Published in final edited form as:
*Nat Rev Microbiol*. 2008 September ; 6(9): 657–666. doi:10.1038/nrmicro1955.

# Revisiting the host as a growth medium

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## Abstract

The ability of the human body to play host to bacterial pathogens has been studied for more than 200 years. Successful pathogenesis relies on the ability to acquire the nutrients that are necessary for growth and survival, yet relatively little is understood about the *in vivo* physiology and metabolism of most human pathogens. This Review discusses how *in vivo* carbon sources can affect disease and highlights the concept that carbon metabolic pathways provide viable targets for antibiotic development.

Billions of dollars are spent annually on research into, and treatment of, bacterial infections, and it is likely that over the course of our lifetimes each of us will be treated for a bacterial infection. As antibiotic resistance continues to increase, it is imperative that researchers not only continue to develop conventional antimicrobials but also pursue more unconventional targets. One under-exploited therapeutic opportunity is based on the simple premise that for a bacterium to cause an infection, it must obtain the nutrients that are necessary for replication from the infection site. This is not a new concept, as Louis Pasteur developed a model that described the body as a culture vessel in the late 1870s as a means of describing immunity[1]. Subsequent work expanded this 'culture-vessel model' as an incomplete explanation for bacterial host and tissue tropism[2,3]. More recently, E. D. Garber presented a paper entitled 'The host as a growth medium' in 1960 (REF. 4), in which he proposed the fundamental importance of understanding the physiology of the bacterium in the infection site. Over the intervening 50 years, a wealth of literature has been generated that details the response of both hosts and pathogens to infection. However, relatively few studies have examined the fundamental question: what makes the human body a good growth medium for bacterial pathogens? In this Review, we outline how the host growth environment affects disease and discuss the potential for targeting metabolic pathways for therapeutic development.

Interest in the host as a growth environment has resurfaced in recent years. More than just a culture vessel, the human body is now considered a chemostat, in which the nutrients that are crucial for bacterial growth are replenished over time. Bacterial pathogens are adept at localizing to and responding to specific nutritional cues within host microenvironments (TABLE 1), and growth within these microenvironments requires specific metabolic pathways. Indeed, some of the first antimicrobial therapeutics targeted bacterial metabolism (BOX 1). More recently, inactivation of lactic acid production in the caries-causing bacterium *Streptococcus mutans* has demonstrated therapeutic promise[5]. Treating infections with defined dietary regimens, such as low-iron diets, has also shown limited success. Iron sequestration is a primary means of defence used by the human body during infection, as



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most bacterial pathogens require iron for growth[6]. As such, dietary supplementation with iron is correlated with increased incidence of human *Mycobacterium tuberculosis* infection as well as increased mortality[7,8]. Conversely, iron chelator therapy and dietary iron restriction diminishes *M. tuberculosis* growth in animal models, and these treatments have been proposed for human use[9]. Iron chelator therapy has also shown promise in individuals with malaria[10]. The rationale is that through dietary restriction one can starve a pathogen of iron, an essential limiting nutrient in the human host[6].

This Review cannot encompass every instance in which the nutritional content of the infection site has been linked to disease. Therefore, we will focus on four general virulence-related phenotypes that are known to be affected by the growth environment: evasion of the host immune system; tissue tropism; niche specialization and resource partitioning; and cell–cell communication.

## Immune evasion

A healthy immune system has the remarkable ability to quickly and effectively identify and eradicate disease-causing bacteria from the host. However, some pathogenic bacteria have developed ways to thwart the host attack and proliferate *in vivo*. Here, we discuss two bacterial species in which specific carbon catabolic pathways are metabolically coupled to immune subversion.

### Neisseria meningitidis

*Neisseria meningitidis*, a Gram-negative commensal of the human oropharynx, can cause septicaemia by crossing the mucosal barrier and entering the bloodstream or meningitis by crossing the blood–brain barrier into the cerebrospinal fluid[11]. In each case, *N. meningitidis* uses molecular mimicry to survive exposure to the innate immune system. The ability of the immune system to distinguish foreign cells from self is mediated in part by sialic acid, which coats the surface of human cells and acts as a ‘self signal’. *N. meningitidis* exploits this feature by decorating its outer surface with sialic acid residues, thus masking itself from human defences[12,13]. Molecular mimicry is a key component of *N. meningitidis* pathogenesis and mutants that are unable to sialylate their outer surface are highly attenuated[14].

The *in vivo* nutritional environment has an impact on *N. meningitidis* host cell mimicry. *N. meningitidis* can use lactate and glucose as carbon and energy sources[15], and lactate and glucose are present in nasopharyngeal tissue, serum and cerebrospinal fluid at 1–2 mM and 6–10 mM levels, respectively[16,17]. Interestingly, although both of these carbon sources are present *in vivo*, *N. meningitidis* catabolizes lactate at a faster rate than it does glucose[16], and mutants that are deficient for lactate transport are defective colonizers of nasopharyngeal tissue[17]. Why might a bacterium preferentially metabolize a more oxidized substrate such as lactate over glucose? One likely explanation is that intermediates of lactate consumption feed directly into the sialylation pathway[16], thus enhancing sialic acid biosynthesis. This, in turn, leads to increased sialylation of the *N. meningitidis* outer membrane (FIG. 1). As expected, an *N. meningitidis* strain that is unable to transport lactate (Δ*lctP*) is highly deficient for sialic acid modification of the outer membrane and is more susceptible to complement-mediated killing[16]. Thus, for *N. meningitidis*, catabolism of a preferred carbon source *in vivo* is coupled to a unique immune-evasion strategy.

### Mycobacterium tuberculosis

*M. tuberculosis*, the causative agent of tuberculosis, infects millions of people worldwide and is a significant health concern in our increasingly globalized society. Upon colonization of the lung, this intracellular respiratory pathogen is engulfed by pulmonary macrophages

and then manipulates the host immune response using elaborate mechanisms, many of which are not fully understood[18]. Ultimately, host cell manipulation results in the creation of an *M. tuberculosis* niche — a granuloma[19] — and chronic infection.

It has been proposed that *M. tuberculosis* uses fatty acids as carbon sources during chronic infection. Evidence supporting this hypothesis includes stimulation of *M. tuberculosis* respiration by fatty acids[20], survival of glycolytic enzyme mutants in murine infection models[21] and a requirement for isocitrate lyase enzymes (which are involved in the glyoxylate shunt) for intracellular growth and virulence[22,23]. Significantly, treatment with an isocitrate lyase inhibitor blocks *M. tuberculosis* growth *in vitro* with fatty acids as well as *in vivo* growth in murine macrophages[22], suggesting that a similar approach could be used to develop human tuberculosis therapies.

*M. tuberculosis* carbon catabolism and virulence have been linked by the flux of a key fatty-acid intermediate within the cell[24]. During infection, *M. tuberculosis* secretes polyketide lipids that interact with components of the host immune system. These lipids are required for immune evasion and pathogenesis[25,26] and are often termed virulence lipids. Interestingly, catabolism of the small fatty acid propionate by *M. tuberculosis* leads to higher production of virulence lipids than observed for growth on glucose or acetate[24]. It has been proposed that high intracellular levels of methyl-malonyl-CoA, an intermediate in propionate catabolism and a biosynthetic precursor of polyketide lipids, mediates this phenotype. The model dictates that during growth on fatty acids such as propionate, intracellular levels of methyl-malonyl-CoA increase, thereby increasing the available substrate for the biosynthesis of polyketide lipids. Consequently, these virulence lipids are produced at higher levels during growth on fatty acids.

More recently, it was reported that *M. tuberculosis* uses cholesterol as a sole carbon source and a transport system that is important for cholesterol uptake was identified[27]. An *M. tuberculosis* mutant deficient for cholesterol uptake was impaired for replication in interferon-γ (IFN-γ)-activated macrophages but not resting macrophages, suggesting that cholesterol metabolism is specifically required for growth in the nutrient-restrictive intracellular conditions that are induced by IFN-γ (BOX 2). In addition, *M. tuberculosis* was found to co-localize with high-cholesterol regions of IFN-γ-activated macrophages. Significantly, oxidation of the cholesterol side chain yields propionate[28,29], and labelled carbon in the cholesterol side chain is incorporated into *M. tuberculosis* virulence lipids[27]. These exciting results provide a direct link between *M. tuberculosis in vivo* carbon catabolism and the production of important immune-evasion factors (FIG. 2).

## Tissue tropism

Primarily on the basis of work by Pasteur[1], bacterial tissue tropism was originally proposed to rely solely on the nutritional conditions within specific host tissues. Of course this is not the case, and tissue localization and specificity are often determined by devoted adhesins such as external appendages (pili and fimbriae) or outer membrane proteins[30]. The molecular interactions between the bacterial and host cell outer surfaces have long been a topic of intense interest; consequently, most tissue tropism studies have focused on interactions between host tissues and bacterial surface components. However, and perhaps not surprisingly, for some bacterial pathogens the nutritional environment at the infection site also influences tissue tropism.

### Escherichia coli

*Escherichia coli* is a pervasive microorganism. A commensal in the human intestinal tract, non-pathogenic *E. coli* is the workhorse of molecular biology and a model system for

microbial geneticists and physiologists. By contrast, its pathogenic counterparts colonize multiple infection sites, generate steep health-care costs and can be fatal[31,32]. In general, pathogenic *E. coli* strains have acquired mobile genetic elements that carry novel virulence determinants such as new toxins and adhesins[33]. Two well-known examples of pathogenic *E. coli* are enterohaemorrhagic (EHEC) and uropathogenic *E. coli* (UPEC). EHEC, an intestinal pathogen that causes severe diarrhoea, has achieved notoriety as a result of highly publicized food-borne outbreaks involving contaminated beef and vegetable products. UPEC is the predominant cause of uncomplicated urinary tract infections[34]; treatment of these infections in women had an estimated cost of ~$1.5 billion in 1995 in the USA alone[35]. Curiously, although UPEC strains benignly inhabit the intestine, they colonize and cause disease in sites outside the intestine, a virulence trait that is not shared by EHEC strains.

Why does UPEC cause infections outside the intestine whereas EHEC presumably does not? The host nutritional environment might have a role. The amino acid D-serine is present in human urine[36] at levels that are potentially toxic to *E. coli*[37]; however, some *E. coli* strains are able to use D-serine as a carbon, nitrogen and energy source. Comparative genomic analyses of *E. coli* strains demonstrated that UPEC strains have the genes that are necessary for D-serine catabolism but that in EHEC these are partially absent and are replaced by sucrose utilization genes[38]. Correspondingly, D-serine catabolism is observed in the majority of UPEC clinical isolates but rarely in EHEC isolates[38]. Finally, the genes that are required for D-serine catabolism are induced during human *in vivo* growth of an asymptomatic bacteriuria *E. coli* strain[39], suggesting that D-serine metabolism is important during urinary tract colonization and infection. These results suggest that EHEC lacks the metabolic capacity to replicate and/or survive in human urine, a capacity that UPEC has retained, and it is this metabolic capability that defines pathogenesis in the urinary tract. Additionally, sucrose catabolism (in lieu of D-serine catabolism) in diarrhoeagenic *E. coli* isolates[40] suggests that this metabolism confers an advantage in the intestinal growth environment.

### Brucella abortus

Spontaneous abortions of cattle caused by the intracellular pathogen *Brucella abortus* are a serious concern to the agriculture industry. Although several industrialized nations have launched historically successful vaccination campaigns against *B. abortus*, these infections still pose a threat in developing countries[41]. An archetypal zoonotic pathogen, *B. abortus* can also cause disease in humans, and countries that lack effective animal disease-control programmes generally have higher rates of *B. abortus* infection[41,42].

*B. abortus* tissue tropism during bovine infection is influenced by nutrition. The naturally occurring sugar alcohol erythritol is the preferred carbon source for *B. abortus*[43]. Extracts derived from ground bovine tissues have been used as *in vitro B. abortus* growth media, and experiments using these media have shown that *B. abortus* growth is promoted by the high levels of erythritol in bovine fetal tissues[44]. Furthermore, injection of calves with erythritol enhances experimental *B. abortus* infection[44]. Interestingly, expression of erythritol catabolism genes in the closely related human pathogen *Brucella suis* increases during intracellular growth in macrophages[45], suggesting that erythritol catabolism (or catabolism of structural analogues) might be conserved during human infection.

## Niche specialization and resource partitioning

Bacterial adaptations to *in vivo* growth have been well documented. Niche specialization refers to the ability of an organism to occupy a specific environment, for example, the adaptation of some bacteria to life in an intracellular compartment. Resource partitioning proposes that bacterial species living within a mixed community can avoid competition by

using a carbon source that is not preferred by other members of the community. In this section, we discuss four examples of niche specialization and resource partitioning in the human body: *Legionella pneumophila* and *Listeria monocytogenes* in macrophages, *Aggregatibacter actinomycetemcomitans* in the oral cavity and *E. coli* in the intestine.

## Legionella pneumophila

Legionellosis, commonly known as Legionnaire's disease, is caused by the intracellular pathogen *L. pneumophila*. This Gram-negative bacterium is found in aquatic environments where it can be internalized and survive digestion by bacteriovorus amoebae[46]. *L. pneumophila* resides within intracellular compartments of the amoeba, and infected amoebae serve as an important environmental reservoir for this bacterium. After internalization, *L. pneumophila* undergoes a two-step life cycle whereby it replicates internally (replication phase) and eventually kills the host, returning to the aquatic environment (transmission phase)[46]. Each phase has a characteristic gene expression profile that is controlled by conditions inside the host cell.

When aerosolized and inhaled, *L. pneumophila* can reach the alveoli of the human lung and be internalized by macrophages, in which it undergoes a life cycle similar to that observed in amoebae[46,47]. Normally, bacteria contained within the phagosome would be destroyed upon phagolysosomal fusion; however, *L. pneumophila* has evolved a unique means of avoiding destruction by coordinating the construction of a specialized compartment that is derived from the host endoplasmic reticulum. This compartment is rich in nutrients and stimulates the bacteria to enter the replication phase. Once nutrients become limiting for growth, *L. pneumophila* changes its gene-expression profile and enters the transmission phase.

What triggers the switch from transmission to replication? *L. pneumophila* uses amino acids as carbon growth substrates, and recent studies suggest that the availability of a specific amino acid, threonine, could be the differentiation switch. Mutants that are unable to acquire threonine can survive within a macrophage but fail to differentiate and do not enter the replication phase. Thus, threonine can serve as both a nutrient source and a differentiation signal during infection. Without threonine acquisition, *L. pneumophila* is unable to replicate, escape from the cell and infect other cells[48]. Therapeutics targeting this pathway could aid in treatment of legionellosis, a disease that affects up to 18,000 people in the United States each year. It is unknown why threonine in particular acts as a differentiation switch, although it is hypothesized that *L. pneumophila* senses the presence of six essential amino acids (threonine, arginine, cysteine, methionine, serine and valine) before entering the replication phase[48].

## Listeria monocytogenes

*L. monocytogenes* is a Grampositive, facultative intracellular bacterium that is responsible for the food-borne illness listeriosis in humans. During infection of a human host, *L. monocytogenes* is internalized by phagocytic host cells where it temporarily resides within phagocytic vacuoles. Normally, the phagocytic vacuole would fuse with a lysosome and kill the bacterium; however, upon entry into the phagocytic vacuole, *L. monocytogenes* produces specific factors that allow it to escape the vacuole and enter the cytoplasm. This escape is controlled by the global virulence regulator PrfA, which is activated upon entry into the host cytosol[49,50]. Once inside the cytoplasm, the bacteria undergo replication and subsequently use host actin to spread to neighbouring cells[51].

Unlike non-pathogenic species of *Listeria*, pathogenic species such as *L. monocytogenes* use glucose phosphates as carbon sources[52]. Interestingly, transcription of the hexose phosphate transporter gene (*hpt*) is dependent upon PrfA[53]. Mutants lacking Hpt can escape the

phagocytic vacuole, but are deficient for replication within the cytoplasm[53,54]. By using a substrate that is available in the host cytoplasm and controlling its consumption with the global virulence regulator PrfA, *L. monocytogenes* effectively couples virulence factor production to specific catabolic pathways.

During residence in the host cytoplasm, *L. monocytogenes* must also scavenge a number of amino acids and vitamins, including the cofactor lipoate. *In vivo* most lipoate is associated with host proteins and is therefore not freely accessible. *L. monocytogenes* possesses two lipoate protein ligases, LplA1 and LplA2. Interestingly, LplA1 is functionally specialized to acquire lipoate from hostderived lipoyl peptides, which are present at low concentrations in the host cytoplasm, whereas LplA2 scavenges free lipoate[55]. Inactivation of LplA1 significantly attenuates *L. monocytogenes* intracellular growth and virulence[56], indicating that the ability to scavenge lipoate directly from host proteins is required for successful pathogenesis. These two examples demonstrate that *L. monocytogenes* has undergone multiple metabolic adaptations to life inside the host cell that allow for efficient replication and dissemination of the bacterium.

## Aggregatibacter actinomycetemcomitans

Periodontitis and heart disease have been linked to the presence of *A. actinomycetemcomitans*, a Gram-negative bacterium that is found exclusively in the mammalian oral cavity[57-59]. More specifically, *A. actinomycetemcomitans* resides in the gingival crevice, the area around the tooth that is bounded by the tooth surface on one side and the epithelium lining the gingiva on the other, in a complex bacterial consortium that includes lactate-producing *Streptococcus* spp.[60,61]. *A. actinomycetemcomitans* has a relatively slow growth rate compared with many other species within the oral cavity, which could limit its competitiveness. However, *A. actinomycetemcomitans* has evolved a novel mechanism of carbon resource partitioning whereby lactate is preferentially consumed over sugars. This preference exists despite higher growth yields and a faster doubling time with the sugars glucose and fructose. It has been demonstrated that the addition of lactate to *A. actinomycetemcomitans* cultures rapidly inhibits glucose transport and metabolism. The current model is that high intracellular levels of pyruvate resulting from lactate consumption inhibit glucose use; however, a detailed mechanism has yet to be elucidated.[62]. Lactate is produced at high levels in the oral cavity, primarily by *Streptococcus* spp., and *A. actinomycetemcomitans* consumes lactate produced by *Streptococcus* spp. during *in vitro* co-culture. These results indicate that by using lactate, *A. actinomycetemcomitans* avoids competition with faster growing species in the oral cavity, thereby enabling it to establish residence within the competitive environment of the human mouth (FIG. 3).

## Escherichia coli

How are resources partitioned in the human intestine, a diverse polymicrobial environment that can support the growth of billions of bacteria[63]? For the study of intestinal pathogens such as EHEC, this question is highly relevant. Once ingested by a potential human host, EHEC must successfully colonize the host intestinal mucosa to cause disease. Colonization, however, has been hypothesized to rely on the availability of an open niche (the nutrient-niche hypothesis[64,65]). As the colon contains high levels of commensal *E. coli*, invading EHEC must either directly compete with commensal *E. coli* for carbon substrates or, according to the nutrient-niche hypothesis, use substrates that are not consumed by commensal strains. The *in vivo* hierarchy of carbon substrate preference has been determined for commensal *E. coli* using murine intestinal mucus as an *in vitro* growth substrate[64]. Interestingly, when compared with the carbon substrate preference of EHEC, important differences are observed[66], lending credence to the nutrient-niche hypothesis. However, both *E. coli* strains undergo simultaneous catabolism during growth with mixed

carbon substrates[64,66], suggesting that a distinct nutrient niche (that is, a niche based on the availability of a single carbon substrate) might not exist. What is clear is that carbon preference is an important component of EHEC-mediated disease and elucidation of the nature of the interactions between commensal and pathogenic *E. coli* might provide new therapeutic strategies to specifically target pathogenic strains.

## Bacterial communication

It is now commonly accepted that bacteria are capable of intraspecies communication using diverse chemical ‘languages’ in a process that is termed quorum sensing. The growing body of research on bacterial cell–cell communication indicates that producing and responding to specific signals allow bacterial populations to coordinate their gene expression and thus their group behaviours. Quorum signals have diverse ecological roles, going beyond the intraspecies level to participate in interspecies[67,68] and even interdomain communications[69,70]. The collective work on bacterial communication to date suggests that bacteria participate in dynamic ‘conversations’ with their microbial neighbours and eukaryotic hosts that intimately influence their physiology. Signal production by bacterial populations can be heavily influenced by nutrients in the growth environment, and for pathogenic bacteria this environment is the host. In this section, we review two well-studied pathogenic relationships in which cell–cell communication, carbon metabolism and pathogenesis intersect.

### Agrobacterium tumefaciens

The Gram-negative α-proteobacterium *Agrobacterium tumefaciens* uses a remarkable mechanism to elicit tumour formation in plants. This soil bacterium, which is recruited by phenolic compounds and sugars that are released by wounded plant tissues[71-73], enters compromised plants and effects DnA transfer to plant cell nuclei through illegitimate recombination[74,75]. The transferred DNA, called T-DNA, originates from the Ti (tumour-inducing) plasmids that are carried by *A. tumefaciens*. The transfer of T-DNA from the bacterial cell to the host plant has revolutionized plant molecular biology and genetic engineering[76,77]. In nature, this process is believed to confer a major growth advantage for *A. tumefaciens*, as T-DNA carries oncogenic genes that promote plant cell proliferation[78] as well as genes that direct the plant to synthesize opines[79]. Opines are carbon substrates that are catabolized by agrobacterial species but not by most other bacteria[80,81]. This phenomenon has been referred to as the ‘opine concept’ hypothesis, and in this way *A. tumefaciens* induces formation of an ecological niche that selects for its own growth[82].

Opines themselves fulfil a twofold purpose, serving as both carbon growth substrates and as inducers of *A. tumefaciens* quorum signal production. Similarly to many Gram-negative bacteria, *A. tumefaciens* produces an acyl-homoserine lactone (AHL) communication signal that is used for density-dependent gene regulation[83-85]. In *A. tumefaciens*, AHL signalling promotes the conjugal transfer of Ti plasmids to other, potentially avirulent *A. tumefaciens* cells in the environment[83]. Thus, host-derived opines promote genetic diversity among *A. tumefaciens* strains by indirectly inducing the conjugative transfer of the Ti plasmid[86]. It was recently demonstrated that *A. tumefaciens* can transfer T-DNA to unwounded plants and elicit plant opine production without tumour formation[87], reminiscent of a commensal relationship. This work suggests that the outcomes of *Agrobacterium*–host interactions could be more complex, with tumour formation being only one of several outcomes. It remains to be seen how this non-tumorigenic interaction will affect agrobacterial plasmid transfer and genetic diversity in the rhizosphere.

### Pseudomonas aeruginosa

The heritable disease cystic fibrosis (CF) is a well-studied model for chronic bacterial infections. Non-CF airways are generally sterile, but inadequate ion transport, combined with defective mucociliary clearance, is thought to predispose CF airways to persistent microbial colonization[88]. Numerous bacterial species are commonly present in the CF lung[89]; however, the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* often predominates. Within the CF lung, *P. aeruginosa* grows within the copious sputum (mucus), where it can reach densities of $10^9$ bacteria per ml of sputum[90,91]. *P. aeruginosa* infections within the CF lung are highly refractory to antimicrobial treatment and are often maintained throughout the life of the individual.

*P. aeruginosa* produces an arsenal of extracellular factors that are crucial for colonization and competition with other bacterial species. The production of these factors is regulated by several cell–cell communication signals, including 2-heptyl-3-hydroxy-4-quinolone, termed the *Pseudomonas quinolone* signal (PQS)[92,93]. Outer membrane vesicles released by *P. aeruginosa* disseminate the hydrophobic PQS and deliver antimicrobial quinolone compounds to potential competitors[94-96]. Interestingly, *P. aeruginosa* initiates PQS production at lower cell densities during *in vitro* growth in CF sputum than in common laboratory medium[97]. Consequently, *P. aeruginosa* exhibits higher production of virulence factors and antimicrobial factors during growth in CF sputum than during growth in normal laboratory media. Use of a synthetic defined medium to nutritionally model CF sputum demonstrated that aromatic amino acids present in CF sputum serve as cues to modulate *P. aeruginosa* PQS production[98]. PQS and aromatic amino acids share biosynthetic precursors, and it is hypothesized that high levels of aromatic amino acids in CF sputum allow these precursors to be assimilated into PQS, thereby enhancing PQS production in CF sputum (FIG. 4a). Thus, it appears that increased flux of metabolic intermediates to PQS — mediated by the presence of aromatic amino acid cues in the CF lung — might enhance the virulence and competitiveness of *P. aeruginosa* in this environment[98]. These results also suggest that development of therapeutics that decrease the levels of these amino acids in the CF lung might affect the ability of *P. aeruginosa* to colonize and initiate disease (FIG. 4b).

## Conclusions and future directions

What is the future of antimicrobial development? Using genetic, proteomic and bioinformatic techniques in serovars of *Salmonella enterica*, Becker *et al*. recently illuminated genetic and metabolic redundancies that could hinder efforts to target *S. enterica* metabolism[99]. In fact, based on their data the authors concluded that most broad-spectrum antimicrobial targets have already been exploited. As disheartening as these results are, it is likely that the future of antimicrobial development lies in novel narrow-spectrum antibiotics and combination therapies, particularly in light of widespread gene-based resistance to many broad-spectrum compounds. By understanding the basic physiology of pathogens during residence in the human body, several novel avenues of drug and treatment development can be explored, including targeting specific enzymes within a bacterial species (such as the isocitrate lyase enzymes of *M. tuberculosis*), specific manipulation of the *in vivo* growth environment (such as targeted destruction of aromatic amino acids in CF sputum), and modification of host diet (such as iron restriction during infection). Although not discussed at length here, the growing body of work on microbial community dynamics in host environments such as the intestine might also lead to appropriate dietary modifications and probiotic prophylactics and treatments. Regardless of the approach used, clever strategies will be necessary to prevent, treat and cure microbial infections in the future.

It is clear that there is interest in revisiting the host as a growth medium. This is most evident from the renewed interest in examining the *in vivo* carbon preference of extracellular

and intracellular pathogens. Unfortunately, the growth conditions in many infection sites are poorly defined, making nutritional modelling of these sites difficult. Perhaps as a result of this, the preferred *in vivo* carbon source of many pathogens is unknown. This observation is troubling as it suggests that, in general, we still do not understand the basic physiology of bacterial pathogens *in vivo*. Using *in vivo*-relevant growth substrates for *in vitro* studies provides a simple and versatile method for examining nutritional effects on bacterial physiology. The use of relevant nutritional substrates has led to significant breakthroughs in the study of several pathogens, including UPEC (human urine), *P. aeruginosa* (lung mucus) and commensal and enterohaemorrhagic *E. coli* strains (intestinal mucus). These *in vitro* studies allow for the development of molecular models that can be assessed in more challenging *in vivo* systems. Ultimately, understanding the nutritional environment of the infection will provide important information for understanding the basis of infection.

## Acknowledgments

We would like to acknowledge members of the Whiteley laboratory for critical discussion and review of this manuscript.

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## Glossary

| | |
|---|---|
| **Molecular mimicry** | Chemical imitation of a host structural component by a foreign body. |
| **Granuloma** | A tissue lesion that occurs when macrophages are unable to clear foreign substances from the body. The centre of a granuloma contains a high density of macrophages (sometimes necrotic), which are surrounded by multiple immune cell types, including polymorphonuclear leukocytes and fibroblasts. |
| **Glyoxylate shunt** | An anaplerotic pathway used to bypass $CO_2$-generating reactions of the tricarboxylic acid cycle to conserve carbon for intermediate biosynthesis; required for growth on fatty acids or acetate. |
| **Polyketide** | A member of a diverse class of secondary metabolites formed by condensation of small fatty acid groups. Polyketides are produced by bacteria, archaea and eukaryotes and can function as natural antimicrobials. |
| **Phagosome** | An intracellular vacuole that is formed by phagocytosis of extracellular components. Phagocytic cells, such as macrophages, are involved in bacterial clearance during infection. Properly trafficked phagosomes fuse with lysosomes — compartments that are filled with degradative enzymes. |
| **Cofactor** | A non-proteinaceous chemical compound that is required for enzyme catalysis. |
| **Illegitimate recombination** | A type of genetic recombination that occurs in spite of little homology between nucleotide sequences. |

Box 1 Targeting bacterial metabolism: a historical perspective

One of the oldest antibiotics has its roots in the textile industry[100]. In 1908, Paul Gelmo synthesized *p*-aminobenzenesulphonamide (sulphanilamide), which was later discovered to make superior textile dyes when mixed with azo dyes. In the 1930s one of these dyes was found to have antimicrobial properties. A French patent in 1934 and a German patent in 1935 were obtained for the newly named Prontosil, which was soon made available for therapeutic use. Prontosil proved to be an effective antimicrobial agent, although turning the patient red was an unfortunate side effect. It was only months later that a team in Paris discovered that the sulphanilamide component of Prontosil was the active portion of the compound, and was, conveniently, colourless. This led to widespread use throughout Europe. The drug did not gain popularity in the United States until late 1936, when the president's son, Franklin D. Roosevelt Jr, was treated successfully for a streptococcal infection. The sulfa drug family went on to play a major part in reducing casualties during the Second World War.

It is now known that sulfa drugs interfere with the folic acid biosynthesis pathway[101], a pathway that is essential for bacterial survival. Since the discovery of sulfa drugs, the development of new antibiotics, along with an increasing level of bacterial resistance, has led to a decrease in their use. Nevertheless, sulfa drugs are still reliable treatment options for bacterial infections today.

Box 2 Two can play this game: IFN-γ and intracellular nutrient depletion

The immunomodulatory compound interferon-γ (IFN-γ) has a crucial role in human immune defence. This chemokine is produced by a host of immune cells in response to pathogen invasion and is a key factor in promoting the antimicrobial activities of macrophages and other cell types[102]. In fact, for intracellular pathogens such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, *in vivo* control of bacterial growth and host survival is dependent on IFN-γ production[103-105].

Arguably, the best-known antimicrobial activity of IFN-γ-stimulated macrophages is the production of reactive nitrogen species such as nitric oxide. However, this activity is not responsible for all of the growth-inhibitory properties of IFN-γ-stimulated cells. Over two decades ago it was discovered that stimulation of human fibroblasts with IFN-γ induces intracellular tryptophan degradation and concomitantly suppresses growth of the intracellular eukaryotic parasite *Toxoplasma gondii*[106]. Mechanistically, IFN-γ stimulation induces production of indoleamine-2,3-dioxygenase[107], an enzyme that degrades tryptophan to kynurenine and *N*-formylkynurenine[108]. Because *T. gondii* is a tryptophan auxotroph and must scavenge tryptophan from the host during infection[106,109], indoleamine-2,3-dioxygenase induction leads to *T. gondii* tryptophan starvation and growth suppression. *In vivo* tryptophan starvation has been observed for other pathogens including *Rickettsia conorii*[110] and group B streptococci[111]. Thus, cellular stimulation by IFN-γ promotes *in vivo* carbon substrate (tryptophan) deprivation as a growth control mechanism for intracellular pathogens.

The effect of IFN-γ on access to *in vivo* carbon substrates might go beyond tryptophan depletion. For example, in resting macrophages, *M. tuberculosis* resides in a maturation-arrested phagosomal compartment that has access to endosomal components such as glycosphingolipids[112], which are potential *in vivo* carbon sources. However, IFN-γ stimulation of macrophages promotes maturation and autophagy of this compartment[113], probably restricting access to host-derived nutrients. In turn, it has been proposed that IFN-γ-dependent mechanisms of *in vivo* nutrient control, beyond tryptophan degradation, are at play during intracellular infection[114].

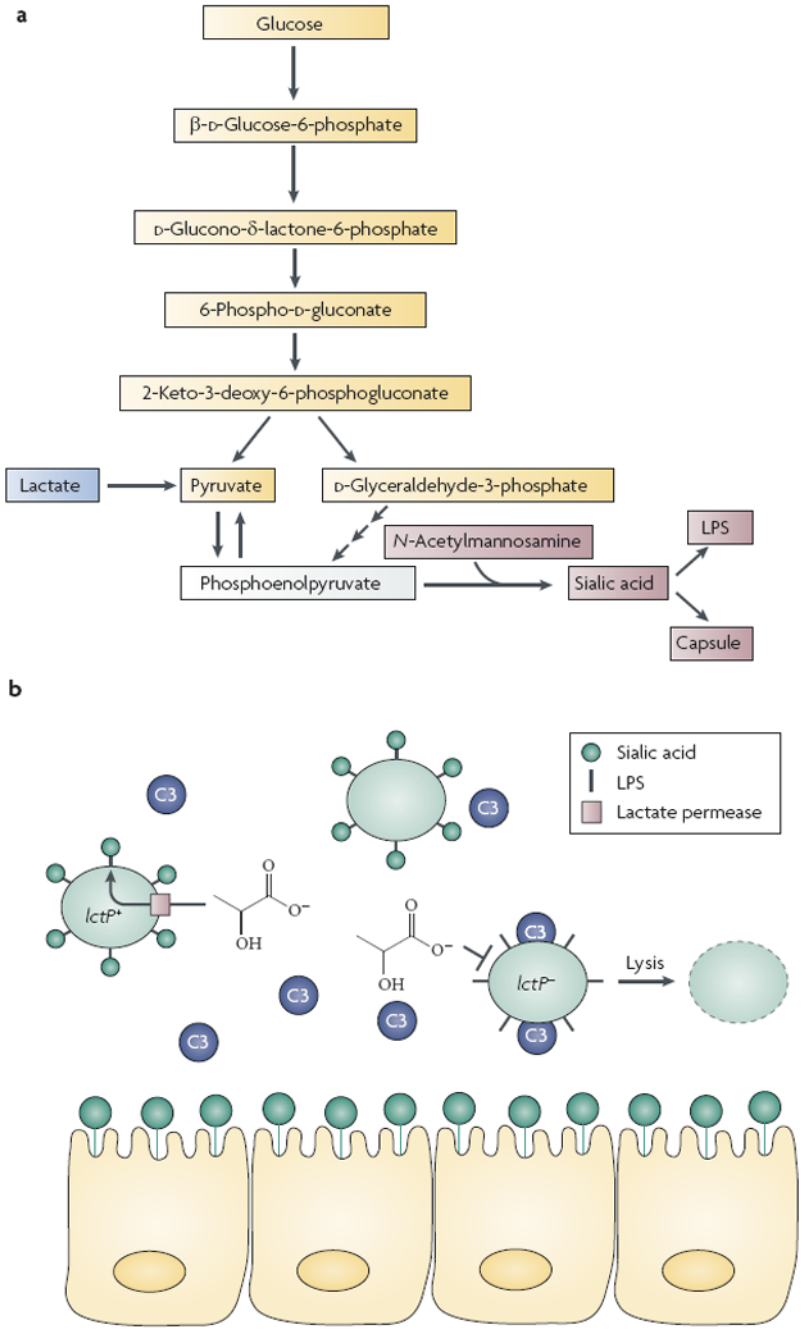


**Figure 1. Molecular mimicry by *Neisseria meningitidis***

**a** Glucose catabolism in *N. meningitidis* proceeds by the Entner–Doudoroff pathway and lactate catabolism feeds directly into the sialic acid pathway[16]. Note the relative number of metabolic steps from glucose to phosphoenolpyruvate compared with that from lactate to phosphoenolpyruvate. **b** Sialylated lipopolysaccharide (LPS) on the *N. meningitidis* surface mimics the surface of eukaryotic cells, preventing deposition of the complement molecule C3. Inactivation of the lactate permease gene *lctP* results in C3-mediated cell lysis[16].

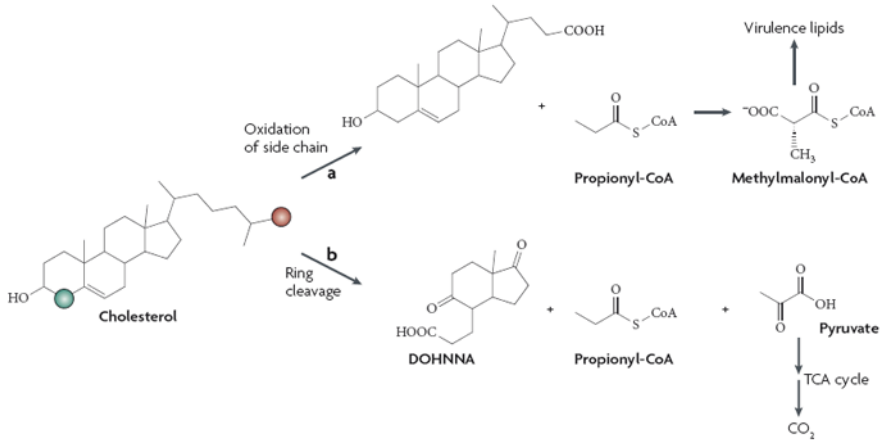


**Figure 2.** ***Mycobacterium tuberculosis*** **cholesterol catabolism and virulence factor production**

A proposed pathway for cholesterol catabolism with relevant gene products has recently been published for the soil bacterium *Rhodococcus* sp. strain RHA1 (Ref. 115). *M. tuberculosis* possesses homologues for almost all of the genes involved in cholesterol degradation[115], several of which are important for intracellular growth[116]. However, definitive *M. tuberculosis* use of cholesterol as a sole source of carbon and energy was reported only recently, and in this study radioactive labelling was used to demonstrate the differential fates of cholesterol carbons[27]. A labelled side-chain carbon (shown in red) was incorporated into the virulence lipid phthiocerol dimycocerosate[27], presumably by conversion of side-chain-derived propionyl-CoA to methylmalonyl-CoA, a precursor to phthiocerol dimycocerosate and other virulence lipids[24]. Successive oxidation of the remaining side chain produces an acetyl-CoA and an additional propionyl-CoA moiety (not shown). Conversely, a labelled ring carbon (shown in green) was released as $CO_2$, indicating that this carbon enters the tricarboxylic acid (TCA) cycle and is mineralized[27]. Some aspects of *M. tuberculosis* cholesterol catabolism remain unclear, including the fates of the degradation product 9,17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-oic acid (DOHNAA)[115] and propionyl-CoA moieties produced through ring cleavage and successive side chain degradation.

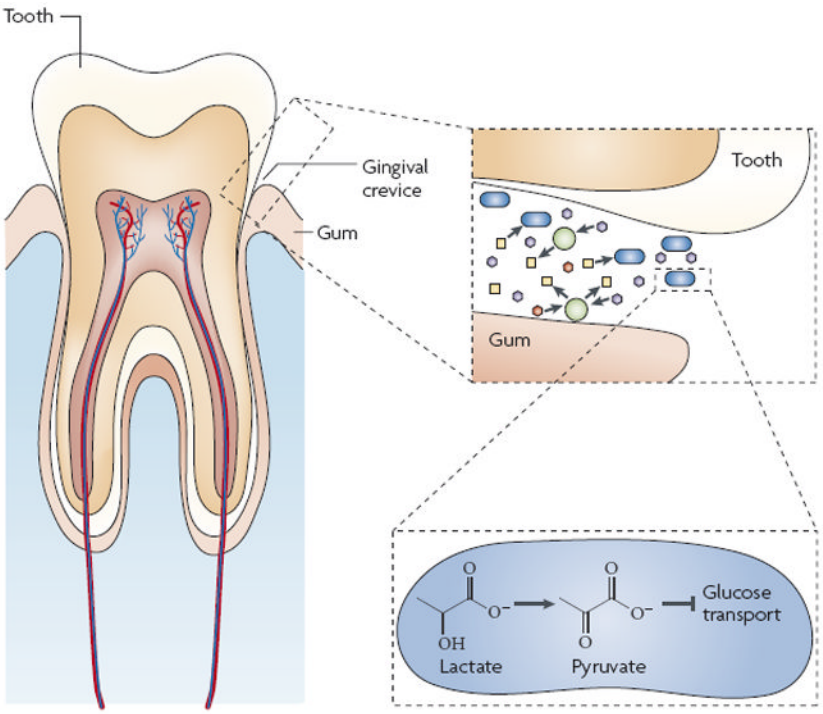


**Figure 3. Resource partitioning by oral bacteria**

The figure shows a view of the microbial community that inhabits the gingival crevice, which is the space between the tooth and gum. Gingival crevicular fluid, which is similar in composition to serum, contains millimolar concentrations of glucose and lactate[117] and micromolar concentrations of other phosphotransferase system (PTS) sugars[118]. *Streptococcus* species (green circles) within the gingival crevice rapidly consume sugars (hexagons) and produce lactate (yellow squares). Despite constitutive expression of PTS sugar and lactate catabolic genes, *Aggregatibacter actinomycetemcomitans* (blue rods) preferentially uses lactate[62]. Lactate metabolism by the lactate dehydrogenase enzyme results in a rapid increase in intracellular pyruvate levels which is hypothesized to inhibit glucose transport or metabolism by *A. actinomycetemcomitans* through an unknown post-transcriptional mechanism[62].

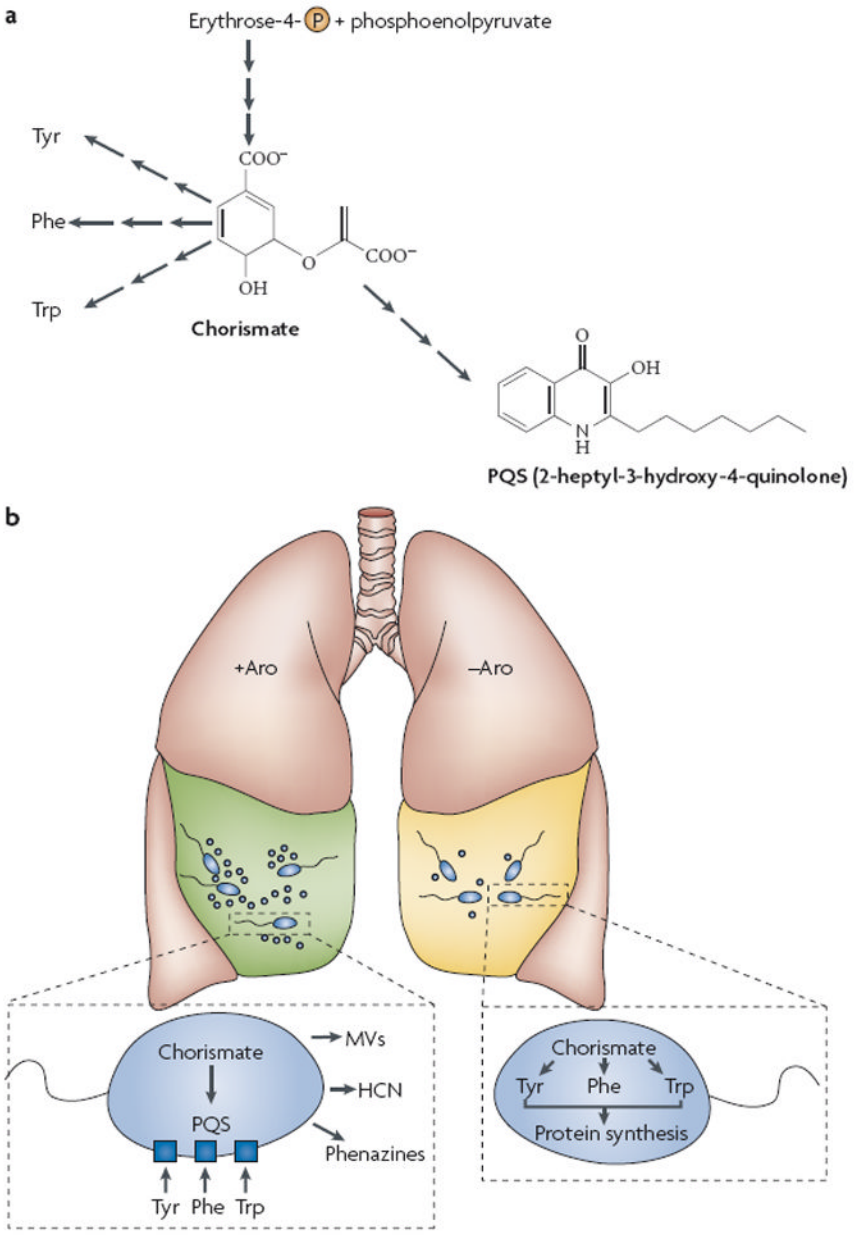


**Figure 4. Cell–cell communication, carbon metabolism and pathogenesis in *Pseudomonas aeruginosa***

**a** A simplified view of the fates of chorismate in *P. aeruginosa*. Chorismate, the biosynthetic precursor of the aromatic amino acids tyrosine (Tyr), phenylalanine (Phe) and tryptophan (Trp), is synthesized from the central metabolites erythrose-4-phosphate and phosphoenolpyruvate. Chorismate synthesis is a highly regulated process in *Escherichia coli*[119] but might be constitutive in *P. aeruginosa*[120]. In addition to the aromatic amino acids, chorismate is also the biosynthetic precursor of 2-heptyl-3-hydroxy-4-quinolone, a cell–cell signal that is unique to *P. aeruginosa* (the *Pseudomonas* quinolone signal; PQS)[121]. **b** A model for the impact of aroxmatic amino acids on *P. aeruginosa* physiology during cystic fibrosis (CF) lung infection. High levels of aromatic amino acids are present in CF sputum[98]. *P. aeruginosa* scavenges these amino acids for protein synthesis, thereby diverting intracellular chorismate pools to PQS biosynthesis. PQS induces virulence factor

production including the blue-green phenazine pigment pyocyanin[92,121], hydrogen cyanide (HCN)[121] and toxin-loaded membrane vesicles (MVs)[94] (+Aro). In the second scenario, depletion of aromatic amino acids from the CF lung (potentially by some novel therapeutic), forces *P. aeruginosa* to use intracellular chorismate pools to support protein biosynthesis (−Aro). In turn, production of PQS and PQS-regulated virulence factors decreases[98].

## Table 1

Impact of carbon substrate on host colonization and disease

| Bacterium | Location | Carbon substrate | Impact |
|---|---|---|---|
| *Aggregatibacter actinomycetemcomitans* | Oral cavity | Lactic acid | Persistence and growth |
| *Escherichia coli* | Intestine | Intestinal mucus sugars | Establishment of infection |
| *Legionella pneumophila* | Macrophage | Threonine | Differentiation signal |
| *Listeria monocytogenes* | Macrophage | Hexose phosphates, lipoate | Persistence and growth |
| *Mycobacterium tuberculosis* | Macrophage | Cholesterol | Immune manipulation |
| *Neisseria meningitidis* | Bloodstream, cerebrospinal fluid | Lactic acid | Molecular mimicry |
| *Pseudomonas aeruginosa* | Lung | Aromatic amino acids | Increased production of virulence factors |
| Uropathogenic *E. coli* | Urinary tract | D-Serine | Tissue localization and growth |