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Modelsystemforgrowingandquantifying*Streptococcuspneumoniae* biofilms insitu andin realtime

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Abstract

By ATR/FTIR, which stands for attenuated total reflection and Fourier transform infrared spectroscopy, it was possible to see how the harmful bacterium Streptococcus pneumoniae forms biofilms on surfaces and multiplies. One of the most typical causes of ear infections in infants and one of the species suspected to create biofilms in those with otitis media, this particular organism was chosen due to its clinical importance (5).ATR/FTIR and Epifluorescence microscopy are two techniques that, When combined, they allow for the simultaneous observation of the S. pneumoniae biofilm's IR spectrum as it forms and offer a way to measure total and viable cell counts at various growth stages. In contrast to earlier ATR/FTIR investigations, this technique investigates how biofilms develop on surfaces. The evolution of the protein and polysaccharide was monitored over time by observing their absorbance bands in the infrared spectrum in order to quantify the cells that are connected with biofilms. Studies by Nivens et al. on Burkholderia (Pseudomonas) cepacia and those by Bremer and Geesey on the biofilm development of an unnamed environmental bacterium ("CP-1") both showed that the protein component grows more quickly than the polysaccharide component.

It was discovered that polysaccharide formation occurs more quickly in S. pneumoniae biofilms than protein does. This rise in the polysaccharide to protein ratio with S. pneumoniae suggests that various organisms may display differential variations in their biofilm structures (8). Additionally, the staining outcomes show that the capsular polysaccharide N-acetylglucosamine makes up at least a portion of S. pneumoniae's EPS. The current study developed a model system for producing and viewing the continuous deposition of a Streptococcus pneumoniae biofilm on a Germanium IRE as well as on several Germanium coupons by combining ATR/FTIR and Epifluorescence imaging. In order to quantify biofilm-associated cells at particular time points, the number of total and live biofilm cells and the growth of the protein and polysaccharide bands in the IR spectra were related.

Introduction

Streptococcus pneumoniae clings to surfaces and develops a biofilm on them. The IR spectrum is tracked using ATR/FTIR as the procedure progresses. It is one among the organisms thought to be responsible for developing biofilms in persons with otitis media (1).To develop and analyse over time the ongoing deposition of a S. pneumoniae biofilm on a Germanium internal reflection element (IRE) as well as on several additional Germanium coupons, a special model system was created. Epifluorescence microscopy and ATR/FTIR, two distinct methods, may be used together to track the formation of the S. pneumoniae biofilm's IR spectra and to count the total and viable cells at various stages of the process. This approach is different from earlier ATR/FTIR investigations looking at how biofilms grow on surfaces. By observing their distinct IR bands in the IR spectrum, the production of the polysaccharide and protein was both time-stamped. Microorganisms cling to solid surfaces and surface coatings in both natural and artificial environments. These organisms can use the solute and/or adsorbates as nutrition, and they then excrete extracellular polymers that form biofilms (2). Both cells and extracellular polymeric materials make up biofilms (EPS). Cells are linked together by the EPS matrix network and then adhere to surfaces (3). These biofilms are dynamic habitats that have the power to alter the chemistry of the aqueous phase, the interfacial phase, and the surface to which they are attached (4). Both positive and negative effects may result from these modifications. On the plus side, biofilms are in charge of removing contaminants from natural waters and maintaining sewage treatment facilities (5). The drawbacks of biofilms include contaminated ultrapure water systems, reduced heat transfer efficiency in heat exchangers, accelerated metal structure corrosion, and increased ship drag (6) (7).

Biocides and antibiotics have less of an impact on the bacteria that form biofilms (9).Upon adhering to a surface, bacteria may produce a biofilm that protects them against antagonistic chemicals that would If not, kill them. The components of bacteria's EPS can also control how cells react to immunological stimuli (10). Standard monitoring techniques are frequently insensitive to the majority of biofilm processes because biofilm formation is a phenomenon that is restricted to solid surfaces (11). Biochemical and microscopic biofilms are frequent. ATR/FTIR provides real-time FTIR spectra at predefined time intervals throughout the film's deposition on a surface, eliminating the need for quantitative film removal. ATR/FTIR infrared (IR) spectroscopy employs multiplex reflected IR beams on the inside surface of an

internal reflection device (IRE) (12). In order to quantify biofilm-associated cells at particular time points, the development of the protein and polysaccharide bands in the IR spectra was correlated with total and viable biofilm cell counts.

Materials and methods

Bench Setup for ATR/FTIR Experiments

The medium Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI) was placed in a 10-L carboy and linked via silicone tubing to a biofilm reactor containing Germanium coupons. The medium was supplied to the reactor by a Master Flex peristaltic pump. Germanium, the same material used to build the IRE, was used to create the coupons. This was done to remove differences between the IRE and the coupon attachment surfaces. The media was transferred from the reactor to the ATR cell using a different set of tubing that was attached to the same pump as before so that it could pass through the IRE on both sides before exiting the cell and returning to the reactor. The reactor was supplied with a filter-sterile mixture of nitrogen, carbon dioxide, and oxygen while being stirred at 75 rpm in a water bath at 34 °C during the experiment. Through the reactor's lid with the coupon holder, coupons might be removed from it on occasion.

PreparationofATRFlowCell

The FT-IR Spectrometer's sample chamber contained an ATR flow cell. While the two intake channels in this cell transport the two outlet channels in this cell transport the media from the cell back to the reactor. Media to both sides of the crystal surface through the flow channels. The biofilm reactor's components were all autoclave-sterilized before the experiment. After being cleaned, the ATR cell was sterilized.

Preparationof*S.pneumoniae*andReactor Inoculation

A 12 hour culture is added to the 500-mL container of BHI broth. Thus generated a healthy culture of S. pneumonia within the reactor’s 500-mL capacity. After 12 hours, the discharge line was released, allowing a constant 500 mL volume to be kept in the reactor while new media was pumped in at the same rate.

Analysis and Processing of Germanium Coupons

Periodically, coupons of germanium were withdrawn by swiftly pushing them through the reactor's cover. Sterile coupons are sonicated three times and homogenized the suspension containing the suspended biofilm. The quantity of cells was then determined using the resulting suspension. The sample was diluted to a volume of 1 mL and cultured using the spread plate method on Trypticase soy agar. After a 24-hour incubation period at 35°C in a

CO2 incubator, colonies were counted. The substance was filtered using a 0.2 m polycarbonate Nucleopore membrane filter with a 7 mL aliquot, which was then put on a glass microscope slide and counted using a calibrated ocular grid over ten fields. The filter was then fixed in 5% formalin for 5 minutes. The filter was then dyed with 2 g/mL 4'6-diamidino-2-phenyl indole for 15 minutes when it was completely dark.

IREProcessing and Analysis

The coupon underwent two or more cleanings in reverse osmosis (filter sterilised) water, was fixed for 5 minutes in formalin solution, stained for fifteen minutes in the dark with 2 g/mL DAPI, dried, mounted in immersion oil, and examined under a microscope with a 100x oil immersion objective and DAPI filter combination.

FTIRSpectra Analysis

Using the ATR/FTIR technique, biofilm protein and polysaccharide concentrations were also measured spectroscopically. To guarantee uniformity in the background, the background spectral profile of the uninoculated medium was collected throughout 12-hour time. After 12 hours, the infected medium was permitted to pass through the ATR cell. At each sample time, there were an average of 100 scans. To get an absorption change at each sample time, the single-beam spectra were ratioed to the original background spectrum. Two well-studied sections, polysaccharide at 1200-1000 cm-1 and protein at 1700-1500 cm-1, were used to monitor the biofilm's development over time and in different environmental settings.

Result and discussion

C=O molecule causes the amide I band. Stretch of the carbonyl group is 1690–1640 cm-1. At 1640 cm-1, this region also absorbs water. The amide II band is created by the N-H bend of amides between 1590 and 1515 cm-1. The broad band at 1650 cm-1 is most likely caused by a hydrated combination of the amide I and amide II bands in the protein component of the biofilm. According to the literature, this region contains the P=O stretch of phosphodiesters as well as the C-O-C, C-O, and ring-stretching vibrations.

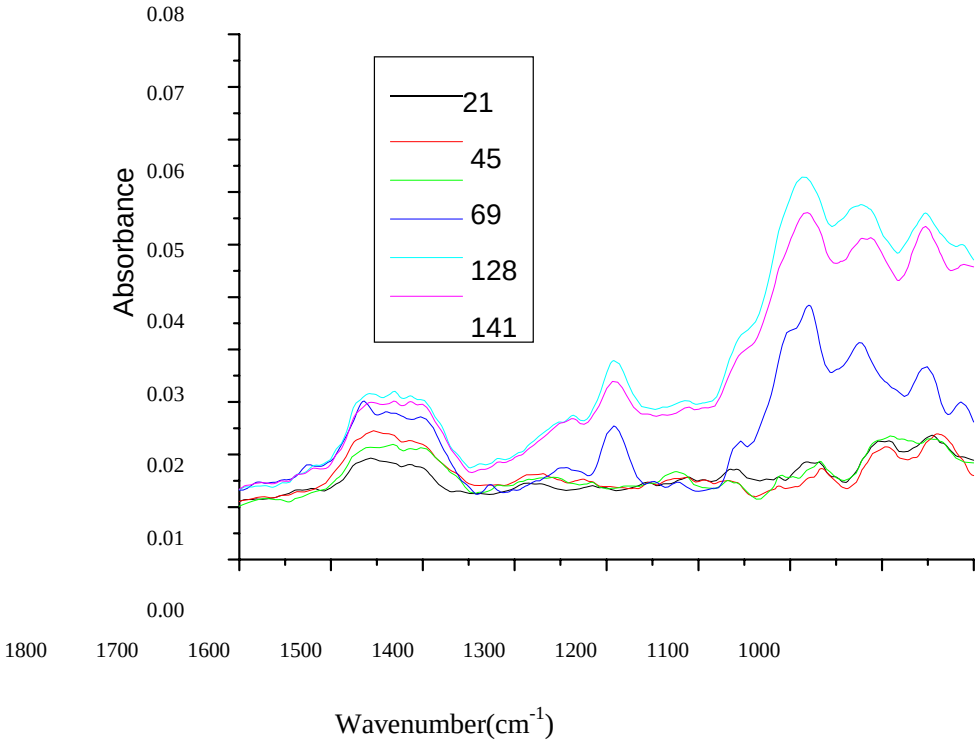

Figure 1: 189 hours after inoculation, the FTIR spectra of S. pneumoniae biofilms.

As a result, it can be seen that the polysaccharide and amide bands appeared on the IRE surface soon after the inoculation, rose for 141 hours, and then decreased for the rest of the exposure time. After 141 hours, the spectrum's overall power started to wane. This is most likely due to the film's tendency to fragment once it reaches a certain thickness and separate from the IRE surface. This S. pneumoniae biofilm showed a significantly faster rise in the polysaccharide band than the amide band region over time. Rather than a rise in biofilm thickness, this increases in spectral intensity over time shows an expansion of the biofilm's covering of the IRE surface. This quicker rate of polysaccharide formation in relation to protein is new.

When compared to a former study, S. pneumoniae, the study's subject, produced more polysaccharides than protein, demonstrating that various organisms may display diverse variations in biofilm construction. After the experiment, more S. pneumoniae biofilms were visible when one side of the IRE surface was examined under a microscope. We noticed dense biofilm growth that was several cells thick.

By gathering coupons at regular intervals throughout the experiment, it was assessed how many cells were embedded in the biofilm and adhered to surfaces. The total number of cells was determined after labelling with DAPI, a dye that identifies both living and dead cells. The quantity of viable cells was determined by counting the amount of cells that formed colonies on blood agar plates. The overall number of biofilms was slightly fewer than the number of biofilms found utilising the germanium coupons placed in the biofilm reactor.

Polysaccharides soon accumulated on the IRE surface for S. pneumoniae, and as time went on, polysaccharide production become more widespread in comparison to protein production. This increase in the polysaccharide to protein ratio in S. pneumoniae raises the possibility that different organisms have varying biofilm architectures (13). Additionally, the staining outcomes show that the capsular polysaccharide N-acetylglucosamine makes up at least a portion of S. pneumonia's EPS.

Conclusion

The ATR cell made it possible to measure the components of biofilms as they were first developing on the IRE surface using spectroscopy. To comprehend the biofilm structure of this organism and its EPS, FTIR spectroscopy and microscopic techniques were combined to describe the biofilm protein and polysaccharide components. The number of cells that are connected with biofilms was determined using viable and total counting techniques. Within hours of the injection, biofilm protein, EPS, and cells we found, and they persisted throughout the experiment.

Inthisstudyit was discovered that polysaccharide formation occurs more quickly in S. pneumoniae biofilms than protein does. This rise in the polysaccharide to protein ratio with S. pneumoniae suggests that various organisms may display differential variations in their biofilm structures (8). Additionally, the staining outcomes show that the capsular polysaccharide N-acetylglucosamine makes up at least a portion of S. pneumoniae's EPS (8).

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