Chapter 3
Omics Approaches: Impact on Bioremediation Techniques

Yordanis Pérez-Llano, Liliana Martínez-Ávila, and Ramón Alberto Batista-García

Contents

3.1 The Uprising of the "Omics"

One major feature of the "omics" techniques is that they are top-down holistic methodologies. This means that the research is focused on the properties of the systems rather than on those of the individual parts. Thus, instead of investigating a single molecular entity, the "omics" approaches aim to characterize a collection of molecules from the same category. Depending on the target molecule under investigation, different technologies have arisen. The genomic and transcriptomic approaches measure nucleic acids (DNA and RNA, respectively) and have advanced rapidly with the advent of next-generation sequencing technologies. Genomics deal with the gene composition of an organism, which shed light on the potentiality for adaptation to different environments and allows the construction of its evolutionary history. Transcriptomics, on the other hand, can unravel the genome function under particular conditions, allowing to determine which genes are expressed and enable the organism to thrive in an environment or circumstance. However, the expression

Y. Pérez-Llano · L. Martínez-Ávila · R. A. Batista-García (✉)
Centro de Investigación en Dinámica Celular-IICBA, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico
e-mail: rabg@uaem.mx

R. Prasad, E. Aranda (eds.), *Approaches in Bioremediation*, Nanotechnology in the Life Sciences, https://doi.org/10.1007/978-3-030-02369-0_3

level of a gene might not correlate with the protein levels or activity, nor it indicates protein localization within the cells or metabolic status. Therefore, recent advances in proteomics have allowed not only the genome-wide identification of proteins but also the characterization of their primary structure (in terms of posttranslational modifications and maturation), their turnover or degradation, their regulation, and their interactions with other proteins (interatomics). Similarly, assessing the metabolic status of cells is also possible by the well-established methodologies that measure low molecular weight organic compounds (metabolomics) and their temporal dynamics (fluxomics).

In environmental research, though, the techniques that analyze the microbial populations and their interactions in a complex sample (e.g., soil, water, organic waste, sludge, etc.) have become a milestone (Fig. 3.1). These techniques avoid the bottleneck of culturing specific microorganisms, thus allowing to recover information of the uncultured microbiota in an environmental sample. Although different terms have been used for these methodologies, the most widely used are the ones that use the preposition "meta" modifying an already established omics technique, i.e., metagenomics, metatranscriptomics, and metaproteomics. Handelsmanl et al. (1998) were the first in the use of the term "metagenomics" to refer to the totality of the genomes found in a certain environment. The main drawback of these methodologies is that recovering the target molecules (nucleic acids or proteins) from a complex source can pose a technical challenge. Another important problem is that the "omics" techniques generate a relatively large amount

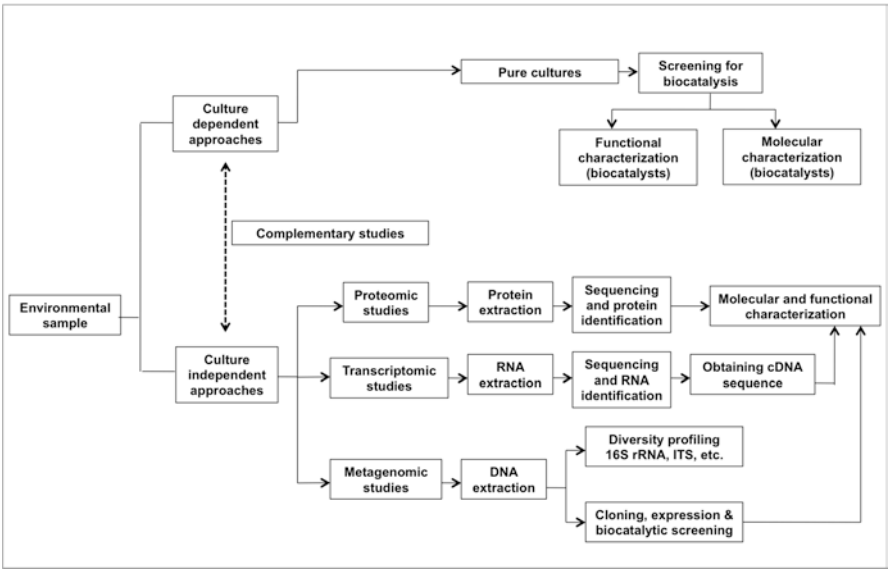

Fig. 3.1 Culture-dependent and culture-independent approaches for environmental sample screening. (Figure was taken from Batista-García et al. 2016)

of information and processing this information requires specific tools and frameworks. Recently, methods and software designed to deal with metagenomic data have become more accessible (or friendly) to biology scientist, who generally lacks professional programming skills. Also, databases gathering "omics" information have been continuously growing and are publicly available, which facilitate the exchange and integration of information.

3.2 The Promises of Metagenomics

The first task of an environmental management project is assessing the contamination level in a site and the extent of the detrimental effect on ecosystem dynamics. Currently, most environments are polluted to some degree with natural or synthetic compounds that are hazardous to animals, plants, and the ecosystems. The traditional approaches during site investigation in environmental risk assessment are determining the physical-chemical properties of the matrix (soil, water, or air) and the presence of suspected contaminants. However, the presence of a toxic compound in a site does not directly implicate that the health of the system is compromised nor that an intervention is required to eliminate the pollutant. In many occasions, the naturally occurring microorganisms are able to degrade the exotic compounds and restore the balance to the ecological unit. The goal in environmental management is to preserve or restore the natural functions of an ecosystem with the minimal intervention and economical cost. Therefore, the bottom line is that a holistic and comprehensive analysis is required to define the most convenient form of intervention in a site-dependent manner.

The discharge of a contaminant can shape the composition of the microbial communities in an ecosystem. Other effects might be the net increase in biomass, the genetic diversity, or the activity (Pulleman et al. 2012). The species able to degrade the pollutant or its intermediary degradation compounds are generally enriched, while the species sensitive to the pollutant are lessened. These species are regarded as bioindicators and have been used widely in the diagnosis of contaminated places (Pulleman et al. 2012). In this way, metagenomics can help to measure the deterioration of the natural function of ecosystems as a result of contamination. By targeting phylogenetic markers, such as 16S rDNA for bacteria and 18S rDNA or ITS for fungi and other eukaryotes, metagenomics can portray the phylogenetic composition of a microbial community. Samples from uncontaminated sites or pre-contamination samples from the same site can serve as controls to determine if particular phyla have varied in the community.

Through a meta-analysis pipeline, Oliveira et al. (2017) analyzed metagenome samples from biomes with crude oil contamination across different geographies and obtained by different research projects. Firstly, they found that distributions of genes involved in the degradation of petroleum hydrocarbons and genes involved in

the biosynthesis of biosurfactants are strongly correlated, which might seem intuitive but is not generally analyzed jointly in metagenomic studies. Also, their results show that terrestrial ecosystems present more degradation-related genes and fewer biosurfactant genes when compared to water biomes. Taking this into consideration, it would imply that treatment strategies to bioremediate oil-contaminated waters would differ from those used for terrestrial spills in terms of the activities that need fostering to achieve a particular rate of hydrocarbon degradation. Lastly, but not less important, these authors found that latitude has a significant influence on the diversity of genes involved in biodegradation and biosurfactant production. According to this, microbiomes near the equator have higher diversity in genes involved in hydrocarbon degradation when compared to other geographical zones. This information can be used in the design of bioremediation techniques, as microbial consortia from these areas can be particularly suited for application to bioaugmentation, enzyme discovery, and degradation pathway analysis.

In addition, the knowledge of the microbial interactions and capabilities of the native microorganisms in a contaminated site has allowed the application of various biostimulation and bioaugmentation strategies. The former pursues the goal of stimulating the capacity of resident microbial communities to degrade pollutants by modifying biochemical parameters such as nutrient availability, carbon sources, pH, temperature, aeration and oxygenation, water content, etc. Here, the systems biology approach is essential to determine the limiting reactions that can affect the biodegradation rates of toxic compounds. Bioaugmentation, on the other hand, is a more intrusive strategy by which nonnative microorganisms are added to the contaminated site, generally to supply pathways that are not present or optimal in the indigenous microbiome. For such purpose, the consortia and synergistic interactions between different microbial species should have been characterized a *priori*. This strategy has some drawback: for example, the foreign microorganisms may not be competitive in the ecological environment that is treated, or the microorganisms degrade the pollutant but remain in the site long after the contaminant has disappeared, modifying the dynamics of the natural ecosystem. In this case, metagenomics comes back into play as a monitoring tool that allows assessing the effect of the bioremediation treatment on the native microbial communities.

Finally, many of the microorganisms that inhabit the heavily contaminated sites represent a source of potential biotechnological products to restore environmental health. Therefore, metagenomics has served to investigate microbial communities that are actively degrading a compound, under the notion that the genes with more variability or representation in the metagenomic libraries might be involved in the biodegradation of such contaminant. This so-called gene-centric approach has gained much attention because it can serve for the prospection of new enzyme activities or new genes that are accessory to the degradative pathways, such as the

aforementioned biosurfactants in the case of hydrocarbon degradation. Also, this gene-centric approach has served as a monitoring tool, because some genes known to be responsive to contamination levels can be screened in the investigated sites to assess the effect of a pollutant in the microbiome without the need to sequence all the genetic content of the samples.

Taking all into consideration, metagenomics has properly served to various purposes on the bioremediation field and is currently a powerful tool during decision-making for treatment strategies and management of contaminated environments. The continuous reduction of the sequencing prices makes these methodologies affordable for industrial applications. Nonetheless, significant efforts remain to be done to change the perception of these techniques in the industrial community and to expand their use to a daily basis.

3.3 Transcriptomics

Transcriptomic approaches are successfully used to obtain insights into microbial functions in contaminated sites. These techniques preferentially study the total environmental mRNA. On the other hand, metatranscriptomic studies the gene expression profile of the entire microbial communities, being the RNA extraction one of the major challenges (Desai et al. 2010). These methods allow analyzing the gene expression dynamic at the transcriptional level of one organism or microbial community inhabiting a specific habitat in a specific time. Transcriptomics also reveal the plasticity of the entire cell repertoire when microorganisms are exposed to pollutants.

Since transcriptomics studies the mRNA profile, it can offer a general idea about the enzymatic mechanisms involved in pollutant degradation. Thus, these omics tools generate information related with subsequent protein profile of the cell. However, the posttranscriptional modification could drastically modulate this information.

Transcriptomic studies are not abundant to understand the enzymatic strategies used for microorganisms. For example, transcriptional analyses associated with the polycyclic aromatic hydrocarbons degradations are scarce. However, the degradative mechanisms involved in asphaltene biotransformation by *Neosartorya fischeri* were studied at the transcriptional level. It has been found that genes encoding for monooxygenase enzymes were upregulated (Hernández-López et al. 2015). Another transcriptional analysis in *Exophiala pisciphila* obtained that glutathione S-transferase genes were upregulated during its growth in soil contaminated with heavy metals (Morel et al. 2013).

3.4 Proteomic in a Degradation Concept

In this chapter, we highlight proteomic approaches since the presence of proteins determines the final phenotype of the cell. In the context of this chapter, it is obligatory that enzymes can finally be detected to fully understand biodegradation pathways.

Proteome refers to the entire protein profiles encoded by the genome, and proteomic, on another hand, is the tool that allows the study of the proteome, their expression, function, and structure, including the posttranslational modifications. The analysis of the proteome plays an important role to fully understand any biological process, including biodegradative processes. In this context, secretome refers to the entire set of extracellular proteins encoded by the genome, and it is very relevant to hypothesize extracellular pathways related with the catabolism of any pollutant. Thus, the analysis of sub-proteomes and secretomes allows the identification of proteins with relevant functions in the degradation or removal of organic contaminants and reveals those differentially expressed proteins involved in the biodegradation. The proteome is very dynamic since the cell quickly regulates its expression according to specific living conditions.

In recent years, proteomics has become important as a discipline in environmental biotechnology. "The application of proteomics to the field of biodegradation is in its infancy" (Chauhan and Jain 2010). However, proteomics is now completely established as a robust and powerful methodology to characterize functional proteins relevant for environmental biotechnology. In general terms, even up today, proteomics is an expensive technique, and it is required to develop a new experimental methodology with reasonable costs for environmental applications. Gel-free approaches are not yet at the hand for many groups around the world, and, in consequence, there are no notable advances in the environmental proteomics. For this reason, gel-based approaches are still useful. Figure 3.2 shows both approaches, 2-DE-based proteomics and gel-free quantitative proteomics (Fig. 3.2 was taken from Loh and Cao 2008).

The information generated by proteomics could be supplemented with information obtained from other global studies such as transcriptomics, metagenomics, and genomics, as well as with those results recovered from other emergent omics such as phenomics, ionomics, lipidomics, culturomics, etc. The synergy of all these molecular and morphological tools could drastically transform the point of view to study the biotransformation of xenobiotics. The new omics era has big challenges to generate new knowledge in the border of the science and, in consequence, propose novel eco-friendly ideas to remove xenobiotics from the environment.

Polycyclic aromatic hydrocarbons (PAHs) are model compounds to study metabolic pathways involved in the xenobiotic biodegradation. Proteomics has been successfully used to elucidate catabolic and regulatory mechanisms involved in PAH degradation (Kim et al. 2011; Vandera et al. 2015; Liu et al. 2017). These mechanisms have been largely studied in bacteria, while the fungal mechanisms are even

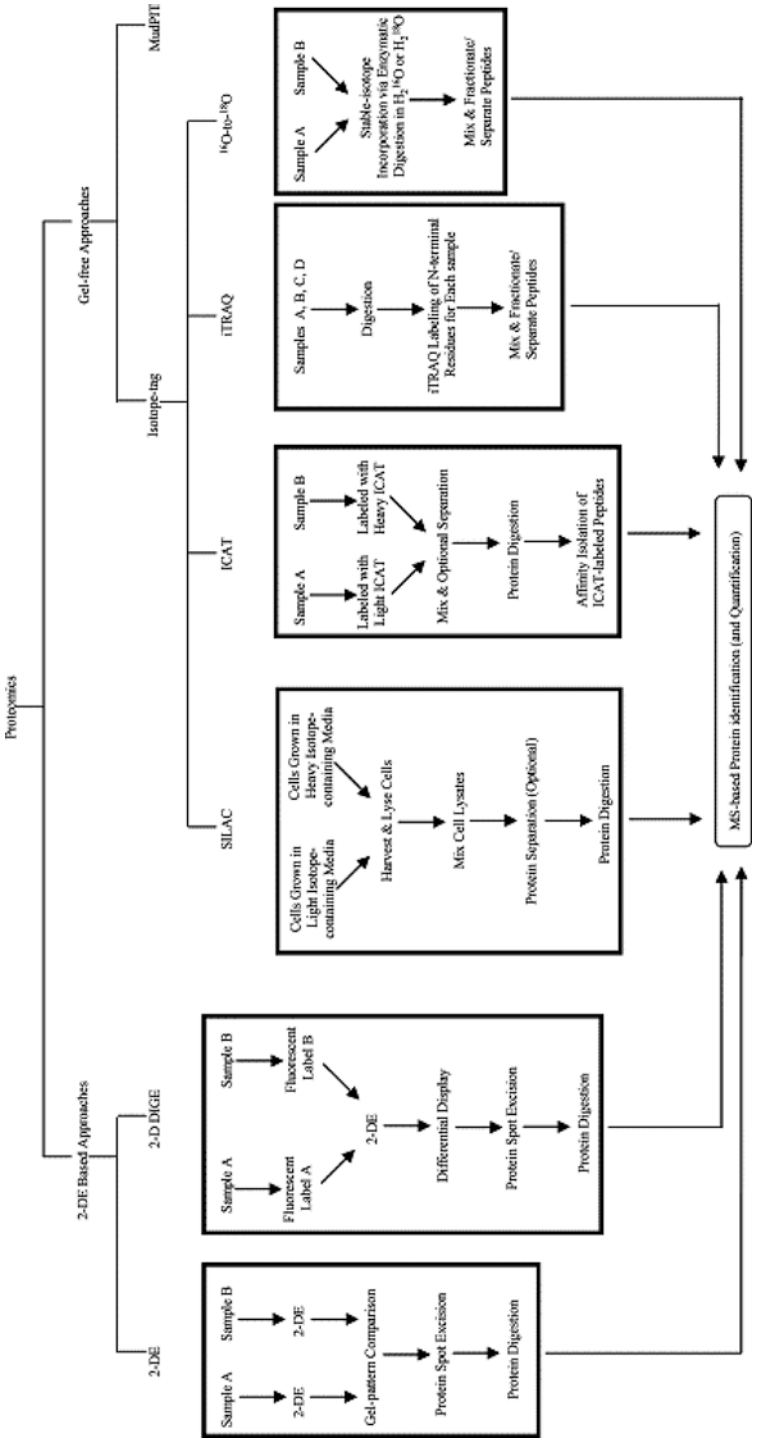

Fig. 3.2 Schematic illustration of representative proteomic approaches. 2-DE-based proteomics and gel-free quantitative proteomic are shown. (Figure and legend were taken from Loh and Cao 2008)

unclear. Figure 3.3 shows a schematic model published by Liu and co-workers (Liu et al. 2017) that was obtained from proteomic studies in *Sphingomonas* sp. strain GY2B during phenanthrene degradation (Fig. 3.3 was taken from Liu et al. 2017). A proteomic analysis conducted in *Achromobacter xylosoxidans* during pyrene biodegradation informed that proteins involved in cell homeostasis, genetic information synthesis and storage, and chemical stress were upregulated. 4-Hydroxyphenylpyruvate dioxygenase and homogentisate 1,2-dioxygenase were particularly important during pyrene transformation (Nzila et al. 2018).

Regarding fungi, the progress in environmental proteomics is discreet with a slight takeoff in the last years. A recent study in *Penicillium oxalicum* identified via shotgun proteomics 158 upregulated and 174 downregulated unique protein species differentially expressed during anthracene biotransformation. Cytochrome P450s, epoxide hydrolases, and transferase enzymes were the main upregulated proteins involved in anthracene biotransformation (Lucero Camacho-Morales et al. 2018). In this study, 77.1% of the proteins were annotated as hypothetical uncharacterized proteins. The protein annotation is crucial during the proteomic analysis. It currently represents an important bottleneck, and it is one of the major challenges of fungal studies. Verdin and co-workers (2005) used a 2-D gel-based approach to study the benzo[a]pyrene degradation by *Fusarium solani*, and an overexpression of P450 was found.

Another proteomic analysis was addressed to understand the metabolic pathway preferentially used by the fungus *Metarhizium robertsii* in presence of 4-*n*-nonylphenol, an endocrine-disrupting compound, as a carbon source (Szewczyk et al. 2014). Oxidation-reduction system related to nitroreductase-like proteins, reactive oxygen species defense systems (peroxiredoxin and superoxide dismutase), the tricarboxylic acid cycle, and energy-related systems were the main proteins and metabolic pathways upregulated in the proteome of *M. robertsii*. In this study, a huge group of unidentified proteins was observed.

Proteomics has also been applied to investigate the fungal degradation of herbicide. The upregulated protein profile of *Paecilomyces marquandii* revealed that in presence of alachlor, an herbicide used to protect plant crops against broadleaf weeds and annual grasses, the proteins related with energy and sugar metabolism, as well as reactive oxygen species, are key enzymes involved in the alachlor biotransformation pathway (Szewczyk et al. 2015). Authors identified cyanide hydratase as the main enzyme implicated in the biotransformation of alachlor.

The previous studies demonstrate that proteomics is a useful and valuable tool to analyze those open reading frames induced by the presence of xenobiotics. But, other aspect related with proteins can also be studied by proteomics since it can answer other questions. For example, what happens with the isoenzymes of key proteins in biotransformation processes? Are the posttranslational modifications important during the xenobiotic biotransformation? How is the dynamic of the protein degradation? These questions remain absolutely understood. Further researches and efforts are needed to clarify these points.

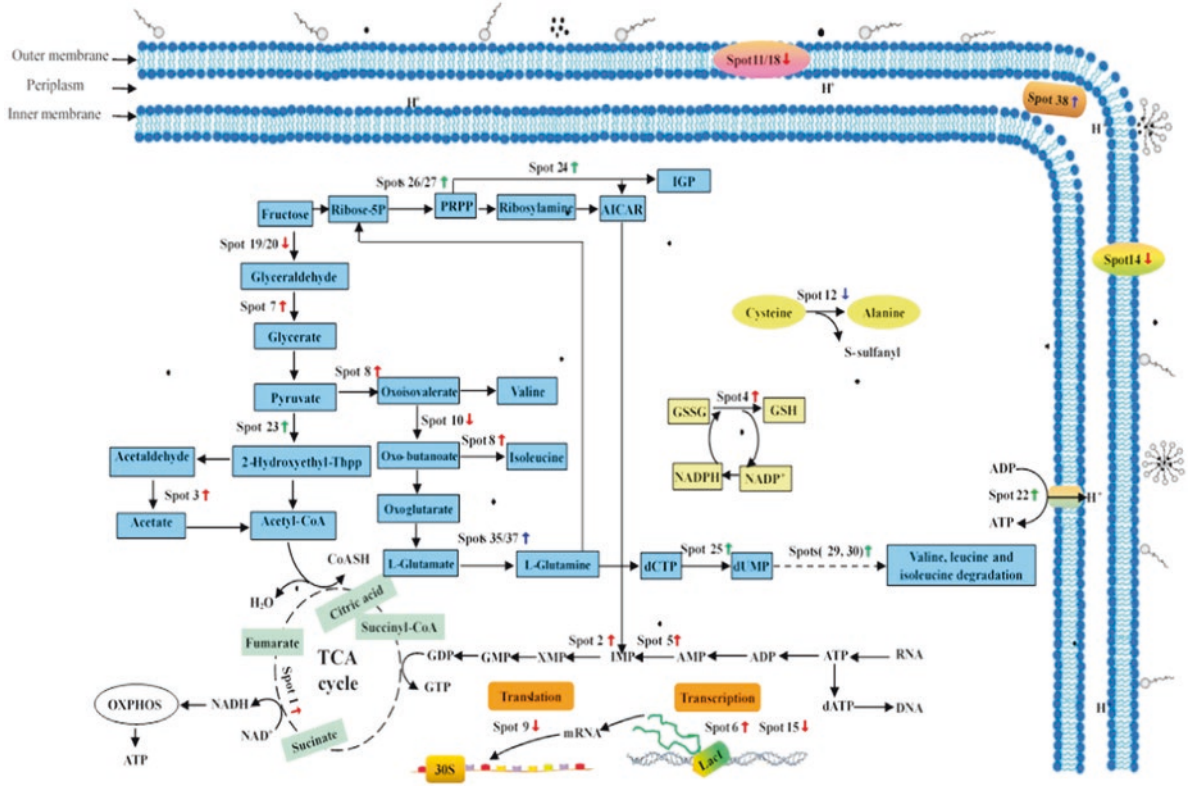

Fig. 3.3 Schematic model of the protein expression of *Sphingomonas* sp. GY2B in response to Tween 80 during phenanthrene (100 mg/L) biodegradation. The upregulated and downregulated proteins are marked by ↑ and ↓, respectively. Red arrows represent the protein with altered expression in all three concentrations of Tween 80 (0.2, 1, and 8 CMC); the green and blue arrows represent the differentially expressed proteins detected in two concentrations (1 and 8 CMC) and only in 8 CMC treated sample, respectively. (Figure and legend were taken from Liu et al. 2017)

Xenobiotics also elicit molecular mechanisms related to stress responses. In this context, proteomics has brought relevant information about the proteins involved in the stress physiology. When microorganisms are exposed at recalcitrant pollutants, changes occur in their protein profiles including membrane proteins and those enzymes related with DNA repair mechanisms, among others. Proteomic approaches have shown in *Pseudomonas putida* KT2440 exposed to phenol that proteins participating in oxidative stress response, general stress response, energetic metabolism, fatty acid biosynthesis, inhibition of cell division, cell envelope biosynthesis, transcriptional regulation, and transport of small molecules are totally upregulated. These proteomic studies have also revealed that the protein profile involved in cell morphology and cell surface hydrophobicity also drastically changes (Santos et al. 2004).

3.4.1 Metaproteomic

Finally, metaproteomic is also a powerful methodology to study the relationship between microorganisms in polluted environments and their possible role in xenobiotic degradation. Metaproteomic is an emerging tool in the post-genomic era and cannot distinguish between proteins from different microorganisms since it analyzes at high-throughput scale, the pull of proteins produced by microorganisms inhabiting a specific environment and time (Keller and Hettich 2009; Wilmes et al. 2008). This tool is based on the extraction, purification, identification, and annotation of all proteins present in an environment, being the first step one of the major challenges. The meta-analysis at protein levels brings relevant information related to metabolic pathways in nature and allows the hypothesis postulation about the ecological role of microbial communities in the xenobiotic degradation. The metaproteomic studies are very scarce in contaminated environments. For example, metaproteomic application to understand the biodegradation processes in wastewater biotreatment is still in its infancy. However, new research efforts are needed due to that metaproteomics could strongly contribute to identifying microbial proteins with key functions in wastewater biotreatments as well as to elucidate the microbial metabolic pathways involved in wastewater biotransformation.

Metaproteomics could also bring knowledge related with the availability to access pollutants to microorganisms, how is the growth control and microbial homeostasis in polluted sites, how xenobiotics could affect the nutrient acquisition in microbes, and how is the dynamic of the stress response at the microbial ecosystem level, among others. As it is supposed that microbial networks are environmentally necessary for xenobiotic degradation (Aydin et al. 2017), these points are crucial for the understanding of the bioprocesses in nature. Thus, the omics era and its molecular

biological tools can be used to understand the dynamics of the microbial networks in the biotransformation of pollutants. Additionally, metaproteomics can also generate information about the regulatory mechanisms in these microbial networks (Aydin et al. 2017) and, interestingly, how is the communication, the chemical talks, between different species and populations in polluted ecosystems. Metagenomics have studied the functioning of microbial networks in soils (Benndorf et al. 2007; Wang et al. 2011), activate sludges (Kuhn et al. 2011; Osman et al. 2007; Wilmes et al. 2008), groundwater (Benndorf et al. 2007; Kan et al. 2005), wool fabrics (Solazzo et al. 2013), wastewater bioreactor (Lacerda et al. 2007), psychrophilic anaerobic wastewater treatment bioreactor (Abram et al. 2011), and thermophilic anaerobic treatment of agriculture biomass (Hanreich et al. 2012). Figure 3.4 represents a schematic model of the active metabolic pathways involved in a swine manure-based thermophilic anaerobic digester obtained from metaproteomic results (Fig. 3.4 was taken from Lin et al. 2016).

Another metaproteomic study also proposed the carbohydrate metabolic pathways of microbial communities during a large-scale aerobic composting plant (Fig. 3.5) (Fig. 3.5 was taken from Liu et al. 2015). Thus, metaproteomics opens new perspectives at the protein level to understand molecular mechanisms of xenobiotic degradation at the community level (El Amrani et al. 2015; Shahi et al. 2016a, b).

In conclusion, (meta)proteomics represent an unique opportunity to study protein profiles produced during pollutant degradations. This approach comprises structural and quantitive analysis. As 90% of the microorganisms in nature cannot be cultivated, a broad range of obtained peptides is probably from those uncultivable microbes. Thus, (meta)proteomics is a source of novel proteins with relevant impact in environmental science.

Finally, the "omics" application to generate novel knowledge related with polluted environments is an area of big opportunities to strongly impact the biotechnological applications. The integrative omics approaches represent a methodological challenge from sampling to analysis. Different disciplines play a key role in omics application. Figure 3.6 shows an overview of molecular and omics technologies applied to contaminated sites (Fig. 3.6 was taken from Desai et al. 2010).

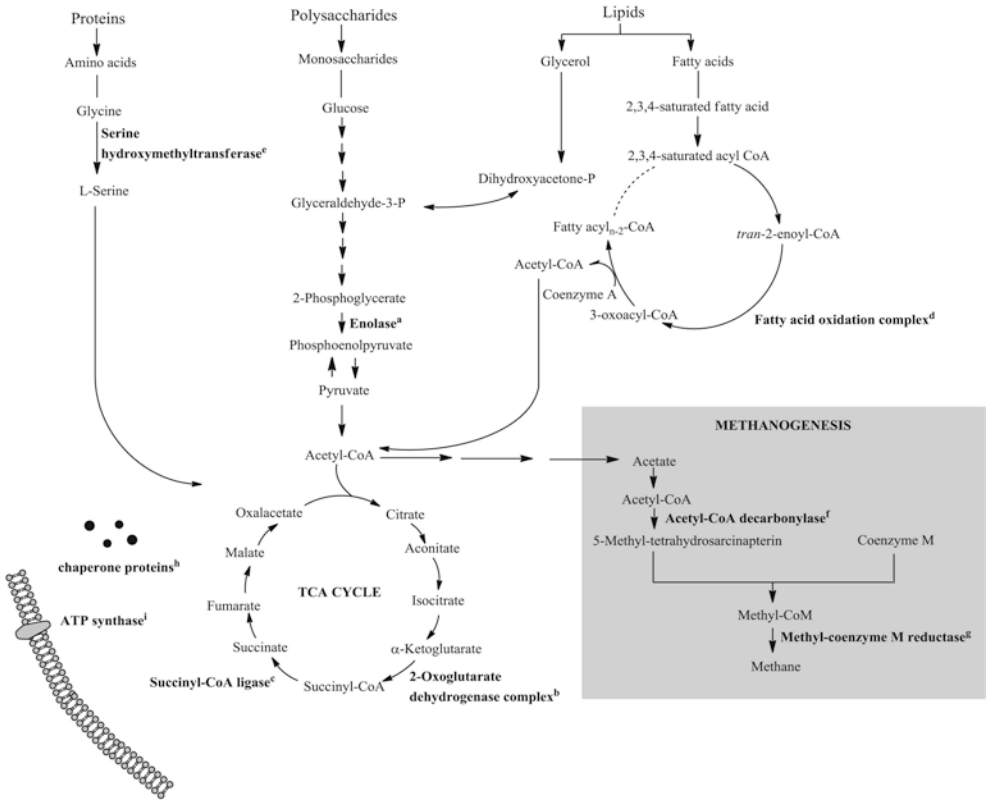

Fig. 3.4 The proposed metabolic pathways active in a swine manure-based thermophilic anaerobic digester as inferred from the metaproteomic results. Enzymes identified in this study are shown in bold. Not all intermediates are shown in this figure. *indicates source organisms that are not confirmed because

Fig. 3.4 (continued) the identified peptide sequences are conserved across many species. The identified proteins belong to eubacteria and archaea. The source organisms are assigned by the identified peptides: [a]*Pseudomonas fluorescens* SBW25; [b]*Bdellovibrio bacteriovorus*, *Buchnera aphidicola* str. Bp, *Clostridium novyi*-NT, *Idiomarina loihiensis*, *Neocallimastix frontalis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Rubrobacter xylanophilus* DSM 9941, or *Thiomicrospira crunogena* XCL-2; [c]*Pseudomonas putida*; [d]**Dictyostelium discoideum*, *Bartonella* sp., or *Pseudomonas aeruginosa*; [e]*Azotobacter vinelandii*, *Buchnera aphidicola*, *Mycobacterium bovis*, *Pseudomonas aeruginosa*, or *Staphylococcus haemolyticus* JCSC1435; [f]*Actinobacillus succinogenes* 130Z, *Acinetobacter* sp. ADP1, *Escherichia coli* BW2952, *Marinobacter aquaeolei* VT8, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas mendocina* ymp, *Pseudomonas putida*, or *Vibrio cholerae*; [g]**Escherichia coli* or *Salmonella typhimurium*; [h]*Colwellia psychrerythraea* 34H; [i]*Pseudomonas aeruginosa*; [j]**Mycobacterium bovis*, *M. marinum*, or *M. tuberculosis*; [k]fatty acid oxidation complex including dodecenoyl-CoA d-isomerase, enoyl-CoA hydratase, 3-hydroxybutyryl-CoA epimerase, and 3-hydroxyacyl-CoA dehydrogenase; [l]**Pseudomonas fluorescens* Pf-5; *Pseudomonas fluorescens* Pf0-1 or *Pseudomonas syringae* sp.; [m]*Pseudomonas aeruginosa*; [n]*Streptococcus pneumonia*; [o]*Pseudomonas fluorescens* or *Pseudomonas putida*; [p]*Methanocaldococcus jannaschii* DSM 2661; [q]*Methanosarcina* sp.; [r]*Methanothermobacter thermautotrophicus* str. Delta H, *Methanosarcina barkeri* str. Fusaro, or *Methanothermus fervidus*; [s]*Acidithiobacillus ferrooxidans*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, *Edwardsiella ictaluri* 93e146, or *Chromobacterium violaceum*; [t]*Pseudomonas putida*; [u]*Candidatus solibacter usitatus* Ellin6076, *Leifsonia xyli* subsp. xyli, *Dechloromonas aromatica* RCB, *Geobacter lovleyi* SZ, *Candidatus blochmannia floridanus*, *Colwellia psychrerythraea* 34H, *Idiomarina loihiensis*, *Pseudomonas fluorescens* Pf0-1, *Pseudomonas fluorescens* Pf-5, *Pseudomonas putida* W619, *Pseudomonas entomophila* L48, *Petrotoga mobilis* SJ95, *Methylobacillus flagellatus* KT, *Methylococcus capsulatus*, *Pelobacter carbinolicus* DSM 2380, *Polaromonas naphthalenivorans* CJ2, *Psychromonas ingrahamii* 37, or *Thioalkalivibrio sulfidophilus* HL-EbGr7; [v]*Bacillus licheniformis* ATCC 14580; [w]*Pseudomonas fluorescens*. (Figure and legend were taken from Lin et al. 2016)

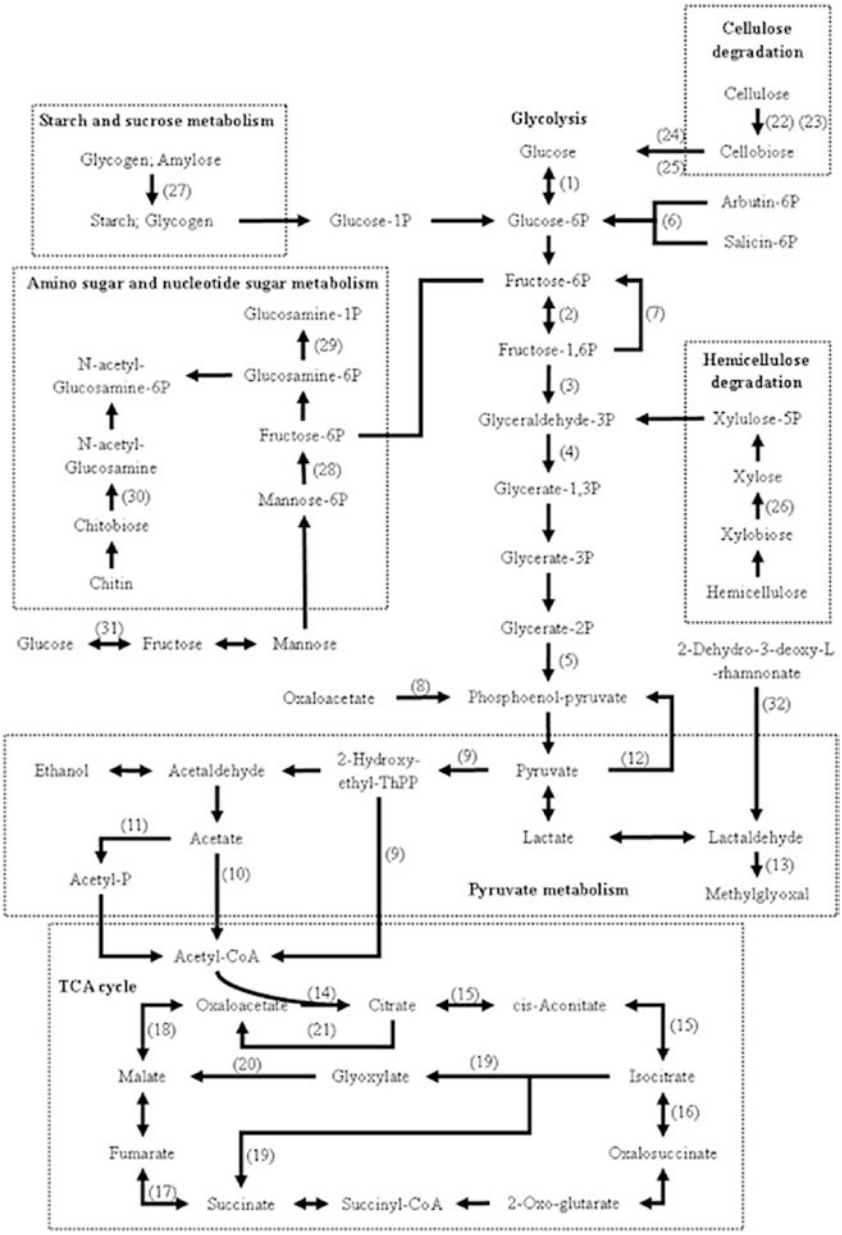

Fig. 3.5 Depiction of the carbohydrate metabolic characteristics of microbial communities inferred from the metaproteome. Proteins shown are (1) hexokinase; (2) 6-phosphofructokinase; (3) triose-phosphate isomerase; (4) glyceraldehyde-3-phosphate dehydrogenase; (5) enolase; (6) aryl-phospho-beta-D-glucosidase; (7) fructose-1,6-bisphosphatase; (8) phosphoenolpyruvate carboxykinase; (9) pyruvate dehydrogenase; (10) acetyl-coenzyme A synthetase; (11) acetate kinase; (12) pyruvate, phosphate dikinase; (13) glyoxylate/hydroxypyruvate reductase; (14) citrate synthase; (15) aconitate hydratase; (16) isocitrate dehydrogenase; (17) succinate dehydrogenase; (18) malate dehydrogenase; (19) isocitrate lyase; (20) malate synthase; (21) citrate lyase; (22) exoglucanase; (23) endoglucanase; (24) cellobiose dehydrogenase; (25) beta-glucosidase; (26) endo-1,4-beta-xylanase B; (27) 1,4-alpha-glucan-branching enzyme GlgB; (28) mannose-6-phosphate isomerase; (29) phosphoglucosamine mutase; (30) beta-N-acetylhexosaminidase; (31) 2-keto-3-deoxy-L-rhamnonate aldolase; (32) xylose isomerase. (Figure and legend were taken from Liu et al. 2015)

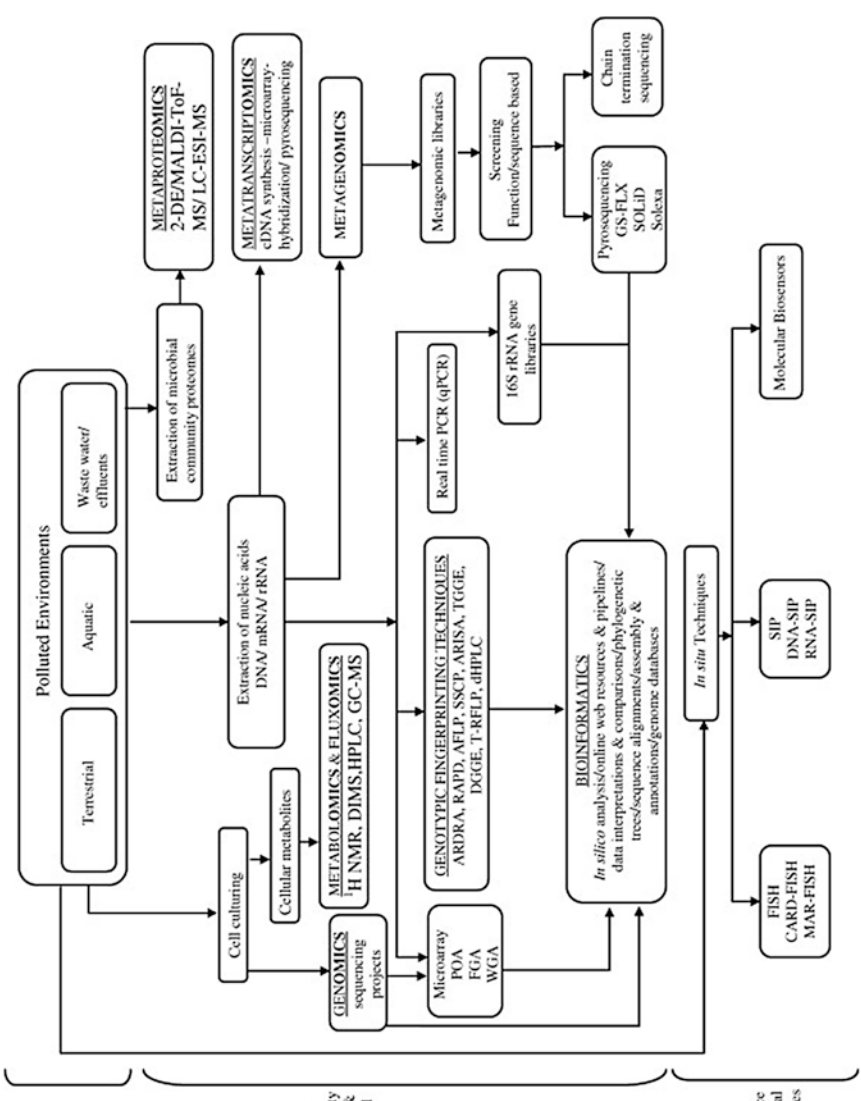

Fig. 3.6 An overview of molecular and omics technologies employed to survey intrinsic microbial communities underlying bioremediation at contaminated sites. (Figure and legend were taken from Desai et al. 2010)

References

Abram F, Enright AM, O'Reilly J, Botting CH, Colling G, O'Flaherty V (2011) A metaproteomic approach gives functional insights into anaerobic digestion. J Appl Microbiol 110:1550–1560

Aydin S, Karaçay HA, Shahi A, Gökçe S, Ince B, Ince O (2017) Aerobic and anaerobic fungal metabolism and Omics insights for increasing polycyclic aromatic hydrocarbons biodegradation. Fungal Biol Rev 31:61–72

Batista-García RA, Sánchez-Carbente MR, Talia P, Jackson SA, O'Leary ND, Dobson ADW, Folch-Mallol JL (2016) From lignocellulosic metagenomes to lignocellulolytic genes: trends, challenges and future prospects. Biofuels, Bioproducts and Biorefining 10 (6):864-882

Benndorf D, Balcke GU, Harms H, von Bergen M (2007) Functional metaproteome analysis of protein extracts from contaminated soil and groundwater. ISME J 1:224–234

Chauhan A, Jain RK (2010) Biodegradation: gaining insight through proteomics. Biodegradation 21:861–879

Desai C, Pathak H, Madamwar D (2010) Advances in molecular and "-omics" technologies to gauge microbial communities and bioremediation at xenobiotic/anthropogen contaminated sites. Bioresour Technol 101:1558–1569

El Amrani A, Dumas AS, Wick LY, Yergeau E, Berthomé R (2015) "Omics" insights into PAH degradation toward improved green remediation biotechnologies. Environ Sci Technol 49:11281–11291

Handelsmanl J, Rondon MR, Goodman RM, Brady SF, Clardy J (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5:245–R249

Hanreich A, Heyer R, Benndorf D, Rapp E, Pioch M, Reichl U, Klocke M (2012) Metaproteome analysis to determine the metabolically active part of a thermophilic microbial community producing biogas from agricultural biomass. Can J Microbiol 58:917–922

Hernández-López EL, Ramírez-Puebla ST, Vazquez-Duhalt R (2015) Microarray analysis of *Neosartorya fischeri* using different carbon sources, petroleum asphaltenes and glucose-peptone. Genomics Data 5:235–237

Kan J, Hanson TE, Ginter JM, Wang K, Chen F (2005) Metaproteomic analysis of Chesapeake Bay microbial communities. Saline Syst 1:7–21

Keller M, Hettich R (2009) Environmental proteomics: a paradigm shift in characterizing microbial activities at the molecular level. Microbiol Mol Biol Rev 73:62–70

Kim SJ, Kweon O, Cerniglia CE (2011) Proteomic applications to elucidate bacterial aromatic hydrocarbon metabolic pathways. Compr Biotechnol 6:105–114

Kuhn R, Benndorf D, Rapp E, Reichl U, Palese LL, Pollice A (2011) Metaproteome analysis of sewage sludge from membrane bioreactors. Proteomics 11:2738–2744

Lacerda CMR, Choe LH, Reardon KF (2007) Metaproteomic analysis of a bacterial community response to cadmium exposure. J Proteome Res 6:1145–1152

Lin YW, Tuan NN, Huang SL (2016) Metaproteomic analysis of the microbial community present in a thermophilic swine manure digester to allow functional characterization: a case study. Int Biodeterior Biodegrad 115:64–73

Liu D, Li M, Xi B, Zhao Y, Wei Z, Song C, Zhu C (2015) Metaproteomics reveals major microbial players and their biodegradation functions in a large-scale aerobic composting plant. Microb Biotechnol 8:950–960

Liu S, Guo C, Dang Z, Liang X (2017) Comparative proteomics reveal the mechanism of Tween80 enhanced phenanthrene biodegradation by *Sphingomonas* sp. GY2B. Ecotoxicol Environ Saf 137:256–264

Loh KC, Cao B (2008) Paradigm in biodegradation using *Pseudomonas putida*-a review of proteomics studies. Enzym Microb Technol 43:1–12

Lucero Camacho-Morales R, García-Fontana C, Fernández-Irigoyen J, Santamaría E, González-López J, Manzanera M, Aranda E (2018) Anthracene drives sub-cellular proteome-wide alterations in the degradative system of *Penicillium oxalicum*. Ecotoxicol Environ Saf 159:127–135

Morel M, Meux E, Mathieu Y, Thuillier A, Chibani K, Harvengt L, Jacquot JP, Gelhaye E (2013) Xenomic networks variability and adaptation traits in wood decaying fungi. Microb Biotechnol 6:248–263

Nzila A, Ramirez CO, Musa MM, Sankara S, Basheer C, Li QX (2018) Pyrene biodegradation and proteomic analysis in *Achromobacter xylosoxidans,* PY4 strain. Int Biodeterior Biodegrad 130:40–47

Oliveira JS, Araújo WJ, Figueiredo RM, Silva-Portela RB, Guerra ADB, Carla S, Minnicelli C, Carlos AC, Tereza A, Vasconcelos RD, Freitas AT, Agnez-Lima L (2017) Biogeographical distribution analysis of hydrocarbon degrading and biosurfactant producing genes suggests that near- equatorial biomes have higher abundance of genes with potential for bioremediation. BMC Microbiol 17:1–10

Osman C, Wilmes C, Tatsuta T, Langer T (2007) Prohibitins interact genetically with Atp23, a novel processing peptidase and chaperone for the F1Fo-ATP synthase. Mol Biol Cell 18:627–635

Pulleman M, Creamer R, Hamer U, Helder J, Pelosi C, Peres G, Rutgers M (2012) Soil biodiversity, biological indicators and soil ecosystem services – an overview of European approaches. Curr Opin Environ Sustain 4:529–538

Santos PM, Benndorf D, Sá-Correia I (2004) Insights into *Pseudomonas putida* KT2440 response to phenol-induced stress by quantitative proteomics. Proteomics 4:2640–2652

Shahi A, Aydin S, Ince B, Ince O (2016a) Evaluation of microbial population and functional genes during the bioremediation of petroleum-contaminated soil as an effective monitoring approach. Ecotoxicol Environ Saf 125:153–160

Shahi A, Aydin S, Ince B, Ince O (2016b) Reconstruction of bacterial community structure and variation for enhanced petroleum hydrocarbons degradation through biostimulation of oil contaminated soil. Chem Eng J 306:60–66

Solazzo C, Dyer JM, Clerens S, Plowman J, Peacock EE, Collins MJ (2013) Proteomic evaluation of the biodegradation of wool fabrics in experimental burials. Int Biodeterior Biodegrad 80:48–59

Szewczyk R, Soboń A, Sylwia R, Dzitko K, Waidelich D, Długoński J (2014) Intracellular proteome expression during 4-*n*-nonylphenol biodegradation by the filamentous fungus Metarhizium robertsii. Int Biodeterior Biodegrad 93:44–53

Szewczyk R, Soboń A, Słaba M, Długoński J (2015) Mechanism study of alachlor biodegradation by Paecilomyces marquandii with proteomic and metabolomic methods. J Hazard Mater 291:52–64

Vandera E, Samiotaki M, Parapouli M, Panayotou G, Koukkou AI (2015) Comparative proteomic analysis of Arthrobacter phenanthrenivorans Sphe3 on phenanthrene, phthalate and glucose. J Proteomics 113:73–89

Verdin A, Lounès-Hadj Sahraoui A, Newsam R, Robinson G, Durand R (2005) Polycyclic aromatic hydrocarbons storage by *Fusarium solani* in intracellular lipid vesicles. Environ Pollut 133:283–291

Wang HB, Zhang ZX, Li H, He HB, Fang CX, Zhang AJ, Li QS, Chen RS, Guo XK, Lin HF, Wu LK, Lin S, Chen T, Lin RY, Peng XX, Lin WX (2011) Characterization of metaproteomics in crop rhizospheric soil. J Proteome Res 10:932–940

Wilmes P, Wexler M, Bond PL (2008) Metaproteomics provides functional insight into activated sludge wastewater treatment. PLoS One 3:e1778