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A method for microbial cell surface fingerprinting based on surface plasmon resonance

Mattias Råvik [a], Christian Cimander [b], Ulla Elofsson [c], Andres Veide [a,*]

[a] Royal Institute of Technology (KTH), School of Biotechnology, Department of Bioprocess Technology, AlbaNova University Center, SE-106 91 Stockholm, Sweden
[b] Novozymes Biopharma AB, Box 965, SE-220 09 Lund, Sweden
[c] YKI, Ytkemiska Institutet AB (Institute for Surface Chemistry), Box 5607, SE-114 86 Stockholm, Sweden

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Abstract

A method for microbial cell surface fingerprinting using surface plasmon resonance (SPR) is suggested. Four different *Escherichia coli* mutants have been used as model cells. Cell surface fingerprints were generated by registration of the interaction between the cell mutants and four different surfaces, with different physical and chemical properties, when a cell suspension was flown over the surface. Significant differences in fingerprint pattern between some of the mutants were observed. At the same time, the physical properties of the cell surfaces were determined using microelectrophoresis, contact angle measurements and aqueous two-phase partitioning and compared to the SPR fingerprints. The generated cell surface fingerprints and the physical property data were evaluated with multivariate data analysis that showed that the cells were separated into individual groups in a similar way using principal component analysis plots (PCA).

Keywords: Microbial cell surface properties; Surface plasmon resonance; Contact angle measurements; Microelectrophoresis; Aqueous two-phase partitioning; Multivariate data analysis

1. Introduction

The surface of microbial cells controls the process of association with other surfaces such as adhesion, biofilm formation, flocculation, flotation and formation of aggregates. The host immune response is also triggered by the cell surface. These type of cell surface related phenomena are important in many fields like biotechnology, process engineering, medicine, environmental protection and material technology. Some more specific examples where cell surface properties are of interest are: water and waste water pipelines, heat exchangers, adhesion to ship hulls, wastewater treatment, bear brewing, immuno responses to pathogenic microbes, formation of dental plaque and many more [1].

In down stream processing of proteins, produced with bacteria or other cells, non-specific adsorption to surfaces also could be an issue. Especially in the primary recovery steps cells are present in the process flow and, since the surface properties of bacterial cells can vary significantly [2–7], it is probable that these could have a great impact on the performance of unit operations like e.g. membrane filtration [8] and expanded bed adsorption [9–11]. Thus, understanding of the relationship between cell surface properties and surface adsorption together with methods for probing cell surface properties are critical for the successful application of primary recovery techniques.

Various methods have been applied to measure physical properties of cell surfaces. Microbial cell surface hydrophobicity is probably the most studied cell surface characteristic and the contact angle measurement has been emphasised as the universal standard for this property [6]. In addition, the electrostatic charge properties of microbial cell surfaces play a role in microbial adhesion. For the characterisation of the charge properties the electrophoretic mobility is measured [12]. Partitioning in aqueous two-phase systems is a surface sensitive extraction technique that has been used for separation and surface analysis of cells. The separation of cells can be obtained on the basis of small differences in surface properties [13].

* Corresponding author. Tel.: +46 8 553 783 14; fax: +46 8 553 783 23.
E-mail address: andres@biotech.kth.se (A. Veide).

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In this work we describe the development of a new method for characterising the differences in surface properties of microbial cells based on the surface plasmon resonance (SPR) technique [14–16]. By flowing the microbial cells over Sensor Chip surfaces with different physicochemical properties the goal is to generate cell surface fingerprints. In the development of the method four *Escherichia coli* (*E. coli*) strains have been used as model cells and data on these are presented. Three of them are K12 mutants, that is, one could expect that their properties are more similar compared to the fourth strain used, which is an ML3 mutant [17]. Two of the K12 mutants are very closely related since they are believed to be isogenic except for the *relA* locus [18]. *E. coli* is characterised by a highly organized outer membrane consisting of a bilayer of phospholipids and lipopolysaccharides (LPS) with porin protein channels. Proteins, many of them lipoproteins, make up about half the weight of the outer membrane and at least 20 of these have been shown to be surface antigens. The outer membrane LPS O-antigenic chains make the cell extremely hydrophilic but proteins outside the LPS in some cases make the surface very hydrophobic [19]. It is known that K12 mutants completely lack the hydrophilic O antigen [20]. The SPR fingerprint data, generated by the *E. coli* cells, are compared with physical properties of the cells obtained by contact angle and electrophoretic mobility (z-potential) measurements. In addition, the cell surface properties were probed by partitioning in aqueous two-phase systems. Multivariate data analysis is used to explore the multiple dimensions and the inter correlation of the different data.

2. Materials and methods

2.1. Chemicals

The detergent CHAPS, (3-[(3-Cholamidopropyl)dimethylammonio]-1-propane-sulfonate) 98%, was purchased from Sigma-Aldrich Gmbh (Steinheim, Germany). Polyethylene glycol (PEG) 6000, average molecular weight 6000 Da was purchased from Merck-Schuchardt (Hohenbrunn, Germany). Dextran T500, average molecular weight 500 000 Dalton was purchased from Pharmacia Fine Chemicals (today GE Healthcare) (Uppsala, Sweden). Diiodomethane 99%, was purchased from Sigma-Aldrich Gmbh (Steinheim, Germany) and formamide 99.5%, from Merck KgaA (Darmstadt, Germany). All other chemicals were of analytical grade.

2.2. Strains and cultivation

The *E. coli* strains used were the three K12 mutants MC4100 [21], AF1000 that is a MC4100 *relA*$^+$ derivative [18] and RV308 [22]. In addition to this an *E. coli* ML3 mutant [17] was used, ML308. For cultivations a salt medium supplemented with trace elements were used [18] with 10.0 g glycerol per L instead of glucose.

For each strain the cultivation procedure was as follows, if not otherwise stated: 1 L shake flask with 100 mL cultivation medium was inoculated with 100–300 μL of a strain stock solution (an OD_{600} of approximately 1) stored at −80 °C in glycerol. The shake flask was incubated at 37 °C and 180 rpm overnight. Three 5 L shake flasks with 500 mL medium each were inoculated with 20–25 mL of the overnight culture, respectively. The shake flasks were incubated at 37 °C and 180 rpm until an OD_{600} value of 2–2.5 was reached (4–8 h). The cell culture was chilled on ice and then centrifuged for 20 min at 4500 rpm (about 3400 ×*g*). The cell sediment was re-suspended in saline (0.9 g NaCl per L), pooled to one bottle and centrifuged for another 10 min at 4500 rpm (about 3400 ×*g*). The cell sediment was re-suspended in 80% (w/w) glycerol. The weight of the added glycerol solution was approximately the same as the weight of the pellet. The cell glycerol stocks were stored at −80 °C in aliquots until further experimental use. For the initial set up tests and the cell fingerprint application test (see below) separate *E. coli* ML308 cell glycerol stocks were used.

2.3. Surface plasmon resonance

The SPR cell surface fingerprint method was developed using the Biacore™ 2000 instrument. The principle of the technique has been described elsewhere [14–16]. A gold covered surface is mounted on a plastic carrier and when placed in the instrument four different flow cells are formed. Through a microfluidic system the sample is flown in a controlled fashion over the sensor surface in each flow cell and the detection is performed on the opposite side of the gold covered surface. Normally the Biacore™ instruments are used for real time measurements with small sample volumes of specific interactions between e.g. proteins and small molecules. The measurements are performed on specifically designed Sensor Chip surfaces grafted with specific ligands. In our experiments we have studied adsorption to four Sensor Chip surfaces (Sensor Chip L1, C1, CM5 and HPA) that differ with respect to properties like charge and charge density, polarity and surface topography (Fig. 1A). Sensor Chip CM5 carries a matrix of carboxylated, unbranched 500 kDa dextran, covalently attached to the gold surface. Under physiological buffer conditions the surface matrix extends about 100 nm from the gold surface and carries negative charges. This surface provides a hydrophilic environment [23]. Sensor Chip L1 has a surface matrix of carboxylated dextran similar to that on Sensor Chip CM5, to which lipophilic alkyl residues have been covalently attached [24]. The charge density of the L1 surface is similar to that of the CM5 surface. Sensor Chip C1 has a flat carboxylated surface with no dextran matrix and with a lower charge density compared to CM5 and L1. The surface lacks the hydrophilic character of the dextran matrix [24]. Sensor Chip HPA presents a flat hydrophobic surface consisting of long-chain self-assembled alkanthiol molecules attached directly to the gold film. There is no dextran or other matrix on the surface [24].

The following was the experimental procedure if not otherwise stated. A washing procedure cleaning all four flow channels was performed before introducing any samples. Then the first injection of the first cell sample was performed on flow channel one, followed by a washing procedure on flow channel