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MINI-REVIEW

Microbial genomics, transcriptomics and proteomics: new discoveries in decomposition research using complementary methods

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Abstract Molecular methods for the analysis of biomolecules have undergone rapid technological development in the last decade. The advent of next-generation sequencing methods and improvements in instrumental resolution enabled the analysis of complex transcriptome, proteome and metabolome data, as well as a detailed annotation of microbial genomes. The mechanisms of decomposition by model fungi have been described in unprecedented detail by the combination of genome sequencing, transcriptomics and proteomics. The increasing number of available genomes for fungi and bacteria shows that the genetic potential for decomposition of organic matter is widespread among taxonomically diverse microbial taxa, while expression studies document the importance of the regulation of expression in decomposition efficiency. Importantly, high-throughput methods of nucleic acid analysis used for the analysis of metagenomes and metatranscriptomes indicate the high diversity of decomposer communities in natural habitats and their taxonomic composition. Today, the metaproteomics of natural habitats is of interest. In combination with advanced analytical techniques to explore the products of decomposition and the accumulation of information on the genomes of environmentally relevant microorganisms, advanced methods in microbial ecophysiology should increase our understanding of the complex processes of organic matter transformation.

Keywords Decomposition · Genomics · Metagenomics · Metatranscriptomics · Metaproteomics · Saprotrophic fungi and bacteria

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Introduction

Recently, rapid technological improvements have allowed the development of molecular methods for the analysis of biomolecules. In particular, the advent of next-generation sequencing methods enabled the scientific community to reduce the costs of large-scale sequencing (Glenn 2011; Mardis 2011), and increases in instrumental resolution enabled the analysis of complex proteome and metabolome data (Keiblinger et al. 2012; Wallenstein et al. 2013).

The potential of these methodological developments is demonstrated by advances in decomposition research. Microbial decomposition receives extraordinary attention for two major reasons: (1) on the level of individual microorganisms, it is driven by the biotechnological need to find microorganisms or enzymes for lignocellulose transformation useful for the production of biofuels, for example, and (2) the interest in complex decomposition processes in the environment by the fact that decomposition represents an important part of the global carbon cycle and affects global climate change through CO_2 release rates. The applications, results and present possibilities of these complementary omics methods in the study and understanding of microbial decomposition are highlighted in this review, focused mainly on fungi and fungal decomposition.

Omics methods for the exploration of decomposition by individual microorganisms

In the early 2000s, after decades of research on fungal decomposition using physiological and biochemical methods, the picture of decomposition pathways was detailed for a limited number of model organisms, especially wood-rotting fungi. There was a good knowledge of the enzymes produced by individual fungi, the corresponding genes and their regulation,

including their activity on lignocellulose (Decelle et al. 2004; Guettler et al. 2003; Martínez et al. 2005). The biochemical exploration of decomposition-related enzymes allowed informed conclusions about the decomposition potential of individual fungi and the properties of their enzymes (Baldrian 2006; Lynd et al. 2002; Baldrian et al. 2008). This knowledge was rapidly advanced in subsequent years, thanks to the novel possibilities associated with genomics, transcriptomics and proteomics. The *Phanerochaete chrysosporium* genome sequence published in 2004 revealed for the first time the impressive complexity of the array of genes encoding secreted oxidases, peroxidases and hydrolytic enzymes that cooperate in wood decay (Martinez et al. 2004). Analysis of the complete genome sequence of *Postia placenta* made it possible to compare the gene pools of ligninolytic (white-rot) and cellulolytic (brown-rot) fungi and to define the genetic features that distinguish these two ecophysiological groups (Martinez et al. 2009).

Comparisons of genome sequences make it theoretically possible to define the physiological potential of individual fungal taxa, taxonomic groups or ecophysiological groups. In this way, the analysis of fungal genomes revealed the presence of multiple multi-copper oxidases (the group of enzymes including laccases) in different ecophysiological groups of fungi, suggesting that they fulfil a wide variety of functions (Baldrian 2006; Hoegger et al. 2006; Kües and Rühl 2011). The redundancy of extracellular enzyme families in fungal genomes was demonstrated to be typical for ligninolytic peroxidases and hydrolytic enzymes (Eastwood et al. 2011; Žifčáková and Baldrian 2012). The presence of multiple gene copies is most likely a result of the complex regulation of expression that is frequently observed (Eastwood et al. 2011; van den Wymelenberg et al. 2011), but it also reflects the evolutionary history of fungi and the events in evolution that led to the development of specific ecophysiological groups of fungi (Floudas et al. 2012; Zhao et al. 2013). Most importantly, the combination of genome sequencing and comparative transcriptomics shows that the presence of genes in genomes and their numerical abundance in individual gene families are insufficient to estimate the potential of individual taxa to produce corresponding genes and perform efficient decomposition of individual compounds.

Secretomes, the pools of secreted proteins, were obtained from *P. chrysosporium* grown on wood by 2-D electrophoresis followed by mass spectrometry. The pool of secreted proteins, mainly represented by oxidases and glycosyl hydrolases, was described in detail on the background of the available genome sequence and indicated complex regulation patterns of protein synthesis (Abbas et al. 2005; Ravalason et al. 2008; Sato et al. 2007). In comparison, the information from proteomes of fungi without genome sequences was moderate (Zorn et al. 2005) due to the limited ability to assign the detected proteins to known genes. The advance of next-generation sequencing methods shifted the preference from proteome to transcriptome analysis. The analysis of tens of thousands of sequences transcribed by *P. chrysosporium* indicated the relative importance of individual gene products for decomposition (Sato et al. 2009). As an alternative, transcriptomes of genome-sequenced fungi can be analysed by RNA hybridisation with probes targeting functional or hypothetical genes. The results obtained from *P. chrysosporium* using microarray analysis combined with proteomics indicated the importance of certain oxidative enzymes and suggested the important role of many new proteins of unknown function, underscoring the complexity of lignocellulose degradation (van den Wymelenberg et al. 2009).

At present, quantitative proteomic techniques such as iTRAQ (Isobaric Tags for Relative and Absolute Quantitation) and LC-MS/MS reveal a picture of the quantitative composition of fungal proteomes that represent a better proxy of actual decomposition processes than do expression studies. This technique, based in the labeling of peptides from protein digestions with isotope-coded covalent tags of varying mass, was used to quantify the secretome of *P. chrysosporium*. During the growth on cellulose, *P. chrysosporium* produces primarily endoglucanases, exoglucanases, β-glucosidases and cellobiose dehydrogenase, suggesting both hydrolytic and oxidative cellulose degradation. When lignin was used as a major carbon source, oxidative enzymes such as copper radical oxidase, isoamyl oxidase, glutathione S-transferase, thioredoxin peroxidase, quinone oxidoreductase, aryl alcohol oxidase, pyranose 2-oxidase, aldehyde dehydrogenase and alcohol dehydrogenase were highly expressed (Manavalan et al. 2011). Transcriptomic studies assisted in the elucidation of the molecular differences in the composition and regulation of the decomposition machinery of various wood-rotting fungi (Eastwood et al. 2011; van den Wymelenberg et al. 2010). These types of studies also revealed that decomposition abilities differ among ectomycorrhizal fungi symbiotic with plant roots. While *Laccaria bicolor* is a less efficient decomposer than related saprotrophic taxa (Martin et al. 2008), *Paxillus involutus* is able to substantially decompose organic matter using a unique enzymatic system (Rineau et al. 2012, 2013). Moreover, transcriptomic analysis has been used for exploring the cellulolytic capacity of *Trichoderma reesei* on lignocellulose biomass, providing the knowledge for the development of effective methods and adequate conditions for biofuel production (Bischof et al. 2013). Biochemical exploration of fungal cultures also led to the recent discovery of novel enzymes or better descriptions of those with previously unknown function. This is the case for novel fungal peroxidases (the dye-decolorizing peroxidases) and unspecific peroxygenases (Hofrichter et al. 2010). The exploration of fungal genomes demonstrated that these novel oxidative enzymes are present in several species of the wood-rotting *Polyporales* (Ruiz-

Dueñas et al. 2013). Biochemical exploration demonstrated that cellulose cleavage by the enzyme that was formerly described as glycosyl hydrolase GH61 (recently reclassified as auxiliary activity family 9, AA9) is not based on hydrolysis but on the reaction of the polysaccharide with molecular oxygen, and the enzyme is thus polysaccharide monooxygenase (Beeson et al. 2011). The AA9 monooxygenase actually belongs to the most widespread enzyme family active in cellulose degradation, harboured in the genomes of saprotrophic and parasitic fungi as well as the mycorrhizal symbionts of plant roots (Žifčáková and Baldrian 2012). Moreover, transcriptomic studies show that polysaccharide monooxygenases are heavily produced during growth on wood by both white-rot and brown-rot fungi (Eastwood et al. 2011; MacDonald et al. 2011) and may perhaps be responsible for the fast degradation of cellulose by the latter.

Although the involvement of bacteria in decomposition processes has attracted much less attention than that of the fungi, the fact that more than 1,500 bacterial genomes are available compared to just over 100 fungal genomes (Větrovský and Baldrian 2013; Zhao et al. 2013) represents an important advantage for genome comparisons in the prokaryota. Recently, studies indicated that there is a widespread potential in multiple bacterial phyla to decompose cellulose or to feed on its degradation products (Berlemont and Martiny 2013). In some bacterial strains, such as *Streptomyces*, the biomass-degrading activity was comparable to a cellulolytic enzyme cocktail from the fungus *T. reesei* (Takasuta et al. 2013). Zimmerman et al. (2013) analysed more than 3,000 annotated bacterial genomes looking for potential production of extracellular enzymes involved in nutrient cycling and decomposition of organic matter. In their study, phosphatases, chitinases and *N*-acetylglucosaminidases were found in nearly half of the genomes analysed. The relative ease of obtaining and annotating genome sequences of bacteria helped to characterise four cellulolytic bacteria associated with woodwasps with respect to their lignocellulose decomposition potential (Adams et al. 2011).

The integration of resources from genome sequencing projects covering both the prokaryotic and eukaryotic microorganisms and exploration of individual genes enabled the establishment of curated databases covering either a wide range of genes, such as the M5 non-redundant protein database (M5Nr; Wilke et al. 2012) or Pfam (Punta et al. 2012) or specific groups of functional genes including those for carbohydrates—CAZy (Cantarel et al. 2009) and lignin-degrading enzymes—FOLY (Levasseur et al. 2008). The database creators started an important work on the classification and categorisation of genes and proteins based either on their 3-D structure (e.g. CAZY) or sequence features (e.g. M5Nr). Although this represents an important step forward to the understanding of both the gene evolution and the genomic potential of individual microorganisms, it has to be noted that the content of genes is not a direct predictor of decomposition abilities.

Exploration of microbial decomposition in complex environments

Understanding decomposition in the environment, especially in plant litter and soil, was previously limited to the knowledge of enzyme activities that can be detected in environmental samples and studies measuring decomposition rates of natural compounds, for example, using respirometry or incubation of litterbags. There was also limited knowledge on enzyme activities in environmental isolates of microorganisms.

With the accumulation of gene sequence data for decomposition-related enzymes in public databases, it became possible in the late 2000s to design degenerate primers capable of amplifying partial sequences of decomposition-related genes from a wide range of fungi. This made it possible to detect genes and transcripts encoding laccase and *cbhI* exocellulase from forest floor DNA and RNA (Edwards et al. 2008; Luis et al. 2005). Subsequently, the diversity of multiple oxidative and hydrolytic enzymes was demonstrated in forest topsoil metatranscriptomes (Kellner and Vandenbol 2010). The combination of targeted metatranscriptomics with next-generation sequencing and stable isotope probing allowed for estimations of the numbers of exocellulase genes present in forest litter and soil and the percentage of genes being transcribed. The results show that forest litter and soil harbours several hundreds of *cbhI* genes, of which 25–40 % are expressed at the same time (Baldrian et al. 2012; Štursová et al. 2012).

While targeted metatranscriptomics can be used to explore the diversity of individual genes, analyses of complete metatranscriptomes make it possible to identify which genes or gene families are responding to a given environment. The first general metatranscriptomic studies showed the possibility of retrieving and characterising eukaryotic genes for decomposition from soil (Bailly et al. 2007) and also the potential of using RNA for the simultaneous analysis of the structure and function of active microbial populations in soils (Urich et al. 2008). Metatranscriptomic exploration of eukaryotic genes in spruce and beech litter demonstrated that extracellular decomposition-related enzymes represent <1 % of all expressed sequences and that multiple families of oxidases and hydrolases are produced (Damon et al. 2012). Although the assignment of environmental transcripts to the Ascomycota or the Basidiomycota can be achieved for several genes, for a closer taxonomic assignment of individual genes to their fungal producers, it is necessary to analyse more fungal

Table 1 Important advances in the research on microbial decomposition obtained using (meta)genomics, (meta) transcriptomics and (meta)proteomics

Decomposition by individual microorganisms	
Knowledge obtained using traditional methods	
Good knowledge of the gene content in model species and enzymes in focus, fragmentary knowledge for non-model species and proteins with putatively lower importance; limited studies on the transcriptomics and protein production by a few key model taxa; decomposition abilities of microbes not exhibiting saprotrophic lifestyle largely absent	
Advances made using modern molecular and analytical methods	
Complete knowledge of gene complement in the first genomes of model saprotrophic and symbiotic fungi	Martin et al. (2008); Martinez et al. (2004, 2009)
Detailed description of *Phanerochaete chrysosporium* transcriptome on the background of whole genome sequence	Sato et al. 2007, 2009
Comparative analysis of the white-rot and brown-rot mode of fungal wood decay using a combination of transcriptomics and proteomics	van den Wymelenberg et al. (2010)
Genome analysis of environmental bacterial isolates demonstrates their ability to decompose cellulose	Adams et al. (2011)
Comparative genomics and transcriptomics show differences among fungal decomposition systems	Eastwood et al. (2011)
Quantitative description of the proteome of *Phanerochaete chrysosporium*	Adav et al. (2012)
Decomposition traits in fungal genomes help to understand the evolution of lignin decomposition	Floudas et al. (2012)
Demonstration of specific decomposition pathways in a mycorrhizal fungus—*Paxillus involutus*	Rineau et al. (2012)
Comparative genomics shows that the traits of cellulose and chitin utilisation are widespread in several bacterial phyla	Berlemont and Martiny (2013); Zimmerman et al. (2013)
Genome-wide transcriptomic and proteomic analysis gives a detailed picture of decomposition abilities of insect-associated bacteria	Takasuka et al. (2013)
Decomposition processes in the environment	
Knowledge obtained using traditional methods	
Decomposition in the environment is mainly characterised in terms of process rates and enzyme activities; scarce information of enzyme production and gene content in a few environmental isolates; first reports of environmental gene diversity are obtained by cloning and sequencing of environmental DNA; the identity of decomposers in complex environments is unknown	
Advances made using modern molecular and analytical methods	
High diversity of genes of fungal laccases and their transcripts is demonstrated in soil	Luis et al. (2005)
Transcripts of several eukaryotic phyla are detected in soil with fungal transcripts prevailing	Bailly et al. (2007)
The structure and function of soil microbial community are linked by the combined high-throughput analysis of rRNA and mRNA	Urich et al. (2008)
Targeted metatranscriptomics of soils demonstrates the diversity of several fungal decomposition enzymes in forest soils and indicates their taxonomic position	Kellner and Vandenbol (2010)
Deep sequencing shows that active and total microbial communities in forests have similar diversity. The gene pool of exocellulase is demonstrated to contain hundreds of molecules with 25–40 % being simultaneously expressed	Baldrian et al. (2012)
Shotgun metatranscriptomics of eukaryotic genes in forest litter shows the composition of the enzymatic complement in a complex environment	Damon et al. (2012)
Environmental metaproteomics shows that protein pool shifts during forest litter decomposition and indicates the contribution of individual groups of organisms	Schneider et al. (2012)
Metabolomics indicates different pathways of litter chemical changes during decomposition depending on location	Wallenstein et al. (2013)

genomes, especially of the taxa inhabiting litter and soil (Marmeisse et al. 2013).

Metaproteomics was first developed for environments of limited complexity, such as contaminated groundwater (Benndorf et al. 2007) or co-cultures of individual microbes (Schneider et al. 2010). Later, in conjunction with the increased amount of information available in public protein and DNA databases, it was possible to use this technique in a complex substrate. The analysis of the proteome of decomposing litter showed the involvement of a complex community of various eukaryotes and bacteria in decomposition (Schneider et al. 2012) and indicated the successive changes of the decomposer community that were later confirmed by the metagenomic analysis of the litter-associated fungal community and by enzyme activity measurements (Voříšková and Baldrian 2013).

Since the advance of the genomics, transcriptomics and proteomics of individual microorganisms and their complex communities, these approaches have dramatically increased our understanding of decomposition (Table 1). Recently, these methods have begun to be complemented with advanced analytical methods, such as analytical pyrolysis or mass spectrometry-based analysis of metabolism of complex compounds (metabolomics). Some of these methods have been used to study plant litter (Šnajdr et al. 2011; Valášková et al. 2007; Wallenstein et al. 2010, 2013) and have helped to describe chemical composition changes at a high resolution.

At the moment, these methods are able to identify the trends in decomposition of various substances and to indicate similarities or differences in decomposition patterns among individual microbial taxa or microbial communities inhabiting various environments (Valášková et al. 2007; Wallenstein et al. 2013).

Future trends and directions

In the future, we can expect that the combination of these advanced analytical methods with the metatranscriptomic and metaproteomic approaches will link the activity of microbial communities and their individual members with biochemical processes in the complex natural environment. Despite progress in the molecular methods, the understanding of environmental processes will never be complete if it is not accompanied by the isolation and characterisation of individual microbial taxa. This task is both laborious and technically demanding, and it should receive much more attention from microbial ecologists than it currently does. However, if successful, the exploration of genomes and transcriptomes combined with physiological research on relevant environmental taxa will represent a significant impetus for understanding the data from complex habitats provided by metagenomics and metatranscriptomics.

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Conflict of interest The authors declare that they have no conflict of interest.

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