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Review

Proteomics boosts translational and clinical microbiology☆,☆☆

F. Del Chierico[a,b], A. Petrucca[a,b,c], P. Vernocchi[a,b,d], G. Bracaglia[a,b], E. Fiscarelli[e], P. Bernaschi[f], M. Muraca[e], A. Urbani[g,h], L. Putignani[a,b,*]

[a]Unit of Parasitology, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy
[b]Unit of Metagenomics, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy
[c]Department of Diagnostic Science, Sant'Andrea Hospital, Via di Grottarossa 1035, 00185 Rome, Italy
[d]Interdipartimental Centre for Industrial Research-CIRI-AGRIFOOD, Alma Mater Studiorum, University of Bologna, Bologna, Italy
[e]Laboratory Medicine, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy
[f]Unit of Microbiology, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy
[g]Department of Experimental Medicine and Surgery, University "Tor Vergata", Rome, Italy
[h]IRCCS-Santa Lucia Foundation, Rome, Italy

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ABSTRACT

The application of proteomics to translational and clinical microbiology is one of the most advanced frontiers in the management and control of infectious diseases and in the understanding of complex microbial systems within human fluids and districts. This new approach aims at providing, by dedicated bioinformatic pipelines, a thorough description of pathogen proteomes and their interactions within the context of human host ecosystems, revolutionizing the vision of infectious diseases in biomedicine and approaching new viewpoints in both diagnostic and clinical management of the patient.

Indeed, in the last few years, many laboratories have matured a series of advanced proteomic applications, aiming at providing individual proteome charts of pathogens, with respect to their morph and/or cell life stages, antimicrobial or antimycotic resistance profiling, epidemiological dispersion. Herein, we aim at reviewing the current state-of-the-art on proteomic protocols designed and set-up for translational and diagnostic microbiological purposes, from axenic pathogens' characterization to microbiota ecosystems' full description. The final goal is to describe applications of the most common MALDI-TOF MS platforms to advanced diagnostic issues related to emerging infections, increasing of fastidious bacteria, and generation of patient-tailored phylotypes.

This article is part of a Special Issue entitled: Trends in Microbial Proteomics.

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* *Corresponding author at:* Units of Parasitology and Metagenomics Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165, Rome, Italy. Tel.: +39 06/68592598 2176; fax: +39 06/68592218.
E-mail addresses: l_putignani@yahoo.com, lorenza.putignani@opbg.com (L. Putignani).

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Contents

1. Introduction

In the last few years, proteomics has dealt with emerging or re-emerging pathogens, including neglected agents, in order to provide support in taxonomic microbial classification, invasion mechanisms, life cycle stage switching, antigen variation, drug target and drug resistance discovery, and infection epidemiology.

Genomic data are currently growing at an extraordinary rate. However, this huge amount of descriptive work has only scraped the surface of the microbial biodiversity, endowed with environmental, opportunistic and pathogenic strain patterns, still lacking comprehensive functional insights into the microbe-host cross-talk. The highly microbial diversity indicates strategies devised to potentiate specialized adaptation mechanisms within host or ecological niches, through genetic material transfers [1], post-transcriptional machineries [2], proteome modulation and controlling in response to specific external stimuli [3]. While genomics may provide a comprehensive snapshot on everything related to nucleic acids, proteomics ensures the actual description of specific proteins or complexes of proteins in their biological milieu. Furthermore, proteomes are much more multiform and dynamic than related up-stream genomes, hence requiring complex experimental pipelines, including separation, detection, and data processing, to highlight either protein content and/or modifications during the complex host–parasite interaction phase (*e.g.*, infection, propagation stages) (Fig. 1).

The successful coupling of multidimensional separations with mass spectrometry (MS) for protein and peptide analyses has been achieved with the advent of the ionization Matrix-assisted laser desorption ionization (MALDI) and Electrospray ionization (ESI) techniques, assisted by the evolvement of new powerful MS instrumentations. While MALDI is usually combined with gel-based separations, ESI is frequently coupled with “on-line” (High-pressure liquid chromatography) HPLC or reversed-phased (RP) LC separations [4,5].

Two main analytical approaches are commonly used for protein analyses for proteome separation and characterization: “top-down” and “bottom-up”. The top-down is an emerging design for the analysis of intact proteins separated by HPLC and identified by MS [6]. In the bottom-up approach, samples require a gel-based purification step, followed by an enzymatic or chemical digestion that provides peptides separated by multidimensional chromatography, such as 2-D-HPLC, and finally identified by tandem MS [7,8]. Otherwise, the crude protein extract may be digested directly, followed by on-line LC or off-line mode coupled-tandem MS (*i.e.*, shotgun proteomics). Top-down approaches allow an extensive protein identification (ID), including amino acid sequence characterization and post-translational modifications since they are based on the intact molecules of interest [9].

Peptide and intact protein analysis can be conducted using either the time of flight (TOF) MS, or the Fourier transform ion cyclotron resonance (FT-ICR), that ensures a performance in a wide mass range and, in the case of FT-ICR, a high mass accuracy. Other types of instruments (*e.g.*, hybrid linear ion trap [LTQ]-orbitraps, LTQ-FTICRs, or quadrupole ion trap) can also be applied to identify proteins [10]. From a general point of view, MALDI-TOF MS is ideal for relatively clean and less complex samples, with the targeted protein resulting dominant in the starting sample. Infusion ESI–MS/MS should be used when the sample is clean and at low complexity, with the scope of characterizing all multiple analytes and possible modifications on them. The complexity item can be managed in terms of appropriate proteomic technological platforms by including in translational and diagnostic microbiology a miscellaneous of methodological pipelines to analyze global proteomes or part of them. This will therefore reflect microorganism status and complexity to respond to specific needs: *i*) proteomic phenotyping supported by MALDI-TOF MS peptide fingerprinting; *ii*) proteome charting by bottom-up shotgun profiling; *iii*) sub-proteome relative and absolute quantitation by differential labelling and targeted analysis, respectively; and *iv*) community based analysis of operational taxonomic units (OTUs) from microbial communities (*e.g.*, gut, airway, salivary microbiota) by LC-ion trap MS/MS (Fig. 2).

Therefore, dealing with pathogens or opportunistic pathogens by proteomic approaches means to design and set-up the proper technology for general microbiological or specific diagnostic purposes [11,12]. Indeed, each diagnostic or translational task needs specific pre- and analytical solutions and proper technological platforms. Therefore, a wide range