Colloids and Surfaces B: Biointerfaces 146 (2016) 459–467

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Investigation of extracellular polymeric substances (EPS) properties of *P. aeruginosa* and *B. subtilis* and their role in bacterial adhesion

Ardiyan Harimawan[a,*], Yen-Peng Ting[b]

[a] Department of Chemical Engineering, Institut Teknologi Bandung, Jalan Ganesha 10 Bandung, 40132, Indonesia
[b] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, 117576, Singapore

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form 21 June 2016
Accepted 22 June 2016
Available online 23 June 2016

Keywords:
Biofilm
Adhesion force
Extracellular polymeric substances
P. aeruginosa
B. subtilis

ABSTRACT

Extracellular polymeric substances (EPS) matrix in biofilm poses important functions such as a diffusion barrier to antimicrobial agents so that biofilm cells are more difficult to completely eliminate. Therefore, biofilm cells exhibit enhanced resilience unlike planktonic cells, and are more difficult to completely eliminate. In order to obtain a comprehensive understanding of bacterial adhesion to surfaces, knowledge of the composition and conformational properties of EPS produced during growth and biofilm formation is required, since their adhesive and conformational properties remain poorly understood at molecular level. Present study has provided further insights into identifying compositional and conformational properties of EPS produced by planktonic and biofilm cells of *B. subtilis* and *P. aeruginosa*. Various spectroscopy analyses showed that EPS produced by the two different species were chemically dissimilar. More proteinaceous compounds were present in EPS from *B. subtilis*, while EPS from *P. aeruginosa* were characterized by greater carbohydrate components. However, relative proportions of polysaccharides and/or proteins constituents varied with the growth mode of the bacteria. AFM was then used to probe the adhesive nature of EPS produced by the bacteria by using Single Molecule Force Spectroscopy (SMFS). Comparison of the two bacterial species indicated that the presence of polysaccharides promoted the adhesion strength of the EPS while proteins had lesser adherence effects. Comparison of the two growth modes for the same bacterial strain also indicated that greater EPS production and enhanced cellular adhesion are associated with biofilm growth.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Extracellular polymeric substances (EPS) is a term used to describe separate classes of organic macromolecules such as polysaccharides, proteins, nucleic acids, lipids and other polymeric compounds found both on the outer surface of cell walls and in the interior of microbial aggregates (biofilm matrix) [1]. EPS associated with the outermost surface layers of bacteria cells often exist as boundary structures, for instance, capsules or sheaths [2].

EPS production in the biofilm poses several important functions. EPS matrix acts as a diffusion barrier to antimicrobial agents and offers protection against unfavorable environmental conditions. Therefore, biofilm cells exhibit enhanced resilience unlike planktonic cells, and are more difficult to completely eliminate [3]. EPS that coats a cell also alters the physiochemical characteristics of the cell such as surface charge, hydrophobicity and polymeric properties. These changes mediate cellular recognition and promote initial cell adhesion and aggregation [4]. EPS also influence the viscoelastic properties of biofilm which affect its structure and its adhesive properties [5]. It has been reported that EPS comprises carboxylate, phosphate and amine functional groups that contribute to bacterial adhesion onto surfaces [4,6]. Production of EPS acts as a trap to increase the availability of nutrients for growth. The polyanionic nature of EPS matrix allows it to concentrate nutrients from the surrounding fluid [7]. EPS is also shown to be required for biofilm development and maintenance of biofilm structure. For instance, glucose and galactose-rich EPS produced by a rugose variant of *Vibrio cholerae* O1 E1 Tor was required for complex structural biofilm development [8–10].

In order to obtain a comprehensive understanding of bacterial adhesion to surfaces, knowledge of the composition and conformational properties of EPS produced by bacteria during growth and biofilm formation is required. Earlier result showed that EPS covers about 45% of the cell surface of Gram-negative bacteria and may protrude 30 nm or more into surrounding media [11]. Many bacterial surfaces also have a layer of extracellular polysaccharides [12].

* Corresponding author.
E-mail addresses: ardiyan@che.itb.ac.id, ardiyan@gmail.com (A. Harimawan).

http://dx.doi.org/10.1016/j.colsurfb.2016.06.039
0927-7765/© 2016 Elsevier B.V. All rights reserved.

The compressibility and affinity of these polysaccharides for solids were thought to determine whether they enhanced or inhibited adhesion [13].

Although much progress has been made in elucidating the chemical structures of cell wall associated polysaccharides, their adhesive and conformational properties remain poorly understood at the molecular level [14,15]. Studying their spatial organization and conformational properties at the molecular level remains a challenge. In this paper, composition and characteristic of EPS produced by *P. aeruginosa* and *B. subtilis* during growth and biofilm formation will be elucidated using several chemical analysis techniques. Furthermore, single molecule force spectroscopy (SMFS) using AFM will be used to relate the composition of EPS with their adhesive properties.

2. Materials and methods

2.1. Microbial strains and growth conditions

Pseudomonas aeruginosa NNRL-B3509 and *Bacillus subtilis* NNRL-NRS762 were obtained from Agricultural Research Service (ARS) Culture Collection, United States Department of Agriculture (USDA). Both bacteria were cultured in TGY medium containing 5 g/L tryptone, 5 g/L yeast extract, 1 g/L glucose, 1 g/L K_2HPO_4 and pH 6.8 for 16–18 h. Strains were grown in 300 ml Erlenmeyer flasks in an orbital shaker at 150 rpm and maintained at 30 °C.

2.2. Stainless-steel coupon preparation

Stainless steel SS-316 coupons (10 mm diameter and 1 mm thickness) were polished using sand paper with various grit size (P400 to P2500), then with 0.3 μm alumina paste until a smooth finish was attained. Coupons were subsequently washed with copious amounts of water before being sonicated at 50 kHz for 5 min with acetone and finally rinsed with 70% ethanol. Cleaned and dried coupons were then stored in 70% ethanol prior use.

2.3. Reactor system and biofilm development

Each overnight culture of bacteria was grown in a 500 ml Duran bottle with 300 ml working volume containing TGY medium for static biofilm development. Polished coupons were hung inside the bottle and subsequently withdrawn for biofilm analysis over a period of time.

2.4. Biofilm dry weight

Coupons were first washed with PBS solution to remove attached microorganisms and residual medium on the metal surface, and dried in an oven to constant weight. Dry coupons were then weighed, followed by sonication using ethanol 70% for 5 min and 50 kHz to remove the biofilm from the metal surface. Coupons were dried again in the oven to constant weight and their weight were measured. Biofilm dry weight was determined gravimetrically by weight difference.

2.5. Extraction of EPS from cell suspension

10 ml of bacterial suspension samples were taken and centrifuged at 5000 G and 4 °C for 20 minutes. After the supernatant was separated, the concentrated biomass was then re-suspended in a 10 ml of aqueous solution containing 0.85% NaCl and 0.22% formaldehyde at 80 °C for 30 min for EPS extraction. The EPS dissolved in the formaldehyde solution was recovered by further centrifugation at 4 °C, 15000 G for 30 min [16].

2.6. Extraction of EPS from biofilm

Biofilm-coated coupons were removed from each reactor after three days and immersed in PBS buffer. Biofilm was scrapped form each coupon using sonicator for 5 minutes and 50 kHz, and then centrifuged at 5000 G and 4 °C for 20 minutes. The concentrated biomass was then re-suspended in a 10 ml of aqueous solution containing 0.85% NaCl and 0.22% formaldehyde at 80 °C for 30 min for EPS extraction. The EPS dissolved in the formaldehyde solution was recovered by further centrifugation at 4 °C, 15000 G for 30 min [16].

2.7. Chemical composition analysis based on ATR-FTIR

FTIR spectroscopy was performed with a Bio-rad FTS-3500ARX FTIR (Excalibur Series) in attenuated total reflection (ATR) mode, in the range of 400–4000 cm^{-1} with a 4 cm^{-1} resolution and 64 scans per spectrum. Measurements were obtained using a trough-style sample holder with a ZnSe internal reflection element (IRE) subjected to a nominal incident beam angle of 45°. Noise from water was automatically filtered out. Pure EPS extracting solution containing 0.85% NaCl and 0.22% formaldehyde was used as background for all ATR spectra.

2.8. Carbohydrate analysis

Anthrone test was used to determine the carbohydrate concentrations in the EPS samples. 0.1% anthrone solution is made up in 75% (v/v) sulphuric acid at least 2 h before use. This reagent was freshly prepared on each day of analysis. Glucose solution of 100 mg/l was used as a standard. 1 ml of the sample and standard solution was transferred into Pyrex test tubes, followed by the addition of 1 ml of cold anthrone reagent. The test tubes were closed with rubber stoppers and immediately shaken. The test tubes were then placed in a water bath at 100 °C for 14 min and cooled in a water bath at 5 °C for 5 min. The absorbance of the samples and standards were then measured using a spectrophotometer at 625 nm [17].

2.9. Protein analysis

Lowry test was used to determine the protein concentration in the EPS samples. Stock solutions of reagent A containing 2.0% Na_2CO_3, 0.4% NaOH, 0.16% sodium tartrate and 1% SDS, and reagent B containing 4% $CuSO_4$·5H,O were prepared. These solutions were stable indefinitely when stored at room temperature. On the day of use, Folin-Ciocalteu phenol reagent was diluted 1:1 with distilled water. 100 parts of reagent A were mixed with 1 part of reagent B to form reagent C, the alkaline copper reagent. A sample volume of 1 ml containing 10–100 pg of protein, plus sucrose or EDTA when indicated, was added to 3 ml of reagent C and incubated at room temperature for 10–60 min without change in the final absorbance. The samples were mixed vigorously with 0.3 ml of diluted phenol reagent and incubated for 45 min at room temperature. The absorbance of the samples on the spectrophotometer were then taken at 660 nm [18].

2.10. Gel permeation chromatography (GPC)

Molecular weights of different components in the EPS extracted samples were chromatographed by GPC using a Waters HPLC system equipped with a model 2414 refractive index detector, a model 1515 isocratic HPLC pump and an Agilent PL aquagel-OH MIXED-M column (8 μm, 7.5 × 300 mm). All measurements were done at 35 °C, using ultrapure water as an eluent at a flow rate of 1.0 ml/min and a sample injection volume of 10 μl. All samples were filtered with 0.45 mm filters (Millipore) prior to injection. The

Download English Version:

https://daneshyari.com/en/article/598771

Download Persian Version:

https://daneshyari.com/article/598771

Daneshyari.com