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Microbial Proteomics and Their Importance in Medical Microbiology

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OUTLINE

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3.1 INTRODUCTION

Microbial infection is a leading cause of death around the world. Most of the infectious diseases are caused by drug-resistant microbes; this may lead to a delay in the administration of microbiologically effective therapy (Chen et al., 2017; Del Chierico et al., 2014). Therefore, exhaustive understanding of microbial physiologies, infection and defense systems, and survival strategies is of great interest in order to actively defeat microbial infection. Microbial proteomics provides complete information of microbial physiology and expression and function of the proteins that are involved in infection and also gives a clue in clinical diagnosis and antimicrobial therapy (Pérez-Llarena and Bou, 2016; Vranakis et al., 2014). Microbial proteomics helps to identify the proteins associated with microbial activity, microbial host-pathogen

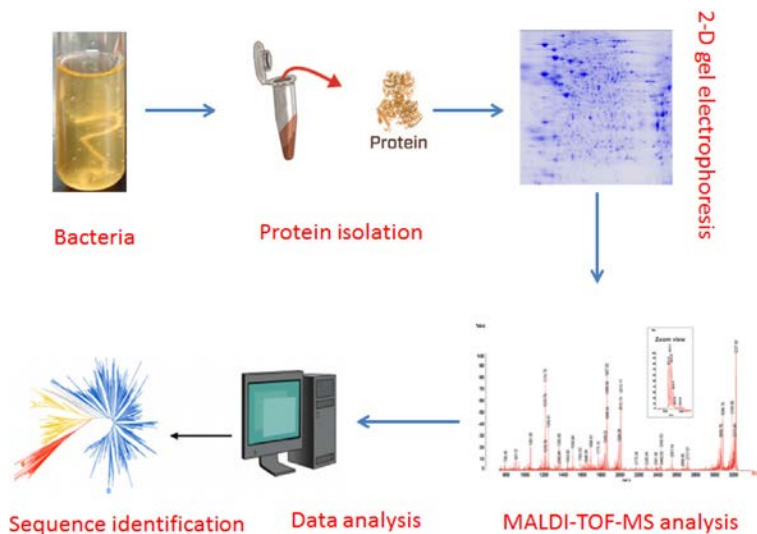


FIG. 3.1 Shotgun proteomic analysis of bacterial sample. Isolation of protein from bacteria, purification of isolated protein sample, separation of the proteins based on isoelectric point and molecular weight of the expressed protein—the new proteins have to be digested with trypsin, before the sample mixed with a suitable matrix for MALDI analysis—and identification with the protein sequence through the database.

interactions, and antimicrobial resistant mechanism. Microbial activity of pathogens can be confirmed by using the 2-D gel-based and gel-free method with the combination of MALDI-TOF-LC-MS/MS. Proteomic analysis of microbial host-pathogen interaction reveals valuable information about the virulence of the pathogen and its resistance; it helps in better understanding of the infection and for developing strategies against microbial infections (Cheng et al., 2016). Fig. 3.1 schematically illustrates the proteomic analysis of the bacterial samples.

3.2 UNDERSTANDING HOST-PATHOGEN INTERACTIONS AND INFECTIOUS DISEASES

Proteomic study of microbial pathogenicity provided invaluable information about the interaction between host and pathogen, captured a clear understanding of infectious process, and identified the proteins expressed by pathogens during infection (Walters and Mobley, 2010). Pathogens affect various molecules for their adhesion and invasion to host cells; infection of neighbor cells, spreading into host systemic circulation; and evasion of host defense mechanisms. Proteomic profiling provides new insights of virulence proteins like proteoglycans; it mediates host-pathogen interactions to affect invasion, progression, and outcome of infection. Host-pathogen interactions of microbes will affect posttranslational modifications, like phosphorylation, glycosylation, and acetylation, which can be rapidly identified by MS (Jean Beltran et al., 2017; Ravikumar et al., 2015).

Many proteomic techniques are used to study the host-pathogen interactions; it may lead to the discovery of important biological insights. Different kinds of methods available for

detecting host-pathogen interactions, that is, yeast 2 hybrid, affinity purification-mass spectrometry, chemical cross-linking mass spectrometry, proximity-dependent labeling strategies-mass spectrometry, and protein microarray-based technologies and protein correlation profiling, have its own advantage and disadvantages (Table 3.1) (Nicod et al., 2017). Viral and bacterial proteins interact with host hub proteins or central proteins and alter the cellular processes. Viral pathogen seems to unavoidably disturb cellular processes as they rely on the transcriptional machinery, whereas bacteria tend to mesh with the immune response to prevent their clearance. Also, the manipulation of the host ubiquitin pathways by viruses and bacterial effector proteins is a recurrent finding. By controlling protein degradation and cell signaling, ubiquitination is a critical regulator of various cellular processes such as inflammatory responses, vesicular trafficking, and cell cycle, altogether making it an ideal target to hijack for bacterial and viral pathogenicity (Martinez-Martin, 2017; Yang et al., 2015). These infection-involved proteins inevitably contribute to the elucidation of the path physiology of pathogen-host interactions. Table 3.1 gives an overview of the main advantages and disadvantages for the commonly used host-pathogen protein-protein interaction (HP-PPI) detection methods.

TABLE 3.1 An Overview of the Main Advantages and Disadvantages for the Commonly Used HP-PPI Detection Methods

S.No.	Technique	Merits	Demerits
1.	Chemical cross-linking mass spectrometry	Provides information on interacting protein domains, residue-to-residue resolution, information on the topology and structural arrangement of protein complexes; can be applied on infected tissues directly	Need for large amounts of purified protein complexes, complex data acquisition and analysis, low sensitivity/resolution
2.	Proximity-dependent labeling strategies-mass spectrometry	Appropriate for weak and transient interactions, sensitive, adequate for resolving the spatial organizations of the tagged proteins	Long reaction times, not suitable for time course experiments, hard to distinguish direct from indirect/proximal interactions
3.	Yeast 2 hybrid (Y2H)	High throughput, existing human and pathogen ORFeome collections, universality—any cDNA from any protein is testable	Need for exhaustive screens, nonphysiological experimental conditions, detects only binary interactions, no PTM information
4.	Affinity purification-mass spectrometry (AP-MS)	High throughput, sensitive, detects entire protein complexes, PTM sensitive, sing antibodies against the bait of interest, can be applied from infected tissues directly	Need for transgenic cell lines, needs additional experimental data to distinguish direct from indirect interactors, identification of PPI depends on the biochemical extraction conditions
5.	Protein correlation profiling	Whole proteome studies, unbiased, stoichiometric and quantitative information readily available	Dynamic range of protein abundances between host and pathogen might be too important, low sensitivity, hard to detect kiss and run interactions

3.3 CLINICAL MICROBIOLOGY AND CURRENT PROBLEMS

Microbial infections are the most common cause of disease development, and it causes millions of deaths around the world. Recurrence and antibiotic resistance of microbial infections demand advanced techniques in medical and diagnostic fields. Timely diagnosis is the most effective approach to prevent diseases, but it requires an accurate and fast identification and characterization of the infecting pathogens, such as bacteria, fungi, viruses, and parasites. New developments in diagnostic techniques not only are for identification purpose but also illuminate bacterial infection mechanisms and bacterial adaptation to the human host. These inputs will help the discovery of new therapeutic approaches to control or eradicate microbial-associated infections.

Clinical diagnosis of microbes is based on, culturing on solid media, molecular and new emerging techniques. Culture-based diagnosis of clinical samples on solid selective enrichment medium is very sensitive, easily contaminated, labor-dependent, and a time-consuming process; there's a chance for error to occur in these diagnosis reports. Currently, some automated identification systems give reliable results in shorter periods of time and also antimicrobial susceptibility testing ([van Belkum et al., 2013](#)). Molecular methods are more accurate, specific, sensitive, and faster than traditional culture-based methods, because they use more stable genotypic characteristics than reversible phenotypic characteristics. Molecular methods are of two types, such as immunologic and nucleic acid-based techniques. Immunologic techniques are based on the binding between diagnostic antibodies and specific antigenic determinants of the target. The human body produces specific antibodies in response to microbial infection; it is possible to use those antibodies to detect an acute or past infection. ELISA is the most commonly used technique in clinical diagnosis due to their speed, capacity, easy handling, and relatively low cost. Molecular methods also have some limitations due to the difficulty to generate selective antibodies and the requirement of large amounts of the respective antigen to quantify bacteria. The development of antibodies with the required degree of specificity is difficult for complex organisms ([Gehring et al., 2015](#); [Mangal et al., 2016](#)).

Nucleic acid techniques are DNA- and RNA-based techniques; DNA-based techniques include DNA microarray; PCR- and RNA-based techniques are RT-PCR and TMA. PCR is one of the greatest inventions discovered by Kary Banks Mullis; it provides great improvements in microbial diagnosis, and the use of species-specific sequences to detect and identify pathogens requires the prediction of the species presence in the clinical samples. Limitations of PCR technique are as follows: There is a limited number of PCR assays that can be performed per clinical sample, infections or diseases are not monocultures being frequently caused by species-species interactions or even strain-strain interactions, and it requires proper standards and controls for valid interpretation ([Maurer, 2011](#)). Nucleic acid-based technologies worked based on entire DNA. The presence of extracellular DNA might influence the DNA-based signals detected; significant amounts of extracellular DNA released by bacterial cells destroyed by immune defenses have been detected. Therefore, DNA-based signals obtained during diagnosis will be influenced by the presence of extracellular DNA that might lead to misidentification ([Maurer, 2011](#)).

3.4 ROLE OF MALDI-TOF- IN DIAGNOSIS

Clinical diagnosis requires rapid, reliable, and cost-effective methods for the identification of potential pathogens in clinical samples so that appropriate antimicrobial therapy may be initiated early. MALDI-TOF MS can be used for early identification of bacteria in blood, urinary tract infections, and other clinical samples. MS-based proteomic approaches have been used in clinical diagnostic procedures, including the characterization, identification, and classification of microorganisms. Matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) has been broadly adopted by many clinical microbiology laboratories over the past decade (Chen et al., 2017). MALDI-TOF MS is the most emerging tool used to identify microorganisms at species level, even strain differentiation based on molecular signatures (Wieser et al., 2012). The successful implementation of this technique in the routine of laboratories is due to its fast, easy, and high-throughput characteristics. Moreover, it generates simple and easily interpretable spectra. Bacterial identification by MALDI-TOF MS process involves different steps, i.e., protein separation, 2-D gel analysis, and gel digestion and analysis. Samples were mixed with suitable matrix and target plate today. Samples were exposed to short laser pulses; it (laser energy) vaporizes the protein sample and leads to ionization; these ions reached the detector at the time-of-flight (TOF) tube. Based on this TOF information, a characteristic spectrum is recorded constituting a specific sample fingerprint, which is unique for a given species. Increased mass and ion charge ratio (m/z) corresponds to lower speed and longer time needs to reach the detector (Tracz et al., 2018). MALDI-TOF MS is a very reproducible and reliable tool for microbial identification and can identify bacterial isolates in a few minutes and with low costs, with high efficiency from both a diagnostic and a cost-per-analysis point of view (Singhal et al., 2015).

Proteomics provides valuable data in the field of microbiology; proteomics characterizes the molecular model systems to investigate specific aspects of microbiological gene expression and metabolism. In medical microbiology, these data help to analyze the disease mechanism and how the pathogen interact with their hosts; these investigations are largely focused on the development of novel approaches for treatment and prevention of infectious diseases (Herschend et al., 2017; Wang et al., 2016).

3.5 ROLE OF PROTEOMICS IN MEDICAL MICROBIOLOGICAL RESEARCH AND DIAGNOSIS

Proteomic tools can be used in different ways to diagnose diseases. Proteomic tactics are being used to improve the screening and early detection of cancer; this is attained by identifying proteins whose expression is affected by the disease process. An individual protein is called a biomarker, whereas a set of proteins with altered expression levels are called protein signature (Kim and Hwang, 2016; Kuppusamy et al., 2017). Applying proteomic technologies may improve the understanding of disease processes, develop new biomarkers for diagnosis and early detection of disease, and accelerate drug development. Emerging techniques create numerous opportunities and challenges to meet the needs for high sensitivity and high throughput required for disease-related probes (Gregorich and Ge, 2014; Mehan et al., 2012).

Biomarker is to be useful as a candidate for early screening and detection of cancer. The in vitro estimation of two pathological proteins (rRv3369 and rRv3874) (Ag) through proteomic technology is a high sensitivity method that diagnosed the tuberculosis in clinical isolates (Geyer et al., 2017; Thomas et al., 2016). Pathogenesis of severe acute respiratory syndrome (SARS) is not well unstated, and a specific diagnostic method is critical for the management and control of this disease. Proteomic analysis of sera from patients with SARS has identified potential protein markers shortened from $\alpha(1)$ -antitrypsin that was consistently found in higher concentrations in the sera of SARS patients related to healthy controls. These markers may be useful as diagnostic tools and therapeutic and potential vaccine targets (Clay et al., 2012; McBride and Fielding, 2012). Proteomics is also used to identify differentially expressed cancer cells, which may reflect differences in insensitivity and tendency in developing resistance to treatment (Mohammad et al., 2015). Proteomics has identified proteins involved in metastasis and multidrug resistance that are highly expressed in glioblastoma multiforme compared with brain tissue. These proteins have potential as diagnostic, drug resistance, and invasiveness markers for glioblastoma tumors. Drug resistance is a major clinical problem in the treatment of many infectious diseases (Chowdhury et al., 2018; Hanif et al., 2017; Olar and Aldape, 2014). Proteomics shows solution for drug development against the resistance. Chloroquine is the most successful drug to treat malaria but has been reduced almost ineffective in many parts of the world by the widespread emergence of chloroquine resistance. Through the help of proteomic technology, the potential therapeutic targets, host-pathogen interaction, and protein-drug interactions in *Plasmodium* species are identified (Bhattacharyya and Chakrabarti, 2015; Hossain et al., 2016; Suazo et al., 2016).

In clinical microbiology, the combination of MALDI-TOF MS plays a key role in bacterial identification, susceptibility testing, and nucleic acid targeting and facilitated a better workflow for the timely management of positive blood cultures and other samples. Detection of proteomic changes in resistant versus susceptible bacterial species and can be performed in the classical MALDI-TOF MS mode (Rahi et al., 2016). Identification and characterization of a bacterial isolate from patient within a transient period of diagnosis have reduced the morbidity and mortality value. Early detection is the key to successful treatment of most diseases; clinical test result is based on chemical composition and man power, so there is a chance of false/error to occur and being late. It's time to reduce errors and improve the experimental design with improved outcomes (Sung et al., 2018). The MS-based biomarker technology enables early detection of cancer and cardiovascular diseases. The field of biomarker research is mainly focused on genomics, transcriptomics, and proteomics and understanding the gene regulation, functions, and how are they involved in disease development. Expectations for the ideal MS-based biomarker should reflect the significant recent and future improvements in its technological basis (Crutchfield et al., 2016). Fig. 3.2 illustrates the research areas in medical microbiology that utilize proteomic technologies.

3.6 IMPORTANCE OF MALDI-TOF MS IN IDENTIFICATION OF PATHOGENIC MICROORGANISMS

In normal methods, the identification of bacteria is based on phenotypic characteristics and biochemical reactions; all of these methods can achieve high accuracies, and it takes longer

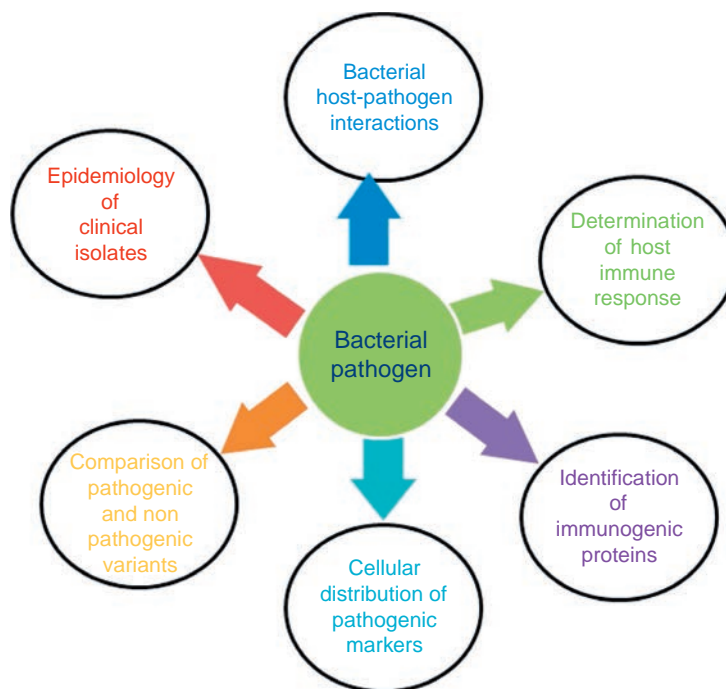


FIG. 3.2 Research areas in medical microbiology that utilize proteomic technologies.

period to total identification process. MALDI-TOF MS is a technical revolution in the microbial field; it is a faster and easier technique for microbial identification of pathogenic microorganisms. Apart from protein extraction from whole-cell bacteria lysates prepared with chemical treatments, direct bacterial analysis is usually adopted for bacterial identification by MALDI-TOF MS; it plays a key role in clinical diagnostic and prognostic workup of biofilms formation and control. Protein profiles can be obtained from a single colony of bacteria directly deposited on the MALDI-TOF MS target plate and overlaid with a matrix solution. MALDI-TOF MS was used to identify microorganisms not only at the species level but also at the subspecies and strain levels and also identify some bacterial toxins (Crutchfield et al., 2016). MALDI-TOF MS is a rapid, easy, relatively inexpensive, and high-throughput method for identifying clinically relevant bacteria and fungi. MALDI-TOF MS methods are implemented in identifying common microorganisms (Cobo, 2013; van Belkum et al., 2017). Characterization of pathogen, based on MALDI-TOF MS fingerprinting profiling, is now a routine in diagnostic microbiology; the entire process is completed in four steps, i.e., detection, ID, susceptibility testing, and epidemiology. The MALDI-TOF MS protein fingerprint employs a pattern-matching algorithm to compare microbial cell extracted peak lists with a reference database, hence creating a consensus “biomarker” peak list, characterized by averaged m/z and intensity. MALDI-TOF MS also has the potential to identify pathogens in direct samples, such as urine and blood cultures. Furthermore, the potential for ID at the serotype or strain level and antibiotic resistance profiling within minutes makes MALDI-TOF MS an ongoing revolution in the clinical microbiology laboratory (Emonet et al., 2010; Suttisunhakul et al., 2017).

3.7 ROLE OF PROTEOMICS IN DEVELOPMENT OF THERAPY AND VACCINE

Proteomics plays a key role in the development of novel therapeutic strategies through the identification of vaccine and antibiotic targets. Antigenic proteins can be identified by an immune response against the infection; these proteins can be used in the development of vaccine. Specific proteins can be selectively identified to optimize the vaccine development. In order to develop vaccine, the bacterial cells were treated with stress or bacterial infection conditions. Then, proteins were analyzed by 2-D electrophoresis, which described the newly expressed proteins with the support of a bioinformatic approach to the identification of vaccine candidate proteins, which took advantage of the extensive gene sequence data now available (Bilgic et al., 2016). Drug resistance of pathogens is the major problem to recurrence of infection or disease. Proteomics can determine the antimicrobial resistance mechanisms through the analysis of microbial proteins; it explains that drug resistance development will lead to improvements in extending the efficacy of current antimicrobial (Li et al., 2010). *Pseudomonas aeruginosa* develops resistant against beta-lactam antibiotics; proteomics can identify the resistance mechanism; it reduces the expression of outer membrane protein against imipenem, ceftazidime, and ampicillin treatment. *Streptococcus pneumoniae* developed resistance against penicillin and erythromycin also, two phenotypes of *S. pneumoniae* specifically the MLS and M phenotypes. Proteomics has been investigating these resistance mechanisms; phenotype M is resistant through the expression of *mefE*, which encodes a membrane transporter protein that reduces the intracellular levels of erythromycin; MLS phenotype owes their resistance to the methylation of rRNA. Antibiotic action can be investigated by locating proteins that show differential expression patterns when bacteria are grown in the presence or absence of antibiotics. All these data gives a deep boost for the development of novel therapeutic compounds (Editorial Mini Focus Issue: Systems Biology, 2014; Li et al., 2010; Wang et al., 2015; Yari et al., 2016).

3.8 FUTURE PERSPECTIVES

Proteomics has made an important impact in medical biology, and it provides valuable data related to host-pathogen interactions and posttranslational modifications and is helpful for better understanding of microbial community functions and microbial physiology. Pathogenic microbial identification and characterization are very difficult before automation; to overcome these difficulties, MALDI-TOF comparative sequencing is very helpful. Therefore, MALDO-TOF MS has become a powerful tool for routine pathogen identifications in clinical laboratories. Before MALDI-TOF, bacterial identification and sequencing approaches were time-consuming and cost-effective process, but now, MALDI-TOF analysis is routine in microbiological laboratories. Proteomics analyzes the host-pathogen resistance mechanism that can be investigated based on the data. Proteomics may serve neither as the primary screening method for antigen detection nor as a complementary method to confirm bioinformatic predictions of immunogenic analysis (Chiang et al., 2018). The ability to rapidly identify pathogens in specific clinical specimens will help us to study the clinical burden resulting from the emergence of these species as human pathogens, definitely suggesting

MALDI-TOF MS as an alternative to molecular methods in clinical laboratories and as a revolutionized bacterial identification in the field of medical microbiology in coming future.

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Conflicts of Interests

The authors declare no relevant competing financial interests to disclose.

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