Creative Proteomics

CREATIVE PROTEOMICS

How Can Proteomics Contribute to Microbial Research?

Importance of Microbial Research

Microorganisms such as bacteria, actinomycetes, and fungi are ubiquitous on our planet. They are widely distributed in soil, water, human body and other environments. Microorganisms and their activities are of great importance to biogeochemical cycles and to all biological systems. There are many excellent outcomes in the study of microbes:

- Study the geochemical cycle of the earth
- Help clinicians determine a certain disease or the stage of disease development
- Identify virulence factors that can be targeted for antimicrobial drug development
- Enable researchers to assess the pollution status of soil, water, and other environments
- Help researchers increase livestock and crop production

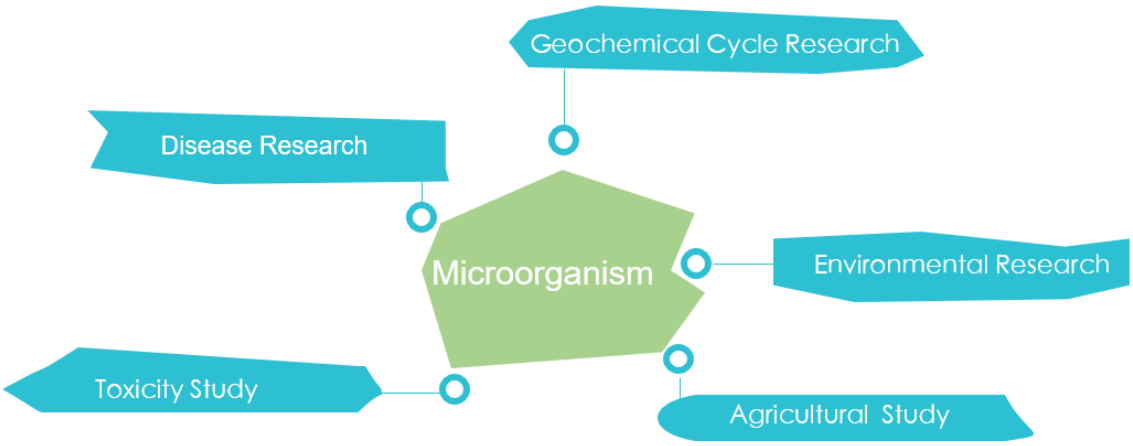


Figure 1. Research fields of microbial research.

Proteomics in Microbial Research

Proteomics was originally defined as a functional genomics approach that allows the study of protein expression pattern in an organism and the construction of a protein map of all proteins expressed by an organism growing under certain conditions. It is currently used not only to obtain protein catalogues giving important information about protein structure and activities in a microbial community, but also to compare protein contents in two different ecosystems by using quantitative proteomics, or to complement or correct genomic data. Besides, the proteomics approaches have many advantages over genomics. Indeed, since proteins are more stable than RNAs (especially those originated from prokaryotes), the proteome content should be less affected by the extraction process, and may give a better insight into the biological functions expressed *in situ*.

Proteomic studies are now being greatly engaged in the microbial field:

- **Environmental proteomics** enables protein cataloging, comparative and semi-quantitative proteomics, protein localization analysis, discovery of post-translational modifications to study structure and function of microbial communities in different environments (such as soil, marine and freshwater, sediments, *etc.*).
- **Pharmaceutical proteomics** can be applied to target identification and validation, identification of efficacy and toxicity biomarkers from readily accessible biological fluids, investigation into mechanisms of drug action or toxicity and identify protein–protein interactions, that major influence the drug development process.
- **Clinical proteomics** can identify proteins associated with microbial activity, which facilitates the discovery of microbial physiological changes and host-pathogen interactions during bacterial infection and antimicrobial therapy, and provides diagnostic and therapeutic support for clinical management.
- **Crop proteomics** usually uses various approaches for proteomic analysis, including protein/peptide separation and identification, but can also provide quantification and characterization of post-translational modifications to study crop-microbe interactions to improve crop productivity and agricultural sustainability.

How Does Proteomics Work in Microbial Research?

Proteomic workflow for studying the structure, distribution, and interactions of proteins from complex microbial communities is described below (Figure 2):

1. **Sample collection and protein extraction**: Microbial samples from different environments can be collected, and specific approaches for sample collection and protein extraction are developed when dealing with different kinds of samples.
2. **Protein separation**: Proteins can be separated prior to identification in order to fractionate very complex protein mixtures and analyze each protein fraction separately. Methods of separation include SDS-PAGE, 2D-PAGE, LC and CE. And the targeted proteins are digested into peptides with trypsin or other enzymes.
3. **Protein quantification**: Protein can be identified and quantified by different methods, including relative and absolute quantification methods.
4. **Bioinformatics analysis**: Including quality assessment of protein, protein statistical analysis, functional annotation and network analysis at protein level, *etc.*

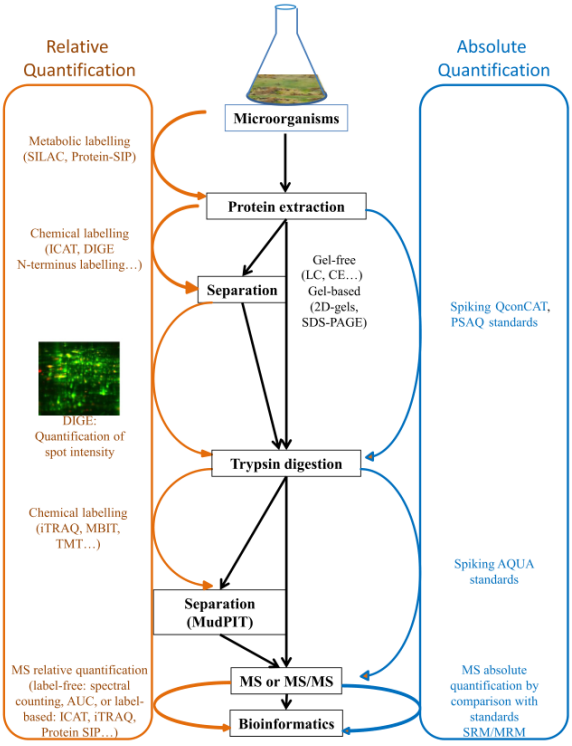


Figure 2. Proteomic workflow for studying the structure, functioning, and interactions in complex communities (Florence *et al.* 2014).

Protein Quantification Methods

With the technology revolution, microbial proteomics has currently developed a series of analytical methods, especially in protein quantification (Figure 3). Protein quantification includes relative quantification and absolute quantification.

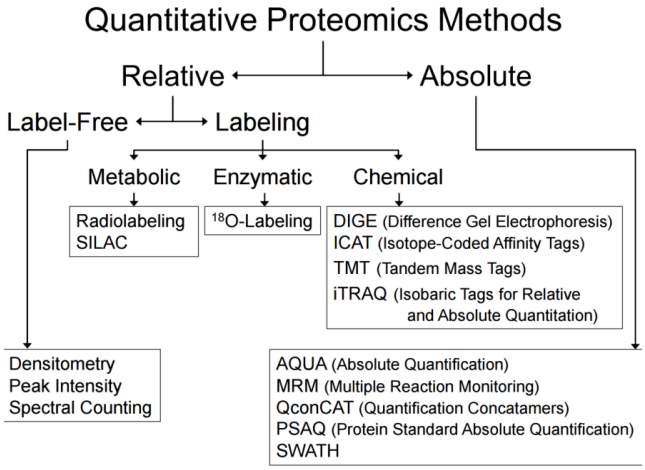


Figure 3. Protein quantification methods

Relative quantification

Relative quantification methods include relative label-based quantification and relative label-free quantification methods. The comparison of some methods is shown in Table 1.

- **Relative label-based quantification method**, often used to compare the relative levels of proteins from two samples, relies on the incorporation of chemical or metabolic labels into the proteins/peptides, and methods such as iTRAQ, TMT, ICAT and SILAC are included.
- **Relative label-free quantification method** does not rely on the incorporation of chemical or metabolic tags but compare peptide spectral counts or peptide intensities between LC-MS/MS runs of samples. It can quantify proteins from metaproteomic samples, quantitate the communities under different conditions, and estimate the efficiency of protein extraction, *etc.*

Table 1. Comparison of relative quantification methods

Method	Advantages	Disadvantages	Applications
Label-free area-under the curve	▪ No special steps required ▪ Cost effective	▪ Poor reproducibility ▪ Requires special software to keep track of everything	▪ Biomarker discovery when labeling is too expensive
SILAC	▪ Reduces technical variability ▪ Biology does the wok	▪ Cells must grow in special media ▪ Only two channels	▪ Any cell culture-based study
TMT	▪ No special cell growth conditions ▪ Can use on primary tissue ▪ Higher multiplex	▪ Noisier than SILAC ▪ Compression ▪ Can be costly ▪ Can interfere with some enrichment strategies	▪ Primary cells/tissue-based studies

Absolute quantification

Absolute quantification methods mainly include shotgun, discovery proteomics and targeted proteomics methods. The comparison of these methods is shown in Figure 4.

- **Shotgun** is a random protein identification and quantitative method designed to hit as many targets as possible, like a machine gun, and then analyze obtained data.
- **Discovery proteomics** enables to quantify and analyze up to thousands of proteins, it is an ideal technology for inventory proteins in a sample or detects differences of proteins in various samples. The available technology platforms include SWATH and msxDIA.
- **Targeted proteomics** is a platform that can absolutely quantify up to 150 target proteins per run. Selected/Multiple Reaction Monitoring (SRM/MRM) and Parallel Reaction Monitoring (PRM) are targeted proteomics techniques that provide highly specific and sensitive quantification of target proteins from complex biological samples.

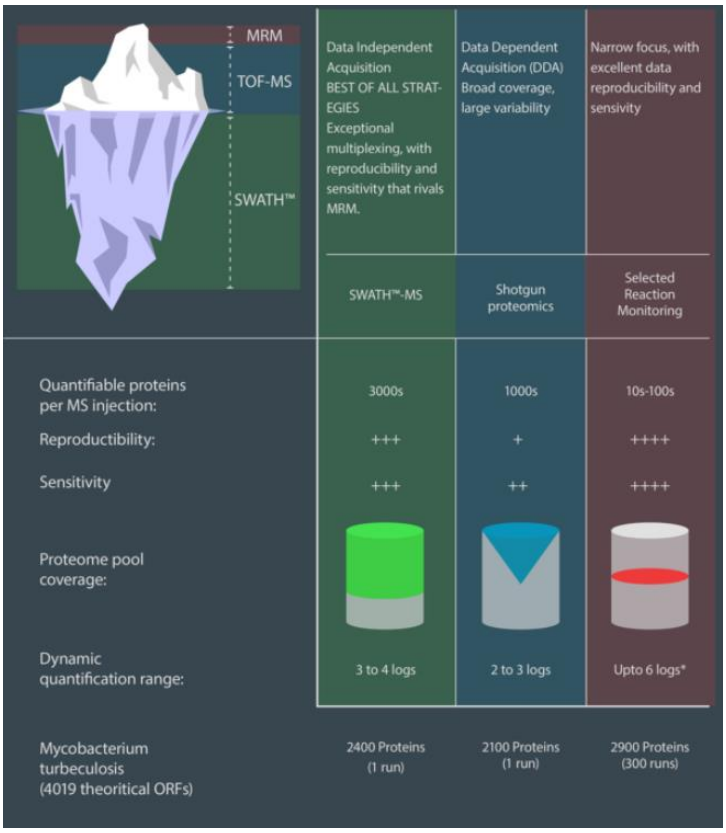
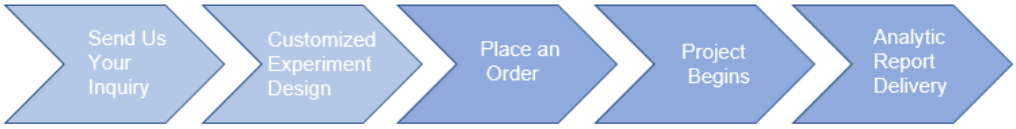


Figure 4. The comparison of absolute protein quantification methods.

Our comprehensive proteomics services

Creative Proteomics provides a one-stop proteomics service from sample collection, protein separation, to protein quantification and bioinformatics analysis. We offer both relative quantification (including iTRAQ, TMT and SILAC) and absolute quantification (such as SRM/MRM and PRM) approaches to help you discover, detect and quantify proteins in a broad array of samples.

Our technical platforms include state-of-the-art NMR, GC-MS, LC-MS, LC-MS/MS, HPLC-UV/FD, UHPLC instruments, *etc*. Our technicians will work closely with you from the experimental design to report delivery. Please feel free to contact us for questions or ordering.



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