

Monitoring curli in bacterial biofilms forming on semi-solid agar

This protocol describes how to use EbbaBiolight to visualise curli in biofilm forming on semi-solid agar in real-time. Curli is a functional amyloid produced by many *Enterobacteriaceae* involved in adhesion to surfaces, cell aggregation, and biofilm formation. EbbaBiolight are versatile molecules that have been reported to target various structures in the cell wall of gram-positive bacteria and the extracellular matrix of gram-negative bacteria. Curli has been identified as one of the major targets for EbbaBiolight in *Salmonella* biofilms using wildtype bacteria as well as curli deficient (Δ csgA) strains. For reference, see Choong et al. (2021) *Biofilms*, 3, 100060. We recommend using fluorescent protein tagged bacteria to be able to distinguish between bacterial cells and curli in the bacterial extracellular matrix. The method described here is a semi-high throughput approach which allows for analysis at a faster pace and larger volume compared to conventional methods.

Note: When adapting this technique, please make sure to include relevant controls to verify that EbbaBiolight does not affect biofilm formation, to confirm curli as EbbaBiolight binding target and to exclude pH effects. Please be aware that fixation might alter the staining pattern of EbbaBiolight.

Materials:

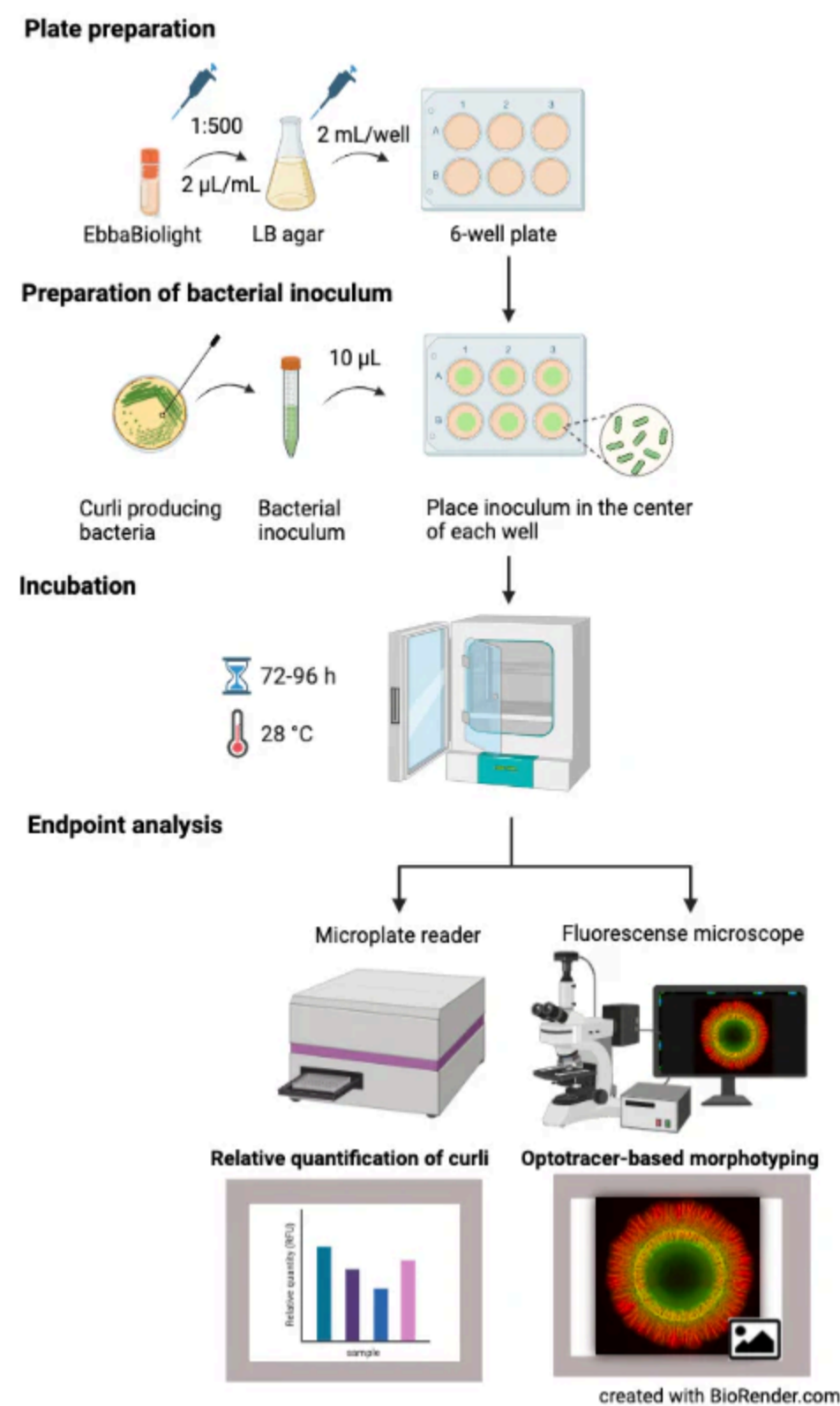
- EbbaBiolight
- LB broth (w/o salt)
- LB agar (w/o salt)
- 6-well plate with cover
- Curli-producing bacteria on standard culture plate

Equipment:

- Incubator (28°C)
- Shaking Incubator (37°C)
- Microwave
- Fluorescence microscope OR Fluorescence Plate Reader

Assay Procedure:

- Prepare plates with EbbaBiolight supplemented LB agar:
 - Microwave LB agar until it is melted.
 - Let the microwaved agar rest at RT for 5-10 min.
 - Add 2 μ L/mL EbbaBiolight.
 - Pour 2 mL of supplemented LB agar per well in a 6-well plate.
 - Cover plates and let cool down at RT.
 - Optional: Store at 4°C.
- Prepare bacterial inoculum:
 - Pick a colony from a standard culture plate.
 - Transfer colony to LB broth.
 - Prepare an overnight or exponential culture under continuous shaking at 37°C.
 - Transfer 10 μ l of bacterial inoculum to the centre of a dedicated well on the previously prepared 6-well plate.
- Incubation:
 - incubate the plate in an incubator at 28°C for as long as required for the biofilm to form.
- Endpoint analysis:
 - Analyse in fluorescent microscope for optotracer based morphotyping OR in Fluorescence Picowell Reader for curli quantification.



Optotracing with EbbaBiolight

EbbaBiolight fluorescent tracer molecules are optotracers. Unlike conventional fluorescent dyes, optotracers bind promiscuously to a range of targets with repetitive motifs. EbbaBiolight has been shown to bind to curli and cellulose in *Salmonella* extracellular matrix^[1,2], peptidoglycan and lipoteichoic acids in the cell envelope of *Staphylococci*^[3], β -glucans from *S. cerevisiae* and Chitin in *C. albicans*^[4]. Upon binding, the fluorescence intensity of the optotracer increases. This property makes it possible to use EbbaBiolight for live fluorescent tracking of microorganisms, without the need to wash away unbound molecules. It is possible to read out fluorescence intensity at the emission maximum ($E_{m_{max}}$) when excited at or close to the excitation maximum ($E_{x_{max}}$). This is useful for microscopy or fluorescence spectroscopy when straight-forward data analysis is required. Yet, due to the unique properties of the optotracers, a unique optical fingerprint is produced reflecting the specific nature of the target (sample composition) and environment (pH, osmolarity, polarity of the medium). This means that depending on the specific properties of the sample, $E_{x_{max}}$ or $E_{m_{max}}$ can shift, or the appearance of double peaks or shoulders might indicate binding to multiple targets. We therefore recommend acquiring fluorescence excitation and emission spectra whenever possible within experimental limitations. EbbaBiolight excitation- and emission spectra can be accessed [here](#).

Table: EbbaBiolight spectral properties with maximum excitation ($E_{x_{max}}$) and emission ($E_{m_{max}}$) when bound and recommended range for acquisition of excitation- and emission spectra as well as recommended filter sets for microscopy.

	$E_{x_{max}}$	$E_{m_{max}}$	Excitation spectrum (detect at $E_{m_{max}}$)	Emission spectrum (excite at $E_{x_{max}}$)	Recommended filter-sets
EbbaBiolight 480	420 nm	480 nm	300 - 450 nm	450 - 800 nm	DAPI
EbbaBiolight 520	460 nm	520 nm	300 - 490 nm	490 - 800 nm	FITC, GFP
EbbaBiolight 540	480 nm	540 nm	300 - 510 nm	510 - 800 nm	FITC, GFP, YFP
EbbaBiolight 630	520 nm	630 nm	300 - 600 nm	550 - 800 nm	PI, Cy3, TxRed, mCherry, Cy3.5
EbbaBiolight 680	530 nm	680 nm	300 - 650 nm	660 - 800 nm	PI, mCherry, Cy3.5

Read More:

1. Choong FX et al. (2016) Real-Time optotracing of curli and cellulose in live *Salmonella* biofilms using luminescent oligothiophenes. *npj Biofilms and Microbiomes*, 2, 16024
2. Choong FX et al. (2021) A semi high-throughput method for real-time monitoring of curli producing *Salmonella* biofilms on air-solid interfaces. *Biofilm*, 3, 100060
3. Butina K. et al. (2020) Optotracing for selective fluorescence-based detection, visualization and quantification of live *S. aureus* in real-time. *npj Biofilms and Microbiomes*, 6(1), 35
4. Kärkkäinen, E. et al. (2022) Optotracing for live selective fluorescence-based detection of *Candida albicans* biofilms. *Frontiers in Cellular and Infection Microbiology*, 12, 2235-2988

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