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Phosphocholine decoration of *Proteus mirabilis* O18 LPS induces hydrophobicity of the cell surface and electrokinetic potential, but does not alter the adhesion to solid surfaces

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ABSTRACT

Proteus mirabilis harbours a variety of O antigens, permitting evasion of the host immune response. LPS decoration with phosphocholine increases cell surface hydrophobicity and decreases electrokinetic potential, which may interfere with antibody interaction and bacterial surface recognition. The decoration does not influence adherence to solid surfaces.

Proteus mirabilis is a Gram-negative, rod-shaped bacterium (Rózalski et al., 1997). *P. mirabilis* is commonly responsible for catheter associated urinary tract infections (CAUTI), especially in long-term hospitalised patients, where *P. mirabilis* is the most frequent aetiological factor (Armbruster et al., 2018). The *P. mirabilis* cell surface is covered by highly diverse lipopolysaccharide (LPS), which provides the basis for classification of *Proteus* strains to serogroups (Drzewiecka et al., 2021; Yu et al., 2017). The one medically important serogroup, O18, harbours a unique decoration based on phosphocholine (ChoP) incorporation into the LPS O antigen (Fudala et al., 2003). The role of this decoration is still unclear. Cell surface decoration with ChoP represents a type of molecular mimicry (Hergott et al., 2015). Many studies have shown that ChoP increases bacterial adherence to epithelial cells by interaction with platelet-activating factor (PAF) receptor. Moreover, choline scavenged from platelet PAF by Pce or GlpQ esterase alternate the PAF functions and provide a substrate for LPS decoration (Young et al., 2013). These events are important in bacterial pathogenesis. Another important virulence factor of *P. mirabilis* is biofilm formation. This mode of growth helps bacteria to colonize an organism, avoid the host immune response, and confers increased resistance to antibiotics by forming a diffusion barrier. Biofilm formation requires cell adhesion.

We have focussed on cell surface modification by decoration with ChoP, with a previously described and recently shown mechanism in *Proteus mirabilis* serogroup O18 (a PrK 34/57 strain) (Czerwonka et al., 2021), and its impact on cell surface properties and its adherence to abiotic surfaces. Bacterial cell adhesion to solid surfaces generally depends on fimbriae/pili. However, the cell surface hydrophobicity (CSH) and electrokinetic potential (zeta) are the main physical parameters that define the cell interaction with the substratum due to the changing environmental conditions and influence the bacterial cell adhesion (van Loosdrecht et al., 1987). The presence of choline in the culture medium resulted in an increase of the cell surface hydrophobicity from 25.35° ± 2.72° to 38.23° ± 5.96°, expressed as a contact angle of water droplet settled on the bacterial film. In our studies an layer of bacterial cells harvested from logarithmic phase of growth was established on the microscopy slide by washing in deionised water and evaporation. The settled droplet of deionised water (2 μl) forms a spherical shape, and the contact angle between the surface and the edge of the droplet corresponds to the hydrophobicity of the tested material (bacterial layer). The electrokinetic potential was decreased by the supplementation of choline from an average of −10.3 mV to −7.5 mV (Fig. 1). The electrokinetic potential (zeta) of the bacterial cell surface was expressed as a electrophoretic mobility of the bacterial cells suspended in liquid medium determined by the laser Doppler velocimetry method. Agglutination of O-antigen phosphocholine decorated strain (PrK 34/57) was evident as a bacterial precipitate. As well, conglomerates of bacteria

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were observed by epi-fluorescence microscopy, where Syto-9 was used (FilmTracer; Invitrogen, Carlsbad, CA, USA). Phosphocholine-depleted strain O17 was used as a negative control and did not exhibited these properties. In contrast, adhesion to polypropylene (hydrophobic) and glass (hydrophilic) (Czerwonka et al., 2016) was not altered compared to the negative control lacking anti-ChoP antibodies. The findings suggest that the presence of ChoP decoration influence the cell surface hydrophobicity and electrokinetic potential, but is not an intermediate in the modification of adhesion to solid surfaces. Additionally, ELISA detected the phosphocholine epitope on the surface of biofilms. The signal was significantly higher compared to strain PrK 61/57 (Fig. 2), which was not decorated with ChoP and which produced similar amounts of biofilm, as determined by crystal violet staining and epi-fluorescence microscopy (Syto-9). The background signal was likely caused by the presence of choline utilised by cells as a substrate for the synthesis of the glycine betaine osmoprotectant encoded by the *bet* gene cluster (Cánovas et al., 2000) present in the *P. mirabilis* genomes (data not shown). Detection of the signal from the surface of biofilms indicated that phosphocholine could be secreted into the extracellular polymeric matrix that forms the bulk of biofilms, based on previous findings indicating that *P. mirabilis* ATCC 49565 does not have a chemically distinct capsule, but rather possesses long O-antigen chains (Dumanski et al., 1994). Our previous research (Czerwonka et al., 2021) showed that the decoration mechanism in *P. mirabilis* O18 is similar to reported earlier in other species, and consists of four genes encoding permease, kinase, cytidylyltransferase and phosphocholine transferase (Young et al., 2013). Presentation of phosphocholine on the bacterial surface is considered an example of molecular mimicry, where bacteria utilise a host-specific molecule to evade the host immune response due to host inability to recognize self-derived molecular structures (Hergott et al., 2015). Molecular mimicry has serious medical impacts, especially in long-term infections and autoimmune disorders, such as rheumatoid arthritis (Ebringer et al., 2010). The electrical charge at the solid–liquid interface (bacterial cell surface and liquid medium) results in the attraction of ions with an opposite charge (counter-ions) and the formation of an electrical double layer. The potential between the solid surface and the outer (mobile) interface represents an electrokinetic (zeta) potential (Kolská et al., 2013). The modification of cell surface charge and hydrophobicity affects the micro-environment on the bacterial cell and interrupts the bioactivity of compounds (i.e., antibody) at the solid–liquid interface, which alters the kinetics between the bioactive compound and its target (i.e., cell surface receptor) (Kolská et al., 2013). Moreover, the increased hydrophobicity of the bacterial surface enables microorganisms to attach to the surface or host cells, or to hydrophobic catheter materials (Krasowska and Sigler, 2014), and agglutinate to each other. Increased hydrophobicity and lower electrokinetic potential may promote aggregation and adherence, and could enable the survival and persistence of the bacteria in environments, such as a catheter.

An experiment was performed to detect ChoP in *P. mirabilis* biofilms. The detection of an available phosphocholine was performed with overnight *P. mirabilis* PrK 34/57 biofilms formed in wells of a 96-well microtiter plate, and was tested by ELISA assay using monoclonal anti-ChoP antibodies and secondary antibodies conjugated with horseradish peroxidase (HRP; Sigma-Aldrich, St Louis, MO, USA). As a control of antibody reactivity, we used *P. mirabilis* PrK 61/57 strain serogroup O17, which is characterized by a similar ability to form biofilms in the absence of phosphocholine-mediated O-antigen decoration. Biofilms were formed using LB medium (Biocorp, Warsaw, Poland) at 37 °C in 96-well microtiter plates (Greiner Bio-One, Monroe, NC, USA), as previously described (Czerwonka et al., 2016). To investigate the electrokinetic potential and CSH, cultures were grown on modified PMSM medium (Armbruster et al., 2013) comprised of (per 1000 ml) 4.5 g KH_2PO_4, 13.8 g K_2HPO_4, 1 g $(NH_4)_2SO_4$, and 0.47 g sodium citrate. The sterilized medium was supplemented with 0.01 mg histidine, 0.01 mg biotin, 0.25 g $MgSO_4$, 2 ml glycerine and (if necessary) 0.01 mg choline. Zeta potential was tested on a Zetasizer Nano ZS apparatus (Malvern Instruments, Malvern, UK) where the laser Doppler velocimetry method was used (Borkowski et al., 2018). For hydrophobicity tests, dense and smooth layers of bacteria were formed on the solid surface by evaporation (Czerwonka et al., 2019). Contact angles of distilled water were measured on a OCA 15EC contact angle goniometer (DataPhysics Instruments, Filderstadt, Germany) at room temperature. To obtain large amounts of cultivated biomass, a BiostatA bioreactor (Sartorius, Gottingen, Germany) was used. Additionally, an adsorption assay was performed. In the assay, the bacteria were covered by TEPC-15 anti-ChoP antibodies diluted 1:100 in phosphate-buffered saline (PBS) and adsorption was determined.

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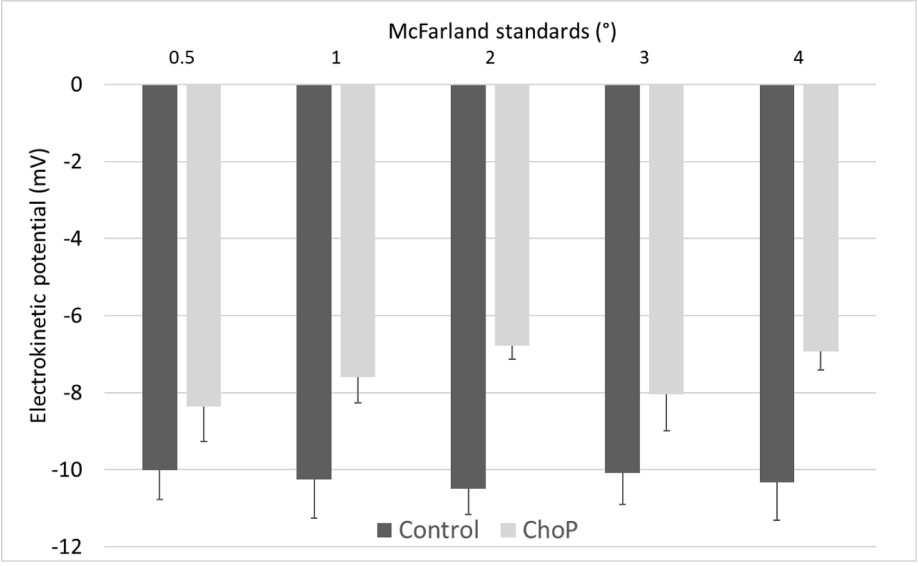

Fig. 1. Zeta potential of ChoP decorated cells compared to control. Cell density was tested at the range from 0.5° to 4° of McFarland standards. Differences between all results compared controls are statistical significant according to One-Way ANOVA.

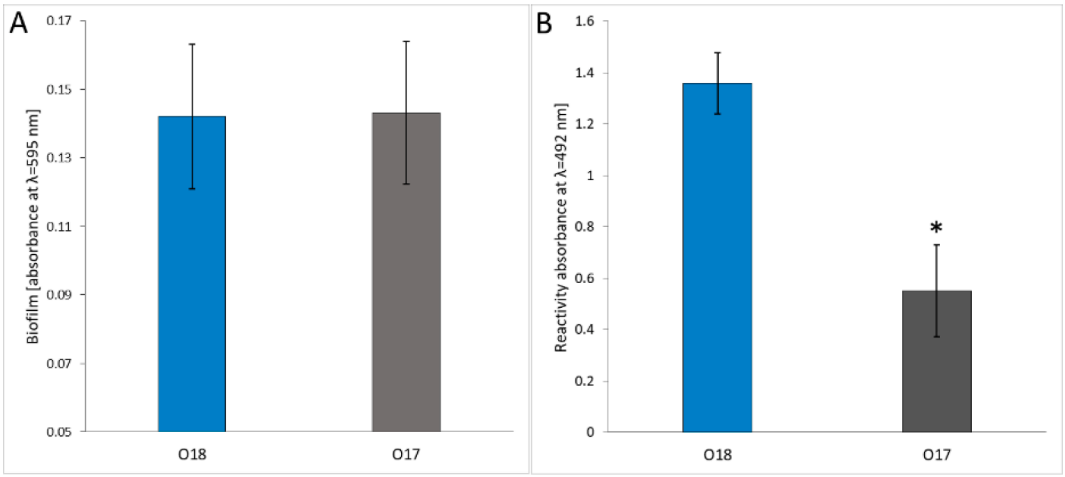

Fig. 2. A) A comparison of biofilm amount between two *P. mirabilis* strains O18 (PrK 34/57), and O17 (PrK 61/57). B) The reactivity of *P. mirabilis* strain O18 (PrK 34/57) exposed in the biofilm mode of growth to monoclonal anti-ChoP antibodies, compared to choline-depleted LPS of O17 biofilm (strain PrK 61/57). * statistical significant difference according to One-Way ANOVA.

CRediT authorship contribution statement

Grzegorz Czerwonka: Conceptualization, Methodology, Writing – review & editing, Data curation, Investigation. **Katarzyna Durlik-Popińska:** Investigation, Conceptualization. **Marcin Drabik:** Investigation. **Martyna Szczerba:** Investigation. **Maria Kwiatkowska:** Investigation. **Wiesław Kaca:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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