ENVIRONMENTAL MICROBIOLOGY AND ECOLOGY

Contents
Adhesion, Microbial
Aeromicrobiology/Air Quality
Algal Blooms
Bacteriophage Ecology
Biofilms, Microbial
Deep Sub-Surface
Deep-Sea Hydrothermal Vents
Ecology, Microbial
Extremophiles: Acidic Environments
Extremophiles: Hot Environments
Extremophiles: Cold Environments
Extremophiles: Dry Environments (including Cryptoendoliths)
Food Webs, Microbial
Freshwater Habitats
Heavy Metals Cycle (Arsenic, Mercury, Selenium, others)
Heavy Metals, Bacterial Resistance
High-Pressure Habitats
Low-Nutrient Environments
Marine Habitats
Mats, Microbial
Methanotrophy/methane oxidation
Nitrogen Cycle
Phosphorus Cycle
Rhizosphere
Sediment Habitats, including Watery
Sulfur Cycle
Adhesion, Microbial
L Cegelski, C L Smith, and S J Hultgren, Washington University, School of Medicine, St. Louis, MO, USA
© 2009 Elsevier Inc. All rights reserved.

Defining Statement
Introduction
Biological Significance of Microbial Adhesion
Mechanisms of Microbial Adhesion
Selected Survey of Specific Adhesion Strategies

Consequences of Microbial Adhesion in Human Disease
Targeting Adhesion to Inhibit Bacterial Virulence
Further Reading

Glossary
biofilm A community of microorganisms associated with a surface.
microbial ecology The study of interactions and relationships between microorganisms and their environment.

planktonic cells Cells grown predominantly in suspension as individual cells in a liquid medium.
teichoic acids A class of negatively charged polymers expressed on the cell surface of Gram-positive bacteria that are either linked covalently to the peptidoglycan (wall teichoic acids) or linked to lipids in the cytoplasmic membrane (lipoteichoic acids).

Abbreviations
CF cystic fibrosis
DAF decay-accelerating factor
ECM extracellular matrix
GbO4 globotetraosylceramide

IBCs intracellular bacterial communities
UPEC uropathogenic E. coli
UTI urinary tract infection
Tafi thin aggregative fimbriae

Defining Statement
Microbial adhesion is crucial to the survival and lifestyle of many microorganisms. Both beneficial and pathogenic relationships forged between microbe and host depend on adhesive events and colonization. This article highlights the highly evolved microbial adhesion mechanisms and discusses the prevalence and implications of adhesion in diverse ecosystems.

Introduction
From the center of the earth and deep-sea vents to plant roots and the human intestine, microorganisms occupy remarkably diverse niches on our planet. These microbes include bacteria, archaea, fungi, and protista, and are found attached to rocks and soil particles, corals, and ocean sponges. Bacteria, for example, symbiotically colonize plants and humans as well as fish and squid, resulting in mutual benefit to both microbe and host. Pathogenic and unwelcome bacteria can egress from their native niche and adhere to and infect other sites and host tissues, leading to cellular injury and disease. Microbes also adhere to the hulls of ships and to machinery in food-processing factories, resulting in contamination and adverse circumstances. Specific adhesion strategies have evolved in order to facilitate microbial attachment to diverse substrata in both symbiotic and pathogenic associations. Understanding the molecular mechanisms and functional implications of microbial adhesion is crucial for generating complete descriptions of our ecosystems, understanding and predicting ecosystem stability due to globalization and climate change, and attempting to control and prevent the unfortunate and often devastating consequences of infectious diseases. Thus, microbial adhesion is a fundamental component of the field of microbial ecology. This article will focus on the adhesive strategies employed specifically by bacteria, though many parallels can be found in the arsenal of adhesive strategies harbored by the other classes of microbes. We will highlight several exciting and up-to-date scientific discoveries as a platform to illustrate the biological significance and implications of microbial adhesion.
Biological Significance of Microbial Adhesion

The propensity for bacteria to associate with surfaces (living or abiotic) in nearly all ecosystems far exceeds the tendency to persist in suspension, living freely in a planktonic state. Attachment to surfaces allows bacteria to persist in advantageous locations where there may be high nutrient concentrations or to provide protection from hostile environments. In numerous instances, bacteria form biofilms – structurally complex and dynamic bacterial communities. The metabolic labor of acquiring nutrients is divided, sometimes according to spatial coordinates in the community, and distribution is promoted through an organized architecture of community members. Protection from harsh environmental conditions is a major benefit of life in a biofilm and the first line of defense is provided by members residing at the edges of the community. Under certain conditions, bacteria disperse from the biofilm, to seek a new environment and potentially readhere and colonize new niches. Adhesion events are crucial to biofilm formation, growth, and development. We set the stage for discussing the mechanisms of microbial adhesion by first illustrating a few examples across a broad landscape in which bacterial adhesion (often followed by biofilm formation) takes place.

Adhesion in the Water

The coral reefs are home to an enormous diversity of marine life, including beautiful fish, mollusks and urchins, and the significantly smaller microorganisms with which they cohabit. Bacteria are, in fact, an integral constituent of the microbiota of healthy corals. They colonize distinct sites in coral tissue including the surface mucus layer and porous components in the coral skeleton where they fix nitrogen, decompose chitin, and provide organic compounds. The molecular mechanisms of adhesion and the sustained interactions between bacteria and their coral hosts are currently not well understood but are key questions being addressed in the emerging field of coral microbiology. Understanding the microbial interactions that promote health versus those that cause disease is important in efforts to preserve and prevent further deterioration and corrosion of the steel surfaces. Understanding the mechanisms of adhesion is key to developing strategies to control and prevent these adverse and costly consequences.

Adhesion to Plants

Rhizobia are Gram-negative soil bacteria that adhere to and colonize the root cells of leguminous plants, including soybeans and alfalfa. Upon entry into a root hair, rhizobia traverse a distance to the center of the root hair cell and together with proliferating plant cells form a nodule. Here, rhizobia fix nitrogen, converting molecular nitrogen (N₂) from the air into ammonia, nitrates, and other nitrogenous compounds to support plant metabolism. Rhizobia are particularly important to plants in nitrogen-deficient soils. In return, rhizobia receive carbon-rich organic compounds, important for their own energy production, from the plant.

Other beneficial symbionts include Bacillus thuringiensis. This bacterium is an important Gram-positive pathogen whose insecticidal properties have gained attention in the development of crops genetically modified to express the bacterium’s potent toxin, now referred to as Bt transgenic crops. In the wild, B. thuringiensis colonizes the surface of some plants and exists naturally in some caterpillars. The bacterium produces a unique kind of endotoxin, a proteinaceous crystal that is lethal to several pests, including flies, mosquitoes, and beetles, upon ingestion. This symbiosis with plants is dependent on initial host–microbe adhesion events.

The attractive chemical signals and ultimate adhesive interactions of Agrobacterium tumefaciens with wounded plants leads to the unfortunate development of tumors on the lower stems and main roots, the hallmark of Crown Gall diseases. Attachment is the first step in the pathogenic cascade and takes place in the soil around the roots—the rhizosphere. In a two-step adhesive process, initial weak binding interactions are followed by the bacterial expression of multiple gene products to synthesize cellulose and anchor the microbe to the host tissue, while enhancing adhesive interactions between bacteria in the microcolony. Adhesive plant proteins called vitronectins are also implicated in the adhesion process. Subsequent DNA transfer and integration of a specific fragment of DNA (the transfer DNA) from the bacterium to a plant cell results in the expression of several oncogenic genes and the formation of tumors.

Adhesion in the Human Host

The normal and healthy human body is composed of approximately ten times more bacterial cells than human cells. These bacteria comprise our microbiota and are colonized in distinct sites throughout the body,
including the skin and mouth, and the small intestine and colon. In the mouth and small intestine, bacterial adhesion is critical to maintenance of microbial populations, where either salivary flow or movement of contents eliminates the nonadherent bacteria. Our microbiota is, in general, beneficial. Bacteria in the gut, for example, attach to undigested by-products and degrade some polysaccharides into carbon and energy sources, for example. Recent results indicate that the balance of bacterial populations in the gut influence caloric intake through complex inter-bacterial metabolic networks and further study may help to understand and potentially control (decrease or increase) caloric uptake. Indeed, the microbiota is dynamic and shifts in balance that alter the sizes of different bacterial populations can also lead to proliferation of disease-causing opportunistic pathogens. In addition, the inoculation of the human host with bacteria from the environment is a common source of infectious disease, particularly in the hospital setting. The consequences of bacterial adhesion in human infectious diseases are numerous and will be addressed in more detail after the description of specific adherence mechanisms.

Mechanisms of Microbial Adhesion

General Physicochemical Factors Affecting Adhesion

Mechanisms of microbial attachment are incredibly diverse and can be generally classified as either general nonspecific interactions or specific molecular-recognition binding events that involve the presentation of specific adhesive proteins on the bacterial cell surface. Of course, multiple mechanisms can act cooperatively to promote adhesion. A successful adhesive event depends on properties of both the bacterium and the substratum. Nonspecific interactions are the primary form of attachment to abiotic surfaces in aquatic and soil environments. Van der Waals interactions are attractive, usually weak, noncovalent forces that can operate at large separation distances (>50 nm) between the bacterium and the surface. At smaller distances (10–20 nm), electrostatic interactions participate and compete as attractive and repulsive forces. The net surface charge of most bacteria is negative due to cell wall and cell membrane components including negatively charged phosphate groups, carboxyls, and other acidic groups, in addition to surface-exposed proteins. Thus, bacteria like to adhere to positively charged surfaces. Typical binding surfaces, however, have a net negative surface charge, creating electrostatic repulsion that must be overcome by other physicochemical factors. The entire binding process is akin to a tug-of-war. The ionic strength of the surrounding medium affects the electrostatic interactions, and the aforementioned repulsion is eliminated, for example, in most aquatic environments due to high ionic strength resulting from high salt concentration. In the range of near contact (0.5–2 nm), hydrophobic interactions are important for bacterial adhesion. Energetically, the association of nonpolar groups on a bacterial surface with hydrophobic surfaces compensates for the unfavorable displacement of water molecules at that surface. When separated by less than 1 nm, stronger interactions including hydrogen bonding and the formation of salt bridges contribute to surface adhesion.

Specific Adhesin-Receptor Mechanisms

On many biotic surfaces, the adhesive forces and interactions described above promote the formation of an initial interface, but require concomitant or subsequent specific adhesive interactions to enable firm adhesion. Adhesin is the term ascribed to the surface-exposed bacterial molecule that mediates specific binding to a receptor or ligand on a target cell. It is not unusual for bacteria to harbor several types of specific adhesive machinery to provide adhesive capacity to multiple receptor molecules or to permit adhesion under changing environmental conditions such as temperature, pH, or nutrient status, where one adhesive strategy may be more effective than another.

Bacteria can produce a diverse array of adhesins with varying specificities for a wide range of host receptor molecules. Adhesion mechanisms can be classified according to the type of adhesin–receptor pair. Many bacterial adhesins function as lectins and the interactions between bacterial lectins and host cell carbohydrates are among the best-characterized attachment processes. Hallmark examples of carbohydrate recognition include *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* adhesion in the respiratory tract, *Escherichia coli* adhesion in the urinary tract and intestine, and *Helicobacter pylori* adhesion in the stomach. Other adhesins recognize specific amino acid-recognition motifs in proteins expressed on host cell surfaces. Extracellular matrix (ECM) proteins that are not directly integrated into the host cell also serve as attractive binding platforms for many bacteria, and numerous adhesins bind to these components in order to indirectly hijack the host signaling pathways, often to enable host cell internalization. Another general category of adhesins includes nonproteaceous molecules such as lipopolysaccharides and teichoic acids, synthesized by Gram-negative and Gram-positive bacteria, respectively.

Most adhesins are incorporated into heteropolymers extracellular fibers called pili or fimbriae. Bacteria invest enormous cellular resources to assemble fimbriae in order to present adhesins at the right time and the right place to initiate attachment when conditions are favorable and to permit detachment when necessary. Indeed, hundreds of such fibers have been described in Gram-negative
organisms, and although they have diverse functions, many appear critical to binding, invasion, and survival of pathogenic microorganisms in the human host. Four distinct assembly mechanisms have emerged as the most well studied and include the chaperone–usher pathway, the general secretion pathway, the extracellular nucleation–precipitation pathway, and the alternate chaperone pathway. Gram-positive pathogens also produce adhesive pili. Unlike their Gram-negative counterparts, Gram-positive pili are formed by covalent polymerization of pilin subunits. A representative set of fimbrial adhesins is provided in Table 1.

Some bacteria present afimbrial adhesins on their surface. These are expressed as monomeric proteins or protein complexes that assemble at the cell surface and recognize host cell surface elements. Adhesins of the Dr family are expressed by E. coli strains and mediate recognition of decay-accelerating factor (DAF). DAF is found in the respiratory, urinary, genital, and digestive tracts, and Dr-mediated adhesion is important for binding in the intestine and urinary tract. Adhesive autotransporters represent a class of afimbrial adhesins expressed by a variety of unrelated microorganisms, including species of Rickettsia, Bordetella, Neisseria, Helicobacter, and many members of the family Enterobacteriaceae. H. influenzae, a causative agent of sinusitis, bronchitis, otitis media, and pneumonia, expresses an adhesive autotransporter termed Hap. Hap mediates binding to laminin, fibronectin, and collagen, all components of the ECM.

The most comprehensive descriptions of bacterial adhesion have emerged from studies of pathogenic bacteria involved in infectious diseases. Examples of these host–microbe interactions as well as some involved in the attachment of bacteria to plants, either as symbionts or as pathogens, are described in more detail below to highlight the remarkable diversity, specificity, and complexity among microbial adhesive strategies.

### Selected Survey of Specific Adhesion Strategies

#### Pilus-Mediated Adhesion to Carbohydrates in the Urinary Tract

Uropathogenic *E. coli* (UPEC) colonize the gut as well as the genitourinary tract and produce numerous important adhesins and adhesive organelles to mediate adhesion in these niches. For example, FimH and PapG adhesins are presented at the tips of type 1 and P pili, respectively. FimH-presenting type 1 pili are required for *E. coli* to cause cystitis, or infection of the bladder, and PapG-presenting P pili are associated with pyelonephritis, infection of the kidney. Type 1 and P pili are composite heteropolymeric structures, with a distal tip fibrillum joined to a thicker rigid helical rod and both are assembled by the chaperone–usher system. More than 100 chaperone–usher systems have been identified through comparative genome analyses and many are

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>Adhesin</th>
<th>Assembly proteins</th>
<th>Associated fiber</th>
<th>Associated disease(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>FimH</td>
<td>FimC/FimD</td>
<td>Type 1 pili</td>
<td>Cystitis</td>
</tr>
<tr>
<td></td>
<td>PapG</td>
<td>PapD/PapC</td>
<td>P pili</td>
<td>Cystitis/pyelonephritis</td>
</tr>
<tr>
<td></td>
<td>PrsG</td>
<td>PrsD/PrsC</td>
<td>Prs pili</td>
<td>Cystitis</td>
</tr>
<tr>
<td></td>
<td>SfaS</td>
<td>SfaE/SfaF</td>
<td>S pili</td>
<td>UTI, newborn meningitis</td>
</tr>
<tr>
<td></td>
<td>CooD</td>
<td>CooB/CooC</td>
<td>CS1 pili</td>
<td>Diarrhea</td>
</tr>
<tr>
<td></td>
<td>CsgA</td>
<td>CsgB (nucleator), CsgE/CsgF (assembly), CsgG (secretion)</td>
<td>Curli</td>
<td>Sepsis</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>AgfA</td>
<td>LpfB/LpfC</td>
<td>Pef pili</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LpfB (nucleator)</td>
<td>Long polar fimbriae</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sef17 (thin aggregative fimbriae)</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>AgfA</td>
<td>AgfB (nucleator)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>MrkD</td>
<td>MrkB/MrkC</td>
<td>MR/K (type 3) pili</td>
<td>Pneumonia</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>FimD</td>
<td>FimB/FimC</td>
<td>Type 2 and 3 pili</td>
<td>Whooping cough</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td></td>
<td>MyfB/MyfC</td>
<td>Myf fimbriae</td>
<td>Enterocolitis</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoea</em></td>
<td></td>
<td></td>
<td>Type 4 pili</td>
<td>Gonorrhea</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em>,</td>
<td>PiIC</td>
<td>General secretion apparatus</td>
<td></td>
<td>Cholera</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em>,</td>
<td>Pilin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium bovis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>HifB</td>
<td>Hif pilus</td>
<td>Otitis media, meningitis</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Representative fimbrial adhesins and disease association
well studied and required for the assembly of extracellular adhesive organelles in pathogens including *Salmonella*, *Haemophilus*, *Klebsiella*, and *Yersinia*. In each chaperone-usher system, pilus assembly requires a unique protein pair (a chaperone and a usher) to facilitate the folding, transport, and ordered assembly of pilus subunits at the cell surface. This process begins in the periplasm, after subunit expression and translocation by the general secretory pathway into the periplasm. Periplasmic pilus chaperones consist of two immunoglobulin (Ig)-like domains and bind to folded subunits to keep their interactive surface capped and prevent nonproductive subunit aggregation. Pilin subunits also have an Ig-like fold, but they lack the seventh β strand, thus exposing the hydrophobic core. In a process termed donor strand complementation, the chaperone’s G1 β strand serves as the pilin’s seventh strand, catalyzing the folding of the subunit. Chaperone–subunit complexes are targeted to an outer membrane usher to facilitate chaperone uncapping, translocation of subunits across the outer membrane, and pilus assembly. This occurs via a process termed donor strand exchange, in which the G1 β strand of the chaperone is replaced by an N-terminal extension of the next pilus subunit. Thus in the mature pilus, each subunit incorporates its neighbor’s N-terminal extension as part of its own Ig fold. Subunits have distinct specificity for other interactive subunits, such as the adhesin, and this confers distinct roles in pilus adhesion, initiation, elongation, termination and regulation.

The FimH adhesin, incorporated at the tip of the type 1 pilus, consists of a pilin subunit and the receptor-binding domain (Figure 1). The primary carbohydrate specificity of FimH is mannose. Interestingly, different *E. coli* isolates present FimH variants (specific allelic variations in protein sequence and structure) that exhibit varying specificities for monomannose and trimannose binding. FimH expressed by most commensal isolates of the intestine exhibit a higher specificity for trimannose-presenting glycoprotein receptors, whereas urinary tract isolates encode for a FimH variant with higher affinity for monomannose. In the latter, FimH mediates adhesion to the monomannose-containing glycoprotein uroplakin Ia that is expressed on the surface of superficial facet cells — the epithelial cells that line the lumen bladder (Figure 1).

Presented at the tips of P pili, the PapG adhesin mediates binding to a different carbohydrate receptor, the α-D-galactopyranosyl-(1–4)-β-D-galactopyranoside moiety of glycolipids presented by cells predominantly in the kidney. PapG variants (G-I, G-II, and G-III) exhibit altered specificities for three Galα(1–4)Gal-containing iso-receptors: globotriaosylceramide, globotetraosylceramide (GbO4), and globopentaosylceramide (the Forssman antigen). The demonstrated allelic variation in PapG and FimH binding specificities supports the notion that,

---

**Figure 1** The FimH adhesin and type 1 pili-mediated adhesion of *E. coli*. (a) The ribbon representation of FimH (from the crystal structure of the FimCH complex). α-mannose is located at the top of the molecule. (b) Molecular surface representation in which the electrostatic potential surface with positively charged residues is shown in blue, negatively charged residues in red, and neutral and hydrophobic residues in white. Residues defining the hydrophobic ridge around the mannose-binding pocket are labeled. (c) The mannose-binding site with FimH residues. Mannose residues are shown with carbon atoms in yellow, oxygen atoms in red, and nitrogen atoms in blue. (d) Type 1 pili-mediated attachment of uropathogenic *E. coli* (UPEC) to the luminal surface of the bladder epithelium. (Top) High-resolution, freeze-fracture, deep-etch electron micrograph is from Mulvey MA, Lopez-Boado YS, Wilson CL, et al. (1998) Induction and evasion of host defenses by type 1-piliated uropathogenic *E. coli*. Science 282: 1494–1497. Reprinted with permission from AAAS. (Bottom) Scanning electron micrograph of a bacterium entering the membrane of bladder epithelial cells is reprinted from Soto GE and Hultgren SJ (1999) Bacterial adhesins: Common themes and variations in architecture and assembly. *Journal of Bacteriology* 181: 1059–1071.
through bacterial evolution, pathoadaptive mutations are selected for increasing the fitness of pathogenic organisms in distinct niches in the host.

**Adhesion to ECM Components**

The ECM contains a diverse array of oligosaccharides, proteoglycans, and proteins and functions to provide structural support and adhesive interactions among cells. Prevalent components include collagen, fibronectin, laminin, and vitronectin, as well as molecules such as heparan sulfate and chondroitin sulfate. Fibronectin is present in most tissues and fluids of the body and helps to create a cross-linked network between cells by presenting binding sites for other ECM components, a process that pathogenic organisms exploit to gain a foothold in host tissue. The ability to adhere to ECM components is a primary adhesion mechanism that contributes to the virulence of many pathogenic microorganisms. *Staphylococcus aureus* is a significant cause of nosocomial and often persistent infections. Among other ECM-binding proteins, *S. aureus* expresses the fibronectin-binding proteins FnBP-A and FnBP-B that permit adherence to fibronectin that are bridged to cellular integrins. This crucial binding event leads to host cell cytoskeletal rearrangements and invasion. *Streptococcus pyogenes* is armed with more than 12 fibronectin- and collagen-binding proteins. Like the FnBP-A adhesin in *S. aureus*, the major *S. pyogenes* adhesin, SfbI, and the *Yersinia* adhesin, YadA, bind to fibronectin and bridge the bacteria to integrins, leading to integrin clustering and eventual internalization. Invasin is a *Yersinia* adhesin that bypasses the ECM and binds directly to integrin transmembrane receptors. Other less-ubiquitous ECM components also serve as binding receptors for bacterial adhesins and their sites of expression often relate to the tissue tropism of a particular bacterial pathogen.

**Curli-Mediated Multipurpose Adhesion**

Curli are a unique class of adhesive extracellular amyloid fibers produced by Gram-negative bacteria, including *E. coli*. The highly homologous fibers produced by *Salmonella* species are called Tafi (thin aggregative fimbriae). The fibers mediate biofilm formation and attachment to host proteins including fibronectin, laminin, and plasminogen, and have been implicated in human sepsis. When expressed together with cellulose, curli and Tafi contribute to a remarkable aggregative phenotype characterized by a patterned assembly of cells radiating from the center when grown on a surface such as agar. Curli are assembled by the nucleation–precipitation pathway, and assembly requires specific molecular machinery encoded by the *csgBA* and *csgDEFG* operons (Figure 2). The major subunit protein (CsgA) and the nucleator

![Figure 2](image-url)
protein (CsgB) are secreted to the cell surface in a CsgG-dependent fashion. CsgE and CsgF are assembly factors required for the stabilization and transport of CsgA and CsgB. Transcriptional regulation of the curli operons is complex and responds to many environmental cues including temperature, pH, and osmolarity. The adhesive functionality is attributed to the main fiber subunit, CsgA.

Curli are also implicated in the binding of *E. coli* strains to plant surfaces and are expressed by many strains associated with food-borne illness, including the prototype strain *E. coli* O157:H7, which has caused several food-borne outbreaks in the United States and around the world. Although the exact nature of binding is still under investigation, curli production is sufficient to permit laboratory strains of *E. coli* to bind plant tissues, such as alfalfa. However, among pathogenic strains such as *E. coli* O157:H7, there appear to be redundant adhesion systems, and under the conditions tested, curli are not required for adhesion. Indeed, external conditions in the environment and in the host may differ as a function of time, and bacteria may depend more on one adhesive system than another in certain circumstances.

The curli bacterial adhesive fiber machinery has gained considerable attention since the discovery of curli as amyloid fibers in 2002. The sticky nature of curli amyloid fibers is like that of amyloid aggregates and plaques associated with eukaryotic amyloid disorders such as Alzheimer’s and Parkinson’s diseases. Thus, ongoing curli research that aims to elucidate structural features of curli assembly and the functional implications of curli-mediated adhesion may also provide valuable information to the exciting field of amyloid fiber biogenesis and aggregation.

**Consequences of Microbial Adhesion in Human Disease**

The critical first step in most infectious diseases requires physical contact between a bacterium and host cell. Bacterial adhesins mediate this binding event through the sophisticated adhesion mechanisms described above and allow the pathogen to gain a foothold in the host, initiating complex signaling cascades in both the pathogen and the host. Binding events can lead to extracellular colonization and invasion into underlying host cells. Adhesion is the first step that promotes the cascading sequelae of infectious diseases, particularly important in the pathogenesis of chronic infections including urinary tract infection (UTI), chronic otitis media (middle ear infection), and chronic lung infections.

**E. coli and UTI**

UPEC engage in an incredibly coordinated and regulated genetic and molecular cascade to assemble type 1 pili, as described above. UTIs are among the most common bacterial infections and nearly 50% of women will be afflicted by at least one UTI in their lifetime, with many experiencing recurrent UTIs. Virtually all clinical UPEC isolates express type 1 pili, enabling them to bind the mannose-containing host receptors, which results in invasion of host bladder epithelial cells. Inside urothelial cells, bacteria form large, densely packed, biofilm-like intracellular bacterial communities (IBCs) of morphologically coccoid bacteria, comprising up to $10^5$ bacteria per superficial facet cell. In this intracellular niche, the pathogens are protected from antibodies, the flow of urine, and other host defenses. Yet, this is only the beginning of a sometimes life-long cycle of interactions between pathogen and host. IBC formation is not an end point or dead end for *E. coli*. Upon entry into superficial facet cells, UPEC activate a complex developmental cascade; UPEC eventually detach and disperse, or flux, from the IBC to initiate another round of IBC formation in other urothelial cells (Figure 3). Some fluxing bacteria form filaments, which are resistant to neutrophil phagocytosis. Filamentation facilitates survival of the bacteria and allows them to invade other epithelial cells. Even after acute infection is resolved and the urothelium is

![Figure 3 Pathogenic cascade of uropathogenic *E. coli* (UPEC). UPEC coordinate highly organized temporal and spatial events to colonize the urinary tract. UPEC bind to and invade the superficial umbrella cells that line the bladder lumen, where they rapidly replicate to form a biofilm-like intracellular bacterial community (IBC). In the IBC, bacteria find a safe haven, are resistant to antibiotics, and subvert clearance by innate host responses. UPEC can persist for months in a quiescent bladder reservoir following acute infection, and challenge current antimicrobial therapies. Quiescent bacteria can reemerge as pathogens from their protected intracellular niche and can be a source of recurrent urinary tract infections (UTIs).](image-url)
seemingly intact, bacteria can remain within the bladder for many days to weeks regardless of standard antibiotic treatments. Thus, the ability of UPEC to adhere to and invade bladder cells appears to facilitate long-term bacterial persistence within the urinary tract.

**P. aeruginosa and CF**

*P. aeruginosa* has emerged as an opportunistic pathogen in several clinical settings, causing nosocomial infections such as pneumonia, UTIs, and bacteremia. *P. aeruginosa* adheres to the respiratory epithelium, leading to chronic lung infections in cystic fibrosis (CF) patients, responsible for the eventual pulmonary failure of most CF patients, typically by 37 years of age. Pili-mediated adhesion is important in the adhesion and early stages of epithelial colonization, and additional virulence factors contribute to the subsequent persistence in the lung. Alginate, for example, is a mucoid exopolysaccharide produced by *P. aeruginosa* that forms a matrix of ‘slime’ to surround a forming biofilm and anchors the cells to each other and to their host. Surrounded by alginate, the bacteria are protected from the host defenses and are often resistant to treatment with antibiotics.

**Targeting Adhesion to Inhibit Bacterial Virulence**

The ability to impair bacterial adhesion represents an ideal strategy to combat bacterial pathogenesis because of its importance early in the infectious process. In addition, adhesion is essential to the long-term persistence of bacteria in the pathogenic cascade of several infectious diseases. Moreover, the adhesion process can be targeted without placing life or death pressure on the bacterium, *per se*. Targeting bacterial virulence in this way is an alternative approach to the development of new therapeutics to disarm pathogens in the host that may offer reduced selection pressure for drug-resistant mutations. In addition, virulence-specific therapeutics could avoid the undesirable dramatic alterations of the host microbiota. Indeed, standard antibiotic treatment regimens may lead to the loss of symbiotic benefits and the proliferation of disease-causing opportunistic pathogens.

As emphasized earlier, pathogens are capable of presenting multiple adhesins that can be expressed differentially to permit binding in specific sites and at specific times over the course of a complex infectious cycle. Thus, it may be difficult to develop a universal class of antiadherence drugs. Nevertheless, several specific pathogenic adhesive strategies have emerged as hallmark requirements for virulence in certain infectious diseases, and represent amenable targets for drug discovery and development. Adhesion is sometimes just the first step of many in pathogenic cycles, yet targeting adhesion holds value even after an infection has been established. In biofilm-associated infections, for example, drug development strategies include attempts to induce the dispersal of bacteria from the biofilm and to inhibit the chemical signaling necessary to encourage new biofilm formation. In UTI, the fluxing bacteria are capable of adhering to new host cells, gaining a foothold and potentially invading a new cell to remain undetected until drug pressure subsides and conditions encourage replication and new intracellular biofilm formation. Thus, strategies to prevent microbial adhesion are being considered in combination therapies to both prevent and treat infectious diseases.

Carbohydrate derivatives of host ligands have demonstrated efficacy in blocking the adhesive properties of *E. coli* expressing type 1 and also P pilus in biophysical and hemagglutination assays. This approach of using soluble carbohydrates or mimics recognized by the bacterial lectin can be readily extended to other adherent organisms by tailoring the antiadhesive compounds to their receptor specificities.

‘Pilicides’ are a class of pilus inhibitors that target chaperone function. A new class of pilicides, based on a bicyclic 2-pyridone scaffold, inhibit the assembly of both type 1 and P pilus in *E. coli* (Figure 4). The potent molecules inhibit an essential protein–protein interaction between chaperone and usher, required for pilus biogenesis. Chaperone–usher systems are highly conserved among various bacteria including *Salmonella, Haemophilus, Klebsiella*, and *Yersinia* and it is possible, although not yet demonstrated, that pilicides may exert broad-spectrum activity and be effective against several Gram-negative pathogens.

![Figure 4](image-url)  
Compounds have been identified that target the two-component signaling system, AlgR2/AlgR1, that controls the synthesis of alginate by *P. aeruginosa*. Alginate is a key component of the protective exopolysaccharide coat, critical to *P. aeruginosa* adherence, biofilm formation, and CF pathogenesis. The inhibitors of alginate synthesis could be therapeutically employed to render the pathogen more susceptible to host defenses or to standard antibiotics currently in use, and thus could be effective also in combination therapy. The ability to inhibit microbial adhesion and thus prevent subsequent pathogenic processes holds enormous therapeutic potential and promises to improve the treatment of numerous infectious diseases.

**Acknowledgments**

L Cegelski is the recipient of a Burroughs Wellcome Fund Career Award at the Scientific Interface.

S J Hultgren acknowledges funding from the National Institutes of Health (Scor P50 DK64540/ORWH, R01AI029549, R01AI048689, and R01DK51406).

See also: Biofilms, Microbial; Food and Waterborne Illnesses; Pili, Fimbriae; Plant Pathogens and Disease: General Introduction

**Further Reading**


