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Perspective paper

Methods for imaging *Shewanella oneidensis* MR-1 nanofilaments

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ABSTRACT

Nanofilament production by *Shewanella oneidensis* MR-1 was evaluated as a function of lifestyle (planktonic vs. sessile) under aerobic and anaerobic conditions using different sample preparation techniques prior to imaging with scanning electron microscopy. Nanofilaments could be imaged on MR-1 cells grown in biofilms or planktonically under both aerobic and anaerobic batch culture conditions after fixation, critical point drying and coating with a conductive metal. Critical point drying was a requirement for imaging nanofilaments attached to planktonically grown MR-1 cells, but not for cells grown in a biofilm. Techniques described in this paper cannot be used to differentiate nanowires from pili or flagella.

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1. Introduction

Extracellular bacterial appendages or nanofilaments, including flagella, fimbriae, curli and pili, can influence motility, aggregation, biofilm formation, host-cell adhesion, cell signaling, DNA uptake and phage attachment. Recent work demonstrated that some bacterial nanofilaments are electrically conducting, raising the question as to whether pili and/or flagella play a role in promoting and extending extracellular electron transfer (EET) beyond the cell membrane (Gorby et al., 2006; Reguera et al., 2005; El-Naggar et al., 2008). The term "nanowire" describes a function of nanofilaments, i.e., transport of electrons, not a particular structure. The precise biological role of extracellular appendages may vary among microorganisms (Craig et al., 2004). Pili, present on the surfaces of all Gram-negative bacteria and some Gram-positive microorganisms, are typically used by bacteria in biofilm formation (Craig et al., 2004; Shime-Hattori et al., 2006; Varga et al., 2008; Van Houdt and Michiels, 2005) or as a conduit for DNA transfer between cells (Harrington and Rogerson, 1999; Marsh and Taylor, 1999; Shu et al., 2008). Throughout this paper the term "nanowire" is used when referring to the published claims of others. The more general term "nanofilament" is used to describe appendages attached to the bacteria.

The list of microorganisms that can produce nanowires is continuously being updated (Choi, 2006). Prior to concluding that a microorganism does or does not have nanowires, conducting measurements via scanning tunneling microscopy (STM) (Gorby et al., 2006) or conducting atomic force microscopy (AFM) (Reguera et al., 2005) must be used to verify the conductivity of observed nanofilaments. The two most important factors that must be considered when concluding the presence or absence of nanofilaments associated with bacteria cells are: 1) the specific growth conditions required for production of nanowire-like filaments by specific organisms and 2) choosing the fixation protocol and imaging technique that will reliably document nanofilaments when they have been produced. TEM has been used for two decades to image bacteria with flagella and pili (Elliott et al., 1991; Bieber et al., 1998; Kirn et al., 2000; Konnov et al., 1999; Collins et al., 2005). TEM images provide consistent data indicating that pili are secreted from all sides of the bacterium and are more numerous and thinner (3–5 nm) than flagella (8–12 nm) which are often polar, emanating from the end of a bacterium (Roine et al., 1997; Grossart et al., 2000; Jin et al., 2001).

For many years, bacterial nanofilaments have been imaged using scanning electron microscopy (SEM) (Bieber et al., 1998; Chandler et al., 1980; Wolfgang et al., 2000). Their appearance in SEM images depends on growth conditions, the imaging method, and the fixation protocol used prior to imaging. Using STM and SEM, Gorby et al. (2006) described *Shewanella oneidensis* MR-1 nanowires as thin single filaments with diameters 3–5 nm or thick bundles of nanowires with diameters of 50 to 100 nm in diameter that were straight and sometimes taut, tightly stretched between two or more cells. Additionally, in their experiments *S. oneidensis* MR-1 cells grown under aerobic conditions were shown to have few if any nanofilaments of any kind, leading to the conclusion that electron acceptor limited conditions were required for nanowire expression (Gorby et al., 2006).

In our laboratory attempts to image nanofilaments attached to MR-1 cells adhered to carbon electrodes using ESEM and SEM were unsuccessful. The suggestion has been made that, "Previous studies may have missed nanowires because bacteria in biofilms are

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14. ABSTRACT
Nanofilament production by Shewanella oneidensis MR-1 was evaluated as a function of lifestyle (planktonic vs. sessile) under aerobic and anaerobic conditions using different sample preparation techniques prior to imaging with scanning electron microscopy. Nanofilaments could be imaged on MR-1 cells grown in biofilms or planktonically under both aerobic and anaerobic batch culture conditions after fixation, critical point drying and coating with a conductive metal. Critical point drying was a requirement for imaging nanofilaments attached to planktonically grown MR-1 cells, but not for cells grown in a biofilm. Techniques described in this paper cannot be used to differentiate nanowires from pili or flagella.

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surrounded by masses of matter that may have obscured the picture" (Choi, 2006). Gorby et al. (2006) provided an alternative explanation for the apparent absence of nanowires in some preparations for SEM. They, "…observed that cells cultivated in nutrient-rich media or under high-agitation conditions that mechanically disrupt fragile extracellular appendages produce samples that contain abundant evidence of cellular debris and relatively few intact structures resembling nanowires." Furthermore, the thickness character (loose/wavy or taut/straight) and cell-to-cell connectivity of nanowires can be distinctly different among publications detailing the physical character of bacterial nanofilaments.

We tested the hypothesis that nanowire-like filaments (thick, taut nanofilaments between cells) could be observed under "non-ideal" culture conditions, including batch cultures with constant exposure to air. In the experiments described in this paper, we used SEM to image MR-1 as a function of lifestyle (planktonic vs. sessile) under aerobic and anaerobic conditions and different sample preparation techniques, including fixation, dehydration and conductive coatings applied prior to imaging.

2. Materials and methods

The following equipment was used in the preparations: an Emitech K850 critical point dryer, a Polaron E5100 sputter coater and an ElectroScan E3 environmental scanning electron microscope (ESEM). When operating in ESEM mode, a Peltier cooling stage was maintained at 4 °C and the specimen chamber was maintained with a water vapor pressure of 4.5 torr to promote condensation to keep the samples wet and moist. When the specimen chamber was maintained at 4.5 torr without using the Peltier cooling stage, the instrument functions as a typical scanning electron microscope (SEM). The experiments described below were repeated three times with independent cultures of MR-1. A summary of methods for sessile and planktonic cells is presented in Table 1. Columns are listed in the order in which the procedure was performed. The glutaraldhyde fixative was not anaerobic. The sputter-coated palladium layer was 15 nm thick.

2.1. Sessile cells

MR-1 cells were grown in Luria-Bertani (LB) broth for 24 h, shaken continuously at 90 revolutions per minute (rpm) under air exposure. These cultures were then concentrated by centrifugation, rinsed 3 times in minimal media [MM], and re-suspended in minimal media [MM] with 18 mM sodium lactate containing 1 cm^2 409 stainless steel (SS) disks. Replicates were grown anaerobically in a sealed Le Parfait jar (maintained with a Gas Pak that generates CO_2, H_2 and N_2) on a bench top shaker. Both aerobic and anaerobic cultures were maintained at 23 °C. Disks were removed after five days and prepared for ESEM and SEM by the methods described below.

1) Rinsed in distilled water to remove salts. Examined in ESEM mode.
2) Fixed in 4% cacodylate buffered glutaraldehyde in a refrigerator overnight at 4 °C. Rinsed in distilled water. Examined in ESEM mode.
3) Fixed in 4% cacodylate buffered glutaraldehyde in a refrigerator overnight at 4 °C. Rinsed in distilled water. Dehydrated through a graduated series of distilled water/acetone/xylene washes. Air-dried and examined in SEM mode.
4) Fixed in 4% cacodylate buffered glutaraldehyde in a refrigerator overnight at 4 °C. Rinsed in distilled water. Dehydrated through a graduated series of distilled water/acetone/xylene washes. Air-dried, sputter-coated with palladium and examined in SEM mode.
5) Fixed in 4% cacodylate buffered glutaraldehyde in a refrigerator overnight at 4 °C. Rinsed in distilled water. Dehydrated through a graduated series of distilled water/acetone washes to 100% acetone. Critical point dried and examined in SEM mode.
6) Fixed in 4% cacodylate buffered glutaraldehyde in a refrigerator overnight at 4 °C. Rinsed in distilled water. Dehydrated through a graduated series of distilled water/acetone washes to 100% acetone. Critical point dried, sputter-coated with palladium and examined in SEM mode.

2.2. Planktonic cells

Planktonic cells in 1 ml MM were removed from both aerobic and anaerobic cultures after three days. Media with cells were transferred to 1.5 ml Eppendorf tubes and centrifuged at 5000 rpm for three minutes, concentrating the cells into a pellet. The supernatant was removed by pipette and the pellet was washed with distilled water and centrifuged for an additional three minutes at 5000 rpm. The supernatant rinse water was removed and the pellet was re-suspended in 0.5 ml distilled water by pipetting up and down. Cells were placed on 0.2 µm pore size filters that had been previously sputter-coated with palladium and treated as described above (Techniques 1–6).

3. Results

3.1. Sessile cells

After 5 days in MM, MR-1 cells produced biofilms on 409 stainless steel discs in both aerobic and anaerobic bulk conditions. Wet cells could be imaged (ESEM) with and without fixation (Techniques 1 and 2). Nanofilaments were not observed in any of the wet preparations. Cells were coated with extracellular polymeric material. Nanofilaments were not obvious in fixed, air-dried aerobic and anaerobic

Table 1
Summary of sample preparation for sessile and planktonic MR-1 cells and SEM observations related to nanofiaments.

Technique	Mode	Fixed in 4% cacodylate buffered glutaraldehyde in a refrigerator overnight 4 °C	Rinsed in distilled water to remove salts	Dehydrated through graduated series of distilled water/ acetone/ xylene washes	Dehydrated through a graduated series of distilled water/acetone washes to 100% acetone	Air-dried	Critical Point Dried	Sputtercoated with palladium	Aerobic sessile nanofilaments	Aerobic planktonic nanofilaments	Anaerobic sessile nanofilaments	Anaerobic planktonic nanofilaments
1	ESEM		✓						−	−	−	−
2	ESEM	✓	✓						−	−	−	−
3	SEM	✓	✓	✓		✓			−	−	−	−
4	SEM	✓	✓	✓		✓		✓	+	−	+	−
5	SEM	✓	✓		✓		✓		−	−	−	−
6	SEM	✓	✓		✓		✓	✓	+	+	+	+

samples (Technique 3) until the specimens were coated in palladium (Technique 4) (Fig. 1a and b). Similarly, nanofilaments were not observed in samples that had been critical point dried (Techniques 5 and 6) until the cells had been coated with palladium (Technique 6) (Fig. 2a and b). After palladium coating it was impossible to distinguish cells grown under aerobic conditions from cells grown under strict anaerobic conditions. It was also impossible to differentiate cells that had been critical point dried from samples that had been air-dried. All had obvious attached nanofilaments. Sputter coating was required for imaging nanofilaments attached to sessile MR-1. Results are summarized in Table 1.

3.2. Planktonic cells

Using either ESEM or SEM, MR-1 cells grown planktonically could be imaged after all treatments (Techniques 1–6). The appearance or absence of nanofilaments depended on the technique used to prepare the cells for imaging, but the cells had the same general appearance as the sessile cells. Nanofilaments were not obvious in air-dried samples even after sputter coating (Technique 4). Attached nanofilaments could be imaged on both aerobically and anaerobically grown cells only after cells had been critical point dried and sputter-coated with palladium (Technique 6) (Fig. 3a and b). Critical point drying was a requirement for imaging nanofilaments attached to planktonically grown MR-1 cells. Results are summarized in Table 1.

4. Discussion

Electrochemically active bacteria, including the metal-reducing bacteria *Shewanella* and *Geobacter* sps., can perform EET to insoluble electron acceptors such as metal oxides and anodes in microbial fuel cells. Direct EET, or electron transfer promoted by membrane contact with an insoluble electron acceptor, has been studied extensively for *Shewanella* and *Geobacter* sps., and has been found to involve an electron cascade from inner to outer surface cytochromes (Reardon et al., 2010; Schuetz et al., 2009). Artificial (quinones, methylene blue, etc.) and biosynthesized (riboflavin) mediators can also be used by these cells to perform indirect EET to insoluble electron acceptors (Baron et al., 2009; Coursolle et al., 2010). Until recently, it was thought that EET was limited to these two pathways. However, conducting AFM and STM have been used to pass electrons through bacterial nanofilaments to a conducting surface, introducing the term "bacterial nanowire."

Production of nanowires associated with bacteria reportedly depends on growth conditions. The SEM images of MR-1 with attached nanowires produced by Gorby et al. (2006) were the result of chemostat cultures grown under O_2-limited conditions (electron acceptor limitation). Gorby et al. (2006) imaged planktonic cells that had been fixed in an anaerobic solution of 2% glutaraldehyde. Cells were "applied to" 0.2 µm pore size membrane filters, gently washed successively with pH 7 phosphate buffer solution (PBS), dilute PBS (50:50 with distilled water) and deionized water. Samples were then dehydrated through a graduated series of ethanol: water washes to ethanol. After three changes in 100% ethanol, samples were critical point dried and coated with evaporated carbon. In the same work (Gorby et al., 2006), few to no nanowires were observed in chemostat cultures when oxygen concentrations exceeded 2% of air saturation. Similarly, Reguera et al. (2005) demonstrated that pili production in *Geobacter sulfurreducens* was growth regulated. Pili were produced when the organisms were grown on Fe(III) oxide or fumarate at suboptimal temperatures under strictly anaerobic conditions.

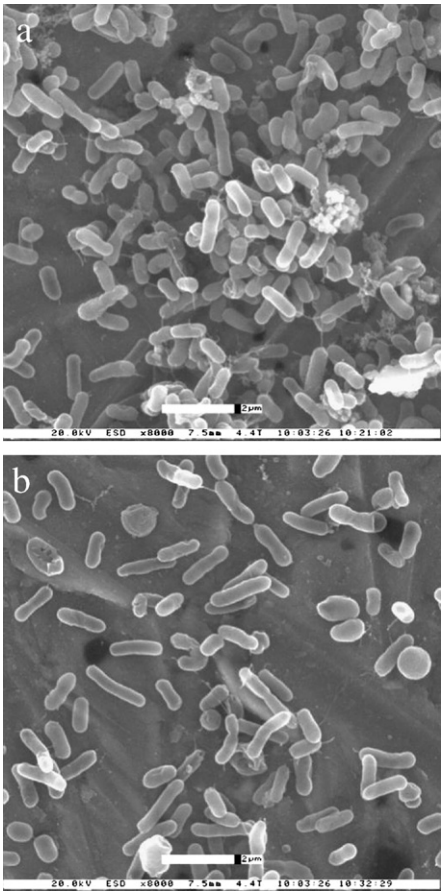

Fig. 1. Sessile Technique #4 on SS substrate with overnight fixation, distilled water rinse, dehydration through a graduated series of distilled water/acetone/xylene washes, air dried, Pd sputter coated, SEM mode, a) aerobic, b) anaerobic.

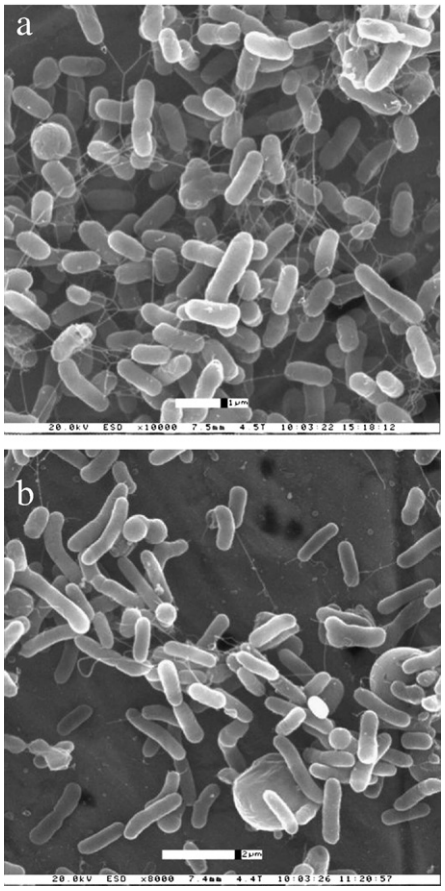

Fig. 2. Sessile Technique #6 on SS substrate with overnight fixation, distilled water rinse, dehydration through a graduated series of distilled water/acetone washes, critical point dried, Pd sputter coated, SEM mode, a) aerobic, b) anaerobic.

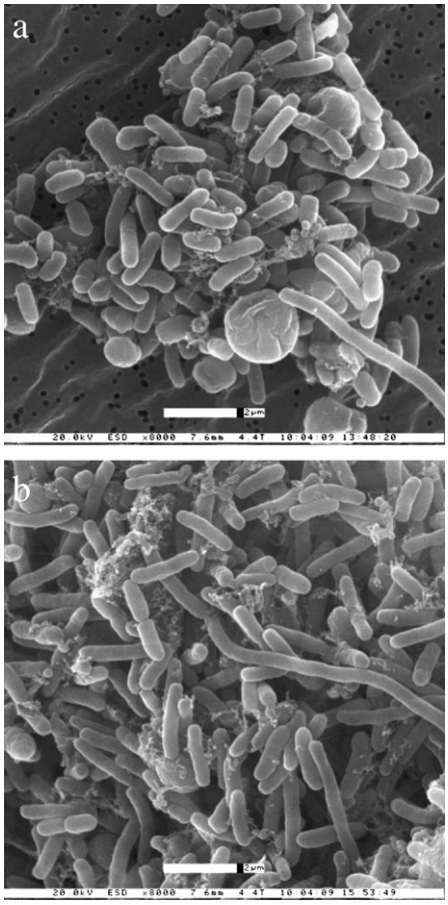

Fig. 3. Planktonic Technique #6 on Au coated 0.2 µm polycarbonate filter with overnight fixation, distilled water rinse, dehydration through a graduated series of distilled water/acetone washes, critical point dried, Pd sputter coated SEM mode, a) aerobic, b) anaerobic.

In our studies, nanofilaments were observed when MR-1 was grown either aerobically or anaerobically, in biofilms or planktonically, provided the cells were fixed, critical-point dried and coated with a conductive film before SEM examination. The nanofilaments were similar in appearance to the single strand nanowires produced by Gorby et al. (2006). The method of drying i.e., air-drying vs. critical point drying, did not influence the ability to image nanofilaments attached to sessile MR-1. Critical point drying did provide taut, thickened MR-1 nanofilaments, similar to those described as nanowires in previous reports. Critical point drying was necessary in preparations of planktonic cells.

The hypothesis at the beginning of this work was that MR-1 will grow nanofilaments under several conditions and that the appearance of those nanofilaments depends on the fixation and imaging method. We observed nanofilaments under sessile and planktonic growth conditions and because only batch cultures were used in this work, it appears that special constant culture (chemostat) conditions are not necessary for MR-1 to express nanofilaments similar to those described as nanowires in previous publications. The distinct advantage of using chemostat cultures is that the electron acceptor (oxygen) concentration can be controlled. Air-exposure during culture does not appear to inhibit the growth of nanofilaments by MR-1.

The presence of nanofilaments on cells grown in bulk aerobic environments does not mean that all cells producing nanofilaments were exposed to oxygen. Anaerobic niches have been identified in clumps of cells, especially biofilms (Lewandowski and Beyenal, 2007), and pili are typically related to biofilm formation. It is possible that some "aerobic" cells grown under our culture conditions were exposed to reduced oxygen conditions (microaerophillic), specifically those cells that reside in clumps (planktonic) or deep in biofilms. We did observe cell clumping after centrifugation of planktonic cells and the biofilms were several cells thick, so under these circumstances a portion of the cells may have been exposed to reduced dissolved oxygen conditions. Previous measurements in our laboratory (Ringeisen et al, 2007) using dissolved oxygen probes determined that MR-1 batch cultures shaken in air sustained concentrations of greater than 8 mg/L dissolved oxygen (air-saturated). If shaking was discontinued, then the dissolved oxygen concentration decreased rapidly (80% reduction within 50 s) and was below the detection limit (<0.1 mg/L) within 400 s. In the work reported here, no special precautions were taken to deoxygenate the glutaraldehyde fixative during sample preparation, so cells were exposed to significant levels of dissolved oxygen during the fixation procedure.

5. Conclusions

Using SEM, nanofilaments could be imaged on MR-1 cells grown in biofilms or planktonically under both aerobic and anaerobic batch culture conditions after fixation, critical point drying and coating with a conductive metal. Critical point drying was a requirement for imaging nanofilaments attached to planktonically grown MR-1 cells, but not for cells grown in a biofilm. Chemostat cultures and controlled electron acceptor limited growth conditions were not necessary to observe nanofilament structures similar to reported MR-1 nanowires. We emphasize that the techniques described in this paper cannot be used to differentiate nanowires from pili or flagella and suggest that this conclusion holds for all types of microscopy.

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References

Baron, D., Labelle, E., Coursolle, D., Gralnick, J.A., Bond, D.R., 2009. Electrochemical measurement of electron transfer kinetics by *Shewanella Oneidensis* MR-1. JBC 284, 28865–28873.

Bieber, D., Ramer, S.W., Wu, C.Y., Murray, W.J., Tobe, T., Fernandez, R., Schoolnik, G.K., 1998. Type iv pili, transient bacterial aggregates, and virulence of enteropathogenic *Escherichia coli*. Science 280, 2114–2118.

Chandler, F.W., Roth, I.L., Callaway, C.S., Thomason, B.M., Weaver, R.E., 1980. Flagella on Legionnaires' disease bacteria: ultrastructural observations. Ann. Intern. Med. 93, 711–714.

Choi, C.Q., 2006. Nanowires Common in Bacteria? The Scientist. www.the-scientist.com/news/display/23924/2006.

Collins, R.F., Frye, S.A., Balasingham, S., Ford, R.C., Tonjum, T., Derrick, J.P., 2005. Interaction with type iv pili induces structural changes in the bacterial outer membrane secretin pilQ. J. Biol. Chem. 280, 18923–19830.

Coursolle, D., Baron, D.B., Bond, D.R., Gralnick*, J.A., 2010. The Mtr respiratory pathway is essential for reducing flavins and electrodes in *Shewanella oneidensis*. J. Bacteriol. 192, 467–474.

Craig, L., Pique, M., Tainer, J.A., 2004. Type IV pilus structure and bacterial pathogenicity. Nat. Rev. Microbiol. 2, 363–378.

Elliott, S.J., Nandapalan, N., Chang, B.J., 1991. Production of type-1 fimbriae by *Escherichia-coli* hb101. Microb. Pathog. 10, 481–486.

El-Naggar, M.Y., Gorby, Y.A., y Wei Xia, Nealson, K.H., 2008. The molecular density of states in bacterial nanowires. Biophys. J. 95, L10–L12.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T. J., Chang, I.S., Kim, B.H., Kim, K.S., Culley, D.E., Reed, S.B., Romine, M.F., Saffarini, D.A., Hill, E.A., Shi, L., Elias, D.A., Kennedy, D.W., Pinchuk, G., Watanabe, K., Ishii, S., Logan, B., Nealson, K.H., Fredrickson, J.K., 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. PNAS 103, 11358–11363.

Grossart, H.-P., Steward, G.F., Martinez, J., Azam, F., 2000. A simple, rapid method for demonstrating bacterial flagella. Appl. Environ. Microbiol. 66, 3632–3636.

Harrington, L.C., Rogerson, A.C., 1999. The f-pilus of *Escherichia coli* appears to support stable DNA transfer in the absence of wall-to-wall contact between cells. J. Bacteriol. 172, 7623–7624.

Jin, Q., Hu, W., Brown, I., McGhee, G., Hart, P., Jones, A.L., He, S.Y., 2001. Visualization of secreted Hrp and Avr proteins along the Hrp pilus during type III secretion in Erwinia amylovora and Pseudomonas syringae. Mol. Microbiol. 40, 1129–1139.

Kirn, T.J., Lafferty, M.J., Sandoe, C.M.P., Taylor, R.K., 2000. Delineation of pilin domains required for bacterial association into microcolonies and intestinal colonization by vibrio cholerae. Mol. Microbiol. 35, 896–910.

Konnov, N.P., Baibyrin, V.B., Zadnova, S.P., Volkov, U.P., 1999. Comparative microscopy study of *Vibrio cholerae* flagella. Scanning and force microscopies for biomedical applications, *proceedings of the* Society of Photo-Optical Instrumentation Engineers, Bellingham, WA, p. 123.

Z. Lewandowski and H. Beyenal ,2007. Fundamentals of Biofilm Research. CRC Press Taylor and Francis Group, p 121.

Marsh, J.W., Taylor, R.K., 1999. Genetic and transcriptional analyses of the *Vibrio cholerae* mannose-sensitive hemagglutinin type 4 pilus gene locus. J. Bacteriol. 181, 1110–1117.

Reardon, C.L., Dohnalkova, A.C., Nachimuthu, P., Kennedy, D.W., Saffarini, D.A., AREY, B. W., Shi, L., Wang, Z., Moore, D., McLean, J.S., Moyles, D., Marshall, M.J., Zachara, J.M., Fredrickson, J.K., Beliaev, A.S., 2010. Role of outer-membrane cytochromes MtrC and OmcA in the biomineralization of ferrihydrite by *Shewanella oneidensis* MR-1. Geobiology 8, 56–68.

Reguera, G., McCarthy, K., Mehta, T., Nicoll, J.S., Tuominen, M.T., Lovely, D., 2005. Extracellular electron transfer via microbial nanowires. Nature 435, 1098–1101.

Ringeisen, B.R., Ray, R., Little, B., 2007. A miniature microbial fuel cell operating with an aerobic anode chamber. J. Power Sources 165, 591–597.

Roine, E., Wei, W., Yuan, J., Nurmiaho-Lassila, E.-L., Kalkkinen, N., Romantschuk, M., He, S.Y., 1997. Hrp pilus: an hrp-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. tomato DC3000. PNAS 94, 3459–3464.

Schuetz, B., Schicklberger, M., Kuermann, J., Spormann, A.M., Gescher, J., 2009. Periplasmic electron transfer via the *c*-type cytochromes MtrA and FccA of *Shewanella oneidensis* MR-1. Appl. Environ. Microbiol. 75, 7789–7796.

Shime-Hattori, A., Iida, T., Arita, M., Park, K.S., Kodama, T., Honda, T., 2006. Two type IV pili of *Vibrio parahaemolyticus* play different roles in biofilm formation. FEMS Microbiol. Lett. 264, 89–97.

Shu, A.C., Wu, C.C., Chen, Y.Y., Peng, H.L., Chang, H.Y., Yew, T.R., 2008. Evidence of DNA transfer through f-pilus channels during *Escherichia coli* conjugation. Langmuir 24, 6796–6802.

Van Houdt, R., Michiels, C.W., 2005. Role of bacterial cell surface structures in *Escherichia coli* biofilm formation. Res. Microbiol. 156, 626–633.

Varga, J.J., Therit, B.H., Melville, S.B., 2008. Type IV pili and the CcpA protein are needed for maximal biofilm formation by the gram-positive anaerobic pathogen *Clostridium perfringens*. Infect. Immun. B 2008, 4944–4951.

Wolfgang, M., van Putten, J.P.M., Hayes, S.F., Dorward, D., Koomey, M., 2000. Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili. EMBO J. 19, 6408–6418.