An improved medium for growing Staphylococcus aureus biofilm

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A medium (Brain Heart Infusion plus 10% human plasma) was developed, tested, and validated for growing Staphylococcus aureus biofilm in vitro. With this medium, S. aureus forms reproducible and robust biofilms in flow chambers under controlled shear flow and with increased viability recovery in static well plates.

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Abstract

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Keywords:
Staphylococcus aureus
Biofilm
Human plasma
Microfluidic

Considered one of the leading causes of human bacterial infections, Staphylococcus aureus, a gram positive organism, is a ubiquitous opportunistic pathogen that commonly colonizes the anterior nasopharynx of humans. The organism readily produces biofilms, a complex microbial community, on tissues or medical indwelling devices (Higashi and Sullam, 2006). Though little is known about the processes regulating the mature S. aureus biofilm, adherence and accumulation of cells are required. The adherence of S. aureus to surfaces and a wide range of plasma as well as extracellular matrix proteins (e.g., fibrinogen, collagen, fibronectin, laminin, and elastin) is mediated by adhesion molecules (Higashi and Sullam, 2006). Identified adhesion molecules include the cell wall associated, negatively charged, teichoic acids and a large number of surface proteins belonging to a family of molecules called microbial surface component recognizing adhesive matrix molecules. Cell accumulation of S. aureus involves the polysaccharide intercellular adhesin, also known as polymeric N acetylglucosamine (Gotz, 2002; Higashi and Sullam, 2006). The bacteria within the biofilm matrix are protected from the host immune system and are resistant to antibiotic therapy. It has been shown that S. aureus biofilms are involved in osteomyelitis; indwelling medical device infections; and peri implantitis, chronic wound infections, chronic rhinosinusitis, endocarditis, and ocular infections (Archer et al., 2011). In addition, emerging evidence indicates that S. aureus biofilms cause healing impairment (Gurjala et al., 2011) in wounds, perhaps by altering the proper course of wound healing. Therefore, biofilm clearance is critical to combat the infections caused by this organism.

To improve biofilm treatment strategies and outcomes, we need to develop in vitro biofilm models for effective screening of antibiofilm agents in modulating S. aureus biofilm development. An important component of an effective anti biofilm screening assay is its ability to generate reproducible robust S. aureus biofilms such that the biofilm burden produced can accurately measure the effectiveness of the test agents and present a reasonable chance that the agents will be effective upon evaluation in vivo. It is also likely to reduce the chance in identifying false positive(s). In our efforts to develop an assay that can produce a consistent S. aureus biofilm burden, we learned that there is a lack of widely accepted media to grow S. aureus biofilms. Different media have been used by the research community for studying S. aureus biofilms. These media consist mainly of complex medium supplemented with different compound(s), such as, Trypticase Soy Broth (TSB) plus glucose (Shanks et al., 2005; Merino et al., 2009); TSB enriched with glucose and sodium chloride (NaCl) (Beenken et al., 2003; Luong et al., 2009); TSB supplemented with glucose and citrate (Shanks et al., 2008); TSB plus yeast extract, glucose, and citrate (Craigin et al., 2011); and Brain Heart Infusion (BHI) broth with glucose or NaCl (Houston et al., 2011). S. aureus formed decent biofilms on microtiter (static well) plates with some of these media. However, in the microfluidic system, only sporadic loose and inconsistent S. aureus biofilm was obtained during a 24 hour incubation period with these media. Here, we report a medium that is suitable for use in growing S. aureus biofilms under both static and flow (microfluidic) conditions with a consistent test biofilm load.

One key factor that S. aureus encounters in vivo is blood. Transcriptome analysis of S. aureus showed that a number of genes encoding known or putative S. aureus virulence factors, such as genes of gamma hemolysin subunits A, B, and C (hlgA, hlgB, hlgC), are up regulated...
Report Documentation Page

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1. REPORT DATE
   01 AUG 2012

2. REPORT TYPE
   N/A

3. DATES COVERED
   -

4. TITLE AND SUBTITLE
   An improved medium for growing Staphylococcus aureus biofilm.

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR’S ACRONYM(S)

11. SPONSOR/MONITOR’S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
   Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:
    a REPORT unclassified
    b ABSTRACT unclassified
    c THIS PAGE unclassified

17. LIMITATION OF ABSTRACT
    UU

18. NUMBER OF PAGES
    4

19a. NAME OF RESPONSIBLE PERSON

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18
during culture in human serum or blood (Malachowa et al., 2011). Therefore, we were interested in testing whether any human blood components can also improve the S. aureus biofilm formation in vitro. For this test, we chose the use of TSB or BHI as the base medium and supplemented it with human plasma (Biological Specialty Corporation, Colmar, PA). We first tested the feasibility of producing S. aureus biofilm by using the plasma containing growth medium in an in vitro biofilm model based on a microfluidic platform (Fluxion Biosciences, South San Francisco, CA). In essence, the system consists of a network of microfluidic laminar flow channels integrated into standard well plates suitable for conventional or confocal laser inverted scanning microscopy (LSM). The growth of biofilms is under controlled shear force and temperature mimicking physiologically relevant conditions. To monitor S. aureus biofilm formation in real time, we used overnight green fluorescein protein (GFP) tagged clinical isolate and 2% NaCl. Citrate: TSB plus 0.6% yeast extract and 0.8% glucose and 0.2% sodium citrate (Craigen et al., 2011). Scale bars=10 μm.

To further characterize the use of these media containing plasma for growing S. aureus biofilms, we also tested other S. aureus strains. Similar results of producing robust biofilms were obtained when other S. aureus strains (ATCC 6538, ATCC 33591, and ATCC 43300) were used (data not shown). These results suggest that both TSB and BHI supplemented with 10% human plasma are good media for growing S. aureus biofilm under fluid dynamic conditions. These human plasma supplemented media also improved the biofilm growth of S. epidermidis, but have adversary effects on the biofilm formation of Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae (data not shown).

To determine whether these two media are also good for S. aureus biofilm formation under static conditions (s), we used two different systems: the microtiter plate system for measuring the formation of total biofilm mass and the Minimum Biofilm Eradication Concentration (MBEC) device for studying biofilm formation and determining total viable cells in the biofilm (Ceri et al., 1999). Mid log growth phase bacteria were harvested, re suspended in corresponding test media, and adjusted to an OD660 of 0.05 (ca. 5 x 10^7 colony forming units per milliliter (CFU/ml)) for inoculation. For the microtiter plate, 200 μl of bacterial suspension per well was added to the desired wells. After approximately 20 h of incubation at 37 °C, media were removed, and biofilms were quantified using the crystal violet staining method and measured spectrophotometrically at a wavelength of 630 nm (Jackson et al., 2002). For the MBEC system, the MBEC plate was inoculated with 150 μl of bacterial suspension per well in the desired wells and incubated for approximately 20 h at 37 °C. The pegs were then rinsed with phosphate buffered saline (PBS) and broken off from the MBEC lid. The biofilms formed on each peg were dislodged into 1 ml of sterile PBS by sonication, and the viable bacterial number was determined by spiral plating. As indicated in Fig. 2, S. aureus formed significantly more biofilms with the media containing supplemented plasma than other media tested. The recovered viability cell numbers from biofilms formed with media containing plasma supplementation are more than 2 log higher than with media without the plasma supplement (Fig. 3). These differences are statistically significant with a P value=0.001. These differences are not resulted simply from increased growth of S. aureus in the presence of the plasma supplement. Planktonic growth experiments showed similar growth yields with or without the supplement of the plasma (data not shown).

Our results showed that S. aureus formed reproducible and robust biofilms with either TSB or BHI plus 10% human plasma media under both fluid dynamic conditions (Fig. 1) and static conditions (Figs. 2 and 3). It has been shown that, compared with TSB medium, BHI supports optimum expression of gamma hemolysin hlgA transcript (Malachowa et al., 2011). Therefore, BHI plus 10% human plasma medium is proposed for use as the medium for growing S. aureus biofilms under both static and flow conditions. The inclusion of plasma in the medium takes the in vitro biofilm assays one step closer to mimicking in vivo environments. At present, the mechanisms by which plasma proteins stimulate the growth of S. aureus biofilms are uncertain. Plasma proteins...
could represent an excellent nutrient source of nitrogen and iron. Alter naturally, some of the plasma proteins, including fibrinogen, could interact with the specific surface adhesins present in S. aureus. These interactions might lead to up regulation of biofilm essential genes such as icaADBC (polysaccharide biosynthesis). Although the mechanism remains to be determined, our data strongly suggest that S. aureus forms robust biofilms with the presence of plasma under both static and fluid dynamic conditions.

Acknowledgments

This work was supported by the US Army Medical Research and Materiel Command, Combat Casualty Care Research Directorate.

DOD disclaimer

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

References


Fig. 3. 20-hour wild type S. aureus UAMS-1 biofilms formed with different media at 37 °C in an uncoated minimum biofilm eradication concentration (MBEC) device with different media measured by total viable cell recovering (Ceri et al., 1999). The pegs were broken off from the MBEC lid, and each peg was placed in a micro-centrifuge tube containing 1 ml of fresh sterile PBS and sonicated (Misonix Microson XL 2000, Newtown, CT) for 2 min each to dislodge the biofilm from the pegs. The bacteria from the biofilm were then diluted and spiral plated (WASP 2, Don Whitley Scientific, Frederick, MD) onto sheep blood agar plates and incubated overnight at 37 °C for viability determination. The results are representative of those from four independent experiments. Statistics were performed using analysis of variance (one-way) software in which all groups were compared with BHI+plasma resulting in a *P value < 0.001. Error bars represent standard deviation. See Fig. 1 legend for media description.