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Review Article

Exploring Bacterial Metabolism and Bacteria-host Interplay by Using Whole Genome Sequencing Techniques

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Abstract

Infectious diseases caused by bacteria are still huge healthcare and economic burdens worldwide. Bacteria employ many different metabolic strategies to obtain nutrients from environment or host for survival and proliferation. A key part of the metabolism of bacterial pathogens is the synthesis and secretion of a variety of virulence factors, which are involved in the bacteria colonizing, adhering to and/or invading host cells, breaking host defense systems, and causing diseases. These virulence factors are encoded by bacterial genomes and controlled by bacteria-host interplay and micro-environments. Whole genome sequencing techniques are valuable tools. The investigation of bacterial metabolisms by using next-generation and third- generation sequencing approaches is important for understanding the molecular mechanisms of bacteria-host interplay, which help to clarify bacterial pathogenesis and host immune response, as well as to identify virulence factors and antibiotic resistance determinant genes. This article aims to elaborate whole genome sequencing approaches that are applied in studying the metabolic activities of pathogenic bacteria-host interaction. The knowledges of bacteria-host interplay, especially the expression and regulation of virulence factors and antibiotic resistance determinants can be used to detect, reduce, and prevent infectious diseases, as well as to trace the infectious sources of outbreaks and develop antibacterial therapeutics.

Keywords: *Pathogenic Bacteria; Metabolism; Virulence Factor; Antibiotic Resistance Determinant Genes; Next-Generation Sequencing; Third-Generation Sequencing*

Introduction

Infectious diseases caused by pathogenic bacteria are still a big concern for human health, and a huge economic burden in the world. Bacterial metabolism is all biochemical activities that occur in a cell including catabolism and anabolism. Bacterial metabolism synthesizes macromolecules from smaller molecules, and breaks down complex organic molecules such as carbohydrates, proteins, and lipids, and generates energy [1,2]. Bacteria use many different metabolic strategies to obtain nutrients and energy from environments or hosts for survival and proliferation. The catabolism or anabolism in a bacterium is not fixed, and have different metabolic pathways, which hinge on bacterial species, available nutrients, and a variety of environmental conditions. Thus, the pathways of bacterial metabolisms inside the human body are significantly different with those in the free living stages of bacteria.

For surviving inside the human body, bacterial pathogens synthesize and secret a variety of virulence factors to cross skin and mucosa barriers, adhere to/invade host cells, deprive nutrients, and counteract host immune response. Bacterial virulence is a term that describes the abilities of pathogenic bacteria colonizing, adhering to and/or invading host cells, fighting against host immune response, and causing diseases. Virulence factors include bacterial toxins, cell surface proteins and carbohydrates, secreted proteins and enzymes, and others [2-5]. These virulence factors are encoded by bacterial genomes, and regulated by regulators, which are controlled by host-pathogen interplay, nutrients, and micro-environment.

Molecular biologic technologies are extensively applied in the investigation of bacterial metabolic activities. Whole Genome sequencing (WGS) technologies bring a new era in bacterial metabolic investigation, especially in the identification of virulence factors and antibiotic resistance determinant genes [6-8]. New sequencing techniques including next-generation sequencing (NGS) and third-generation sequencing (TGS) show great advantages over first- generation sequencing by generating high throughput and speed at a cost-effective way [9,10]. Bacterial survival in the human body depends on the capability of activating the synthesis of virulence factors, which are encoded by bacterial genomes to counteract host defense system, to adhere, invade, and colonize in the human body, as well as to assist the organisms depriving nutrients from host. WGS approaches are great tools for investigation of the metabolic alternation.

Bacterial pathogen and host interplay may result in some genomic changes and adaptions in both sides of bacteria and host. The studies of the bacterial metabolic changes and adaptions help to clarify bacterial pathogenesis, host immune response, and the molecular basis of host specificity, as well as find out promising bacterial or host genes for developing new antibacterial agents. Although important development has been obtained, the molecular information of bacterial metabolic pathways, especially that of pathogenic bacteria-host interplay is still limited. From the view of bacterial metabolisms, investigating the altered synthesis and regulation of virulence factors in responding to varied micro-environments and nutrient availability inside the human body has not fully accomplished. This article aims to elaborate WGS approaches that are applied in studies of the metabolic activities of pathogenic bacteria-host interplay. The knowledge of virulence factors and antibiotic resistance determinants can be used to detect, reduce, and prevent bacterial diseases, as well as to trace outbreak sources and develop antimicrobial agents.

Metabolic activity between pathogenic bacteria and host

For successful colonization and survival inside the human body, pathogenic bacteria activate a variety of metabolic activities including the synthesis and secretion of virulence factors to circumvent host immune response and survival inside the human body. Bacterial virulence factors are encoded by genes, and assist the organisms to adhere to, invade, multiply, and survive in host cells. Various studies have reported that bacterial virulence factors include bacterial toxins, cell surface proteins and carbohydrates, secreted proteins/enzymes, plasmids, adhesins, invasion-related factors, and biofilms [2,4,5,11].

Toxins: Bacterial toxins are composed of exotoxins and endotoxins. Exotoxins are proteins produced and released from viable pathogenic bacteria including cytotoxins, neurotoxins, and enterotoxins. Famous exotoxins are tetanus toxin and botulinum neurotoxin, which have been applied in several toxin/toxoid vaccines [12]. Cytolethal distending toxin (CDT) found in *Campylobacter* [13] blocks target eukaryotic cells in the G2 phase and leads to cell apoptosis. Endotoxins are lipopolysaccharides (LPS) located on the outer membranes of Gram-negative bacteria, which cause fever, inflammation, lethal shock, and other symptoms.

Flagella and motility: Flagella are required for bacterial motility and chemotaxis, which also contribute to bacterial adherence to their host cells. Flagellin is also an agonist of toll-like receptor (TLR) 5, and the binding of flagellin and TLR5 activates innate immune responses [14].

Capsules: Many pathogenic bacteria are surrounded by capsules that protect the organisms from phagocytosis. The polysaccharide Vi capsule of *Salmonella typhi* is a key virulence factor, and can inhibit rapid neutrophil recruitment by blocking LPS recognition [15-17].

Important metal ions: Iron, zinc, manganese ions, and other metal ions are cofactors of many proteins and enzymes involving in the regulation of gene expression, oxidative stress resistance, and virulence [18-20]. Siderophores are iron-chelating small molecules secreted by some bacteria, and transport iron across cell membranes. Siderophores can also compete with hosts for iron absorption and transport because iron is the important cofactors of human hemoglobin, transferrin, and lactoferrin.

Fimbriae, pili, and outer membrane vesicles (OMVs): Many pathogenic bacteria colonize mucosa by using pili and fimbriae to adhere to the mucous cells [21]. The OMVs of Gram-negative bacteria consist of LPS, phospholipids, and proteins, which can stimulate the innate immune response [22,23].

Bacterial adherence and invasion: Pathogenic bacteria express diverse surface adhesins and invasion-related proteins and other factors that interact with epithelial and mucous cells, and help bacteria across skin and mucosa barriers into deeper tissues to spread infections [4,5,21].

Intracellular bacteria including *Salmonella, Shigella, Yersinia, Campylobacter* and other bacteria communicate with host cells via biochemical crosstalk and signal transduction pathways, resulting in bacterial entry into host cells. For example, effector proteins encoded by the genes of type 3 secretion system are involved in *Salmonella* invasion and survival. T3SS is a molecule machine, and functions as a needle to inject effector proteins into host cells. T3SS1 is linked to the bacterial internalization of non-phagocytic cells and activation of proinflammatory responses [22-25]. T3SS2 is required for intramacrophage survival, as well as systemic infection in mice [26-28].

Bacterial survival in macrophages

At early stage of infection, some pathogenic bacteria are effectively killed by monocytes and macrophages, but other bacteria can fight against the bactericidal components of macrophages and survive in the cells [11,29,30]. Pathogenic bacteria such as *Salmonella*, *Yersinia*, and *Campylobacter* are capable of resisting host immune response. For example, after internalized by macrophages and monocytes, *S. typhi* can survives in the cells. *Staphylococcus aureus* protects themselves by producing coagulates and clumping factors to avoid phagocytosis and killing [31].

Biofilm formation

Many bacteria can form biofilms, which are associated with the ability of bacterial persistence in harsh environment. Bacteria growing in biofilms gain more resistant to antimicrobial agents over their planktonic counterparts. *Salmonella* biofilms are composed of cellulose, curli, fimbriae, BapA, and polysaccharides [32-34]. *Cronobacter* also form biofilm which provide the organisms more resistant to dryness, heat, osmotic stresses, and antibacterial agents, and may contribute their long-term survival and catheter-associated wound infection [35,36].

Therefore, understanding metabolic activities between bacteria and their host can identify key virulence factors. Bacterial sequencing data provide valuable molecular information about bacterial pathogenesis. Capsule, flagella, adherins, invasion-related components, etc. are important virulence factors, and they play key roles in bacterial adhere, invasion, colonization, and breach of host defense system for growth and multiplication. The synthesis and regulation of virulence factors in the interaction of bacteria and host are not fixed, and depend on the specific micro-environment and available nutrients in host. Investigation of the synthesis and regulation of the virulence genes in different micro-environments and nutrient availability will bring new understanding of bacterial pathogenesis, and find efficient preventing and treating strategies.

The advantages of whole genome sequencing

Genomic analysis is an important tool for exploring bacterial metabolic alterations, especially for investigating the metastatic activities between pathogenic bacteria and host. WGS and computational analysis can be employed to the in-depth exploration and comparison of bacterial genomes with high accuracy [8-10,37-39]. Genomic variation between two or among multiple bacterial species, subspecies or strains can be identified and analyzed to find virulence genes and drug resistance determinants. The genome-scale data related bacterial metabolism are also good at tracing strain sources in epidemical screening and outbreak analysis.

The general workflows of WGS are (1) obtaining bacterial pure cultures or appropriate samples; (2) DNA extraction and quality control; (3) DNA library preparation that may include fragmenting DNA into appropriate lengths by mechanical or enzymatic treatment, blunting the ends, adaptor ligation, and amplification; (4) sequencing; (5) data processing [37-39].

Comparing with other molecular approaches, WGS technologies with computational analysis are good for: (1) demonstrating genetic variations such as single nucleotide polymorphism (SNP), low frequency mutations, copy number changes, deletions, and insertions in a bacterial genome; (2) identifying unknown or novel organisms; (3) bacterial typing and subtyping; (4) aligning and comparing sequences from multiple species and subspecies, strains, or same strains isolated from different hosts or environments to explore bacterial metabolic alternation and adaption.

Sequence data can be applied in bacterial metabolic studies to (1) detect bacterial species or subspecies based on specific metabolic capabilities; (2) identify virulence factor for developing bacterial detection methods, pathogenesis studying, and disease diagnosis; (3) identify antibiotic determinants to develop antimicrobial therapeutics [40]; (4) analyze and compare multiple bacterial genomes in complex microbial communities, fixed host cells or tissues for understanding complex bacteria-host interaction *in situ*; (5) examine the evolution of bacterial metabolism.

In first-generation (Sanger sequencing) sequencing, multiple short fragments are sequenced, and assembled by overlapping or comparing with a known reference [37-39]. Although first-generation sequencing techniques provide remarkable knowledge about nucleic acids, the need of cloning each DNA fragment and high costs limit their application.

Next-generation sequencing (NGS) (also called second-generation sequencing) technologies have largely improved throughput and speed with reduced cost. The advantages of NGS over first-generation sequencing are sequencing millions of short read DNA fragments in a single run without the need of cloning. NGS techniques simplify the general workflow and reduce the time and cost, and can identify low frequency variants and genome rearrangements. However, the sequence assembly and analysis by NGS require heavy computer work and well-trained personnel [41].

Third-generation (single molecule) sequencing (TGS) technologies generate longer read length fragments (1,000 bp reads or > 10,000 bp), and skip library preparation step, which makes it easy to sequence and assemble of repeated and complex DNA [42-44]. Moreover, in TGS approaches, individual nucleic acid of target DNA molecules is directly sequenced without an amplification step [37-53]. These three sequencing approaches are summarized in general in table 1 to help to understand the features, advantages, drawbacks, and potential applications of the approaches.

Characteristics	First-generation sequencing	Next-generation sequencing	Third-generation sequencing
Read length	~500 - 1000 bp	50 - 100 bp or other read lengths	> 500 - 1000 bp (60 - 100 kb)
Time to result	Slow	Fast	Fast
Accuracy	> 98%	> 98%	> 85%
Speed	Low	High	High
Cost	High	Cost efficient	Cost efficient
Throughput	Low	High	High
Sequencing reaction	Sanger method	Pyrosequencing, reverse dye terminator, sequencing by ligation or synthesis	Signal-molecule sequencing, ironic current sensing
Sequencer	ABI 3730xl (Applied Biosystems)	GS FLX+ (Roche 454); Ion Torrent PGM (Life Technologies); SOLiD (Life Technologies); MiSeq, NextSeq, and HiSeq (Illumina)	PacBio RS (the Pacific Biosciences); Heliscore single molecule sequencer (Helicos Bioscience Cooperation); GridION and MinION (Oxford Nanopore Technologies);
Advantage	High quality sequences	Without the need of cloning, high quality sequences	Long and high-quality read length, real time sequencing, portability, high speed
Drawback	Low throughput, low speed, and high cost	Short read, the need of heavy computer work and well-trained personnel	High error rate, expensive equipments

Table 1: *Comparison of first-, next-, and third-generation sequencing techniques.*

Recently, some authors use fourth-generation sequencing to describe *in situ* sequencing approaches which perform genome sequencing directly in fixed cells or tissues. The sequencing approaches base on second generation sequencing technologies and enable to conduct sequencing *in situ* [45-47].

Conclusion

Understanding the molecular mechanisms of bacterial metabolism is important, and can help to clarify bacterial pathogenesis, host immune response, virulence factors, and antibiotic resistance determinants. Biological molecular technologies have provided valuable information about bacteria-host interplay.

Over the last two decades, WGS has been identified as one of the most promising tools in the studying of bacterial metabolism. Rapid progresses in sequencing technologies and computer software have largely improved the throughput and speed, and lowered down the cost. WGS and computational analysis can be used to the in-depth exploration and comparison of bacterial genomes fast and accurately. Next- and third-generation sequencing techniques provide us not only a detailed picture of one bacterial genome, but also very full pictures of the bacteria-host interplay and bacterial population *in situ*.

WGS techniques can be applied in studies on how genomic change and adaption affecting the bacterial metabolism. The bacterial genome-scale data are good at studying bacterial metabolism and bacteria-host interplay for identifying virulence factors and antibiotic resistance determinant genes for developing bacterial detection methods, pathogenesis studies, and disease diagnosis. WGS can be also used to analyze multiple bacterial genomes in complex microbial communities, fixed host cells or tissues for understanding bacteria-host interaction *in situ*.

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