# A sticky bacterium versus antiadhesive surfaces

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**Abstract:** While microorganisms have evolved to adhere and form biofilms on surfaces, various materials with antiadhesive surfaces have been developed. *Acinetobacter* sp. Tol 5 exhibits high adhesiveness to various surfaces through AtaA, a member of the trimeric autotransporter adhesin (TAA) family. We examined the adhesion of Tol 5 and other bacteria expressing different TAAs to antiadhesive surfaces. The results highlighted Tol 5's stickiness through AtaA, which enables cells to adhere even to antiadhesive materials including polytetrafluoroethylene with a low surface free energy, a hydrophilic polymer brush exerting steric hindrance, and mica with an ultrasmooth surface. Tol 5 cells also adhered to a zwitterionic 2-methacryloyloxyethyl-phosphorylcholine-polymer-coated surface but were exfoliated by a weak shear stress, suggesting that exchangeable bound water molecules contribute to AtaA's interaction with materials.

**One Sentence Summary:** *Acinetobacter* sp. Tol 5 can adhere to typical antiadhesive surfaces but its preference suggests its adhesion mechanism.

**Main Text:** The COVID-19 pandemic caused by a virus reminded us of the threat of infectious diseases. Pathogenic bacteria also cause infectious disease, but bacteria are not as much of a threat as viruses because antibiotics are effective against them. This is changing, however, with the emergence of antibiotic-resistant bacteria. The global expansion of multidrug-resistant bacteria has become a clinical problem (*1*), and the threat of bacterial infection might come back in the near future. The overuse of antibiotics amplifies the opportunity for resistant bacteria to emerge and spread (*2*). The increased antibiotic use during this COVID-19 pandemic could also increase the threat of resistant bacteria (*3*). As an alternative to antibiotics, antiadhesive (antibiofouling) surfaces have drawn intensive research interest because bacterial adhesion is the initial step of infection by pathogens and biofouling of equipment (*4-7*). As a result of extensive efforts, various antiadhesive surfaces have been developed and characterized, such as fluoropolymers, polymer brushes, highly hydrophilic zwitterionic polymers, and ultrasmooth or nano/micro-topographical patterned surfaces (*8-12*).

*Acinetobacter* sp. Tol 5, which is a toluene-degrading bacterium that we previously isolated from a biofiltration system, exhibits autoagglutination and high adhesiveness to solid surfaces (*13, 14*). Tol 5 cells quickly adhere to various material surfaces from hydrophobic plastics to hydrophilic glasses and metals independently of biofilm formation (*13*). This characteristic nonspecific adhesiveness of Tol 5 cells is mediated by AtaA, a member of the trimeric autotransporter adhesin (TAA) family (*15-17*). TAAs are outer membrane proteins of Gram-negative bacteria and have been well-studied as virulence factors because each TAA shows an ability to bind to biotic molecules of mammalian host cells and occasionally to some kinds of abiotic surfaces (*18, 19*). Although they have a variety of lengths from several hundreds to several thousands of amino acids, they have a common structure that includes an N-terminal passenger domain (PSD), which is secreted onto the cell surface and is responsible for its function, and a C-terminal transmembrane domain, which anchors the PSD onto the outer membrane (*19*). AtaA is one of the largest TAAs consisting of 3,630 amino acids but shares common structural features with other TAAs, (*15, 20*). However, there have been no reports of TAA-mediated adhesion similar to Tol 5 cells through AtaA in terms of nonspecificity and high stickiness.

In a proverb known as the “shield-spear contradiction” derived from an ancient Chinese text *Han Feizi*, a merchant first boasts that, “this shield is strong enough to prevent anything,” and then,

"this spear is sharp enough to pierce anything." In response to the merchant's boasting, one person from the crowd asks the merchant, "What would happen if you attack your shield with your spear?" The merchant could not answer. Similarly, we also don't know what would happen if highly adhesive Tol 5 cells encounter an antiadhesive surface. In this study, we investigated the interaction of Tol 5 and some other TAA-expressing bacterial cells with various surfaces including antiadhesive surfaces that have different repelling mechanisms.

First, we compared the adhesiveness of Tol 5 and its Δ*ataA* mutant (negative control) with that of *Yersinia enterocolitica* and *Bartonella henselae*, which have also been reported to adhere to an abiotic surface through their TAAs (*18*), YadA and BadA, respectively, by shaking each cell suspension in the presence of a polyurethane support for 30 min. The production of these TAAs in the bacteria was confirmed by western blotting (see Supplementary Figure S1). The result showed the overwhelming stickiness of cells expressing AtaA compared with that of cells expressing the other TAAs (Fig. 1). Most of the Tol 5 cells adhered to the support and the cell suspension became abundantly clear. In contrast, the cell suspensions of *Y. enterocolitica, B. henselae,* and Tol 5 Δ*ataA* mutant remained cloudy, which indicated that many of the cells did not adhere to the polyurethane support.

Next, we quantitatively investigated the adhesiveness of bacterial cells that express TAAs to various material surfaces. Cell suspensions were placed and incubated on polystyrene (PS), glass, stainless steel, and polytetrafluoroethylene (PTFE, known as Teflon) surfaces for 10 min, the unadhered cells were removed by washing with a fresh medium and the adhered cells on the material surface were quantified by crystal violet staining. As shown in Figure 2, in a short time (10 min), Tol 5 could adhere to not only PS, glass, and stainless steel but also to PTFE, which has antiadhesive properties derived from its low surface energy (*8*). On the other hand, Tol 5 Δ*ataA* mutant and *Y. enterocolitica* hardly adhered to all the material surfaces. Although *B. henselae,* of which BadA mediates relatively high adhesiveness to abiotic surfaces among TAAs (*18*), showed measurable adhesiveness; the amount of adhered cells was much smaller than that of Tol 5. These results quantitatively demonstrated that Tol 5 cells exhibit remarkably higher adhesiveness to various material surfaces through AtaA than bacterial cells expressing other TAAs.

Subsequently, to investigate whether Tol 5 cells adhere to various other antiadhesive surfaces in addition to PTFE, we performed adhesion assays with mica, poly(oligo(ethylene glycol) methyl ether methacrylate) (poly(mOEGMA)) brush, and 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer surfaces. Mica is a phyllosilicate mineral of aluminum and potassium, and its surface after cleaving is atomically flat (*21*). A poly(mOEGMA) brush is a neutral hydrophilic polymer brush and exerts steric repulsion (*10*). An MPC polymer is a zwitterionic hydrophilic polymer and possesses a high free water fraction (*22*). These surfaces have been reported to have antiadhesive properties against bacterial cells (*9, 11, 23*). After incubation of bacterial cells on the antiadhesive surfaces for 10 min, Tol 5 cells adhered to PTFE but not to the mica, poly(mOEGMA) brush, and MPC polymer surface (Fig. 3A). After incubation for 2 h, Tol 5 cells adhered to not only PTFE but also to the mica and poly(mOEGMA) brush surface, but hardly adhered to the MPC polymer (Fig. 3A). After a 2 h incubation, a control strain, *B. henselae* adhered to PTFE and mica but not to the poly(mOEGMA) brush and the MPC polymer (Fig. 3B). These results emphasized that Tol 5 cells were the only cells that adhered to the poly(mOEGMA) brush and showed that even sticky Tol 5 cells hardly adhered to the MPC polymer under these experimental conditions.

To investigate how the MPC polymer repels Tol 5 cells, we observed the behavior of Tol 5 cells on the polymer surface by using a flow cell system with a square glass tube (Fig. 4A) (*24*). The glass tube with or without the MPC polymer coating was filled with a Tol 5 cell suspension and incubated for 10 min. Then, the cell suspension was replaced with fresh BS-N medium by slow flowing at 10 μL/min for rinsing, and the flow rate was increased stepwise, as shown in Figure 4B, while observing the inner surface of the bottom of the glass tube under a microscope. Unexpectedly, Tol 5 cells adhered to the MPC-polymer-coated glass as much as the bare (non-coated) glass under static conditions and remained adhered after rinsing at 10 μL/min (Fig 4C initial). When the flow rate was increased to 20 μL/min, a small fraction of previously adhered cell clumps started to move and slip on the surface (see Supplementary Movie S1), but many cells still resisted detachment after 10 min of flowing (Fig. 4C, 20 μL/min). At a high flow rate of 50 μL/min or more, the Tol 5 cells firmly adhered to the bare glass whereas the cells attached on the MPC polymer were exfoliated, rolled, and washed off from the surface by the shear stress (Fig. 4C, >50 μL/min and see Supplementary Movie S1).

So far, various antiadhesive materials have been developed on the basis of repelling mechanisms. Fluoropolymers with a low surface free energy are widely used in cookware and medical equipment although their hydrophobicity is also said to cause protein adsorption that hiders cell attachment by contraries (*25*). Polymer brushes with a high grafting density have been especially studied as powerful antiadhesive surfaces for cell adhesion (*26*). However, the finding that *Acinetobacter* sp. Tol 5 is able to adhere to these antiadhesive materials makes us realize the marvel of microbial diversity and evolution. In addition, AtaA could mediate cell adhesion to poly(mOEGMA) brush but BadA could not. Note that BadA is similar to AtaA in size and abundance on the cell surface; it consists of 3,082 amino acids and its fibrous molecules peritrichately cover over bacterial cells (*18*). Therefore, their difference in adhesiveness demonstrates the functional diversity of the TAA family as a result of protein evolution.

Tol 5 cells even adhered to an MPC-polymer-coated surface but their interaction was so weak that the cells could be exfoliated by a weak shear stress. The exfoliated and rolling cell clumps seemed to involve and remove cell clumps that were still adhered owing to the autoagglutinating property of Tol 5 cells (*24*), self-cleaning the surface coated with the MPC polymer. In an adhesion assay using a microwell, the Tol 5 cells should have been detached by the washing step. MPC is a methacrylate monomer with a phosphorylcholine (PC) group, which is a hydrophilic polar head group of phospholipids comprising a eukaryotic cell membrane (*27*). MPC polymers are known to significantly suppress adhesion of proteins and cells because there are lots of free water molecules (*22*) but capture few bound water molecules on their PC group (*27-29*). The fact that Tol 5 cells can adhere to the poly(mOEGMA) brush and the mica, but can only interact very weakly with a surface coated with MPC polymers, despite similar levels of hydrophilicity, as shown by the static contact angles of air in water (Table S2), suggests that exchangeable bound water molecules contribute to the interaction between AtaA and material surfaces (*28*).

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**Supplementary Materials:**

Materials and Methods

Figures S1-S2

Tables S1-S2

Movie S1

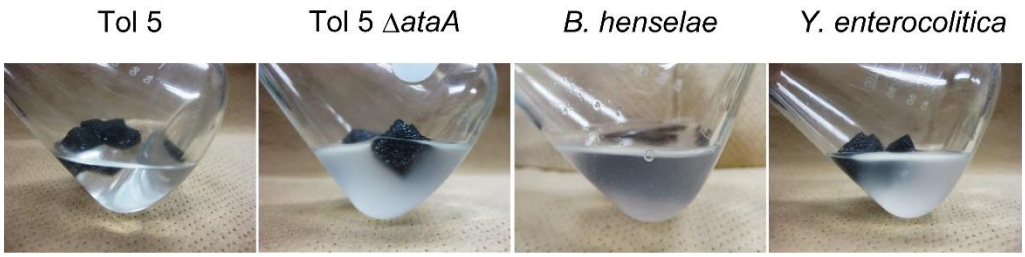


**Fig. 1.** Adhesion of bacterial cells to a polyurethane surface. Each panel shows the bacterial cell suspension after shaking for 30 min with a polyurethane foam support.

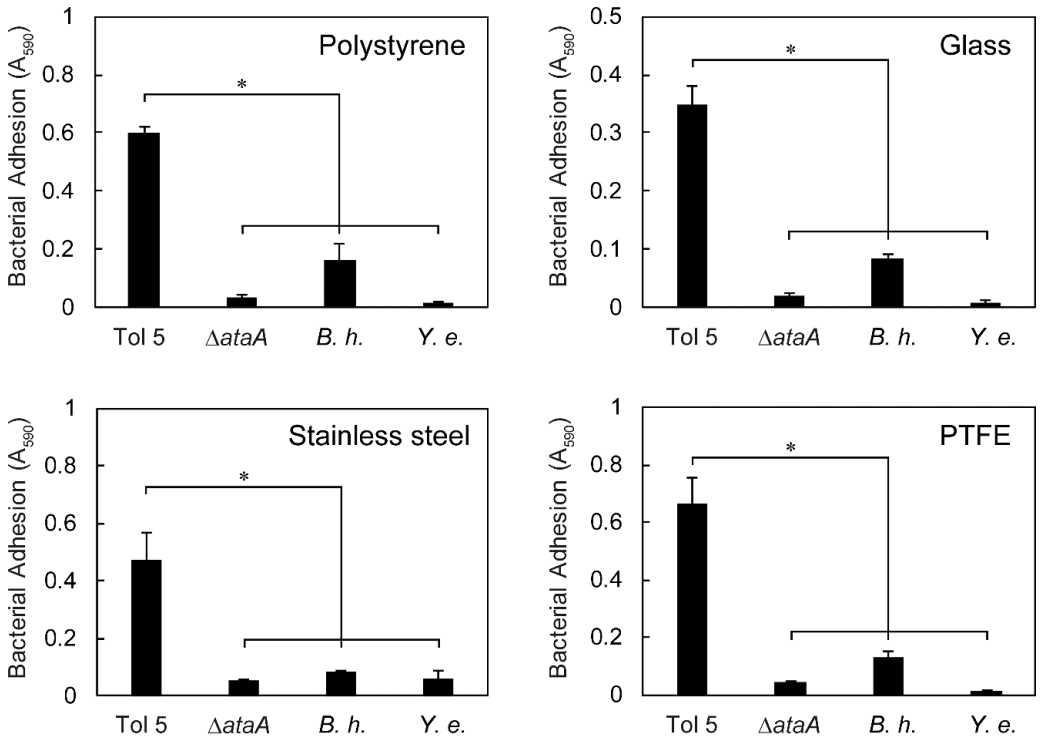


**Fig. 2.** Adhesion of bacterial cells to various materials. Adhesion of *Acinetobacter* sp. Tol 5, Tol 5 Δ*ataA*, *B. henselae* (*B. h.*), and *Y. enterocolitica* (*Y. e.*) to polystyrene, glass, stainless steel, and PTFE was assessed by microwell adhesion assays. Data are expressed as the mean ± SEM (n=3). Significant differences from the result of Tol 5, analyzed using Student's t-test, are indicated by an asterisk ($p<0.05$).

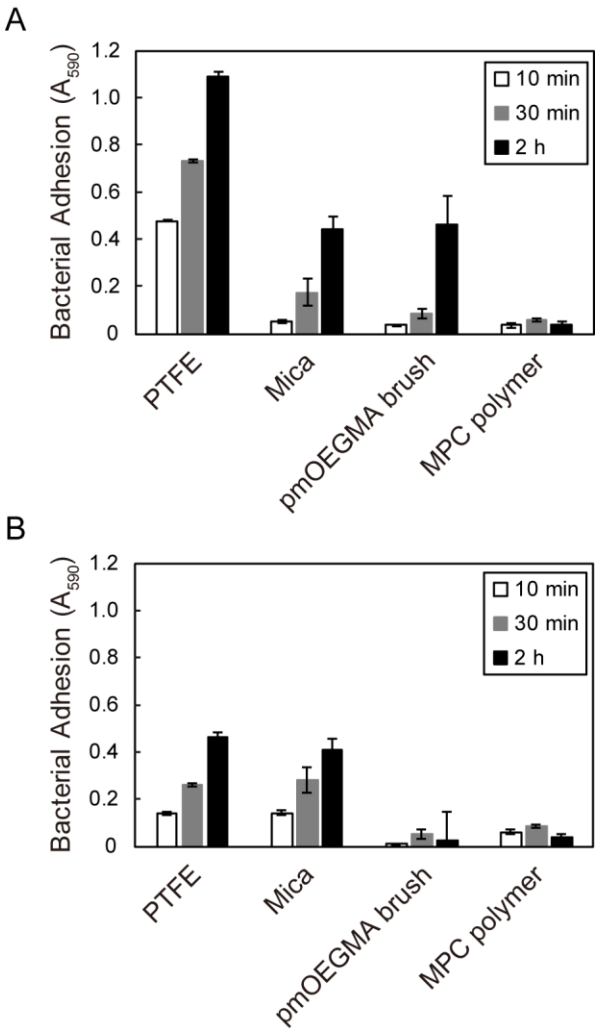


**Fig. 3.** Adhesion of bacterial cells to antiadhesive surfaces. Adhesion of Tol 5 (A) and *B. henselae* (B) to PTFE, mica, poly(mOEGMA) brush on glass, and MPC-polymer-coated glass, was assessed by microwell adhesion assays. Data are expressed as the mean ± SEM (n=3).

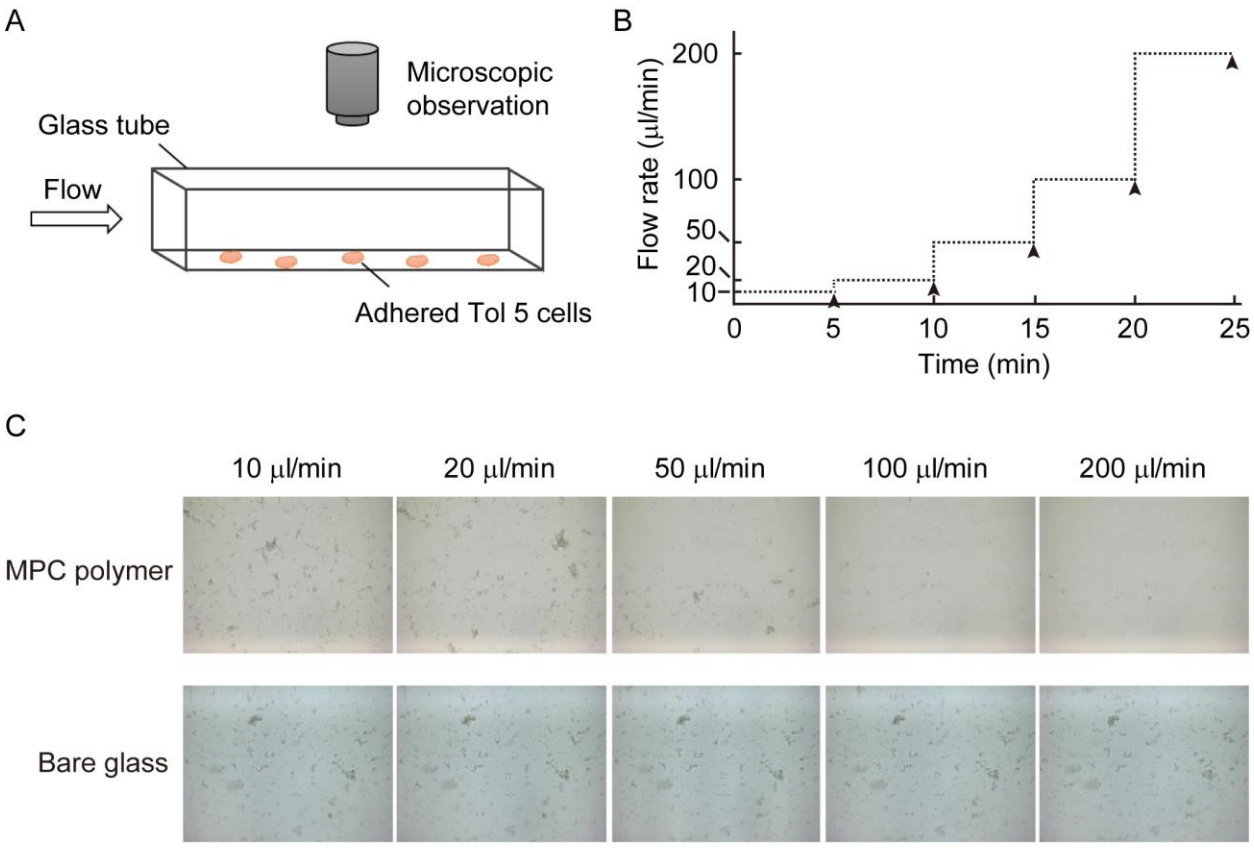


**Fig. 4.** Observation of the behavior of Tol 5 cells that were adhered to the MPC polymer surface beforehand. (A) Schematic representation of the flow cell system used in this study. (B) Transition of the flow rate. The flow rate was increased stepwise every 5 min. The black arrowheads indicate the time at which snapshots of the inner surface at the bottom of the glass tubes were captured. (C) The snapshots captured as described in (B).