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Amino acid biosynthesis deficiency in bacteria associated with human and animal hosts

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Abstract

Opportunistic pathogenic bacteria continuously live in humans and obligately pathogenic bacteria associate with humans only in the case of diseases. A mystery is whether pathogens can live outside the hosts. We showed here that most human pathogens have lost biosynthetic pathways for amino acids. This condition suggests that most microbial pathogens are obligately host-dependent and they cannot multiply outside their hosts. Further analysis of the genome sizes revealed that the genomes of host-dependent bacteria are smaller than those of free living bacteria, suggesting that reductive evolution of genomes occurs broadly in bacterial pathogens and non-pathogens closely associated with human and animal hosts. The extent of genome reduction appears to depend on the environment in which they reside. The richer the nutrients are in the environment the smaller the genome is in the bacteria.

Introduction

Pathogenic bacteria of humans can be classified into potential (opportunistic) pathogens and obligate pathogens according to their relationship with the hosts. The potential pathogens include the normal bacterial flora (e.g., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*) that do not cause disease in humans unless they have an opportunity when host immunity or anatomical barriers are weakened. The obligate pathogens (e.g., *Clostridium tetani* and *Rickettsia rickettsii*) do not associate with humans except in the case of disease. We hypothesize that both opportunistic and obligate pathogens have co-evolved with humans or animals and they became obligately host-dependent parasites by loss of genes whose products are available in their host environments. We tested our hypothesis by analysis of pathogenic bacteria for the loss of the genes for biosynthesis of amino acids because bacteria that are deficient in amino acids cannot multiply outside the living organisms due to the limitation of availability of amino acids in the environment.

Material and methods

We analyzed biosynthetic pathways of bacteria using the KEGG pathway database (<http://www.genome.ad.jp/kegg/pathway.html>) and MetaCyc pathway (<http://metacyc.org>) for amino acids: arginine (R), histidine (H), isoleucine (I), leucine (L), lysine (K), methionine (M), phenylalanine (F), threonine (T), tryptophan (W), and valine (V). For convenience of description, we classified bacteria into the following categories: intracellular bacteria that

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reside in the cells of arthropods, protozoa, humans, or animals; bacteria that live on the mucous membrane of humans or animals; saprophytic bacteria that live on decaying organic matter; and others that do not fit to the above categories (Table 1).

Results and discussion

Deficiency of amino acid biosynthesis in bacteria

Bacteria associated with arthropods, regardless of whether intracellular or extracellular, usually lose most genes required for biosynthesis of amino acids. These bacteria include *Anaplasma phagocytophilum*, *Bartonella henselae*, *Bartonella quintana*, *Borrelia burgdorferi*, *Coxiella burnetii*, *Francisella tularensis*, *Ehrlichia chaffeensis*, and *Rickettsia typhi*. Similarly, the protozoa-associated *Legionella pneumophila* has also lost most of the genes for biosynthesis of amino acids. Thus, it seems that bacteria residing in arthropods and protozoa easily lose genes for amino acid biosynthetic pathways. This situation suggests that not only the arthropod cells but also the hemolymph of arthropods is rich in amino acids. Because bacteria can acquire amino acids from the arthropod cells or hemolymph, they can afford to delete the genes involved in the biosynthesis of amino acids.

It was unexpected that many bacteria residing in the respiratory and gastrointestinal tracts have lost genes for amino acid biosynthesis. The bacteria in the respiratory tract that have lost amino acid biosynthetic pathways include *Bordetella pertussis*, *Corynebacterium diphtheriae*, *Haemophilus influenzae*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. The bacteria in the gastrointestinal tract that have lost amino acid biosynthetic pathways include *Campylobacter jejuni*, *Helicobacter pylori*, *Enterococcus faecalis* and *Streptococcus agalactiae*. These findings suggest that the mucous membranes in the respiratory tract and gastrointestinal tracts may be rich in amino acids.

It is also surprising that the pathogenic *Clostridium* have lost most genes required for amino acid biosynthesis, in contrast to the free living *C. acetobutylicum*, which has a complete set of genes for amino acid biosynthesis¹.

It is well known that because of genome reduction bacteria with small genomes (less than 1.5 Mb in most cases) such as *Mycoplasma*, *Borrelia burgdorferi*, and obligately intracellular bacteria (*Rickettsia* and *Chlamydia*) have lost genes encoding accessory proteins or regulatory products involved in universal cellular processes, replication, transcription, and translation^{2, 3}. However, our results suggest that not only bacteria with small genomes, but also bacteria with moderate genome sizes (2 – 4 Mb) have lost genes for biosynthetic pathways for amino acids. Our findings show that it is a universal phenomenon for bacteria residing in an animal host to lose gene for metabolic pathways. These findings imply that most bacteria associated with humans and animals are obligate host-dependent parasites because they are deficient in amino acid biosynthesis and they cannot multiply in an environment where the amino acid supply is limited.

Lifestyle and the genome size of bacteria

By comparing the genome sizes of bacteria, we found that typically bacteria that are deficient in amino acid biosynthesis have smaller genomes and most of them are less than 4 Mb. In general, the smaller the genome, the more pathways for biosynthesis of amino acids have been lost in the bacteria (Figure 1 and table 1). This situation suggests that the parasitic bacteria that are associated with humans and/or animals are undergoing genome reduction. The basis for genome reduction is that bacteria living continuously in hosts can obtain many compounds of

intermediate metabolism from host cytoplasm or tissue and they can discard the corresponding biosynthetic pathways and genes ².

Loss of genes required for synthesis of an amino acid has locked the bacteria onto the hosts and the bacteria will co-evolve with the host. The common wisdom for evolution of bacteria associated with animal hosts is that evolution of the host-pathogen interaction will select bacteria that reduce virulence and eventually become commensal or symbiotic bacteria ⁴. However, this hypothesis cannot explain why many microorganisms are highly virulent. We believe that evolution may have gone in two directions to become the commensal bacteria and virulent bacteria. The bacteria continuously residing in an animal host have lost pathogenicity and evolved toward commensalism because the bacteria that are less harmful to the host can coexist with the host, and in contrast the highly virulent bacteria that kill the host will cease to exist. However, bacteria that co-evolved with an animal host and became commensal bacteria in that host could be pathogens for humans. For example, the human pathogen *E. coli* 0157:H7 is the normal flora of animals; *Salmonella* and *Campylobacter jejuni* may be the normal flora of poultry ^{5,6}. For the bacteria that spend most of the time outside the host to wait for next host, evolution favors virulence rather than commensalism for effective transmission of the bacteria. For example, our results showed that *Clostridium* spp. depend on the host for nutrients to multiply, but they evolved toward high virulence rather than commensalism because pathogenic *Clostridium* bacteria rely on its toxin to rapidly kill their hosts, and upon release into the soil after the death of the host, they are resistant to the environmental conditions by forming dormant spores. Pathogenic enteric bacteria that need a toxin to stimulate diarrhea to discharge the bacteria into the environment for transmission may be another example of evolutionary selection of virulence in bacteria.

The question is why evolution favors a small genome in bacteria associated with animal hosts. A proposed explanation for genome reduction is that selection has favored small genome size for the sake of growth efficiency or competitiveness within the host. Changes in DNA content, on a scale corresponding to individual genes, do not affect the rate of bacterial cell replication². Moreover, the generation time of the small genome organism *Ehrlichia chaffeensis* is 12 hours, which reflects much slower growth than *E. coli*⁷. Thus, genome reduction may not have been selected for efficient growth. We believe genome reduction is selected by competitiveness within the host because bacteria with small genomes have smaller size that is energy efficient. For example, the volume of a free living *E. coli* ($0.8 \times 2 \mu\text{m}$) is 256 times larger than the smallest bacterium, *Mycoplasma genitalium* (0.2 to $0.3 \mu\text{m}$). Thus, it is obvious that the smaller the genome, the less building materials are required for a bacterium. The small size of these bacteria may have advantages over larger organisms because small organisms can produce more units of the organisms in a limited nutrient environment such as inside a cell or on the mucous membrane.

By analysis of biosynthetic pathways of ten amino acids, we found that most pathogenic bacteria have lost the ability to synthesize many amino acids. We believe that additional pathogenic bacteria will be revealed to be obligately host-dependent if more biosynthetic pathways are analyzed. For example, *Brucella* appears not to be host cell dependent from our analysis because they are not deficient in amino acid biosynthesis. However, they are intracellular organisms and are relatively fastidious in terms of growth in the laboratory. They are actually auxotrophic for nicotinic acid that is the only precursor for the synthesis of nicotinamide in *Brucella* organisms. Genome sequence analysis demonstrates that *de novo* synthesis of quinolinate and NAD is absent in *Brucella*, confirming the auxotrophy for nicotinic acid in these organisms^{8,9}. Thus, we conclude that most bacteria that are associated with human or animal hosts are obligately host-dependent parasites and they cannot multiply outside their hosts.

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Fig 1. Comparison of genome sizes of bacteria that are potential pathogens or obligate pathogens for humans. * indicate bacteria that are deficiency in biosynthesis of at least one amino acid.

Table 1**Amino acid biosynthesis deficiency in bacteria**

Bacteria	Reservoir and host	Deficiency in amino acid biosynthesis
Saprophyte	<i>Bacillus anthracis</i>	No deficiency
	<i>Burkholderia pseudomallei</i>	No deficiency
	<i>Clostridium botulinum</i>	F,H,I, K, L,M, W,V
	<i>Clostridium perfringens</i>	H,I, K, L,M, T, W,V
	<i>Clostridium tetani</i>	F,H,I, K, L, M, W, V
	<i>Pseudomonas aeruginosa</i>	No deficiency
Bacteria residing inside the host cells	<i>Anaplasma phagocytophilum</i>	F,H,I,K,L,M,R,T,W,V
	<i>Bartonella quintana</i>	F,H,I,K,L,M,R,T,W,V
	<i>Bartonella henselae</i>	F,H,K,L,M,R,W
	<i>Brucella abortus</i>	No deficiency
	<i>Chlamydia pneumoniae</i>	F,H,I,K,L,M,R,T,W,V
	<i>Coxiella burnetii</i>	F,H,I,K,L,M,R,T,W,V
	<i>Ehrlichia chaffeensis</i>	F,H,I,K,M,R,T,W,V
	<i>Francisella tularensis</i>	H, M, R
	<i>Legionella pneumophila</i>	F,I,K,L,M,T,V
	<i>Mycobacterium tuberculosis</i>	H
	<i>Rickettsia typhi</i>	F,H,I,K,L,M,R,T,W,V
Bacteria residing on respiratory tract mucous membrane	<i>Bordetella pertussis</i>	M
	<i>Corynebacterium diphtheriae</i>	F, K
	<i>Haemophilus influenzae</i>	K, M, R
	<i>Mycoplasma pneumoniae</i>	F,H,I,K,L,M,R,T,W,V
	<i>Neisseria meningitidis</i>	No deficiency
	<i>Staphylococcus aureus</i>	K, M
	<i>Staphylococcus haemolyticus</i>	F, H, K, W, V
	<i>Streptococcus pneumoniae</i>	H, K, R
	<i>Streptococcus pyogenes</i>	F,H,I,K,L,M,R,T,W,V
Bacteria residing on gastrointestinal track mucous membrane	<i>Campylobacter jejuni</i>	K, R
	<i>Enterococcus faecalis</i>	A,F,H,I,L,V,W
	<i>Escherichia coli K12</i>	No deficiency
	<i>Escherichia coli O157</i>	No deficiency
	<i>Helicobacter pylori</i>	F, H, I, L, M, R, V
	<i>Salmonella typhi</i>	No deficiency
	<i>Shigella flexneri</i>	No deficiency

Bacteria	Reservoir and host	Deficiency in amino acid biosynthesis
<i>Streptococcus agalactiae</i>		A,F,H,I,K,L,M,V,W,
Others	<i>Borrelia burgdorferi</i>	Tick
	<i>Burkholderia mallei</i>	Horse
	<i>Neisseria gonorrhoeae</i>	Human urethral mucosal cells
	<i>Yersinia pestis</i>	Rodent and flea
	<i>Vibrio cholerae</i>	Zooplankton, Chironomid egg masses ¹⁰
	<i>Vibrio vulnificus</i>	Shellfish, eel ^{11,12}