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Microbial Proteomics: Approaches, Advances, and Applications

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Abstract

At present, whole genome sequences are reasonably available for many organisms. However, genomics alone is insufficient in providing comprehensive information on the mechanisms underlying the biological processes of an organism. With the development of various proteomic technologies, proteomics has enhanced our understanding of biological processes on a global scale. Enormous efforts have been exerted to deeply inspect microbial systems using proteomic technologies. Inspiring progress and achievements have facilitated our understanding of the cellular physiology of microorganisms in many aspects. In this review, we present different proteomic technologies and advances, and enumerate their significant applications in microbial studies. Proteomic studies provide identification or/and quantitative measurement for proteins from microorganisms. This review discussed the molecular physiology of microbial systems when facing external environmental stimuli, and illustrated the pathogenic mechanisms of microbial pathogens and their significance to clinical vaccine selection on a proteomic scale. Last but not least, the review quoted some important studies of microbial metaproteomics.

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Introduction

Trillions of microbes flourish in our surroundings; however, most of these microbes are invisible to naked eyes. Given their simple cellular structure and less-demanding condition of culture, microorganisms have been subjected to extensive studies. Compared with "immense" species such as plants and animals, microbes possess relatively tiny genomes, which are more feasible for sequencing. Proteomics has been widely used in both fundamental and applied research to uncover metabolic mechanisms underlying cellular processes. Many studies utilized the diverse tools of proteomics to probe bacterial protein expression profile in specific conditions. Microbial proteomics has been entirely applied to study hotspot issues of interest, such as stress responses, extreme environment adaptation, microbial pathogenicity, and metabolic engineering.

Many completely sequenced microbial genomes of different organisms are available to date. A genome comprises an inventory of genes; it provides complete gene sequence information of an organism. Genome sequencing is no longer a challenge to researchers. However, the existences of incomplete and error-prone annotations in microbial genomes are pervasive. Genomics alone is obviously insufficient in providing comprehensive understanding of the biological mechanisms of an organism. In the past decade, the genome has been accepted to only represent the first layer of complexity in an organism. According to de Hoog and Mann[1], biological function is mainly carried out by the dynamic population of proteins, which reflects the interplay of gene and protein regulation with extracellular influences rather than the static genome. Conversion from genes to proteins is complicated. This process involves a range of post-transcriptional processes (e.g., alternative splicing) and post-translational modifications. Numerous high-throughput technologies have evolved into considerably mature and powerful tools for genomic and transcriptomic applications. However, these technologies are incapable to capture the biological mechanisms of highly dynamic cellular physiology. Under

this circumstance, proteomics is crucial in revealing functional mechanisms on a global level.

Proteins embody the biological functions transmitted from genes in most cases. These amazing cellular functions mainly rely on elaborate protein interaction networks that cannot be elucidated by a single protein or a small set of proteins. In 1996, Wilkins and Williams[2] introduced the term "proteome", which refers to the protein complement in a cell, tissue, or an organism. Proteomics elucidates the biological systems of microorganisms in large-scale investigation, providing invaluable information about protein abundances, post-translational modifications, localizations, interactions, and their changes. The capability of proteomics to address crucial issues in microbial field is largely dependent on the sustained development of numerous proteomic technologies, which respectively show their talent in proteomic research either qualitatively or/and quantitatively[3]. Proteomic studies systematically provide identification or/and quantitative information on proteins from microorganisms. Currently, proteomes can be expediently predicted from genome sequencing data, but they mostly remain unverified[4]. This phenomenon promptly aroused numerous proteomic studies[5]. In 1975, Patrick O'Farrell[6] designed two-dimensional gel electrophoresis (2-DE), which was used to separate proteins of *Escherichia coli*. Technologies based on 2-DE are continuously improving; nevertheless, 2-DE is still generally exploited by many laboratories as the primary tool to separate proteins[7]. By contrast, gel-free methodology directly tackling the peptide mixture digested from protein mixture has been proven efficient in separating proteins[8,9]. Liquid chromatography (LC) is regarded as a dominant approach in gel-free methodology[10]. Since 1988, mass spectrometry (MS) has established a solid position in large-scale measurement of proteins[11]. Coupled with separation technologies, currently LC in most cases, MS/MS has evolved into a versatile approach of identifying a dynamic range of proteins and their various physicochemical properties in microbes. Along with the identification information obtained from MS analyses, quantitative data are urgently required to better characterize fundamental mechanisms and regulatory pathways by capturing the concentrations of proteins associated with different states[12]. Generally speaking, there are two strategies for quantitation: 1) metabolic or chemical protein labeling with differential mass tags and 2) label-free methods with MS spectral information from a given protein[1,13]. The specific approaches for the former category include stable isotope labeling with amino acids in cell culture (SILAC)[14], isotope-coded affinity tags (ICAT)[15], and isobaric tagging for relative and absolute quantitation (iTRAQ)[16]. These technologies can be performed thoroughly to determine protein abundance in relative aspects.

Applications of proteomic technologies in microorganisms

Instead of focusing on a single pathway or simple protein complexes of an organism through traditional biochemical experiments, proteomic studies consider the metabolic states of an organism on a global scale. In this trend, large-scale proteomic technologies emerge and develop prosperously. Proteomic studies provide genome-wide identification or/and quantitative measurement for proteins from microorganisms.

Protein identification

As a cornerstone of proteomic studies, protein identification lays a firm foundation for further proteomic exploration, such as protein quantitation, cellular localization, and protein-protein interactions. MS is the most comprehensive and universal tool in large-scale proteomics, especially in the application of protein identification[12]. Prior to MS analyses, separation technologies are required to isolate incredibly complex protein samples whose performance are extremely associated with the identification results. Major separation technologies can be divided into two categories: gel-based and gel-free methods.

2-DE, the original separation technology, separates proteins based on their isoelectric point and molecular weight in the first and second dimensions, respectively[2]. Dos Santos, et al.[17] used 2-DE and MALDI-MS/MS to identify eight under-expressed and eight under-phosphorylated proteins, respectively, from approximately 1,600 spots in the eukaryotic model *Saccharomyces cerevisiae* with or without the presence of imatinib mesylate (IM). All proteins of interest have been found to possess human functional homologs and play roles in protein folding, nucleotide and amino acid metabolism, glycolysis, and translation, providing new insights into the mechanisms of adaptation and tolerance to IM. Fuchs, et al.[18] successfully integrated 2-DE data into the Protecs database, which revealed that *Staphylococcus aureus* responds to different anaerobiotic experimental setups with a general anaerobiosis response. Two-dimensional difference gel electrophoresis (2D-DIGE), which allows analysis of multiple samples on a single gel, has wider dynamic range and higher sensitivity than the original 2-DE. Eliminating the variance existing between different gels, 2D-DIGE is well suited for quantifying differentially expressed proteins under different conditions[8]. 2-DE is currently employed to accomplish differential expressions and quantitative analyses, not only identification.

Among gel-free methods, LC plays a dominant role in separation prior to MS analyses. The sufficient resolution and high detection capacity of the classic coupled approach LC-ESI-MS/MS immensely contribute to the identification of proteins[19,20]. Multidimensional protein information technology (MudPIT) is another popular method that addresses the separation problem by integrating several LC technologies. MudPIT is utilized for high-complexity proteomic samples containing proteins with large dynamic range[21]. However, the identification status relies ultimately on the performance of the subsequent mass spectrometer. Linear trap quadrupole (LTQ) was smoothly utilized in a large-scale proteomic analysis of *Mycobacterium tuberculosis*, in which the protein identification results were used to improve gene annotations in Sanger and The Institute for Genomic Research (TIGR) databases[22]. Shotgun approaches, which are incorporated methods of MudPIT and MS analyses, were employed to inspect the proteomes of *Scheffersomyces stipitis* during xylose fermentation under oxygen restriction[23]. Huang, et al. identified 958 non-redundant proteins, from which unique expression patterns were found in biological processes and metabolic pathways, including alternative respiration salicylhydroxamic acid pathway, activation of glyoxylate cycle, and expression of galactose enzymes.

Both gel-based and gel-free methods have their own advantages and limitations when coupled with MS to identify proteins. 2-DE-MS has an evident difficulty in detecting membrane and hydrophobic proteins; hence, its detection range needs to be improved. On the other hand, the use of high-throughput

LC/MS strategy is always limited by its high cost. Some studies used a combination of gel-based and gel-free strategies to identify more proteins. Furthermore, subcellular fractionation followed by protein enrichment technique can provide a higher resolution in proteomic identification, but may also introduce some bias.

Quantitative proteomics

Quantitative proteomic studies are crucial to understand the functional behavior of an organism. From a relative perspective, quantitative proteomics primarily aims at comparing different cell states or cellular localizations to explain the relative changes in parallel experiments. Quantitative proteomic studies can either use label-based or label-free methods.

Label-based methods metabolically or chemically incorporate differential mass tags at the protein or peptide level. The introduction of stable isotope only changes the molecular weight of the targets but not the other behavior in MS[1]. Metabolic incorporation represents early labeled proteins with stable isotopes of elements or amino acids by culturing cells in a medium enriched in stable isotope-containing precursors[24,25]. For instance, a stable isotope labeled by SILAC first labels products in isotope-enriched cell culture media. Protein levels can be inferred from statistical evaluation of peptide relative-abundance ratios, which are measured by comparing heavy/light peptide pairs[12]. Soufi, et al.[26] applied SILAC to analyze the relative protein changes in *Bacillus subtilis* under two physiological conditions. With a high identification rate, they detected and quantified the dynamics of 35 Ser/Thr/Tyr phosphorylation sites under growth on succinate, and 10 phosphorylation sites under phosphate starvation, proving the great site-specific detection and quantification capabilities of this method. ICAT and iTRAQ are intensive chemical quantitative techniques. The former labels proteins by reacting with cysteine, whereas the latter marks proteins with free amines[15,16]. Based on a thiol-labeling approach with ICAT reagents, Santamarina, et al.[27] characterized the disulfide proteome of fission yeast in response to the addition of H_2O_2. They identified multitudinous proteins, including Pap1, which is also present in other redox proteomic reports. Pap1 is not only a transcription factor of the adaptive signaling pathway in fission yeast but also a sensor of H_2O_2 for cell survival enhancement. This technique can compare two disulfide proteomes with high sensitivity and specificity. Florian, et al.[28] adopted iTRAQ in conjunction with LC-MS/MS to perform relative quantitative comparisons of secretomes from interactions resulting in susceptibility and basal and gene-specific resistance using different genotypes of *Pseudomonas* on the same host. Quantitative information indicated complex patterns of accumulation and further provided the inference that the pathogen can manipulate host secretion to facilitate the successful invasion of plants.

Recently, many studies have resorted to label-free methods to quantification. Rather than labeling targets with stable isotopes, label-free techniques directly compare the peak information from the MS dataset to estimate protein abundances[8]. Spectral counts, which are now increasingly used, are proportional to the relative abundance of the protein in the sample[29].

Conclusively, metabolic labeling is mostly restricted to microbes and cell culture, whereas chemical labeling is specially limited to a few amino acids that can be tagged. Although label-free methods also suffer from run-to-run variations in separate experiments[9], they can determine the absolute level of proteins in a complete sample[8].

Advancement of proteomics in cellular physiology of microorganisms

The abilities of microorganisms to endure severe environmental stresses, such as extreme temperature, extreme pH, hyperosmosis, radioaction, dry, and toxic compounds or pollutants, and to infect their hosts, are of great value in both basic and applied research. To date, microbial proteomics has been successfully applied to certain hotspot issues of interest, such as stress responses, extreme environment adaptation, and microbial pathogenicity.

Cellular physiology under environmental stress conditions

Despite the fact that the proliferation of microorganisms requires specific environmental conditions, elastic cellular constructions enable these organisms to survive even under unfitted environment. Under adverse conditions, such as fierce changes in temperature, acidity, or oxygen density, microbes manage to operate a stress-reaction system to enhance their chances to adapt successfully to environmental changes[3]. In such environmental changes, microorganisms undergo sophisticated physiological changes to survive, which are reflected by protein expression profile alterations. Proteomic studies elucidate mysterious physiological phenomena under a particular condition for a microbe by providing information on protein abundance, subcellular localization, post-translational modifications, and protein-protein interactions. Comparative proteomics can be used to qualitatively or quantitatively acquire information on biological processes by analyzing protein expression differences. Proteins closely related to environmental stimuli assist in screening resistant or sensitive strains[3].

Proteomic studies have directed many scholars to thoroughly investigate the mechanisms underlying the physiological adaptations and responses of microbes to extensive external stresses, especially in areas of high[30] and low temperatures[31], hyperosmosis[32], oxidizing chemicals[33], and high acidity[34]. In this review, we emphasize the physiological adaptations and responses of microbes to extreme temperatures, acid, and oxidative stimuli respectively.

High temperature is a notable stress factor to most mesophilic and psychrophilic bacteria. The drastic increase in temperature triggers the temperature-response system in bacteria to synthetize an array of heat shock proteins (HSPs) to cope with survival challenge to the greatest extent. The highly conserved property of HSPs, ranging from microbes to human, indicates that this ubiquitous type of proteins has relevance to certain critical physiological functions. Although this inference has yet to be verified, HSPs as molecular chaperones are known to ensure proper protein-folding processes, prevent protein aggregation, and degrade badly damaged proteins[3]. The mechanism by which microbes respond to high temperature has been widely explored in many proteomic studies, especially in *E. coli*[35,36]. In 1978, HSPs were first recognized to have high abundance through 2-DE analyses[35]. Wu, et al.[37] used a proteomic approach to inquire the responses and survival strategies of Bacillus *thuringiensis* YBT-1520 under prolonged high temperature (42°C) condition. Comparative proteomic analysis of the

physiological changes in the strain revealed that this bacterium survives long-term heat stress by changing many metabolic enzymes and by continuously accumulating poly 3-hydroxybutyrate. In addition, small HSPs, an unclear category of HSPs with less-conserved sequences, may perform specific functions for the adaptation of microbes to specific environmental stress[38]. Contrary to mesophilic and psychrophilic bacteria, thermophilic bacteria have an extraordinary appeal to high temperature environment. This characteristic has prompted several studies to focus on the molecular mechanisms of protein folding and conformational stability of thermophiles. One of such works, Meng, et al. extracted and identified the temperature-dependent thermophilic protein complexes from *Thermoanaerobacter tengcongensis*[39]. A complete sequence of *T. tengcongensis* has been released in 2002, which contains 2,689,445 base pairs with 2,588 predicted open reading frames[40]. These Gram-negative and rod-shaped bacteria thrive in anaerobic environment under high temperatures ranging from 50 to 80°C. With the combination of LC-MS/MS and MALDI-TOF/TOF-MS, Meng, et al. detected six temperature-dependent complexes from *T. tengcongensis* cultured at three temperatures (55, 75 and 80°C) and verified 92 unique proteins in these complexes. Interestingly, the abundances of two chaperones, HSP60 and HSP10, consistently increased corresponding to the raised temperatures. All these proteomic studies focusing on heat stress responses have paved a way for revealing the mechanism for the adaptation of microorganisms to high temperature stress.

Low temperature is also a thermal stress to microbes. However, we know little about the functions of proteins responded to the reduction in temperature[41]. Recently, the change of proteins after cold stress response of microbes, such as *Lactococcus piscium*, has attracted attention because of potential applications in food biopreservation[42]. Subjecting a bacterium to temperatures lower than its optimal growth temperature triggers an immediate and transient synthesis of cold shock proteins (CSPs)[43]. CSPs induced by low temperature reduce the synthesis of macromolecules by interacting with DNA and RNA directly or indirectly, such as helicases and nucleases[6]. Strocchi, et al.[44] introduced a new method of examining essential proteins for cell viability at low temperatures with a transgenic strain of *E. coli*. By screening proteome of the cells incubated at 4°C, Strocchi, et al. identified 22 housekeeping proteins involved in the adaptation system of *E. coli* under low temperature. Similar to thermophilic bacteria, psychrophilic bacteria thrive in environments with low temperatures. Compared with mesophilic bacteria, psychrophilic bacteria have evolved to naturally adapt to cold stress. This characteristic has motivated scientists to further identify the distinguishing properties of psychrotrophic bacteria. Garnier, et al.[42] carried out a comparative proteomic analysis using 2-DE in a psychrotrophic *L. piscium* strain to analyze protein responses to both cold shock and cold acclimation. LC-MS/MS was used to identify up-regulated proteins, which were proven involved in general and oxidative stress responses, as well as fatty acid and energetic metabolism. Generally, proteins that are not normally expressed in cells are used to confer cold-shock resistance in the environment[41]. Further exploration on this respondent set of proteins will surely uncover physiological mechanisms and be of vast biotechnological significance.

Due to its highlighted importance in food safety and pathogenicity, acid tolerance response (ATR) in microorganisms has drawn considerable attention on a proteomic scale. When exposed to acidic conditions, microbes swiftly initiate the ATR system to promote survival under such adverse environment. Considering that ATR may prolong the survival of some pathogens in various food systems, determining their physiological adaptation strategies to acid stress is significant to enhance food safety. Acid adaptation and increased resistance to acid stress have been observed in various organisms, including *E. coli*[45], *Salmonela typhimurium*[46], and *Listeria monocytogenes*[47], which are major foodborne pathogens. In *E. coli*, pH distinctively regulates a large amount of periplasmic and outer membrane proteins along with enzymes participated in several pH-dependent amino acid and carbohydrate catabolic pathways[48,49]. *L. monocytogenes* is a gram-positive bacterium that can flourish at temperatures low to 0°C and can stay alive in the presence of extensive ranges of salt concentrations and pH values[50,51]. The remarkable ability of *L. monocytogenes* to thrive in diverse environments associated with various food products makes it tough to alleviate the threat to human[32]. Bowman, et al.[52] investigated the global proteomic responses of *L. monocytogenes* strain Scott A to gradually more acidic circumstances resulted from the addition of lactic acid and chlorhydric acid. The label-free LC-MS/MS approach has been proven effective in quantitatively accessing approximately 56% of the Scott A proteome. The relation of ATR to the growth phase transition indicated by the data showed that the maintenance of growth rate was related to the activation of cytoplasmic pH homeostatic mechanisms. Meanwhile, cell component turnover and cellular reproductive-related proteins were found to be more abundant in acid-stressed cultures. Interestingly, acidification led to a transformation from heterofermentation to an oxidatively stressed condition, where ATP seems to be produced chiefly through the pyruvate dehydrogenase/pyruvate oxidase/acetate kinase and branched chain acid dehydrogenase pathways. It is thrilling that 2D-LC-MS/MS is effective in characterizing the bacterial proteome in a high coverage, laying a foundation to further probe the metabolic changes imposed by acidic conditions in bacteria. Finally, it is worth mentioning that ATR is a complicated global cellular regulation that cross-protects organisms against other stresses, such as high temperature and oxygenation.

Oxidation is one of the most deleterious stress factors to the cell because of its damage to DNA and other cellular macromolecules via the production of reactive oxygen species (ROS). ROS are principally derived from the reduction of dioxygen to superoxide (O^{2-}), H_2O_2, and the hydroxyl radical (OH); they primarily account for damages to nucleic acids, membrane lipids, and proteins[53]. Oxidative stresses caused by ROS accumulation are associated with human aging and carcinogenesis. Therefore, comprehensive proteomic studies on cellular mechanisms of oxidative stress response will be of great value for medicine and public health. Chuang, et al.[54] used high-resolution 2-DE and MALDI-TOF-MS to compare the protein expression profiles of *Helicobacter pylori* under normal and oxidative stress conditions. Among the 11 proteins differentially expressed under oxidative stress, urease accessory protein E (an indispensable metallochaperone for urease activity) and alkylhydroperoxide reductase with antioxidant potential are greatly declined under stress conditions.

Proteomic studies on microbial pathogenicity

Infectious diseases are mainly responsible for human morbidity and mortality all over the world. The unambiguous knowledge of microbial pathogenicity can provide invaluable information about the interaction between pathogenic microbes and human host cells. Various systematic genome- and transcriptome-wide approaches have been exploited to capture a clear understanding of infectious processes. As an indispensable complement of other omics approaches, proteomics has a highlighted sensitivity for identifying proteins expressed by pathogens during infection[55]. These infection-involved proteins inevitably contribute to the elucidation of the path physiology of pathogen-host interactions. Appropriate vaccination antigen selection still holds a great promise for decreasing the rapid spread of infectious diseases. Proteomic technologies serve as an efficient approach of identifying proteins with vaccine and diagnostic applications, as well as determining potential targets for drug design and the resistance of pathogens to these drugs[56].

Vaccines are biological or biochemical agents that manage to improve immunity to a certain disease. Then the subsequent contact with the pathogen has little possibility to result in diseases[55]. The current endeavors that deal with microbial pathogenicity are still vastly based on the development of vaccines. Thus, the effective discovery of desirable vaccine targets of diverse infectious pathogens is urgently needed. Proteomic technologies have a good performance efficient in characterizing these sub-proteomes, such as outer membrane proteomes, which represent an enriched proteomic fraction of potential vaccine candidates[57,58]. *S. aureus* is a severe infectious pathogen commonly related to bacteremia, pneumonia, acute endocarditis, meningitis, osteomyelitis, toxic shock syndrome, and fatal invasive diseases[59,60]. The resistance of *S. aureus* strains to methicillin generates a new challenge to produce novel therapeutic agents against this strain. Monteiro, et al.[60] used 2-DE and MALDI-TOF-MS/MS to determine the cytoplasm proteome of a clinical MRSA strain of *S. aureus*, resulting in 227 identified proteins. Proteins related to antibiotic resistance have been successfully detected, indicating that the catalase may be a significant staphylococcal virulence factor. Becher, et al.[61] provided deeper insights into *S. aureus* by combining four sub-proteomic fractions: cytosolic, membrane-bound, cell surface-associated, and extracellular proteins. Given the comprehensive coverage of the entire proteome of *S. aureus*, they succeeded in targeting extracellular and surface-exposed virulence factors as well as in determining staphylococcal survival and adaptation capabilities.

Quantitatively measuring outer membrane protein expression has been recognized as a credible strategy for vaccine antigen selection[62]. Conceivably, sub-proteomic fractions, especially for membrane-bound proteins, which are believed to be highly associated with microbial pathogenicity, have been nearly integrally delineated by proteomics. Based on the respective advantages of multiple omics technologies in depicting the pathogen-host interactions, the integration of genomics, transcriptomics, and proteomics provides a process that can select possible vaccine candidates. Nevertheless, the determination of a newly developed vaccine calls for stringent clinical tests and repeated validations, which is definitely an interminable and slow process. The novel vaccine candidates identified by these techniques must be subjected to *in vitro* (e.g., bactericidal assay) and *in vivo* (e.g., animal protection experiments) validations[63].

Metaproteomics

Instead of mining the independent behavior of a single microbe, metaproteomics aims to investigate the property of microbial community through proteomic methods. Metaproteomics or whole-community proteomics aims to completely identify proteins expressed by the microbial community. The rapid rise of metaproteomics in recent years has been promoted by the wide availability of extensive metagenomic sequences from diverse ecosystems[64]. The investigation of whole-community proteomics requires the microbes in the environment to be uncultured, distinct from the isolated microorganisms cultured in unnatural environment. The detailed information provided by metaproteomics facilitates better understanding of microbial community structures, metabolic activities, competition for nutrients, and community development[65]. One of the large-scale studies of metaproteomic measurements exploited an uncultured microbial community from acid mine drainage[66], a less complex environment. Thus, the community complexity and wide range of protein expressions pose principal challenges in entire protein characterization. Recent studies have focused on microbe–host ecosystems, which appear to be the most complicated level of microbial metaproteomics.

Outlook

Microbial proteomics witnessed the birth of 2-DE technology to separate the proteins of *E. coli* in 1975. In the last few decades, proteomics has exploited a comprehensive and splendid arena, where various microorganisms can be widely explored with fundamental, pathogenic, and biotechnological values of interest. Utilizing different technologies ranging from mass spectrometry to protein chip, proteomics has unraveled an inventory of protein contents of microbial cells with massive information on protein expression, abundance, modification, localization, and interaction. The abilities of microorganisms to endure severe environmental stresses, such as extreme temperature, toxic compounds, or pollutants, and to infect their hosts, are valuable resources in fundamental and applied research. A myriad of proteomic technologies are playing a pivotal part in elucidating the intricate molecular mechanisms of microbial stress responses and pathogenicity. Meanwhile, the fruitful application of microbial proteomics in metabolic engineering has resulted in enhanced recombinant protein products of biotechnological interest. The integration of genomic and proteomic data helps to improve the annotation of genome by providing alternative splicing and even post-translational information.

We have witnessed the vibrant success and headway of microbial proteomics during the recent decades. However, technical limitations have brought intractable challenges in the effort to apply proteomic methods to address microbial issues. Likewise, the low identification coverage of microbial proteome and the less than satisfactory reproducibility of large-scale proteomic studies are the vital factors that hinder us from achieving further progress. Multi-omics integration is a strategy that can compensate for the weaknesses of proteomics in some aspects. Considerable achievements have been made with the help of proteomics in combination of other omics, such as genomics and transcriptomics, to better survey the cellular processes of microbes on a systematic level. In addition, specific bioinformatics tools are extremely needed to construct powerful proteome databases, optimize mass spectrometry searching algorithms, and

inspect protein changes in microbial cells.

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