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Review Article

Metaproteomics approaches and techniques: A review

Shahid Raza and Ayesha Ameen^{*}

Department of Biological Sciences, University of South Asia, Lahore Pakistan



*Correspondence Info:

Ms. Ayesha Ameen Department of Biological Sciences, University of South Asia, Lahore Pakistan

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Abstract

Extensive studies have been done on metagenomics for various microbial communities. The advancements in metagenomics level led to the need of metaproteomics approaches. Metaproteomics involve the identification, function and expression of various proteins present in microbial community, it also involves the identification and expression analysis of stress related proteins. The concepts of metaproteomics come with advancement in proteomics techniques which includes 2D gel electrophoresis for the identification of proteins and peptides in a particular microbial community. Mass spectrometry which is used to separate the proteins by desorption and ionization using a gas on a liquid medium. MALDI use for protein identification and separation, connected with TOF to give better results. The metaproteomics approaches become more advanced when HPLC and LC were used for peptides and protein with computational tools to sequence the peptide and protein. It is concluded that there is a requirement of research in metaproteomics. Many scientists have done research on these approaches but there is lack of better quality and desirable results.

Keywords: Metagenomics, Metaproteomics, MALDI.

1. Introduction

The field of environmental biotechnology has been widely extended by the recent advances in metagenomics levels. It also extended the post genomic area of research. Little research have done on metaproteomics, it reveals the expression and function of various proteins. These studies highlighted the need of metaproteomics studies for the microbial consortia. However, the application of proteomic investigations to complex microbial assemblages such as seawater and soil still presents considerable challenges. Nonetheless, metaproteomics will enhance the understanding of the microbial world and link microbial community composition to function [1].

A clear recognition is given by nucleic acid based methods for information on functions expressed by microbial communities in the post genomic level. Meta proteome provide the information of the functioning microbial proteins, it is the large scale study of indigenous microbial communities [2]. Characterization of the metaproteome is expected to provide data linking genetic and functional diversity of microbial communities. Studies on the metaproteome together with those on the metagenome and the metatranscriptome will contribute to progress in our knowledge of microbial communities and their contribution in ecosystem functioning. Effectiveness of the metaproteomic approach will be improved as increasing metagenomic information is made available thanks to the environmental sequencing projects currently running. More specifically, analysis of metaproteome in contrasted environmental situations should allow (1) tracking new functional genes and metabolic pathways and (2) identifying proteins preferentially associated with specific stresses. These proteins considered as functional bioindicators should contribute, in the future, to help policy makers in defining strategies for sustainable management of our environment [3].

Proteomics is constantly evolving field. This field is diversified and complex without any measurement platform, experimental approaches and results. The proteomics studies were started with the invention of 2D gel electrophoresis. This technique was initially limited because of lack of quality identification of proteins. The best separation of protein is provided by 2D gel electrophoresis, it also provides the visualization of 1000 proteins in a given sample on single gel. The new approaches in proteomics led to Mass spectrometry also termed as MS- based proteomics. This technique provides the more desirable protein identification. The development and modification in mass spectrometry located this technique to be very well suited for protein analysis or proteomics. Large biomolecules such as proteins and peptide liberated by the development of soft ionization techniques. Direct desorption and ionization of peptides in to the gas phase by liquid matrix can be performed by electrospray ionization. MALDI is used for the direct desorption and ionization of proteins and peptides in to the gas phase by using a solid matrix. MALDI is connected to TOF which is well suited for the pulsed form of ions produced by MALDI. The second major key in proteomics is the sequencing of peptides and proteins by the development of rapid scanning tandem mass spectrometers. The sequencing of proteins and peptides can be completed by tandem mass spectrometry or MS/MS by utilizing CID (Collisional induced dissociation). The development of low flow single and multi-dimensional HPLC high performance liquid chromatography and low flow liquid chromatography was considered necessary to couple with Nano electrospray and mass spectrometry in order to give greater sensitivity and high resolution. This is the third key of proteomics advancement along with computational technology for sequencing of peptides and proteins [4].

2. 2D- Gel Electrophoresis

The coupling of 1D or 2D gel electrophoresis to the mass spectrometry id the initial objective for the high quality separation of proteins. The digestion of proteins can be done within excised gel slices. The digestion is processed by analysis and peptide extraction using mass spectrometry along with peptide mass finger printing and MALDI-TOF or alternatively direct analysis using nanospray. Whole microbial protein analysis is widely performed by 2D-PAGE and mass spectrometry but this technique have some limitations such as difficulties in resolving basic and membrane proteins. The liquid separation and digestion of peptides is termed as shotgun proteomics. The combination of 2D-gel electrophoresis and mass spectrometry is using now a days for rapid analysis. This is the comparison of gel based analysis with liquid based approach [4].

The whole microbial proteome can be studied with greater depth and accuracy by using multi-dimensional liquid based method coupled with Rapid scanning tandem mass spectrometers. With minimal false results it is possible to identify and 1500-2500 proteins from a microbe IJASR/VOL 03/ISSUE 05/2017

with active growth rate and these proteins can be compared as well. 50-90% proteome can be identified and compared in case of multiple growth rates. The complex samples are characterized by mass spectrometry due to advancement in this technique the quality of result is better. Three dimensional ion trap is connected to the LC for high quality results. This technique can identify and analyse complex protein even a ribosomal subunit sequence could be analysed by using three dimensional ion trap system with LC [5].

3. Shot Gun Proteomics

Shot gun method is of great interest because of the simplicity of the technique used. Lysis of cell takes place in the presence of denaturing agent and protease inhibitors. Raditional methods of lysis are used e.g. sonication, freez thaw, bead beating and french press. Addition of some enzymes must be avoided to bring downstrem complxity e.g lysozymes. The denatured proteins are peptides of length ranging from 7-30 amino acids. These peptides are desalted by solid phase extraction. Quality digestion is important to promote good mass spectrometry results [6].

4. Proteome Bioinformatics

To handle the large datasets there is a need of proteome bioinformatics. Peptides are digested by using MS and MS/MS spectra. Identification of peptides for each tandem mass spectra with search engine e.g. SEQUEST, MASCOT and X! Tandem [7]. Archival of datasets in to data base systems such as Oracle or MySQL [4].

5. Conclusion

The recent availability of extensive metagenomic from various environmental microbial sequences communities has extended the postgenomic era to the field of environmental microbiology. Although still restricted to a small number of studies, metaproteomic investigations have revealed interesting aspects of functional gene expression within microbial habitats that contain limited microbial diversity. These studies highlight the potential of proteomics for the study of microbial consortia. However, the application of proteomic investigations to complex microbial assemblages such as seawater and soil still presents considerable challenges.

Nonetheless, metaproteomics will enhance the understanding of the microbial world and link microbial community composition to function. Much research has been done on halophile metagenomics and soil microbial community. The whole genome extraction has been done at molecular level. Many bacterial genomes have been sequenced so far. Many new bioinformatics tools have introduced by keeping the importance of metagenomics. There is a need for extensive research on metaproteomics as this area lacks quality and desirable results. The identification and characterization of complex protein still need modified techniques of 2D and MS. Bioinformatics tools have been introduced for proteomics and protein sequencing. But still there is need for extensive research in metaproteomics.

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