacterial species did not fluoresce red.
- Red fluorescence was also observed from **porphyrin-producing bacteria grown in biofilms** (confirmed by SEM) supplemented with nutrients to support porphyrin production and illuminated with violet light.

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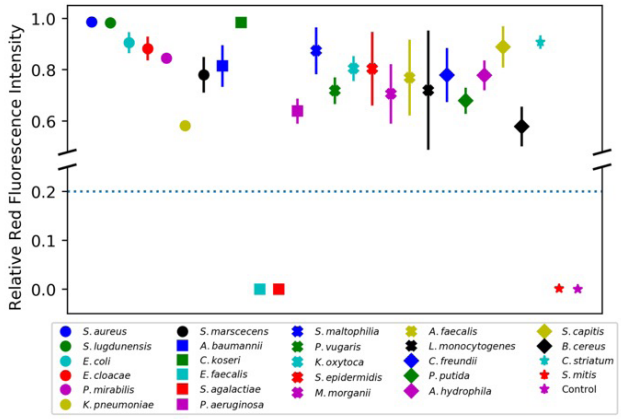
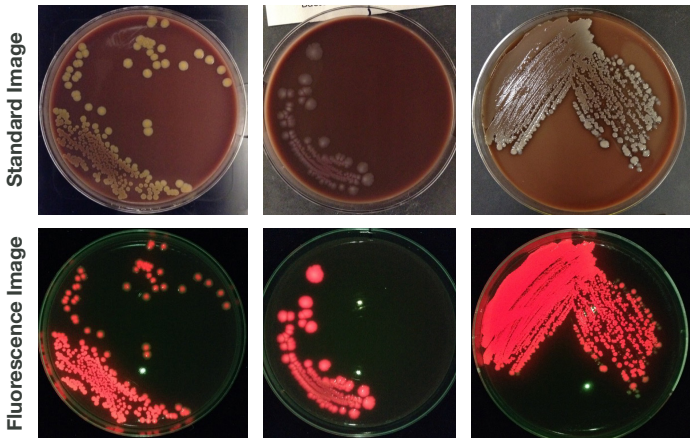
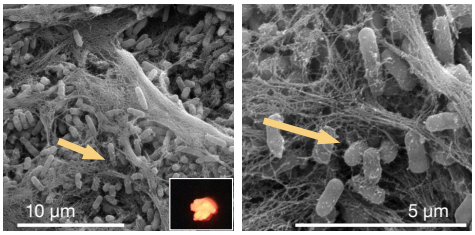
Results







Clockwise from top left: Relative red fluorescence intensity of all 26 aerobic species after 40 h of incubation, determined using an automated image analysis algorithm. Representative standard and fluorescence images of an aerobic porphyrin producing bacterial species (*S. aureus*) and an anaerobic bacterial species (*B. fragilis*) are shown. Red fluorescence is also detected from a polymicrobial culture of porphyrin-producing (*S. aureus*. 1 part) and non-porphyrin producing (*E. faecalis*, 4 parts) bacterial species. Scanning electron microscopy (SEM) images confirm the presence of biofilm from polymicrobial bacterial cultures that fluoresced red (inset). Arrows point to regions of bacteria closely associated with EPS matrix that comprise biofilm at magnifications of 3,500x and 10,000x, respectively.

- Time course analysis of *S. aureus* and *E. coli* revealed detectable red fluorescence >24 hours of incubation, and an increase in red fluorescence intensity over time.
- 3/4 yeast species did not produce red fluorescence. Only *Candida guillermondii* produced some detectable, weak red fluorescence under violet light illumination.
- Red fluorescence is detectable in polymicrobial cultures where non-porphyrin producing bacterial species are predominant. Non-porphyrin producing bacteria occur monomicrobially in <1% of wounds.[5]

Conclusion

Fluorescence imaging using the MolecuLight ***i:X*** imaging device can detect both planktonic and biofilm bacterial cultures. Our findings confirm porphyrin production as the main biological source of red fluorescence detected from the majority (88%) of clinically relevant wound bacteria.

Clinical Implications

The MolecuLight ***i:X*** can help to overcome challenges in treating chronic infections by visualizing the presence of bacteria at point-of-care and providing diagnostic feedback on treatment selection. Biofilm is present in more than 70% of chronic wounds[6,7] and it contributes to delayed healing. Using fluorescence imaging to localize bacteria, whether planktonic or biofilm, may facilitate targeted disruption of biofilm and bacterial removal.

Study Citation

Jones LM, Dunham D, Rennie MY, Kirman J, Lopez AJ, Keim KC, Little W, Gomez A, Bourke J, Ng H, DaCosta RS, Smith AC. *In vitro* detection of porphyrin-producing wound bacteria with real-time fluorescence imaging. Future Microbiology. http://doi.org/10.2217/fmb-2019-0279

References
1. Rennie et al. J Wound Care, 2018; **2.** Hurley et al. J Wound Care, 2019; **3.** Nitzan et al. Photochem. Photobiol. Sci. 2004; **4.** Phillip-Dormston et al. Enzyme, 1973; **5.** Wolcott et al. Wound Repair and Regeneration, 2016; **6.** Attinger et al. Advances in Wound Care, 2012; **7.** Malone et al. J Wound Care, 2017