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Dirk Linke
Adrian Goldman *Editors*

# Bacterial Adhesion

## Chemistry, Biology and Physics

Springer

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**ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY**



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Editors

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## Chemistry, Biology and Physics

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# Introduction

Why a book on bacterial adhesion? Adhesion plays a major role in the bacterial lifestyle. Bacteria adhere to all surfaces and did so long before the first eukaryotes were around; stromatolites, which are calcium-based rocks in shallow seawaters formed and inhabited by cyanobacteria, are among the oldest fossils found (Battistuzzi et al., 2004). Bacteria can adhere to each other, a phenomenon referred to as autoagglutination, which is generally viewed as one of the first steps towards biofilm formation. Bacteria can also form more complex and defined structures, such as the *Myxococcus* fruiting bodies – *Myxococcus* is generally seen as a "social" bacterium with complex inter-cell interactions, and as a model for the early evolution of multicellularity (Konovalova et al., 2010). Last but not least, bacteria can adhere to other cells: different prokaryotic species in the formation of complex biofilms, or eukaryotic cells during disease. Adhesion to eukaryotic cells can serve different purposes in commensalism, symbiosis, and pathogenesis. The general principle, the expression of surface molecules to adhere to other structures, stays the same.

But why this particular book when reviews on bacterial pathogenesis are common, if not quite a dime a dozen? Our focus is: how are such adhesion phenomena best studied? Microbial genetics experiments have greatly enhanced our knowledge of what bacterial factors are involved in adhesion. For numerous reasons, though, biochemical and structural biology knowledge of the molecular interactions involved in adhesion is limited. Moreover, many of the most powerful biophysical methods available are not frequently used in adhesion research, meaning that the time dimension – the evolution of adhesion during biofilm formation remains poorly explored. The reason for this is, we believe, on the one hand microbiologists, who are experts at handling and manipulating the frequently pathogenic bacterial organisms in which adhesion is studied, lack detailed knowledge of the biophysical possibilities and have limited access to the frequently expensive instrumentation involved. On the other hand, the experts in these methods frequently do not have access to the biological materials, nor do they necessarily understand the biological questions to be answered. The purpose of this book is thus to overcome this gap in communication between researchers in biology, chemistry, and physics, and to display the many ways and means to address the topic of bacterial adhesion.

Thus, the book consists of three loosely connected parts. The first Chapters 1 to 7 deal, broadly speaking, with bacterial adhesion from a biological perspective, where different bacterial species and their repertoire of adhesion molecules are described. The chemistry section includes the biochemistry and structural biology knowledge which have been obtained on some of the adhesin systems. The physics section contains examples of biophysical methods that have been successfully applied to bacterial adhesion. For obvious reasons, we had to limit ourselves in the choice of systems and methods described in this book. The biological systems described are only examples, and mostly come from genera containing the better-studied human pathogens. We tried nonetheless to cover a broad spectrum of organisms, both Gram-positive and Gram-negative bacteria. Chapters 1 and 9 also put specific Gram-negative and Gram-positive systems into a historical perspective and describe the development of the field of infectious diseases. Many of the findings also apply to bacteria that are either non-pathogenic (Chapter 13) or pathogenic on different species and kingdoms, and Chapter 5 nicely shows that in plant pathogens, adhesins similar to those of human pathogens exist and serve comparable functions.

The chemistry section (Chapters 8 to 15), contains examples of molecular structures of the very different types of adhesins found. These are mostly from the human pathogens discussed in the biology section, again from both Gram-negative and Gram-positive bacteria. We have also included two chapters on carbohydrate structures (13 and 14), as these structures are at least as important as the proteins in bacterial pathogenesis. One pattern that emerges is that most of these adhesins contain repetitive elements, which make them long and fibrous, but which might also allow for easy recombination and thus evolution in the face of the host immune system.

The physics section (Chapters 16 to 22) originally seemed the hardest to fill: how should we identify methods useful in adhesion research, but infrequently used? Discussions with colleagues and literature searches led us to authors on such diverse methods as force measurements, electron microscopy, NMR, and optical tweezers, as well as a chapter on how bacteria adhere to medical devices and how this can be studied (Chapter 22). Moreover, the enthusiastic response of these authors showed to us that indeed, there is a need for a forum to display the panel of technical possibilities to the researchers who struggle with unsolved biological questions.

Now that the book is finished and out of our hands, we hope that it will achieve our goals – that it will be of broad interest to researchers from different fields all working on different aspects of bacterial adhesion. We hope it provides an advanced but jargon-free introduction to the state of adhesion research in 2010, one that will bring researchers together in new, exciting, and most importantly, interdisciplinary projects. The struggle for new therapies against bacterial infections is not made easier by the "Red Queen Principle" – the fact that pathogens evolve and adapt quickly in the face of new challenges (van Valen, 1973). We strongly believe that only interdisciplinary research can tackle the growing problems of multidrug

resistance, hospital-acquired infections, and other adhesion- and biofilm-related topics in human health that require new drugs, disinfectants, or vaccines.

We thank all of our authors for their hard work and Thijs van Vlijmen of Springer for being always available to answer our questions.

Tübingen Dirk Linke
Helsinki Adrian Goldman
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# Chapter 1
# Adhesins of Human Pathogens from the Genus *Yersinia*

**Jack C. Leo and Mikael Skurnik**

**Abstract** Bacteria of the Gram-negative genus *Yersinia* are environmentally ubiquitous. Three species are of medical importance: the intestinal pathogens *Y. enterocolitica* and *Y. pseudotuberculosis*, and the plague bacillus *Y. pestis*. The two former species, spread by contaminated food or water, cause a range of gastrointestinal symptoms and, rarely, sepsis. On occasion, the primary infection is followed by autoimmune sequelae such as reactive arthritis. Plague is a systemic disease with high mortality. It is a zoonosis spread by fleas, or more rarely by droplets from individuals suffering from pneumonic plague. *Y. pestis* is one of the most virulent of bacteria, and recent findings of antibiotic-resistant strains together with its potential use as a bioweapon have increased interest in the species. In addition to being significant pathogens in their own right, the yersiniae have been used as model systems for a number of aspects of pathogenicity. This chapter reviews the molecular mechanisms of adhesion in yersiniae. The enteropathogenic species share three adhesins: invasin, YadA and Ail. Invasin is the first adhesin required for enteric infection; it binds to $\beta_1$ integrins on microfold cells in the distal ileum, leading to the ingestion of the bacteria and allows them to cross the intestinal epithelium. YadA is the major adhesin in host tissues. It is a multifunctional protein, conferring adherence to cells and extracellular matrix components, serum and phagocytosis resistance, and the ability to autoagglutinate. Ail has a minor role in adhesion and serum resistance. *Y. pestis* lacks both invasin and YadA, but expresses several other adhesins. These include the pH 6 antigen and autotransporter adhesins. Also the plasminogen activator of *Y. pestis* can mediate adherence to host cells. Although the adhesins of the pathogenic yersiniae have been studied extensively, their exact roles in the biology of infection remain elusive.

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## 1.1 Introduction

Plague is arguably the most notorious of all diseases. This calamitous affliction is particularly virulent, and has shaped the course of history. It is estimated that the Black Death of fourteenth century Europe wiped out approximately 30% of the population (Perry and Fetherston, 1997). In 1894, Alexandre Yersin discovered the causative agent of plague to be a Gram-negative bacillus. Later, this bacterium was named *Yersinia pestis* in his honour. In addition to this infamous pathogen, two other members of the genus, *Y. enterocolitica* and *Y. pseudotuberculosis*, are known to cause human diseases.

*Y. enterocolitica* and *Y. pseudotuberculosis* cause food poisoning and are relatively abundant in the environment. Plague is still endemic in several regions of the world, including the Western USA and many regions in Africa, Asia and Latin America. Between 1000 and 5000 cases of human plague have been reported to the World Health Organisation per year, 100–200 leading to death, but a significant number of cases probably go unreported. Worryingly, antibiotic-resistant stains of *Y. pestis* have emerged, including some which are resistant to multiple drugs (Prentice and Rahalison, 2007).

Thus, the genus *Yersinia* is a medically important one, being prevalent and responsible for several human diseases. In addition, bacteria of the genus serve as important model organisms for various aspects of pathogenicity, including adhesion, invasion, immune evasion and effector protein delivery. This chapter gives a short overview of the biology of the human pathogenic yersiniae, followed by a more detailed discussion of the adhesins expressed by this family of bacteria.

## 1.2 The Human Pathogenic Yersiniae

### *1.2.1 Enteropathogenic Yersiniae*

The yersiniae are facultative anaerobic Gram-negative pleiomorphic rods of the family Enterobacteriaceae. The genus contains 15 recognised species, with environmental, commensal and pathogenic representatives. Pathogenicity to humans correlates with the presence of the *Yersinia* 70-kb virulence plasmid pYV, found in disease-causing strains of *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis*, but absent from the other species.

The two most commonly encountered human pathogenic species are *Y. enterocolitica* and *Y. pseudotuberculosis*. Like most other *Yersinia* species, both are ubiquitously found in aquatic environments, soil, and animals. Infections caused by both organisms have been reported worldwide. Although rather distantly related, *Y. enterocolitica* and *Y. pseudotuberculosis* share a number of features.

Though regarded as a single species, *Y. enterocolitica* is heterogeneous and is now considered to consist of two genetically distinguishable subspecies, *Y. enterocolitica* subsp. *enterocolitica* and *Y. enterocolitica* subsp. *palearctica* (Neubauer

et al., 2000). In addition, the species comprises 6 biogroups (1A, 1B, 2, 3, 4 and 5), based on biochemical variability, which are further subdivided into approximately 60 serotypes (Bottone, 1997). *Y. enterocolitica* has been isolated from a number of mammalian hosts, with swine being a significant reservoir for pathogenic strains of this organism. *Y. enterocolitica* is responsible for the majority of human cases of yersiniosis, and undercooked pork products have been implicated in a large number of outbreaks (Bottone, 1997).

*Y. pseudotuberculosis* derives its name from the tuberculosis-like granulomatous abscesses it causes in the spleen and liver of infected animals. A less common human pathogen than *Y. enterocolitica*, *Y. pseudotuberculosis* is associated with outbreaks from fresh produce like lettuce and carrots (Jalava et al., 2006). *Y. pseudotuberculosis* infections are generally more severe than those of *Y. enterocolitica*, and are more likely to require hospitalisation (Long et al., 2010). In addition to gastrointestinal infections, *Y. pseudotuberculosis* is implicated as the cause of Far East scarlet-like fever and Kawasaki disease. The former mimics symptoms often seen in scarlet fever caused by group A streptococci, including widespread scarlatinoid rash and toxic shock syndrome (Eppinger et al., 2007). The latter is an inflammatory syndrome affecting the blood vessels, lymphatics, skin, mucous membranes and heart. Though the aetiology of Kawasaki disease has not been established, epidemiological data suggest *Y. pseudotuberculosis* as a possible agent in the development of the syndrome (Vincent et al., 2007).

Infection by either organism follows a similar course. The bacteria are ingested with contaminated food or water. The bacteria then traverse the gastrointestinal tract until they reach the terminal ileum, where they cross the intestinal mucosa. Crossing is facilitated by microfold (M) cells in the intestinal epithelium (Miller et al., 2007). M cells are transcytotic epithelial cells associated with Peyer's patches, the lymphoid follicles of the intestine. They function in sampling the luminal solution for immunogenic substances, which are then transported by transcytosis to the underlying immune cells of the follicle. Yersiniae and several other enteropathogens, including *Salmonella* and *Shigella*, can hijack this transport process to gain entry to the submucosa.

Once in the follicle, yersinae replicate extracellularly. Growth of these bacteria leads to destruction of the follicle (Autenrieth and Firsching, 1996). The bacteria can then disseminate to the mesenteric lymph nodes. Usually the infection is self-limiting, but in severe cases bacteria can spread to other organs (the liver, spleen, kidneys and lungs), leading to systemic infection and bacteraemia. In addition to this infection route, it is probable that bacteria from a pool replicating in the intestinal lumen can infect the liver and spleen by some other means, possibly by disseminating through the hepatic portal vein (Barnes et al., 2006).

The symptoms of yersiniosis are varied. Cases range from mild gastroenteritis and diarrhoea to pseudoappendicular syndrome (Bottone, 1997). Enterocolitis is a typical manifestation of yersinioisis in young children, whereas terminal ileitis and mesenteric lymphadenitis (the causes of pseudoappendicitis) are usual for adults. Diarrhoea, occasionally bloody, is associated with most cases of *Y. enterocolitica* infection but is less usual for *Y. pseudotuberculosis*. Sepsis is an uncommon result

of yersiniosis. Primary infections by enteropathogenic yersiniae are infrequently followed by sequelae such as reactive arthritis (inflammation of joints), erythema nodosum (localised skin inflammation), iritis or glomerulonephritis (inflammation of the kidney) (Bottone, 1997).

### *1.2.2 Yersinia pestis*

Three major plague pandemics have blighted recorded human history (Perry and Fetherston, 1997). The first, referred to as the Justinian plague, spread around the Mediterranean in the sixth century AD. The most famous was the second pandemic, the Black Death of Europe, which started in the fourteenth century and continued intermittently for a further 300 years. Although there is some debate as to whether *Y. pestis* was in fact the pathogen behind these historical pandemics, there is considerable evidence linking the bacterium to the Black Death (Stenseth et al., 2008). The third pandemic ("modern plague") initiated in China in the mid-nineteenth century and has since spread across the world to continue to the present, albeit at a low incidence.

*Y. pestis* appears to have diverged from its parent species *Y. pseudotuberculosis* within the last 20,000 years. In contrast, the *Y. pseudotuberculosis* and *Y. enterocolitica* lineages diverged between approximately 150 and 200 million years ago (Achtman et al., 1999). *Y. pestis* is thus very closely related to *Y. pseudotuberculosis*, and in fact can be considered a pathovar of this species. However, due to its historical importance and public health considerations *Y. pestis* has not been reclassified as belonging to its parent species.

*Y. pestis* is one of the most virulent organisms known. It is highly invasive and proliferates rapidly in host tissues. Like its enteropathogenic relatives, *Y. pestis* replicates extracellularly and the first sites for replication are within lymphatic tissues, normally lymph nodes. However, *Y. pestis* is also able to survive and replicate within macrophages (Prentice and Rahalison, 2007). The swift replication of plague bacilli in lymph nodes quickly leads to their spread into the blood stream resulting in massive bacteraemia ($\sim 10^8$ bacteria/ml blood). The mortality of plague is staggering; untreated, the disease is fatal in 40–70% of cases (Stenseth et al., 2008).

Plague is a zoonosis. The primary hosts for *Y. pestis* are rodents. Fleas, usually of the genus *Xenopsylla*, act as the vector transporting the pathogen from host to host. This form of plague (sylvatic plague) is endemic to many regions of the world (Perry and Fetherston, 1997). However, in inhabited areas of poor hygiene where rodents, particularly rats, and humans interact, the disease can be transmitted to humans (urban plague). *Xenopsylla* fleas will take blood meals from humans, and so the disease can spread from rodent to human or human to human aided by the flea vector.

When a flea takes a blood meal from a host infected with *Y. pestis* it ingests a significant number of bacteria. Once inside the flea, *Y. pestis* adheres to the spines of the proventriculus, a compartment at the beginning of the digestive tract, and