Pathogenicity Mechanisms of Prokaryotic Cells: An Evolutionary View

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The success of pathogenic microbes depends on their ability to colonize host tissues and to counter host defense mechanisms. Microorganisms can produce overwhelming infection because of their relatively short generation times, and because they have evolved powerful mechanisms for generating phenotypic diversity as an efficient strategy for adapting to rapidly responding immune system defenses and the broad range of polymorphisms characteristic of different host tissues. Bacterial evolution may not be a continuous process, but more of a succession of temporally spaced major events. These events cause a non-gradual sequence of adaptations to a given environment. The pathogenicity islands may be genetically unstable elements, and many of the genes coding for the adhesins, toxins and other virulence factors are present in pathogenicity islands, which almost certainly had former lives as accessory elements or as parts thereof, or were borne on functional accessory elements. Novel genes are also acquired by transduction (mediated by bacteriophages, plasmids or transposons), by conjugation (DNA transfer between cells) or by transformation (natural DNA uptake). Horizontal gene transfer from other species is a major source of variation and is fundamental to the genetic theory of adaptive evolution in prokaryotes.

Key Words: Pathogenicity, prokaryotic evolution, genetic variation, bacterial virulence.

Prokaryotic cells are capable of coexisting with eukaryotic organisms, through various types of interrelations. One type of relationship does not cause damage to the host or there may be benefits, such as found for the normal biota of mammals with bowels. In this case, the host-pathogen interaction does not always result in disease. The other type of relationship could result in damage to the host, with different degrees of severity. The extent of loss is a function of host health and of the virulence of the bacteria [1,2]. Bacterial virulence can be enhanced through specialized virulence factors. These include capsules, enzymes, colonization factors (adhesins), invasins, and toxins. In extreme cases of interaction, a bacterium could insert its own genome into the eukaryotic genome, causing alteration of genetic activity to favor the pathogen.

Bacterial illness is a result of complex interactions between bacteria and the host. During evolution, humans developed many ways to protect themselves against bacterial pathogens. On the other hand, bacteria have developed strategies to evade, subvert or circumvent these defenses. One of the most important characteristics of bacterial pathogenicity is the various strategies developed by prokaryotic organisms to use host molecules for their own benefit [3]. To accomplish this, bacteria have evolved elaborate control mechanisms to turn genes on and off, varying the transcriptional activator or protein repressors of systems that act at the structural level of the genetic material [1].

Bacteria that have the ability to colonize a host must have specialized attributes that enable them to do this: adhesins promote adhesion to host surface cells; a capsule can hide and protect; invasion factors (invasins) enable microorganisms to invade cells and other factors help bacteria to survive in an intracellular habitat; specialized enzymes are able to capacitate bacteria to evade host defenses or to move from one cellular

Received on 21 February 2002; revised 09 January 2003.
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The Brazilian Journal of Infectious Diseases 2003;7(1):23-31

compartment to another or from one cell to other; toxins can assist in the colonization process at different infection stages and they facilitate the liberation of nutrients from the lysed cell host; and specialized chelators help the bacteria to gain access to the abundant but normally unavailable sequestered iron from transferrin, lactoferrin and related compounds. Some pathogens have the capacity to mimic characteristics of the host in order to evade its immune system.

All these abilities are genetically coded in each bacterium and many genes are subject to regulation in response to environmental changes or are controlled by mutational events that alter the expression profile in a bacterial population. Numerous factors determine the outcome of the host-bacterium relationship. Though the majority of bacteria are largely harmless to man, attention is inevitably focused on those microorganisms that cause disease.

Spontaneous Genetic Variation in Bacteria

Genes can act as variation generators or as modulators of the frequency of genetic variation. In general, the generation of genetic variation occurs independently of functional requirements related to particular living conditions [4].

Biological constituents of the process of evolution are likely to undergo biological evolution themselves. Since genes do not provide a direct substrate for natural selection in each individual cell of microbial populations, selection for their evolutionary improvement must occur at the level of populations based on directly selectable genes, or second order selection [5].

One can distinguish three major natural strategies in the spontaneous generation of genetic variants in bacteria on the basis of established knowledge of microbial genetics: i) small local changes in the nucleotide sequence of the genome; ii) intragenomic reshuffling of segments of genome sequences and iii) the acquisition of DNA sequences from other organisms [4]. During bacterial evolution, the ability of bacteria to exploit new environments and to respond to new selective pressures can be most easily explained by the acquisition of new genes by horizontal transfer [6].

The three mechanisms of gene transfer in bacteria have been identified [7]: i) transduction (whereby host DNA is encapsulated into a bacteriophage, which acts as the vector for its injection into a recipient cell), ii) transformation (involving the uptake and incorporation of naked DNA) and iii) conjugation (a cell contact-dependent DNA transfer mechanism found to occur in most bacterial genera).

Among the mechanisms of acquisition of DNA sequences, lysogenic conversion by bacteriophages appears to be advantageous [8]. Lysogenic transduction is efficient and does not require intimate contact between bacteria as in conjugation, nor does it require natural DNA uptake by transformation. Bacteriophages can carry large blocks of DNA and can survive harsh conditions that eliminate bacterial populations. They can also spread DNA directly to an entire population of bacteria, eliminating the need for clonal expansion of a specific population. Virulence factors encoded in bacteriophages may allow the bacterium to enlarge its host range and to increase its fitness in an environmental niche by promoting evasion of host immune defenses or providing mechanisms to breach host structural barriers [8]. A variety of major bacterial toxins associated with epidemics are carried on bacteriophages, such as the diphtheria and cholera toxins [9] and an *E. coli* toxin 0157:H7.

Along with a number of non-genetic factors, various specific gene products are involved in the generation of genetic variation and in the modulation of the frequency of genetic variation. The involvement of bacterial viruses and of plasmids in DNA reshuffling and in horizontal gene transfer are hints to their evolutionary functions [4].

Prokaryotic microorganisms are usually haploid and they thus rapidly reveal phenotypic consequences of genetic variation. Natural means to obtain temporary or more permanent partial diploidy in bacteria by transformation, conjugation or virus-mediated transduction have been used extensively to study both functional complementation and genetic recombination between genetic variants.

Spontaneous genetic variants are normally substitution mutants, or more generally, point mutants

[4]. There is however a class of spontaneous mutations that often exerts polar effects and which turn out to be caused by the insertion of mobile genetic elements [10]. Not all microbial recombination occurs between identical DNA sequences. Site-specific recombination can also occur, although at lower frequencies, at DNA sequences other than the normal recombination site.

Both DNA rearrangement activities, transposition and site-specific recombination, can also give rise to deletions, as well as DNA inversions. Additionally, both of these events can bring about an association of segments of chromosomal DNA with viral genomes and plasmids, which act as natural gene vectors.

The cellular and molecular mechanisms of microbial pathogenesis are currently being defined, with increasingly precise knowledge of both strategies used by pathogenic bacteria and the unique strategies developed by individual species that enable them to establish infection [11]. A pathogen can be defined as a microbe capable of causing host damage; this definition can encompass classical and opportunistic pathogens. Host damage can result from either direct microbial action or from the host immune response [12]. Damage can be used to characterize host-microbe interactions [2], since it is a relevant and potentially quantifiable outcome that can serve as the common denominator for analysis of the consequence of host-pathogen interactions.

Bacterial Pathogenesis

Knowledge concerning microbial pathogenesis and the molecular basis of bacterial disease has been increasing in recent years. Many pathogens have common mechanisms of interaction with the host, but each species has developed a unique strategy that enables it to exploit the eukaryotic cell [13].

Genetic techniques used to identify genes that are induced when bacteria are introduced into an animal or into a culture media, or to identify specific virulence genes, are essential to the comprehension of virulence [14]. Virulence is determined by sophisticated mechanisms for gaining access to environments and to the nutrients sequestered within them, for releasing these nutrients in a usable form, and finally for moving to new hosts when these nutrients are expended.

A key enzyme, DNA adenine methylase (Dam), plays a central role in the virulence of various bacterial species. This versatility stems from the biochemical activity of Dam, which acts as a global regulator of gene expression and affects a wide range of critical cellular functions, such as DNA replication and repair, as well as the transposition and segregation of chromosomal DNA.

Dam alters the interactions of some regulatory proteins with their designated gene targets by adding methyl groups to various sites along the cellular DNA, and in the process, effectively controls expression of those genes. Such changes can both modulate bacterial virulence and elicit protective immune responses in host organisms. Mutations of Dam and of other adenine methyl-transferases can attenuate virulence in a variety of pathogens, suggesting that these enzymes are essential in bacterial pathogenesis [15].

The pathogenic bacteria can be classified as primary and opportunistic. The primary pathogenic bacteria are capable of causing illness in healthy people and the opportunistic bacteria generally cause disease in immune-deprived individuals.

The pathogenic character of bacteria is conferred by colonization and lesion factors. Colonization factors enable the bacterium to colonize the host, that is, they can proliferate and survive in the organism. Adhesins, invasins, evasins and nutritional factors are important colonization factors [16].

Colonization Factors

Adhesins are molecules that have the ability to promote the adhesion of bacteria to the tissue surface. In Gram-negative bacteria lipopolyssacharides (LPS) can also participate in the adhesion process. The receptors to fimbrial and non-fimbrial adhesins are carbohydrates of glycoproteins and glycolipids in the epithelial eukaryotic cells

Invasins are molecules that promote the invasion process. The invasion of epithelial cells is an active process, induced by the pathogenic bacteria. Two main

mechanisms of invasion are known: macropinocytosis, as seen in *Salmonella* spp. and *Shigella* spp., and phagocytosis in *Listeria* spp. and *Yersinia* spp.

Evasins are substances or bacterial structures that enable the bacteria to evade phagocytosis, the complement system and antibodies such as SIgA protease, capsules, K antigen, LPS, peptidase C5a, toxins, antigenic variation and apoptosis.

Iron is an important mineral used by bacteria to synthesize cytochromes and other proteins. In the human body, iron is not directly available to bacteria because it is complexed with lactoferrin, ferritin, transferrin and hemin. Bacteria use siderophores to extract iron from these complexes. Siderophore production is stimulated by low iron concentrations.

Lesion Factors

There are multiple virulence factors that promote lesion in the organism: i) exotoxins; ii) hydrolytic enzymes; iii) super antigens; iv) endotoxins and v) antigens that induce self-immune illness [16].

Exotoxins can be divided into three groups according to action site: i) toxins that are active in the cytoplasmic membrane, and can interfere with cell signaling mechanisms; ii) toxins that alter cellular membrane permeability, or pore forming toxins and iii) toxins that act inside the cell enzymatically, modifying cytosolic targets.

Many bacteria produce hydrolytic enzymes, such as hyaluronidase and proteases that lyse extra cellular matrix compounds, causing tissue disorganization. This matrix disorganization liberates nutrients that can be used by bacteria.

Endotoxins can be LPS of Gram-negative bacteria. These toxins are responsible for clinical manifestations, such as fever, inflammation and shock. The cellular receptors to endotoxins on the surface of macrophages are CD14 molecules.

Super antigens are not processed and are presented to macrophages in association with histocompatibility complex class II molecules (MHC). They can interact with this type of molecule and with the lymphocyte receptors that recognize them. Super antigens are proteins produced principally by *Streptococcus* spp. and *Staphylococcus* spp.

Bacteria can stimulate self-immune disease by various mechanisms: i) production of antigens similar to those existing in the host cell and ii) production of heat-shock proteins when bacteria encounter stress conditions, such as high temperatures, attack by macrophages and extreme pH.

Definition of Virulence Genes

The properties that pathogenic bacteria require to survive, multiply, and cause damage to a host are: i) capacity to compete inside in the host; ii) ability to gain a foothold within a specific host; iii) ability to avoid normal host defense mechanisms: iv) ability to multiply once established, and in the course of this process, and v) ability to damage the host.

Wassenaar and Gaastra, 2001 [17] proposed some interesting divisions: i) virulence genes from bacteria that are exclusively pathogenic (true virulence genes) such as cholera, anthrax and botulinum toxin; ii) virulence genes from bacteria displaying host-dependent pathogenicity (colonization factors) such adhesins, fimbriae, intimin, invasins and defense system evasion genes, such as immunoglobulin-specific proteases, cytotoxins directed against immune cells, surface layers, slime polysaccharides; and iii) virulence genes from opportunistic pathogens, such as specific proteases, methylases, chaperonins, glycosyltransferases, with virulence life-style genes as a substrate and secretory virulence genes, such as type I and III secretion machinery and virulence housekeeping genes, such as urease, catalase, superoxide dismutase and siderophores, and regulatory genes, such as alternative sigma factors, global regulators, specific transcription activators and regulators of phase variation by gene/promoter inversion.

The role and function of specific genes and the factors that they encode in bacterial virulence summarize the changing concept of bacterial virulence and the detection and identification strategies used to recognize these genes. The definitions of bacterial

virulence and virulence factors have been summarized elsewhere [12].

The Evolution of Pathogenic Bacteria

Pathogens are constantly evolving, because the bacterial and the host population, as well as the ecological conditions that affect the survival and interaction of both, undergo constant changes. A shift towards pathogenicity can be caused by changes in the bacteria, or alternatively by changes in the susceptible hosts or in the success of bacterial survival and contamination routes *ex vivo*.

Recent data [13] suggest that pathogenic bacteria evolved from related nonpathogenic organisms by genetically acquiring relatively large blocks of genetic material that encode virulence factors, rather than by slow, adaptive evolution of preexisting genes. A disproportionate number of essential virulence determinants, particularly toxins and adherence factors, are found on mobile genetic elements, which can be disseminated to other bacteria by conjugation, transformation and transduction events [13].

An important part of adaptive evolution in bacteria is through genes borne on, transmitted by, and sequestered from external sources. From this perspective, the population and evolutionary dynamics of these elements form an integral part of the process of adaptive evolution in bacteria [18].

A common thread has been observed in a number of distinct microbial pathogens that suggests a major evolutionary mechanism by which pathogenic bacteria have evolved. In many cases, virulence genes are found in large contiguous blocks known as chromosomal inserts or pathogenicity islands [19,20](Table 1).

A variety of bacterial virulence factors are encoded on DNA elements that show evidence of more recent acquisition when compared with genes encoding highly conserved essential metabolic functions. In some cases, this horizontal gene transfer is obvious because the virulence factors are encoded within highly mobile genetic elements, such as transposons, plasmids, and bacteriophages. In other cases, the site of chromosomal insertion and/or the nucleotide composition of virulence factors indicate that they were acquired by horizontal gene transfer, without any evidence of currently functional transfer machinery [21].

Many of the genes coding for the adhesins, toxins and other virulence factors either are present in clusters known as pathogenicity islands [13,20-22], which almost certainly had former lives as accessory elements or as parts thereof, or are borne on functional accessory elements, such as plasmids and viruses [18].

Virulence is often multifactorial and coordinately regulated, and virulence genes could be clustered in the genome or in pathogenicity islands. Pathogenicity islands are defined as large regions of DNA encoding for virulence determinants and could be a major mechanism in pathogen evolution. These regions are present in pathogenic bacteria but absent in phylogenetically related non-pathogenic bacteria [21,22]. The addition of pathogenicity islands is being recognized as an important element in the evolution of bacterial pathogens through horizontal spread of virulence genes, similar to the horizontal transfer mediated by plasmids and bacteriophages [23]. In many cases pathogenicity islands have different G+C content than that of the host organism, and are often flanked by specific DNA sequences such as direct repeats, or insertion sequences of transposons or phages. These features indicate genetic horizontal transfer mechanisms as the basis for pathogenicity islands formation. Insertion sites for many pathogenicity islands are tRNA loci, which may contain sequences that are target sites for extra chromosomal elements. In some cases pathogenicity islands may be unstable regions, although some show unusually high excision frequencies. This instability appears to be mediated by the flanking direct repeats [21]. Acquisition of pathogenicity islands provides the receptor organisms with traits that allow them to colonize new environmental niches. Expression of the acquired DNA necessitates that it be under regulatory control in order to be stabilized [22].

These islands are frequently bound by sequences that suggest that the DNA segment was acquired by an illegitimate recombination event that resembles transposition or phage insertion. These DNA blocks are inserted into hot spots in the chromosome that are presumably more susceptible to incursion by foreign DNA or that are a phage attachment site [13].

Table 1. Examples of pathogenicity islands

Bacterium	Name	Size (Kb)	Point of insertion	Function	Ref.
EPEC	LEE	35	selC (82 min)	attachment and effacement, pedestal formation (encodes type III secretion system)	[26]
Samonella typhimurium	SPI1	40 (60 min)	fhlA mutS	invasion into non phagocytic cells (encodes type III secretion system)	[27]
	SPI2	40	ydhE-pykF (31 min)	bacterial survival in macrophages (encodes type III secretion system) intracellular proliferation	[28] [29]
	SPI3	82	selC	survival in macrophages	[30]
	SPI4	92	ssb-yjcB	probably survival in macrophages	[31]
	SPI5	20	sertT	enteropathogenesis	[32]
Shigella flexnery	PAI	30	ipa/mxi-spa	entry into epithelial cells and macrophage apoptosis	[33]
ETEC	LEE	35	selC (82 min)	attachment and effacement, pedestal formation (encodes type III secretion system)	[26]

The chromosomal organizations of *E. coli* and *SaImonella typhimurium* are generally similar. Probably these large DNA insertions were acquired during evolution by an ancestor shared with *E. coli* that gave to the prototype *Salmonella* the ability to transpose the mucosal barrier and the genes necessary to survive within the host in particular target organs. These regions of chromosomal DNA that encode virulence genes are common among various other pathogens [24].

Bacterial chromosomes are not constant but are continually changing. Such dynamic changes also may modify the evolution of pathogenicity within different clonal variants of a pathogenic species. For example, the chromosomes of S. *typhi* (a human-specific pathogen) have undergone major genomic rearrangements during its evolution when compared to other Salmonellae, including inversions, transpositions and insertions, through homologous recombination events [25]. Liu and Sanderson [25] proposed that some of these events might affect *S. typhi* virulence, and probably had a contribution towards its human specific host adaptation. Shuffling of chromosomal genes also could affect both, the regulation and expression of chromosomal virulence genes.

Duplicity of function appears to be of advantage for bacterial virulence. This duplication can confer an ability to resist host cell defenses. For example, *L. monocytogenes* has two phospholipase genes, *plcA* and *plcB*, and a hemolysin that enable the bacterium to escape from the intracellular vacuole. Deletion of one of these genes does not significantly affect virulence, but when both genes are deleted virulence is lost [34,35].

Bacterial virulence factors can often encode multiple functionality in evolutionary terms. Acquisition of a single virulence factor may bestow several functional attributes, as seen for the filamentous hemagglutinin (FHA) of *B. pertussis*, which encodes several functions [36]. The same is true to *Yersinia,* in which the invasin protein acts as an adhesin, and also mediates invasion [13].

It is possible that pathogenic organisms evolve in quantum leaps, by acquiring a genetic segment from an unrelated organism that encodes multiple virulence factors, possibly even eukaryotic sequences. This genetic information is then integrated into the chromosome or into a stable plasmid. Appropriate selection for virulence factors ensures that pathogens maintain such sequences.

By sharing this genetic information via mobile genetic elements, these organisms enable other organisms to acquire selective advantages [37]. Organisms can also vary this genetic information, altering it for a specific purpose [38]. Conserved gene families, including secretion mechanisms, are examples. Chromosomal shuffling and rearrangements provide an alternate way to alter and shuffle the genetic expression of various virulence factors.

Conclusions

The objective of the evolution of genes is thought to be to ensure a rich biodiversity, which helps guarantee a long-lasting maintenance and development of life on earth. Distinct pathogenic signatures are examples of how microorganisms have crafted the effectors of virulence for their own particular use.

Pathogenic bacteria use only a handful of motifs to survive within their preferred host, and the various species share many mechanisms that cause infection and disease. This is because virulence determinants of bacteria tend to be clustered in discrete regions of the chromosome, as well as on plasmids, pathogenicity islands and in bacteriophages.

We can consider the genes of an ancient ancestral organism. Over millions of years mutations will occur in the DNA sequences of its genes, at a slow but steady rate. Most mutations will be selected against because they are detrimental, but some will survive. Most mutations that are incorporated permanently into the genes will be neutral mutations with no harmful or beneficial effects on the organism. Occasionally mutations that improve the function of a gene and/or the protein encoded by it will occur. These are very rare. Sometimes a mutation that was originally harmful may turn out to be beneficial under new environmental conditions [39].

What matters in most cases is how well the protein encoded by a gene functions. If the protein can still operate normally, a mutation may be acceptable. In practice, many of the amino acids making up a protein chain can be varied, within reasonable limits, without damaging the function of the protein.

Genetic information can be passed horizontally, for example when antibiotic resistance genes are carried on plasmids they can be passed between unrelated types of bacteria. Since genes carried on plasmids are sometimes incorporated into the chromosome, a gene can easily move from one organism to an unrelated one.

Horizontal gene transfer is a form of change and is the key to bacterial evolution. Horizontal gene transfer is part and parcel of the adaptive dynamics of the biosphere, and the way the biosphere responds to major disruptions is through macroevolution [40]. Horizontal gene transfer depends on elements that cross the boundaries from one species to another. Viruses, plasmids and transposons are all involved in such sideways movement of genes. The compositions of bacterial genomes can be changed rapidly and dramatically through this process.

In bacterial evolution a succession of temporally spaced major events cause a non -gradual sequence of bacterial adaptations to a given environment [41]. Pathogenicity islands may be genetically unstable elements that are present or absent in a prokaryotic cell in near-random fashion [22,24,42]. Genes are also acquired by transduction mediated by bacteriophages, by the introduction of plasmids or transposons, by conjugative DNA transfer between matching cells, or by natural DNA-mediated transformation. Microbial variability due to switching of gene expression modulated by activation of silent genes as a consequence of genetic rearrangements also contribute to microbial evolution in which major changes is induced very rapidly [43]. The environment can influence this speed of evolution, and bacterial species take successful advantage of this mode of high-speed evolution to gain selective profits.

The natural transfer of genetic material between bacteria in the environment is necessary for the generation of genetic diversity and provides the raw material of natural selection and evolution.

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