Bacterial Metabolic Fitness During Pathogenesis

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Abstract

Pathogenic bacteria encounter hostile environments and experience diverse stresses from their initial moment of contact with the host. To survive within the host, bacteria improved metabolic fitness and altered virulence character. As in the case of respiratory pathogens, bacteria respond against an array of host-derived antimicrobial mediators, nitrosative stress, hyperosmolarity, and oxygen limitation. Further, in case of enteric pathogens, after ingestion, they must survive the acidic pH. Moreover, enteric pathogens living within the intestinal lumen encounter host-generated antimicrobial peptides, reactive oxygen radicals, bile salts, and free fatty acids and enhanced osmolality. Subsequently,

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inflammatory responses against pathogen recruit macrophages, neutrophils, and other phagocytic cells. These defense mechanisms increase oxidative and nitrosative stresses as well as sequestration of essential metals and nutrients. Bacteria respond to these by developing exquisite systems that sense these stresses and trigger appropriate mechanism for successful survival. Different environmental stimuli not only trigger adaptive responses but also modulate virulent genes at particular condition. An array of stress responses and their mechanism is consequently critical and necessary to understand bacterial pathogenesis.

In an effort of emphasizing the complexity of bacterial adaptation during the host infection, this chapter will focus on different stress-sensing and stress response mechanisms that finally improvise the bacterial metabolic fitness.

Keywords
Bacterial pathogens · Immunity · Autophagy · Defense strategies · Cell cycle

12.1 Introduction

Disease-causing pathogenic bacteria while thriving within host encounter diverse stresses. All successful pathogens are well equipped with its genetic, biochemical, and structural features that enable them to overcome the host-derived stresses and cause disease. These capabilities allow pathogens to not only withstand against precise stress situations but additionally to express their pathogenicity with the aid of modulating immune and survival signaling in a sophisticated way. The pathogens also have a series of adaptive mechanisms that safeguard from the host defense mechanisms. A comprehensive understanding of bacterial adaptation to host stress responses offers better understanding of host microenvironments, bacterial virulence, and stress resistance.

Pathogenic bacteria experience nutritional challenges, oxidative and nitrosative stress, envelope stress, oxygen limitation, hyperosmolarity, DNA damage, and change in temperature during infection and colonization within mammalian hosts. Bacteria not only counterattack these stresses and immune challenges but additionally activate their defense mechanisms that allow survival under these hostile environments. This chapter explains adaptation mechanisms of pathogenic bacteria during infection within the host.

12.2 Metabolic Adaptation Mechanisms in Intracellular Pathogenic Bacterium

The pathogens are protected against phagocytes and other extracellular immune defense. Though intracellular and extracellular pathogens often have no well-defined distinction, most pathogens live within infected host cells in some stages of their life cycle. For instance, the *Streptococcus pneumoniae* is an extracellular pathogen, but it can regularly be found intracellularly. The intracellular life does have its challenges. The host encountering intracellular pathogens fights back with innate and adaptive immunity and autophagy. After internalization, intracellular bacteria evade the immune system and replicate in specialized non-acidic vacuoles, for example, *Salmonella*, *Mycobacteria*, *Brucella*, *Legionella*, *Coxiella*, and *Chlamydia*. Some pathogens after escaping from their internalization vacuole reside in cytosol. This group of intracellular bacteria includes *Shigella*, *Listeria*, *Rickettsia*, and *Francisella* and escapes into the host cytosol and actively replicates (Ray et al. 2009).

After phagocytosis numerous pathogens use macrophages as their intracellular niche for survival and replication (Thakur et al. 2019). During evolution, pathogens have built up the capacity to instigate their own internalization even by non-phagocytic cells, along with epithelial and endothelial cells.

In general, the nutrient trafficking for intravacuolar bacteria is dependent on transporter proteins of host of pathogen origin, directly inserted into the membrane of vacuole or by fusion of endocytic vesicles. Majority of these vacuolar bacteria utilizes substrate-level phosphorylation to generate ATP by converting pyruvate to acetate. However, *Coxiella* and *Chlamydia* being obligate pathogens are unable to perform this ATP-generating reaction using pyruvate as a substrate in acidic pH range of 4.5–4.7. Alternatively, they harbor two ATP/ADP translocases that allow them to uptake ATPs from the host cells.

Additionally, several pathogenic bacteria successfully thrive in non-acidic vacuoles including *Salmonella*. *Salmonella*-containing vacuoles are remodeled by the several effectors secreted by the type 3 secretion system (T3SS) of the bacterium. These effector molecules allow the polymerization of an actin basket around the vacuoles by rearranging the actin cytoskeleton and thus help in regulation of bacterial virulence. Also these effectors are able to block the recruitment of NADPH oxidase to inhibit the production of a superoxide free radical. *Mycobacterium tuberculosis* has developed strategies to replicate successfully in macrophages because of having an exceptionally hydrophobic cell wall. As a consequence, it evades immune responses and persists within the host (Awuh and Flo 2017). It prevents fusion of phagosome and lysosome and gets nutrients from macrophages to persist intracellularly (BoseDasgupta and Pieters 2018).

12.2.1 Life in the Vacuole

Organisms survive from lysosomal degradation by resisting the endosome development pathway. Pathogens subvert phosphoinositide digestion to readjust character of endosomal layer, thus inhibiting endocytic pathway. *Legionella pneumophila* inhibits lysosomal execution by making ER-like compartment by obtaining ER-derived vesicles, while *Mycobacterium tuberculosis* and *Salmonella* resist endosomal maturation pathway (Xu and Luo 2013). Similarly, the intracellular pathogen *Chlamydia/Chlamydophila*, causing genital, visual, and respiratory diseases, redirect early vacuole from the endocytic pathway. They form metabolically idle rudimentary bodies for transmission, which subsequently change into metabolically dynamic reticulate bodies that, lastly, multiply in endosome-inferred vacuole or *Chlamydia*-containing vacuole (Wong et al. 2019) which diverge from the endocytic pathway (Nickel et al. 1999). *Chlamydia*-containing vacuole recruits Rab GTPases in some chlamydial species (Aeberhard et al. 2015).

Though escape from phago-lysosomal maturation is key mechanism for pathogen survival, some bacteria follow alternate mechanism as in the case of quasi-obligate intracellular pathogen *Coxiella burnetii*, causing Q fever. *C. burnetii* spreads among hosts through inhalation of contaminated aerosol (Gurtler et al. 2014). *Coxiella* infects macrophages, in addition to infecting epithelial cells, monocytes, and other cell types, thereby multiplying in an acidic parasitophorous vacuole (Stein et al. 2005). *Coxiella* has a way to escape the parasitophorous vacuole microbicidal conditions and its acidic pH 5.0 (Ghigo et al. 2012).

12.2.2 Escaping from Vacuole

To avoid the vacuolar acidification and subsequent destruction of inhabiting pathogen, immediate escape of pathogens from the phagosome to cytosol is necessary. Usually, pathogens have been detected in the cytosol within 30 min of internalization (de Souza Santos and Orth 2014). Escape of the pathogen is primarily driven by pH of the vacuole. Pathogens during escape use membrane-damaging toxins/enzymes to escape successfully (Radoshevich and Cossart 2018). For example, in *Shigella flexneri*, the plasmid-encoded T3SS effector proteins IpaB and IpaC are associated with escape from the phagosome, forming a IpaB-IpaC mediated by pore-forming complex (Mattock and Blocker 2017; Picking and Picking 2016).

12.2.3 Life in the Cytosol

Pathogens have ways to escape lysosomal degradation soon after engulfment. In the process, they ensure their intracellular survival in unfavorable microenvironment. As in the case of *Shigella*, pore-shaping effectors interfere with the phagosome

(Mattock and Blocker 2017). Free-living mycobacteria *Mycobacterium marinum* escape by exploiting Esx-1 secretion system and utilize the cytoplasm for replication (Bosserman et al. 2019). Thus, some pathogens have evolved the strategies to be exclusively cytosolic. Proficient cytosolic pathogens have evolved escape mechanisms exploiting actin-interceded motility and cell-to-cell communication junctions to spread (Wiesel and Oxenius 2012). By manipulating the host actin cytoskeleton, microbes impel themselves through the cell-cell junction, venture into contiguous cells, and spread without leaving the limits of the defensive intracellular compartment (Lamason and Welch 2017). By commandeering the host cell actin cytoskeletal apparatus, microscopic organisms can impel themselves via the cytosol, venture themselves into contiguous cells, and spread without leaving the limits of the defensive intracellular compartment (Colonne et al. 2016). In summary, in contrast with survival of cytosolic pathogens, the vacuolar survival seems to be safer choice for successful life cycle.

12.2.4 Evasion from Autophagy

Autophagy is a cellular destructive process which disposes cytoplasmic materials by lysosomal degradation (Hu et al. 2019). The autophagy pathway is modulated by pathogens, for example, the *Listeria* pore-forming toxin LLO triggers the autophagy. The PlcA and PlcB phospholipases support the *Listeria monocytogenes* to protect from decimation in autophagosomes (Cheng et al. 2018). Further, *L. monocytogenes* avoids autophagy in the cytosol; it might select the autophagic assembly to make an extra/intracellular compartment and cause disease progression. Also, during the early phases of infection, LLO of *L. monocytogenes* dephosphorylates histone H3 and deacetylates H4 that is independent of its pore-forming activity. These epigenetic modifications downregulate host inflammatory response (Hamon et al. 2007). By action of LLO, *Listeria* converts phagosome to *Listeria*-containing phagosome in which they can sustain (Mitchell et al. 2015; Vdovikova et al. 2017).

12.2.5 Cytoskeleton-Based Cell Motility

Cytosolic pathogens have been developed to activate host F-actin polymerization on their surface, delivering mechanical power that impels them into neighboring cells (Ireton 2013). For example, a surface protein in *Burkholderia*, *Shigella*, *Listeria*, and *Rickettsia* triggers actin-based motility by promoting actin polymerization. The F-actin fibers form cross-connecting structures as an unbending meshwork. This meshwork utilizes formation and engulfment of bacterial protrusions causing cell-to-cell spread of bacterium. The depolymerization of actin meshwork at the distal end appears as comet tail (Truong et al. 2014).

12.2.6 Modulation of Host Cell Autophagy

Modulation of host cell death pathway drives the pathogen survival within host. Intracellular pathogens evade extracellular immune surveillance by subverting host autophagy to save their replicative capability (Stewart and Cookson 2016). Organisms trigger cell survival pathways, for example, the NF-κB pathway by *Legionella* or the PI3K/Akt pathway by *Salmonella* (Creasey and Isberg 2012; Knodler et al. 2005), *Listeria* (Mansell et al. 2001), or *Rickettsia* (Joshi et al. 2004), by downregulation of apoptotic genes and the upregulation of key survival genes. This enables microbes to escape autophagy-mediated death and encourage their replication and disease spread.

12.2.7 Reprogramming of Host Cell Cycle

As another defense, intracellular pathogens regulate host cell cycle for their proliferation within host. Numerous pathogens including *Shigella* and *Salmonella* produce secretory toxins called cytolethal distending poisons, homologous to mammalian DNase I that can hinder the cell cycle at the G2/M phase (Lara-Tejero and Galan 2000). Also, *Chlamydia trachomatis* destabilizes the cell cycle proteins associated with the G2/M phase (Gargi et al. 2012) and controls the host cell cycle (Gargi et al. 2012). Genome-wide transcriptomic profiling demonstrated that intracellular pathogens make changes in host gene expression (Jenner and Young 2005). A large number of these changes impact infection-induced innate immune response as in the case of *Salmonella*. *M. tuberculosis* infection upregulates histone deacetylase which in turns causes the inhibition of IFN-γ-induced articulation of host macrophages (Chandran et al. 2015). Similarly, pathogens reprogram host cell cycle by epigenetic regulation. *Shigella* inhibits mitogen-activated protein kinase (MAPK) action by phosphatase action of its T3SS effector OspF (Schroeder and Hilbi 2008).

12.3 Metabolic Adaptation Mechanisms in Extracellular Pathogenic Bacterium

Bacterial pathogens can be characterized as either extracellular or intracellular on the basis of their life cycle in the host. Extracellular pathogens replicate outside of host cells. However, the condition seems more multifaceted with a dual way of life of bacterial pathogens.

12.3.1 Survival Measures for the Extracellular Pathogens: Defensive Strategies

To thrive inside the host, extracellular bacterial pathogens must defeat the host immune system. Pathogens devise a variety of mechanisms to get away with host defenses.

(a) *Molecular camouflage*

Bacterial pathogens hide their pathogenic properties from the host by molecular camouflage. This involves capsule generation, biofilm formation, and alteration of bacterial surface antigenic variation at bacterial surface. Capsule of extracellular polysaccharide coatings gives pathogens a physical barrier to protect them from host defense as in the case of both gram-positive and gram-negative pathogens. Certain bacterial pathogens contain capsules with host similar molecules and thus suppress immune response against them. Biofilms are microbial aggregation forms at the interface of solid-solid, solid-liquid, liquid-liquid, or liquid-gas and make infection more tolerant to antibacterial. *Pseudomonas aeruginosa* (causing cystic fibrosis (CF) in lungs) and *S. aureus* (forming biofilms on medical implants) are one of the most studied biofilm-forming microorganisms (Heidari et al. 2018). Extracellular bacteria can escape from recognition by hiding inside host (Nobbs et al. 2009). *S. aureus* exhibits a classic case of defense by generating a coagulase, where two of non-proteolytically prothrombins cause the polymerization of fibrin, thus resulting in clump arrangement (Abamecha et al. 2015; Liesenborghs et al. 2018). Inside this complex macromolecular structure, *S. aureus* can hide and escape phagocytic degradation. Similarly, bacteria such as *S. pneumonia* may change capsule, to skip from humoral or cell-mediated immune response. Antigenic variations of these proteins and the capsules enable *S. pneumonia* to keep away from host immune recognition. The facultative intracellular pathogens *N. gonorrhoeae* causing gonorrhea, similar to *N. meningitidis* causing meningitis, change surface proteins and display antigenic variabilities (Coureuil et al. 2013).

(b) *Pathogen modulates host immune system*

Extracellular bacterial pathogens guard themselves with the aid of altering host immune defenses for their survival. Pathogens summon pathways to meddle with the typical movement of supplement enactment, counteracting agent official, AMPs, and phagocytosis. The supplement framework is perplexing, and microorganisms have advanced various approaches to get away from its strong impacts; these incorporate tweak of supplement administrative proteins, direct association with supplement segments to forestall enactment, and enzymatic corruption of antibodies or supplement factors. At long last, some bacterial pathogens control the invulnerable framework by balancing the ordinary genius fiery reaction that is produced by the natural safe framework within the sight of a culpable creature, for example, the *Y. enterocolitica*-encoded protein, LcrV, modifies cytokine generation to guarantee *Y. enterocolitica* survival in vivo (Reithmeier-Rost et al. 2007).

12.3.2 Survival Measures for the Extracellular Pathogens: Offensive Strategies

Extracellular bacterial pathogens are loaded with hostile weapon to harm the host cells, tissues, and organs to guarantee their proliferation. The most noteworthy

mechanisms of secretion of microbial toxins by extracellular pathogens are either discharge (by bacterial pathogens upon cell lysis) or release straightforwardly into the targeted host cell. Generation of toxins by extracellular bacterial pathogens increases the level of degradative proteins that impose hostile factors significant for the survival of bacterial pathogens in vivo.

(a) *Microbial toxins:* Microbial toxins are harmful to human host. They can function in two ways: first, during bacterial cell lysis, the toxins are re-released extracellularly (endotoxins), and second, pathogenic bacteria secrete toxins extracellularly into host cell (exotoxins). Numerous bacterial pathogens produce various toxins with these mechanism.
(b) *Degradative enzymes:* Degradative enzymes are not traditionally considered as toxins; they provide an important survival mechanism for extracellular pathogens. Large numbers of these degradative proteins can redirect host immune response by modulating immunoglobulins, extracellular matrix, cell membranes, and fibrin network, thus providing organism a chance to move away from site of disease with active immune challenges (Karlsson et al. 2018).

Extracellular pathogens escape from phagocytic events and thrive in the extracellular spaces, for example, *S. aureus*, *P. aeruginosa*, *or S. pyogenes*. However, the dual way of life in both intracellular and extracellular bacterial pathogens is quite complicated (Silva 2012), although many bacterial pathogens live extracellular life as second phase of their life cycle after establishing intracellular infection.

12.4 Impact of Metabolic Dynamics in Virulence and Pathogenesis of Bacteria

12.4.1 Metabolic Dynamics

Bacterial pathogens invade and thrive in the variable and hostile environment of their host and they rely on their proficient metabolic adaptation. The variable environment within the host ranges from oxidative stress due to host immune system, free radical oxygen, and nitrogen intermediates, various carbon sources to be used as a substrate for energy to the changes in the pH. These conditions thus make it necessary to eternally monitor and respond to the microenvironment in an appropriate way in order to establish the successful sustenance.

The important factor is to understand the functional and genomic condition of pathogen metabolism that exploits utilization of host's nutrient. Horizontal gene acquisition drives the metabolic functions to provide a selective advantage to the pathogen residing within the host. Some loss-of-function mutations that alter the metabolic abilities may also provide the selective advantage to the pathogen in the host.

12.4.2 Fight for Survival Among Invading Pathogen and Gut Microbiota

Within the host microenvironment, the occurrence of competition is very first and common step in order for the pathogen to thrive. One of the prerequisites to succeed within host and establish the pathogenesis is the ability of the pathogen to sense the available carbon sources. Carbon catabolite repression (CCR) is triggered in response to readily available energy source (glucose), and variation in amino acid sources is used to adopt to and regulate the virulence factor via stringent response through (p)ppGpp (Zhang et al. 2016). Hence, efficient processing of the available nutrients in the limiting conditions would help one species to outgrow compared to the other species (Rohmer et al. 2011). In order for the pathogen to adopt successfully in the host environment, they need to invade the niche that is already perfectly adopted by resident microbiota since the resident bacteria have their own mechanisms to protect their niche from the competitive bacterial species.

12.4.3 Challenging Environment Within the Host

One of the most important challenges pathogen faces is variation in the pH range within the host gut. Usually this variation ranges as low as pH 1.0 in the stomach to as high as pH 8.0 in the urine. Secondly, invading pathogen has to struggle with the already existing niches of the microbes which are perfectly adopted and more than ten times the number of human cells themselves in the body. The new estimates by Hans-Curt Flemming in their Nature Microbiology review in 2019 reveal that there is one to one ratio of host cells to microbiome are present in the human gut. A predominant aerobic bacterium *S. epidermidis* produces antimicrobial peptides which are toxic to invading pathogens *S. aureus* and *S. pyogenes* (Otto, M. 2010). Additionally, the resident microbes of the intestine form a heterogeneous complex ecosystem and ultimately affect the cytokine production by the spleen and bone marrow macrophages, promoting the defense against invading pathogens (Nicaise et al. 1999). Lactic acid-generating *Lactobacilli* species in the woman vagina is another example of this type which maintains the acidic pH of the vagina to inhibit the invasion of many pathogenic colonizers (Amabebe and Anumba 2018). Except for the mucosal surface within the host which is well oxygenated, the pathogens encounter oxygen-deficit environments in buccal cavity, the large intestine, female genital tract, abscesses, and damaged tissues. Bacteria need iron in sufficient amount to thrive within the host which is quite below the desired level within the host, subjecting bacteria to devise their own strategy to sustain in the iron scavenging conditions by upregulating the iron transporting genes (Moumene et al. 2017). Other environmental factors which are used in addition to nutrient sensing include temperature, ion concentration, pH, and oxygen, and these altogether determine the location of the pathogens within the host to adjust and successfully adjust the pathogenesis.

12.4.4 Metabolic Interaction Between Pathogen and Host: Dynamism

Metabolic modulation of the host is utilized by the pathogen to regulate the expression profile of their virulence determinants since the nutrient availability within the host is not always constant (Schaible and Kaufmann 2004). For example, iron availability within the host varies during the infection and after the production of host factors interacting during iron metabolism (Ibraim et al. 2019). Inflammation-induced sequestration of the iron occurs by host lactoferrin and lipochellin-2 (Raffatellu et al. 2009). These host proteins sequester the iron in response to infection to prevent iron acquisition by the pathogens (Skaar 2010). Bacterial pathogens thereby sense the iron depletion as a signal, and thus they subsequently modulate the production of virulence factors (Skaar 2010). For example, iron-activated global repressors Dtxr and Fur of the *Corynebacterium diphtheriae* and *Shigella* species, respectively, are well-studied examples of inhibition of diphtheria and *Shigella* toxin expression (Tao et al. 1994; Schaible and Kaufmann 2004). Invading pathogens sense the variation in nutrient availability within the host, and thus they accordingly modulate the expression of their virulence factors. Pathogens acquire the metabolic genes in the same way as the virulence genes in order to establish the successful infection within the host.

12.4.5 Colonizing New Territories: Contribution of Metabolic Genes

Pathogens need virulence factors to adapt to new niches (Schmidt and Hensel 2004), and they need new metabolic pathways to help them thrive in the new environment and to exploit the available host resources. Pathogenic genes helping to adapt to new metabolic environment are often located in the pathogenicity islands. These pathogenicity islands are absent in the non-pathogenic relatives but present in the pathogenic species, and they show the evidence of lateral transfer (Hacker and Kaper 2000).

Well-studied example of this is the tetrathionate respiration (as a terminal respiratory electron acceptor) in *S. enterica* serovar *typhimurium* infecting the intestinal lumen. This pathogen produces hydrogen sulfide (H_2S) in large amount, and the cecal mucosa protects itself by converting the highly toxic gas to thiosulfate ($S_2O_3^{2-}$). Intestinal inflammation is induced by *Salmonella* virulence factors which are located on pathogenicity islands, SPI1 and SPI2, causing the production of large quantity of reactive oxygen and nitrogen species. As a result, thiosulfate gets oxidized to tetrathionate ($S_2O_6^{2-}$), thereby selectively inhibiting the growth of coliforms. *Salmonella* now becomes able to use tetrathionate to utilize ethanolamine of 1, 2-propanediol as a source of carbon for the anaerobic growth in the intestinal lumen, thereby achieving a competitive edge over the gut microbes, allowing the

pathogen to successfully establish itself within the host (Winter et al. 2010; Price-Carter et al. 2001).

12.4.6 Metabolic Adaptation Within the Host Microenvironment: A Characteristic to Pathogenicity

Different hosts have varying nutrient availability that makes it necessary for pathogens circulating among many hosts to adapt metabolically. For the pathogen, metabolic requirements are directly proportional to host infected and route of infection. The status of conservation of metabolic gene depends on its habitat. Nevertheless, metabolic potential of the pathogen is also dependent on effect of loss-of-function mutations offering survival benefits for a given niche (Rohmer et al. 2011).

12.4.7 Genome Reduction and Gene Loss: Common Adaptation Mechanisms

When a pathogen establishes itself a new niche that offers improved nutritional resources, detrimental metabolic pathways either get suppressed or can be even lost. It is also likely that virulence character can be altered because of loss of functional mutation in non-essential metabolic genes that reduces the metabolic demands of bacterium. Functional complementation of pseudogenes has proven that loss-of-function mutations could be enhancing the virulence, thereby increasing survival capacity. *Shigella* lacks lysine decarboxylase activity, but its closely related bacterium *E. coli* does have the functional lysine decarboxylase. When *Shigella* is complemented with lysine decarboxylase, its virulence capacity is attenuated due to reduction in the level of *Shigella* enterotoxin caused by lysine decarboxylase action (Maurelli et al. 1998).

Genome reduction is a known evolutionary mechanism in some bacteria offering them improved metabolic fitness and enhanced virulence. Pseudomonads infecting the airways of the cystic fibrosis patients have been shown to lose a variety of metabolic pathways. This is attributed to greater nutrients availability in the host airway microenvironment compared to the soil and water (Smith et al. 2006; Barth and Pitt 1996). A very interesting review was published by Ahmed et al. in 2008 in Nature Reviews Microbiology where he has shown that evolutionary success of *Mycobacterium tuberculosis* causing TB depends on the vertical genome reduction whereas *Helicobacter pylori* causing gastric cancer and ulcer depends on horizontal gene acquisition. These examples indicate how loss-of-function mutation, genome reduction, and horizontal gene acquisition by pathogen can impact its metabolic fitness and virulence.

12.5 Metabolic Adaptation and Bacterial Population Behavior

Why considering the population behavior is important than individual bacterial behavior in fitness dynamics? During infection when bacteria kill the host and when not depends on the metabolic fitness it offers. When killing the host ceases the chance of bacterial propagation, it will prefer not to kill the host. Its virulence will decrease and pathogen will not kill the host. Thus, sustenance of host increases the chance of bacterial propagation and evolutionary survival. The evolutionary success drives the direction of metabolic adaptation. This also emphasizes that population choose metabolic adaptation for their evolutionary success.

Further, cooperativity in the bacterial population is fortified by cell-cell communication. In bacterial population, cells collaborate with one another by exchange of signaling molecules, even in heterogeneous bacterial population in host like gut microbiome. In gut microbiome, multicellular behavior is regularly seen as constrained of resources, which may cause colony or biofilm formation which is an inclusive procedure for unicellular life forms to overcome environment challenges in collective and cooperative manner.

12.5.1 Synchronize Multicellular Behavior of Bacteria

E. coli colonies show impressive spatial association. Surface electron microscopy (SEM) uncovers zones inside colonies described by cells of particular sizes, shapes, and multicellular course of action (Shapiro 1987). Vertical areas through colony reveal stratification into layers of cells with various protein substances, a large number of which seem to be nonviable (Lyons and Kolter 2015; Shapiro 1992). Apoptosis of myxobacteria creates the zones of fruiting bodies leading to its rapid growth (Allocati et al. 2015). This shows an unforeseen limit with respect to cell division and making specific zones of differentiated cells in *E. coli* K12 colonies. *B. subtilis* can emerge with the potential to divide in different cell types when stimulated by extracellular signals from external environment. This capacity of *B. subtilis* to differentiate into different cell types offers survival advantages to the bacterium (Vlamakis et al. 2013).

Swarming is a group phenomenon far reaching among flagellated bacteria wherein the microbes relocate by and large over a strong surface and show expanded protection from antimicrobials. Swarming microbes have been grouped into two classes: strong swarmers that can explore over any agar surface, especially crosswise over hard agar (1.5% agar or more), and mild swarmers having capacity to swarm on the surface of relatively soft agar (0.5% to 0.8% agar). The swarming motility of any bacterial population is dependent on their aggregation. Single isolated bacterium cannot swarm over agar, but population of swarmers can do that. The capsule made up of polysaccharide in *P. mirabilis* facilitates the swarming, whereas mutants lacking capsule formation cannot swarm (Zablotni et al. 2018). *S. liquefaciens* swarmer cell differentiation is regulated by ectopic expression of the FlhDC (Soo et al. 2008). A second degree of swarming control in *Serratia* includes acyl-homoserine lactones (AHL) signaling. Mutant lacking functional swrI

homologue of luxI loses swarming capabilities (Liu et al. 2011). *Serratia* swarm colonies produce cyclic peptide surfactants basic to motility (Daniels et al. 2004).

12.5.2 Adjustive Preferred Position from Multicellular Participation

As per developmental view point, bacteria are diffusely disseminated over numerous metabolic, regulatory as well as micro-evolutionary and genome modification includes just as favorable circumstances of development rate and population size. Ordinarily in culture media, microbes are provided with basic substrates. In nature, numerous bacteria catabolize large organic polymers, requiring the purposeful activity of numerous cells. For instance, myxobacteria use a "wolf pack" methodology to assault and lyse their prey life forms by freeing stomach-related extracellular proteins and engrossing the cell substance (Munoz-Dorado et al. 2016). *Myxococcus xanthus* shows autoaggregation caused due to environmental stress. When energy resources are plentiful, myxobacteria show single species swarm formation, while under resource-limiting condition, it shows the multicellular development (Vaksman and Kaplan 2015; Bretl and Kirby 2016).

Numerous agents can adequately cause secluded bacterial cells in suspension however are ineffectual against thick or dense population of similar bacteria. For instance, *S. aureus* is one of the most widely recognized hospital-acquired gram-positive bacteria (Tong et al. 2015). Biofilm development gives bacterium a survival benefit, for example, mechanism of quorum sensing or horizontal gene acquisition may increase tolerance to antimicrobials and cause immune evasion (Gebreyohannes et al. 2019). Multicellular defense has wide impact in survival of pathogens. Numerous microbes produce anti-toxins under the influence of intercellular communication and quorum sensing, in general.

12.5.3 Infection Dynamics and Impact on Host

Bacterial infection is great cause of worry for human health particularly with increasing antibiotic resistance (Ventola 2015). Clinical manifestation of diseases caused by pathogenic microorganisms shows interchange of factors between organisms and host. While the inborn and adaptive immune system effectively contains the disease, the acquisition of new factors by microbes makes significant impact on their fitness. Macrophages are primary phagocytes that are regularly targeted by pathogenic microorganisms for intracellular growth (Mitchell et al. 2015). A few species have advanced the mechanisms to modulate the host determinants for intrusion, replication, and proliferation within host (Bourdonnay and Henry 2016). The intracellular way of life protects bacteria from the adaptive immune response of the host (Thakur et al. 2019; Uribe-Querol and Rosales 2017). Intracellular bacteria additionally have better availability of micronutrients compared with other bacteria. Bacterial species living inside professional phagocytes like macrophages incorporate *Salmonella* (Di Russo and Samuel 2016; Hu et al. 2013), *L. monocytogenes* (Hanawa et al.1995), *M. tuberculosis*

(Mehra et al. 2013), *Shigella*, and some *E. coli* strains. Either acute or persistent infection can be established in the host without any clinical symptoms.

There are models based on fitness to explain infection outcomes:

(a) *Containment, growth inhibition, and bacterial clearance:* This occurs as a consequence of exceeding microbial death compared to their growth as well as replication inside macrophages. Here, growth is insufficient compared to clearance during the infection in the host.
(b) *Growth followed by persistent infection:* When bacterial growth and clearance within host are balanced in a way that maintains the state of infection, the bacteria harboring macrophages behave similar to Lotka-Volterra prey-predator dynamics, where oscillations of frequencies occur for both prey and predator.
(c) *Exhaustion of bacteria and its resource cells causing:* The third case of acute infection appears when bacterial growth is very high followed by sharp decline because of exhaustion of resource. In an extreme condition, both macrophage and, thus extinction of macrophage caused by bacterial overabundance diminish bacterial population.

The cross talk between host factors and pathogenic determinants makes it difficult to map the mechanism of pathogen infection. Microbial populations infect or colonize hosts and are subjected to selection pressure, and thus their metabolic adaptive dynamics evolve (Webb and Blaser 2002). Active research in bacterial metabolic adaptation has mostly focused on the genetics, biochemistry, and physiology of the interactions between microbe and host (Levin et al. 1999). The host metabolic adaptation dynamics are difficult to measure as there are no direct interactions.

12.6 Drug Resistance Menace, Reason, and Current Approaches

Emergence of antibiotic resistance and its spread in the nature is a huge threat to mankind and the wilderness. Use of antibiotics as a growth promoter in livestock in excess, increased international travel, poor hygiene, lack of best practices in use of antibiotics, and polluting water bodies by industrial effluent having antibiotics and household discharge containing remains of antibiotics accelerate the antibiotic resistance development over the globe. These factors collectively impart to the selection pressure which ultimately leads to the emergence of superbugs (multidrug and extreme drug resistance) infection (Hawkey et al. 2018). Moreover, certain pathogenic infections like pneumonia, tuberculosis, and salmonellosis are becoming hard to treat as the pathogenic strains are getting resistant and the antibiotics lose their effect. The sincere intervention efforts are required to address the issue of antibiotic resistance.

Most of the global pharmaceuticals assume that research for developing new antimicrobials are not financially advantageous as any new antibiotic will eventually lose its effect due to development of resistance. Long-term strategies are needed

to devise the approaches for preventing drug resistance like improvising hygiene, running vaccination programs, devising alternative medicines, and making the public aware against misuse/overuse of antibiotics (Hughes and Karlen 2014).

Drug resistance is a sort of ecological calamity with enormous damage potential for humans and livestock. Various global organizations such as the Food and Agriculture Organization (FAO) and Centers for Disease Control and Prevention (CDC) are putting serious efforts at the global level to combat the calamity of drug resistance. Global Health Security Agenda (GHSA) and Antimicrobial Resistance Action Package are other such programs dealing with the global threat of developing drug resistance (Aslam et al. 2018; Brown et al. 2017).

Some countries are getting success in combating drug resistance by adopting certain approaches, viz., advancement in healthcare setup, restricted drug promotions, adopting health insurance policies, cautious use of antibiotics, and developing consistence disease control strategies (Sakeena et al. 2018; Van Boeckel et al. 2015).

12.7 Conclusion

In the last decade, impressive development has been made in our understanding of mechanisms of infection biology, though we only know the tip of the iceberg till yet. Every pathogen cooperates differently with its host. Pathogens utilize a mixed technique for intracellular survival and hijack the host defense mechanism in a sophisticated manner.

In a nutshell, bacterial evolutionary forces drive the direction of metabolic adaptation. This metabolic adaptation is quite fluidic and easily reshapes itself with appearance of new challenges. Also, the collective behavior of heterogeneous bacterial population and their interaction will contribute significantly in the metabolic adaptation. The better understanding of bacterial adaptation mechanisms can help us developing new treatments that instead of targeting single pathway which is a source of drug resistance, can provide a way to redirect the bacterial adaptation in a way decreasing their pathogenicity thereby weakening the infection born challenges for the host.

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