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Towards Microbial Metabolism in Future

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Introduction

The use of organisms as biopharmaceutical production factories offers several advantages over chemical synthesis. Microbial metabolism consists of networks of reactions that bacteria use to convert nutrients into molecules that make up the cell and release energy for cell processes. Such networks have an enormous potential to produce a huge range of molecules that might be useful, like biofuels or biopharmaceuticals. Microorganisms can produce high molecular weight compounds such as proteins [1] and carry out highly enantio- and regio-selective reactions by their native enzymatic machinery – these reactions are hard to achieve by chemical synthesis. The use of microorganisms also enables repeated implementation of immobilized enzymes or cells resulting in the reduction of the overall production costs [2]. However, the high complexity of bacterial metabolism has considerably limited such efforts. This calls for new approaches that can incorporate the complexity of metabolic systems and predict appropriate modifications. This article provides insight into microbial metabolism, approaches and caveats of their use.

Understanding of the microbial metabolism is not just defining and cataloging the metabolic processes. The models will be helpful to get better knowledge about metabolism. Historically, model organisms have been used to generate knowledge about the biological pathways. For decades, ideal bacteria for studying microbial metabolism are *Escherichia coli* and *Salmonella typhimurium* [3]. These organisms are serving as a solid foundation for work in other organisms. The current technological advantages have started considering the metabolism as a system [4]. Based on the system, number of mathematical models and metabolic maps has been generated which allows visualization of various metabolic connections which in turn may predict behaviors [5]. For example, a thermodynamic-kinetic model describe the metabolic reaction network where it provides a physically consistent systems-level view of the central carbon and energy metabolism of *E. coli* and its regulation [6]. However, it is important that, these models should be potential enough, descriptive and provide information about the components and connections being modeled. Carefully defining the components and connections may offer the comprehensive model building. Additional data on protein function and metabolic interaction may gives significant advantage to these theoretical models.

Well studied microorganism also has limitation and unable to provide the complete knowledge. As discussed earlier, the understanding of metabolism requires the defining of functional contribution of components and connections. The components and connections represent the function of whole system and hence observation of complete cell is the best way to investigate function. Advancement in this field has increased using of whole cell approach for understanding the metabolism. This has helped to observe the various parameters on global scale through various technologies, development and identification of diverse mutation and expression and purification of components. This in turn results in reduction in technical barrier and obtaining needed data. Most important result of this progress is probing the grey areas of metabolism. Understanding the grey areas is critical to expand our knowledge of metabolism over basic

considerations. Even though the emerging approaches are continuously developing but any single approach may not be able to provide the understanding of microbial metabolism. The knowledge and recognition of its strength and limitation will help for choice of approaches. At the same time, any approach should possess the strong knowledge of basic principles outline in central dogma. Few of the approaches with strength and limitations are briefed in the below text.

Various approaches for studying the microbial metabolism

Bioinformatics approach

Bioinformatics, which aims to discover new biological concepts and laws based on large-scale data, is now expected to accelerate discovery in unexamined areas of the microbial universe. Advancing bioinformatics capabilities provides the means to cluster these data in informative ways and mine sequence data to identify probable homologs/orthologs/paralogs, promoters, regulatory binding sites, signaling networks, and enzymatic motifs [7,8]. The data deluge has made bioinformatics indispensable in modern research; recent innovative technologies are producing large amounts of data at an unprecedented pace. Observations are key to science; for example, optical and electron microscopies are important methods of observation combined with various staining methods. Among recent observational technologies, high-throughput DNA sequencing technologies have rapidly produced vast amounts of genetic information at low cost, making available thousands of microbial genomes. These genome sequences provide a comprehensive catalog of the microbial genetic elements underlying diverse microbial physiology, and also assist in weaving a massive tapestry of microbial evolutionary histories [9]. Despite the fast paced global effort, the current analysis is limited by the lack of available gene-functionality from the wet-lab data, the lack of computer algorithms to explore vast amount of data with unknown functionality, limited availability of protein-protein and protein-DNA interactions, and the lack of knowledge of temporal and transient behavior of genes and pathways. The bioinformatics technologies do not provide fundamentally new functional information, or definitive mechanistic data.

Biochemical approach

There are many ways to detect, characterize, and identify microorganisms. Some methods rely on phenotypic biochemical characteristics, while others use genotypic identification. The biochemical characteristics of a bacterium provide many traits that are useful for classification and identification. However, more modern systems such as the one developed by Biolog, Inc., are based on panels of biochemical reactions performed simultaneously and analyzed by software. Biolog's system identifies cells based on their ability to metabolize certain biochemicals and on their physiological properties, including pH and chemical sensitivity. It uses all major classes of biochemicals in its analysis. Rigorous biochemical analyses of cellular processes will remain a critical aspect of studies on metabolism for the foreseeable future. Biochemical analysis of enzymes, cell membrane and other processes in a defined *in vitro* system has been considered the hallmark of understanding a molecular process. For example, Ion-pumping rhodopsins bacteriorhodopsins and halorhodopsins are identified and characterized using biochemical approach in the 1970s [10]. Ion-pumping rhodopsins transfer ions across the microbial cell membrane in a light-dependent manner. If the ion translocated is H+, then the resulting ion gradient can contribute to the proton motive force (PMF) used to synthesize ATP or power flagellar rotation) [11,12]. The strength of a biochemical approach is that it tests directly the function of a component and thus eliminates complications caused by indirect effects present in the natural system. However, without complementary *in vivo* data, biochemical results do not define the cellular role of a protein, nor do they detect side reactions, regulatory controls, or accessory components that could be relevant *in vivo*.

The classical and molecular genetics approach

The classical approaches make use of morphological, physiological, biochemical, ecological and genetic characteristics. They are quite useful in routine identification and may provide phylogenetic information as well. It will be useful to study and analyze the eukaryotic microorganisms and the more complex prokaryotes, the nature and activity of microbial enzymes and transport proteins, life-cycle

patterns, ability to cause disease, symbiotic relationships, energy sources, motility, photosynthetic pigments etc. The molecular genetics is the approach of determining the genetic basis responsible for a phenotype. This was initially done by using naturally occurring mutations or inducing mutants with radiation, chemicals, or insertional mutagenesis (e.g. transposable elements). A genetic approach requires rigorous, creative thought and integration of known facts to generate testable hypotheses. For example, bacteria behave differently in space compared to earth. It result from reduced mass transport in the local extracellular environment, where movement of molecules consumed and excreted by the cell is limited to diffusion in the absence of gravity-dependent convection. However, to date neither empirical nor computational approaches have been able to provide sufficient evidence to confirm this explanation. Molecular genetic analysis findings, conducted as part of a recent spaceflight investigation, support the proposed model. This investigation indicated an overexpression of genes associated with starvation, the search for alternative energy sources, increased metabolism, enhanced acetate production, and other systematic responses to acidity-all of which can be associated with reduced extracellular mass transport [13]. Like other approaches, when used in isolation, genetic analyses have significant weaknesses. While genetics has the potential to uncover new functions and subtle interactions, it does not allow conclusions about mechanism nor does it easily differentiate between direct and indirect effects.

Constraint in interpretation of results in microbial metabolism

Interpretation of the data obtained during the microbial metabolism is very challenging and have certain caveats in it such as:

Wild-type strains are not metabolically stable

Experiments or research work depends on the comparison of standard with the new concept. The standard in the experimental biology is called as wild-type. The wild type strain provide stability and other component can be compared with it. With an isolated component, this assumption is generally valid. However, when experiments involve analysis of metabolism, this assumption is flawed. For example, Cyanobacteria have evolved over a long period of time to be successful in their native dynamic environments. Many research groups are interested in engineering the metabolism of cyanobacteria with the objective to convert solar energy, CO_2, and water (perhaps also N_2) into commercially valuable products. But it is challenging because of genetic instability [14]. The metabolic instability of strains not only applicable for experiments in the same lab on day to day basis, but also for the comparison and reproducibility of results in different labs. Bluetongue is a major infectious disease of ruminants caused by bluetongue virus (BTV), an arbovirus transmitted by *Culicoides*. Researcher investigated how mammalian host species, breed, age, BTV serotypes, and strains within a serotype affect the clinical course of bluetongue. They observed striking differences in virulence between closely related strains of the same serotype collected toward the beginning and the end of the European BTV-8 outbreak. As observed previously, differences in disease severity were also observed when animals were infected with either blood from a BTV-infected animal or from the same virus isolated in cell culture [15]. The validity of interpretation from metabolic studies can be increased by the generation and use of isogenic strains and the application of statistical methods. The researcher should put efforts to control critical aspects of the experiments and results should be statistically supported.

Enzyme promiscuity

Enzyme promiscuity is the ability of an enzyme to catalyse a fortuitous side reaction in addition to its main reaction. Although enzymes are remarkably specific catalysts, they can often perform side reactions in addition to their main, native catalytic activity [16]. The anticipated prevalence of promiscuous enzymes in metabolism has implications for interpreting genetic experiments. Minor activities can be amplified by multi-copy expression, regulation, or mutations affecting the enzyme directly. For example, Hydration of coenzyme NADH or NADPH is common, occurring either non-enzymatically or as a side reaction of promiscuous enzymes such as glyceraldehyde 3-phosphate dehydrogenase. The resulting R and S hydrated forms of NAD(P)H display high structural similarity with the cofactors but are non-functional and inhibit various dehydrogenase reactions [17]. Hence multiple activities during microbial metabolism should be considered while interpreting the results or outcome.

Positive and negative effects of metabolites

Regulation is a critical component of metabolism. While the prevalence of transcriptional regulation and the significance of the resulting regulons are widely appreciated, allosteric and metabolic regulation also permeate the system. Cellular metabolites (i.e. intermediates or byproducts of a biochemical pathway) can affect metabolism in positive or negative ways. For instances, scientists have discovered a crucial factor in the spread of cancer. A team has demonstrated that the metabolism of macrophages, a particular type of white blood cell, can be attuned to prevent the spread of cancer. The key is in making these macrophages more prone to 'steal' sugar from the cells forming the tumor's blood vessels. As a result, these blood vessels will be structured more tightly, which can prevent cancer cells from spreading to other organs. On top of their positive effect on pathogens, macrophages can also play a negative role in cancer biology. Indeed, tumors contain a lot of specific macrophages that play a decisive role in the formation of blood vessels. In tumors, these vessels traditionally have a chaotic and dysfunctional buildup. As a result, cancer cells are more likely to escape through the vessels, enter the bloodstream and invade other organs [18].

Identification of various metabolites

The ability to identify and quantify the entire set of intracellular and extracellular metabolites with molecular mass lower than 1,000 daltons [19]. The numbers of these compounds vary among different organisms, from hundreds to hundreds of thousands and in many cases their identity maybe unknown. In contrast to genome, transcriptome and proteome analyses, products generated from metabolic reactions are highly variable in their chemical structures and properties [20]. This makes simultaneously determining the entire set of metabolites at a given physiological state extremely difficult. Further complicating the analyses is the dynamic nature of these metabolites. Constantly in a state of flux, their concentrations and compositions change rapidly in response to environmental stimuli. Correlation of results may not be straightforward, since a direct link between genes and metabolites sometimes does not exist (for example, microorganisms have fewer metabolites than genes) [21]. All these factors contribute to the complexity and difficulty of metabolomics research.

Emerging areas in Microbial Metabolism

Most natural microbial systems have evolved to function in environments with temporal and spatial variations. A major limitation to understanding such complex systems is the lack of modeling frameworks that connect the genomes of individual species and temporal and spatial variations in the environment to system behavior. These can be prompted and facilitated with the evolution of new genomic, bioinformatics and mathematical tools. For example, the emerging field of spatiotemporal metabolic modeling based on genome-scale reconstructions of microbial metabolisms is the next frontier for microbial metabolic modeling and a rapid increase in methods development and system applications is anticipated [22]. The organization of the various "omics" in a hierarchical fashion enables an integration approach which becomes the foundation of systems biology. There are multiple benefits but most importantly, integration and correlation of data sets provide insights not obtainable from other techniques. A number of annotation and statistical tools as well as network analysis software and databases have been developed such as Kyoto Encyclopedia of Genes and Genomics, KEGG; Clusters of Orthologous Groups, COG; Entrez Gene, Transport DB and UniProtKB etc [23-25]. Technologies for mining genomic data continue to progress and offer a means to fill in missing but predicted functions; suggest function by coinheritance; cluster genes by expression, location, and proposed function; and predict metabolic roles and regulatory characteristics. The integration of physical and biological sciences is further evidenced by the evolving efforts to model biological systems with in silico analyses. Continued description of components with experimental science will facilitate the generation of more comprehensive models that provide not only a catalog of data but also a valuable predictive feature.

Conclusion

The next decade of genomics will continue to emphasize function analyses and promote a systematic and integrated approach for life

science studies. Microbiological research has already adopted this perspective, yielding results and insights not possible with traditional methodology. Microbes are no longer regarded as isolated organisms existing in a system, but rather an integrated component for understanding functional biology. The most pressing need in the field of metabolism is the identification of new functional paradigms and the resulting expansion of the data on which computational programs and mathematical modeling efforts are based. The number of genes that cannot be functionally assigned, despite modern computational programs, emphasizes that fundamentally new functional insights are needed. Efficient interdisciplinary collaboration is paramount to the advancement of systems biology. The proven success of classic logical thought combined with continuing technological advances makes this an era of great potential and excitement for understanding microbial metabolism.

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