UDK 582.284:577.21 *https://doi.org/10.31861/biosystems2025.01.059*

GENETIC STUDIES OF PECTINOLYTIC ENZYMES OF BASIDIOMYCETES

P. ZUBYK, I. KLECHAK, L. TITOVA, O. YALOVENKO

National Technical University of Ukraine
«Igor Sikorsky Kyiv Polytechnic Institute»,
3 Akademika Yangelya Street, Building 4, Kyiv, 03056
e-mail: pv.zubyk@i.ua

The article presents an overview of current ideas about the genetic organisation and functional characteristics of pectinolytic enzyme systems in basidiomycetes. The main families of enzymes involved in the degradation of pectin compounds are analysed, in particular glycosyl hydrolases (GH), polysaccharide lyases (PL) and carbohydrate esterases (CE), as well as the corresponding genes found in representatives of the genera Agaricus, Armillaria, Flammulina, Laccaria, Lentinula, Pleurotus, Schizophyllum and Trametes. A comparative characterisation of the genomes of these fungi is provided in terms of the quantitative indicators of pectinase genes, their structural affiliation and potential functional activity. Methodological approaches to studying pectinolytic potential are considered, in particular sequencing, transcriptomics, biochemical methods and comparative genomics tools. Prospects for further research in the context of the biotechnological application of enzymes of this group are outlined.

Keywords: pectinolytic enzymes, basidiomycetes, sequencing, transcriptomics, CAZy

Introduction. Pectin, as a general term, covers a heterogeneous group of carbohydrates with different molecular weights (Lara-Espinoza et al., 2018). Nevertheless, most pectins consist of a backbone represented by galacturonic acid residues linked by α-1,4-glycosidic bonds, which may be methylated or acetylated (Danalache et al., 2018). The main structural components are homogalacturonans and rhamnogalacturonans with side branches represented by arabinans, galactans, and arabinogalactans (Kaczmarska et al., 2022). This structure perfectly ensures the functional properties of pectin as a component of plant cell walls, which determines their strength, elasticity and water-holding capacity (Voragen et al., 2009).

The breakdown of pectin is catalysed by specific enzymes called pectinases (pectinolytic enzymes). This process can be carried out by depolymerisation (under the action of hydrolases and lyases) and de-esterification (by esterases) (de Souza & Kawaguti, 2021). Pectinases are synthesised by many groups of microorganisms, including bacteria, yeasts, actinomycetes and fungi (Shet et al., 2018). In nature, these enzymes are involved in the pathogenesis and destruction of plants, mainly caused by fungi. That is why the first commercial pectinases were obtained using fungal producers (Haile & Ayele, 2022).

Traditionally, research on producers of pectinolytic enzymes has focused on ascomycetes (Benoit et al., 2012). However, there has been growing interest in basidiomycetes, which are also capable of effectively breaking down plant polymers, including pectin (Berger & Ersoy, 2022; Shankar Naik et al., 2019). With the spread of genomics and transcriptomics, new opportunities have emerged for identifying pectinolytic enzyme genes, even without classical activity screening (Peng & de Vries, 2021). This opens up new prospects for finding producers with properties valuable for biotechnology and industry among representatives of different trophic groups and ecological niches.

The genera *Pleurotus*, *Lentinula* and *Trametes* belong to biotechnologically significant saprotrophs that demonstrate active degradation of higher plant cell wall components, including pectin, and are widely used in applied research in enzymatic biotechnology (Floudas et al., 2012; Moen et al., 2018). *Flammulina velutipes*, as a typical xylotroph, has a developed set of hydrolases and lyases, which ensures the effective mobilisation of wood polysaccharides, including pectin components (Yu et al., 2020). The genus *Armillaria* is represented by necrotrophic wood pathogens, whose genomes contain an expanded set of genes involved in the degradation of complex plant cell wall polysaccharides, in particular pectin (Sipos et al., 2017). *Agaricus bisporus* belongs to the humus-type saprotrophs with a high degree of genomic characterisation, which allows for the accurate identification of genes involved in the degradation of organic matter (Morin et al., 2012). *Schizophyllum commune* is a model basidiomycete with known potential for the biodegradation of plant polymers, as well as a fully sequenced genome that is actively used in functional genomics (Ohm et al., 2010). Finally, *Laccaria bicolor* is a representative of

ectomycorrhizal symbionts with a reduced hydrolytic profile, which was included in the analysis to compare the pectinolytic potential of saprotrophic and symbiotrophic macromycetes (Martin et al., 2008).

The relevance of the study is due to the growing demand for effective enzyme systems for biotechnological processes, such as plant biomass processing, juice production, winemaking, textile and paper industries, as well as bioremediation (Berger & Ersoy, 2022; Haile & Ayele, 2022). Basidiomycetes, in particular representatives of the genera *Agaricus*, *Armillaria*, *Flammulina*, *Laccaria*, *Lentinula*, *Pleurotus*, *Schizophyllum* and *Trametes*, demonstrate significant pectinolytic potential, which remains understudied compared to ascomycetes (Sahu et al., 2022). The development of genomic and transcriptomic technologies opens up new opportunities for identifying pectinase genes without traditional activity screening, which contributes to the search for new biotechnologically valuable producers (Peng & de Vries, 2021). In addition, the study of pectinolytic enzymes of basidiomycetes is important for understanding their ecological role in saprotrophic, pathogenic and symbiotic interactions, which is important for the development of strategies for the sustainable use of natural resources (Sipos et al., 2017).

The aim of the study is to systematise current data on the genetic organisation and methodological approaches to the study of pectinolytic enzymes of basidiomycete macromycetes, in particular the analysis of GH, PL and CE family genes in representatives of the genera Agaricus, Armillaria, Flammulina, Laccaria, Lentinula, Pleurotus, Schizophyllum and Trametes, as well as determining the prospects for their biotechnological application. To achieve this goal, the classification of pectinolytic enzymes, the organisation of their genes in genomes, modern methods of sequencing, transcriptomics and comparative genomics are considered, and directions for further research are outlined.

1. Classification and biological role of pectinolytic enzymes

Pectinases are a functionally diverse group of enzymes involved in the degradation of pectin substrates. Depending on the type of catalytic reaction and the target of enzymatic action, pectinases belong to three main types of enzymes: hydrolases, lyases and esterases (Table 1). This division is based on the generally accepted enzyme classification system proposed by the International Enzyme Commission (EC) (KC et al., 2020; Ozojiofor & Rasheed, 2023; Yüksel et al., 2024).

Table 1.

Classification of pectinases (Patidar et al., 2018; Zheng, Xu, et al., 2021)

Enzyme	EC number	Reaction products
Exopolygalacturonase	3.2.1.15	Oligogalacturonates
Endopolygalacturonase	3.2.1.67, 3.2.1.82	Mono-, di-, oligogalacturonates
Endoligalacturonatlyase	4.2.2.2	Unsaturated oligogalacturonates
Endolymethyl galacturonatlyase	4.2.2.10	Unsaturated methyloligogalacturonates
Pectin methylesterase	3.1.1.11	Pectates, methanol
Pectyacetylesterase	3.1.1.6	Pectates, ethanol

Hydrolases (EC 3.2.1.x) include those pectinolytic enzymes that hydrolytically cleave α-1,4-glycosidic bonds in the polygalacturon chain – polygalacturonases. (Roman-Benn et al., 2023). Polygalacturonases themselves are divided depending on the localization of the substrate attack site into endopolygalacturonase (cleaves internal bonds) and exopolygalacturonase (cleaves monomers from the terminal regions of polymers) (KC et al., 2020; Roman-Benn et al., 2023).

Lyases (EC 4.2.2.x), such as pectin lyases and pectate lyases, catalyze the cleavage of pectin of various degrees of esterification, mainly highly esterified) without the participation of water by the β-elimination mechanism with the formation of unsaturated hydrolysis products (Zheng, Guo, et al., 2021). The reaction produces 4,5-unsaturated unmethylated or methylated oligogalacturonides without the accumulation of toxic methanol (Saharan & Sharma, 2019; Samreen et al., 2019; Zheng, Xu, et al., 2021). Lyases involved in the breakdown of pectin are also known as polymethylgalacturonate lyase and polygalacturonate lyase (Zheng, Xu, et al., 2021).

Pectinesterases catalyze the deesterification of esterified groups of pectin to form pectic acid (Samreen et al., 2019). The main enzymes of this class, represented in the decomposition of pectin, are pectin methylesterase and pectin acetylesterase, which cleave methyl and acetyl residues, respectively (Yüksel et al., 2024). Fungal enzymes of this class act randomly, removing esterified

groups by a multi-chain mechanism (Patidar et al., 2018).

Pectinolytic enzymes ensure the growth of both phytopathogenic and saprotrophic basidiomycetes (Baldrian, 2008). Polygalacturonases are involved in the destruction of polygalacturonan, which provides access to other structural polysaccharides of the cell wall (Safran et al., 2023). Pectate lyases are also synthesised by pathogenic fungi when colonising pectin-rich substrates. However, endopolygalacturonate lyases are more common for them than exoforms of this enzyme (Patidar et al., 2018). In turn, the presence of pectinases is important for the activity of other pectinolytic enzymes, since non-esterified or low-esterified substrates are more easily subjected to enzymatic hydrolysis (Bonnin & Pelloux, 2020).

2. Organisation of the genome and gene family of pectinolytic enzymes in basidiomycetes

The molecular biology of basidiomycetes is a key area of fundamental mycology, providing a deep understanding of genome organisation and functional regulation in fungi (Yang, 2011). Basidiomycetes, including saprotrophic (*Agaricus*, *Flammulina*, *Lentinula*, *Pleurotus*, *Schizophyllum*, *Trametes*), pathogenic (*Armillaria*) and symbiotic (*Laccaria*) species, exhibit significant genomic diversity, reflecting their ecological strategies. Genome sizes range from compact (about 30 Mb) in saprotrophs to larger (up to 60 Mb) in pathogens and symbionts, which is associated with adaptations to plant material degradation, pathogenesis or symbiosis (Table 2.) (Morin et al., 2012; Sahu et al., 2022).

Table 2.

Characterisation of the basidiomycete genome (Park et al., 2018; Ruiz-Dueñas et al., 2021; Sipos et al., 2017; Zhang et al., 2020)

Genera	Approximate total genome size, Mb	Approximate size of the pectinase genome, kb
Agaricus	30,4	68-144
Armillaria	58,2	158-324
Flammulina	35,6	174-324
Laccaria	60,7	20-69
Lentinula	46,1	168-252
Pleurotus	34,3	134-228
Schizophyllum	38,5	72-321
Trametes	44,8	22-141

Notes: The size of the genome attributable to pectinolytic genes was calculated based on the approximate size of the gene (2-3 kb) and the number of these genes (Kües, 2000)

Saprotrophs have a developed set of carbohydrate-active enzyme (CAZymes) genes, in particular pectinases, while symbionts are characterised by their reduction (Ruiz-Dueñas et al., 2021; Sipos et al., 2017).

Pectinases, which include glycosyl hydrolases (GH), pectate lyases (PL) and carbohydrate esterases (CE), play a key role in the breakdown of pectin components of plant cell walls (Table 3.).

Table 3.

Distribution of pectinase genes by families in some macromycetes (Park et al., 2018; Ruiz-Dueñas et al., 2021; Sipos et al., 2017; Zhang et al., 2020)

Genera	Gene families		
	GH	PL	CE
Agaricus	GH28	PL1, PL3, PL4, PL9	CE8, CE12
Armillaria	GH28	PL1, PL3, PL4, PL9	CE8, CE12
Flammulina	GH28	PL1, PL3, PL4, PL9	CE8, CE12
Laccaria	GH28	-	CE8, CE12
Lentines	GH28	PL1, PL4	CE8, CE12
Pleurotus	GH28	PL1, PL3, PL4	CE8, CE12
Schizophyllum	GH28	PL1, PL3, PL4, PL9	CE8, CE12
Trametes	GH28	PL4	CE8, CE12

Their proportion in basidiomycete genomes remains low (less than 1%), but varies depending on the ecological niche. Saprotrophic species, such as *Flammulina* and *Pleurotus*, have a higher proportion of pectinases, which ensures effective degradation of lignocellulosic substrates, while pathogenic species, such as *Armillaria*, demonstrate an expanded set of pectinolytic genes associated with pathogenesis. Symbiotic species, such as *Laccaria*, have minimal pectinases, reflecting their dependence on their host (Park et al., 2018; Sipos et al., 2017). Among saprotrophs, *Schizophyllum* and *Trametes* are distinguished by the variability of pectinases, which makes them promising for biotechnological applications such as bioremediation and processing of plant biomass (Park et al., 2018; Zhang et al., 2020).

Genes encoding GH28 family pectinases are key to pectin degradation by basidiomycetes, although their characterisation is somewhat limited compared to ascomycetes (Gacura et al., 2016). The enzymes synthesized as a result of the expression of these genes are aimed at breaking down the backbone of the pectin molecule, while the side chains can be broken down by the products of the GH3, GH43 and other gene families (Wefers et al., 2017). The number of glycosyl hydrolase, polysaccharide lyase, and esterase genes involved in the decomposition of pectin in some macromycetes is shown in Figure 1.

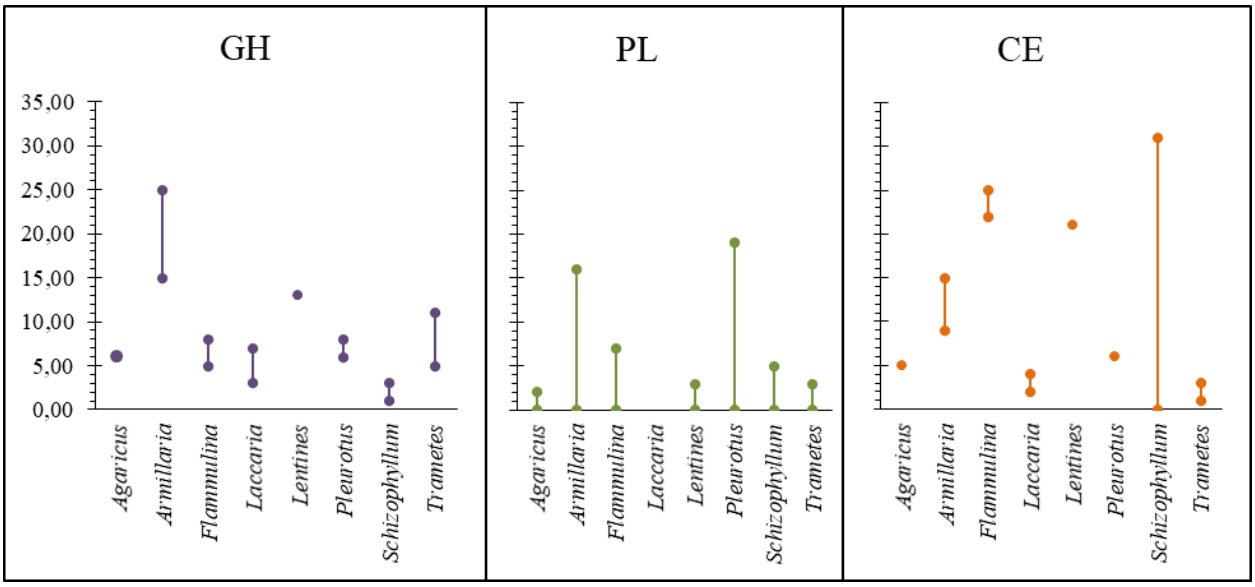

Fig. 1. Number of pectinase-encoding genes in some macromycetes (Park et al., 2018; Ruiz-Dueñas et al., 2021; Sipos et al., 2017; Zhang et al., 2020)

According to Floudas et al., the number of glycosyl hydrolase (GH28) genes in brown rot fungi ranges from 7 to 13 (Floudas et al., 2012). The GH28 family genes are most fully described in phytopathogens. For example, *Chondrostereum purpureum* has five isoforms corresponding to five cloned GH28 genes (Reina et al., 2019). At the same time, representatives of the *Armillaria* family are characterised by a significant number of genes from this family, while in *Schizophyllum* they are practically absent (Park et al., 2018; Sipos et al., 2017). Similar heterogeneity in gene distribution was observed among esterase genes. There is significant variation in the number of these genes even among representatives of the same genus (Ruiz-Dueñas et al., 2021). At the same time, the smallest number of genes among the studied CAZy families was recorded among polysaccharide lyases. Representatives of the *Armillaria* and *Pleurotus* genera demonstrate a larger number of genes of this family (Sipos et al., 2017). Similar variability in the number of genes indicates the evolutionary adaptation of basidiomycetes to specific substrates. For example, wood-decaying species (*Trametes*, *Schizophyllum*) are characterised by a smaller set of GH28, while pathogenic species (*Armillaria*) have an expanded set of pectinases, which is consistent with their life cycle and mode of infection (Ruiz-Dueñas et al., 2021; Sahu et al., 2021).

3. Methods for studying pectinolytic enzymes of basidiomycete

Pectinolytic enzymes of basidiomycetes, encoded by genes of the GH, PL and CE families, are key to pectin degradation and have significant biotechnological potential (Rytioja et al., 2014). The genomes of these fungi show significant variability in the size and composition of CAZymes genes, reflecting their adaptation to different ecological

niches – from wood-decaying saprotrophs to pathogens (Floudas et al., 2012).

The study of pectinolytic enzymes in basidiomycetes is based on a complex of modern molecular genetic methods, including nucleic acid isolation, whole-genome sequencing, genome annotation, transcriptomic analysis, enzyme identification using CAZy databases, and comparative genomics.

Sample preparation involves the use of DNA and RNA extraction methods, including the phenol-chloroform method, CTAB protocols, and commercial kits such as the PowerMax MOBIO DNA Isolation Kit and RNeasy Midi Kit (Martin et al., 2008; Morin et al., 2012; Park et al., 2019; Sipos et al., 2017).

Whole-genome sequencing is performed using Sanger, Illumina HiSeq, PacBio RS II platforms or combinations thereof. Genome assembly is performed in environments such as Velvet, JAZZ, HGAP3, Falcon and others, which ensures high-quality genomic sequences (Martin et al., 2008; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017). Genome annotation is performed using automated tools such as JGI Annotation Pipeline, AUGUSTUS, GeneMark-ES, DIAMOND, Fgenesh, Genewise, and EuGène. Pectinolytic enzymes are identified based on the CAZy, dbCAN, Pfam, and InterProScan databases, and the secretory properties of proteins are determined using SignalP 5.0 (Martin et al., 2008; Morin et al., 2012; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017).

Comparative genome analysis includes the identification of orthologs and clustering of protein sequences using MCL, OrthoFinder, Tribe-MCL, and FastOrtho tools. Phylogenetic studies are performed based on MAFFT alignments, with cleaning in Gblocks and tree construction in RAxML, PRANK, FastTree; time calibration is performed using r8s (Martin et al., 2008; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017). The evolution of the PL1 and GH28 gene families is analysed using CAFE, and quantitative differences in copy number are analysed using statistical tests, including Bonferroni-corrected binomial tests and Fisher's exact test (Ruiz-Dueñas et al., 2021).

Transcriptomic analysis methods, including RNA-seq and microchips based on Agilent technologies, are used to assess the expression levels of pectinolytic genes. Expression data analysis is performed using software tools such as CyberT, edgeR, limma, and EPCLUST (Martin et al., 2008; Morin et al., 2012; Sipos et al., 2017).

Generalised information on the latest approaches to studying the genes of pectinolytic enzymes of basidiomycetes is presented in Table 4.

Table 4.

Approaches for studying pectinase genes of basidiomycetes

Method	Platform/tool	Target genera	References
Sample preparation: cultivation and isolation of genetic material	CTAB, Phenol-chloroform, PowerMax MOBIO, RNeasy Midi Kit	*Flammulina, Armillaria*	(Park et al., 2019; Sipos et al., 2017)
Genome assembly and annotation retrieval via online databases	JGI MycoCosm, NCBI SRA, JGI Portal, GenBank et al.	*Pleurotus, Agaricus, Armillaria, Trametes, Laccaria*	(Martin et al., 2008; Morin et al., 2012; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017)
Whole genome sequencing	Illumina, PacBio, Sanger	*Pleurotus, Flammulina, Agaricus, Armillaria, Trametes*	(Martin et al., 2008; Morin et al., 2012; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017)
Genome annotation	AUGUSTUS, GeneMark, InterProScan	*Agaricus, Flammulina, Lentinula, Pleurotus, Schizophyllum, Trametes, Armillaria, Laccaria*	(Martin et al., 2008; Morin et al., 2012; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017)
Genome quality assessment	BUSCO 4.1.1	*Agaricus, Pleurotus, Trametes*	(Ruiz-Dueñas et al., 2021)
Protein clustering	MCL, OrthoFinder	*Pleurotus, Armillaria, Laccaria*	(Martin et al., 2008; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017)
Phylogenomic analysis	RAxML, MAFFT, FastTree	*Pleurotus, Armillaria, Trametes*	(Martin et al., 2008; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017)

CAZymes annotation	CAZy, dbCAN, Pfam	*Pleurotus, Schizophyllum, Laccaria, Trametes*	(Martin et al., 2008; Morin et al., 2012; Park et al., 2019; Riley et al., 2014; Ruiz-Dueñas et al., 2021; Sipos et al., 2017)
Microarray expression analysis	Agilent Microarrays	*Agaricus*	(Morin et al., 2012)
Transcriptomic analysis	Illumina RNA-seq	*Armillaria, Laccaria*	(Martin et al., 2008; Sipos et al., 2017)
Signal peptide prediction	SignalP 5.0	*Flammulina, Laccaria*	(Martin et al., 2008; Park et al., 2019)
Analysis of gene family evolution	CAFE 4.1	*Trametes, Laccaria, Armillaria*	(Ruiz-Dueñas et al., 2021)
Statistical analysis of copy number	Fisher's test, Bonferroni	*Agaricus, Flammulina, Lentinula, Pleurotus, Schizophyllum, Trametes, Armillaria, Laccaria*	(Ruiz-Dueñas et al., 2021)

4. Prospects for research into the genetic studies of pectinolytic enzymes of basidiomycetes

Further development of genetic research on pectinolytic enzymes in basidium macromycetes opens up new opportunities for improving analysis methods and expanding their application. One of the key areas is the introduction of third-generation sequencing technologies, such as Oxford Nanopore, which provide longer reads and higher accuracy in detecting complex genomic regions, including introns and regulatory sequences of pectinolytic genes (Ruiz-Dueñas et al., 2021) This will allow us to refine the annotation of the PL1, GH28, and CE8–CE12 genes in species with limited data, such as *Lentinula* and *Trametes* (Riley et al., 2014; Ruiz-Dueñas et al., 2021).

Another promising direction is the integration of multi-omic approaches combining genomics, transcriptomics, and proteomics. For example, the use of single-cell RNA-seq can help investigate the expression of pectinolytic genes in specific fungal tissues, such as hyphae or fruiting bodies, in *Agaricus* or *Pleurotus* (Morin et al., 2012). Supplementing transcriptomic data with mass spectrometry will confirm the presence and activity of enzymes encoded by the PL1 and GH28 genes under different environmental conditions (Sipos et al., 2017).

Improvements in algorithms for annotating CAZymes, such as updated versions of dbCAN with machine learning, may increase the accuracy of identifying pectinolytic genes in genomes with low assembly quality, such as *Armillaria* (Park et al., 2019; Sipos et al., 2017). In addition, the creation of specialised databases, such as JGI MycoCosm, focusing on specific enzyme genes, including pectinolytic ones, will facilitate comparative analysis between saprotrophic, pathogenic and symbiotic species (Ruiz-Dueñas et al., 2021).

The use of genome editing technologies such as CRISPR/Cas9 for functional analysis of pectinolytic genes is also promising. For example, knockout or overexpression of PL1 genes in *Flammulina* or *Pleurotus* may clarify their role in the degradation of plant cell walls (Park et al., 2019; Riley et al., 2014). This opens up opportunities for biotechnological applications, particularly in the development of enzyme preparations for industrial biomass processing.

Conclusions. Genomic and functional studies of pectinolytic enzymes in basidiomycetes indicate a high degree of structural and functional diversity. Representatives of the genera *Agaricus*, *Armillaria*, *Flammulina*, *Laccaria*, *Lentinula*, *Pleurotus*, *Schizophyllum*, and *Trametes* demonstrate the presence of different subfamilies of genes from the GH, PL, and CE families involved in the hydrolysis and β-elimination of pectin polysaccharides. The specificity of the genetic set and probable enzymatic activity varies between genera and is closely related to the ecological niche of the species and the type of wood or plant substrate.

Given the limited number of studies, especially experimentally confirmed functions of the identified genes, promising areas for further research include comprehensive genome annotation using metatranscriptomics, proteomics, and functional validation of pectinolytic enzyme activity. Special attention should also be paid to studying the conditions for inducing the expression of such enzymes when growing fungi on pectin-rich agricultural waste.

Deepening knowledge about the pectinolytic potential of basidiomycetes creates the basis for the biotechnological application of their enzymatic systems in the food industry, winemaking, textile and pulp and paper industries, as well as in environmental bioremediation.

Conflict of interest: *The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest*

References:

1. Baldrian, P. (2008). *Chapter 2 Enzymes of saprotrophic basidiomycetes* (pp. 19–41). https://doi.org/10.1016/S0275-0287(08)80004-5
2. Benoit, I., Coutinho, P. M., Schols, H. A., Gerlach, J. P., Henrissat, B. & de Vries, R. P. (2012). Degradation of different pectins by fungi: correlations and contrasts between the pectinolytic enzyme sets identified in genomes and the growth on pectins of different origin. *BMC Genomics*, *13*(1), 321. https://doi.org/10.1186/1471-2164-13-321
3. Berger, R. G. & Ersoy, F. (2022). Improved Foods Using Enzymes from Basidiomycetes. *Processes*, *10*(4), 726. https://doi.org/10.3390/pr10040726
4. Bonnin, E. & Pelloux, J. (2020). Pectin Degrading Enzymes. In *Pectin: Technological and Physiological Properties* (pp. 37–60). Springer International Publishing. https://doi.org/10.1007/978-3-030-53421-9_3
5. Danalache, F., Mata, P., Alves, V. D. & Moldão-Martins, M. (2018). Enzyme-Assisted Extraction of Fruit Juices. In *Fruit Juices* (pp. 183–200). Elsevier. https://doi.org/10.1016/B978-0-12-802230-6.00010-2
6. de Souza, T. S. P. & Kawaguti, H. Y. (2021). Cellulases, Hemicellulases, and Pectinases: Applications in the Food and Beverage Industry. *Food and Bioprocess Technology*, *14*(8), 1446–1477. https://doi.org/10.1007/s11947-021-02678-z
7. Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R. A., Henrissat, B., Martínez, A. T., Otillar, R., Spatafora, J. W., Yadav, J. S., Aerts, A., Benoit, I., Boyd, A., Carlson, A., Copeland, A., Coutinho, P. M., de Vries, R. P., Ferreira, P., Findley, K., … Hibbett, D. S. (2012). The Paleozoic Origin of Enzymatic Lignin Decomposition Reconstructed from 31 Fungal Genomes. *Science*, *336*(6089), 1715–1719. https://doi.org/10.1126/science.1221748
8. Gacura, M. D., Sprockett, D. D., Heidenreich, B. & Blackwood, C. B. (2016). Comparison of pectin-degrading fungal communities in temperate forests using glycosyl hydrolase family 28 pectinase primers targeting Ascomycete fungi. *Journal of Microbiological Methods*, *123*, 108–113. https://doi.org/10.1016/j.mimet.2016.02.013
9. Haile, S. & Ayele, A. (2022). Pectinase from Microorganisms and Its Industrial Applications. *The Scientific World Journal*, *2022*, 1–15. https://doi.org/10.1155/2022/1881305
10. Kaczmarska, A., Pieczywek, P. M., Cybulska, J. & Zdunek, A. (2022). Structure and functionality of Rhamnogalacturonan I in the cell wall and in solution: A review. *Carbohydrate Polymers*, *278*, 118909. https://doi.org/10.1016/j.carbpol.2021.118909
11. KC, S., Upadhyaya, J., Joshi, D. R., Lekhak, B., Kumar Chaudhary, D., Raj Pant, B., Raj Bajgai, T., Dhital, R., Khanal, S., Koirala, N. & Raghavan, V. (2020). Production, Characterization, and Industrial Application of Pectinase Enzyme Isolated from Fungal Strains. *Fermentation*, *6*(2), 59. https://doi.org/10.3390/fermentation6020059
12. Kües, U. (2000). Life History and Developmental Processes in the Basidiomycete *Coprinus cinereus*. *Microbiology and Molecular Biology Reviews*, *64*(2), 316–353. https://doi.org/10.1128/MMBR.64.2.316-353.2000
13. Lara-Espinoza, C., Carvajal-Millán, E., Balandrán-Quintana, R., López-Franco, Y. & Rascón-Chu, A. (2018). Pectin and Pectin-Based Composite Materials: Beyond Food Texture. *Molecules*, *23*(4), 942. https://doi.org/10.3390/molecules23040942
14. Martin, F., Aerts, A., Ahrén, D., Brun, A., Danchin, E. G. J., Duchaussoy, F., Gibon, J., Kohler, A., Lindquist, E., Pereda, V., Salamov, A., Shapiro, H. J., Wuyts, J., Blaudez, D., Buée, M., Brokstein, P., Canbäck, B., Cohen, D., Courty, P. E., … Grigoriev, I. V. (2008). The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature*, *452*(7183), 88–92. https://doi.org/10.1038/nature06556
15. Moen, V. P., Drageset, J., Eide, G. E. & Gjesdal, S. (2018). Dimensions and predictors of disability—A baseline study of patients entering somatic rehabilitation in secondary care. *PLOS ONE*, *13*(3), e0193761. https://doi.org/10.1371/journal.pone.0193761
16. Morin, E., Kohler, A., Baker, A. R., Foulongne-Oriol, M., Lombard, V., Nagye, L. G., Ohm, R. A., Patyshakuliyeva, A., Brun, A., Aerts, A. L., Bailey, A. M., Billette, C., Coutinho, P. M., Deakin, G., Doddapaneni, H., Floudas, D., Grimwood, J., Hildén, K., Kües, U., … Martin, F. (2012). Genome sequence of the button mushroom *Agaricus* bisporus reveals mechanisms governing adaptation to a humic-rich ecological niche. *Proceedings of the National Academy of Sciences*, *109*(43), 17501–17506. https://doi.org/10.1073/pnas.1206847109
17. Ohm, R. A., de Jong, J. F., Lugones, L. G., Aerts, A., Kothe, E., Stajich, J. E., de Vries, R. P., Record, E., Levasseur, A., Baker, S. E., Bartholomew, K. A., Coutinho, P. M., Erdmann, S., Fowler, T. J., Gathman, A. C., Lombard, V., Henrissat, B., Knabe, N., Kües, U., … Wösten, H. A. B. (2010). Genome sequence of the model mushroom *Schizophyllum* commune. *Nature Biotechnology*, *28*(9), 957–963. https://doi.org/10.1038/nbt.1643
18. Ozojiofor, U. O. & Rasheed, Z. A. (2023). Pectinases: structure, functions and biotechnological applications. *Journal of Natural and Applied Sciences Pakistan*, *5*(2), 1448–1464.
19. Park, Y.-J., Jeong, Y.-U. & Kong, W.-S. (2018). Genome Sequencing and Carbohydrate-Active Enzyme (CAZyme) Repertoire of the White Rot Fungus *Flammulina* elastica. *International Journal of Molecular Sciences*, *19*(8), 2379. https://doi.org/10.3390/ijms19082379
20. Park, Y.-J., Lee, C.-S. & Kong, W.-S. (2019). Genomic Insights into the Fungal Lignocellulolytic Machinery of *Flammulina* rossica. *Microorganisms*, *7*(10), 421. https://doi.org/10.3390/microorganisms7100421

21. Patidar, M. K., Nighojkar, S., Kumar, A. & Nighojkar, A. (2018). Pectinolytic enzymes-solid state fermentation, assay methods and applications in fruit juice industries: a review. *3 Biotech*, *8*(4), 199. https://doi.org/10.1007/s13205-018-1220-4
22. Peng, M. & de Vries, R. P. (2021). Machine learning prediction of novel pectinolytic enzymes in *Aspergillus niger* through integrating heterogeneous (post-) genomics data. *Microbial Genomics*, *7*(12). https://doi.org/10.1099/mgen.0.000674
23. Reina, R., Kellner, H., Hess, J., Jehmlich, N., García-Romera, I., Aranda, E., Hofrichter, M. & Liers, C. (2019). Genome and secretome of *Chondrostereum purpureum* correspond to saprotrophic and phytopathogenic life styles. *PLOS ONE*, *14*(3), e0212769. https://doi.org/10.1371/journal.pone.0212769
24. Riley, R., Salamov, A. A., Brown, D. W., Nagy, L. G., Floudas, D., Held, B. W., Levasseur, A., Lombard, V., Morin, E., Otillar, R., Lindquist, E. A., Sun, H., LaButti, K. M., Schmutz, J., Jabbour, D., Luo, H., Baker, S. E., Pisabarro, A. G., Walton, J. D., … Grigoriev, I. V. (2014). Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. *Proceedings of the National Academy of Sciences*, *111*(27), 9923–9928. https://doi.org/10.1073/pnas.1400592111
25. Roman-Benn, A., Contador, C. A., Li, M.-W., Lam, H.-M., Ah-Hen, K., Ulloa, P. E. & Ravanal, M. C. (2023). Pectin: An overview of sources, extraction and applications in food products, biomedical, pharmaceutical and environmental issues. *Food Chemistry Advances*, *2*, 100192. https://doi.org/10.1016/j.focha.2023.100192
26. Ruiz-Dueñas, F. J., Barrasa, J. M., Sánchez-García, M., Camarero, S., Miyauchi, S., Serrano, A., Linde, D., Babiker, R., Drula, E., Ayuso-Fernández, I., Pacheco, R., Padilla, G., Ferreira, P., Barriuso, J., Kellner, H., Castanera, R., Alfaro, M., Ramírez, L., Pisabarro, A. G., … Martínez, A. T. (2021). Genomic Analysis Enlightens Agaricales Lifestyle Evolution and Increasing Peroxidase Diversity. *Molecular Biology and Evolution*, *38*(4), 1428–1446. https://doi.org/10.1093/molbev/msaa301
27. Rytioja, J., Hildén, K., Yuzon, J., Hatakka, A., de Vries, R. P. & Mäkelä, M. R. (2014). Plant-Polysaccharide-Degrading Enzymes from Basidiomycetes. *Microbiology and Molecular Biology Reviews*, *78*(4), 614–649. https://doi.org/10.1128/MMBR.00035-14
28. Safran, J., Tabi, W., Ung, V., Lemaire, A., Habrylo, O., Bouckaert, J., Rouffle, M., Voxeur, A., Pongrac, P., Bassard, S., Molinié, R., Fontaine, J.-X., Pilard, S., Pau-Roblot, C., Bonnin, E., Larsen, D. S., Morel-Rouhier, M., Girardet, J.-M., Lefebvre, V., … Pelloux, J. (2023). Plant polygalacturonase structures specify enzyme dynamics and processivities to fine-tune cell wall pectins. *The Plant Cell*, *35*(8), 3073–3091. https://doi.org/10.1093/plcell/koad134
29. Saharan, R. & Sharma, K. P. (2019). Production, purification and characterization of pectin lyase from Bacillus subtilis isolated from moong beans leaves (*Vigna radiata*). *Biocatalysis and Agricultural Biotechnology*, *21*, 101306. https://doi.org/10.1016/j.bcab.2019.101306
30. Sahu, N., Indic, B., Wong-Bajracharya, J., Merényi, Z., Ke, H.-M., Ahrendt, S., Monk, T.-L., Kocsubé, S., Drula, E., Lipzen, A., Bálint, B., Henrissat, B., Andreopoulos, B., Martin, F. M., Harder, C. B., Rigling, D., Ford, K. L., Foster, G. D., Pangilinan, J., … Nagy, L. G. (2022). *Genomic innovation and horizontal gene transfer shaped plant colonization and biomass degradation strategies of a globally prevalent fungal pathogen*. https://doi.org/10.1101/2022.11.10.515791
31. Sahu, N., Merényi, Z., Bálint, B., Kiss, B., Sipos, G., Owens, R. A. & Nagy, L. G. (2021). Hallmarks of Basidiomycete Soft- and White-Rot in Wood-Decay - Omics Data of Two *Armillaria* Species. *Microorganisms*, *9*(1), 149. https://doi.org/10.3390/microorganisms9010149
32. Samreen, P., Mangipudi, M., Grover, S., Rajan, H. & G, S. (2019). Production of Pectinases and Pectinolytic Enzymes: Microorganisms, Cultural Conditions and Substrates. *Advances in Biotechnology & Microbiology*, *14*(2). https://doi.org/10.19080/AIBM.2019.14.555884
33. Shankar Naik, B., Abrar, S. & Krishnappa, M. (2019). *Industrially Important Enzymes from Fungal Endophytes* (pp. 263–280). https://doi.org/10.1007/978-3-030-10480-1_7
34. Shet, A. R., Desai, S. . & Achappa, S. (2018). Pectinolytic enzymes: classification, production, purification and applications. *Life Science Informatics Publications*, *4*(3), 337. https://doi.org/http://doi.org/10.26479/2018.0403.30
35. Sipos, G., Prasanna, A. N., Walter, M. C., O'Connor, E., Bálint, B., Krizsán, K., Kiss, B., Hess, J., Varga, T., Slot, J., Riley, R., Bóka, B., Rigling, D., Barry, K., Lee, J., Mihaltcheva, S., LaButti, K., Lipzen, A., Waldron, R., … Nagy, L. G. (2017). Genome expansion and lineage-specific genetic innovations in the forest pathogenic fungi *Armillaria*. *Nature Ecology & Evolution*, *1*(12), 1931–1941. https://doi.org/10.1038/s41559-017-0347-8
36. Voragen, A. G. J., Coenen, G.-J., Verhoef, R. P. & Schols, H. A. (2009). Pectin, a versatile polysaccharide present in plant cell walls. *Structural Chemistry*, *20*(2), 263–275. https://doi.org/10.1007/s11224-009-9442-z
37. Wefers, D., Dong, J., Abdel-Hamid, A. M., Paul, H. M., Pereira, G. V., Han, Y., Dodd, D., Baskaran, R., Mayer, B., Mackie, R. I. & Cann, I. (2017). Enzymatic Mechanism for Arabinan Degradation and Transport in the Thermophilic Bacterium *Caldanaerobius polysaccharolyticus*. *Applied and Environmental Microbiology*, *83*(18). https://doi.org/10.1128/AEM.00794-17
38. Yang, Z. L. (2011). Molecular techniques revolutionize knowledge of basidiomycete evolution. *Fungal Diversity*, *50*(1), 47–58. https://doi.org/10.1007/s13225-011-0121-1
39. Yu, H.-W., Im, J.-H., Kong, W.-S. & Park, Y.-J.

(2020). Comparative Analysis of Carbohydrate Active Enzymes in the *Flammulina velutipes* var. *lupinicola* Genome. *Microorganisms*, *9*(1), 20. https://doi.org/10.3390/microorganisms9010020
40. Yüksel, E., Kort, R. & Voragen, A. G. J. (2024). Structure and degradation dynamics of dietary pectin. *Critical Reviews in Food Science and Nutrition*, 1–20. https://doi.org/10.1080/10408398.2024.2437573
41. Zhang, Y., Wang, J., Yajun, C., Zhou, M., Wang, W., Geng, M., Xu, D. & Xu, Z. (2020). Comparative Genomics Uncovers the Genetic Diversity and Synthetic Biology of Secondary Metabolite Production of *Trametes*. *Mycobiology*, *48*(2), 104–114. https://doi.org/10.1080/12298093.2020.1725361
42. Zheng, L., Guo, Z., Cao, S. & Zhu, B. (2021). Elucidating the degradation pattern of a new cold-tolerant pectate lyase used for efficient preparation of pectin oligosaccharides. *Bioresources and Bioprocessing*, *8*(1), 121. https://doi.org/10.1186/s40643-021-00475-2
43. Zheng, L., Xu, Y., Li, Q. & Zhu, B. (2021). Pectinolytic lyases: a comprehensive review of sources, category, property, structure, and catalytic mechanism of pectate lyases and pectin lyases. *Bioresources and Bioprocessing*, *8*(1), 79. https://doi.org/10.1186/s40643-021-00432-z

ГЕНЕТИЧНІ ДОСЛІДЖЕННЯ ПЕКТИНОЛІТИЧНИХ ФЕРМЕНТІВ БАЗИДІЄВИХ МАКРОМІЦЕТІВ

П. Р. Зубик, І. Р. Клечак, Л. О. Тітова, О. І. Яловенко

Національний технічний університет України
«Київський політехнічний інститут імені Ігоря Сікорського»,
пр-т. Берестейський, 37, к. 4, м. Київ, 03056
e-mail: pv.zubyk@i.ua

У статті представлено огляд сучасних уявлень про генетичну організацію та функціональні особливості пектинолітичних ферментативних систем базидієвих макроміцетів. Проаналізовано основні родини ферментів, залучених до деградації пектинових сполук, зокрема глікозилгідролази (GH), полісахаридліази (PL) та карбогідратні естерази (CE), а також відповідні гени, виявлені у представників родів Agaricus, Armillaria, Flammulina, Laccaria, Lentinula, Pleurotus, Schizophyllum і Trametes. Наведено порівняльну характеристику геномів зазначених грибів за кількісними показниками генів пектиназ, їхньою структурною належністю та потенційною функціональною активністю. Розглянуто методологічні підходи до вивчення пектинолітичного потенціалу, зокрема секвенування, транскриптоміку, біохімічні методи та інструменти порівняльної геноміки. Окреслено перспективи подальших досліджень у контексті біотехнологічного застосування ферментів цієї групи.

Ключові слова: пектинолітичні ферменти, базидієві макроміцети, секвенування, транскриптоміка, CAZy.

Отримано редколегією 01.06.2025 р.

ORCID ID
Павло Зубик: https://orcid.org/0000-0003-0435-0254
Інна Клечак: https://orcid.org/0000-0002-7382-0259
Лариса Тітова: https://orcid.org/0000-0003-4564-9674
Олена Яловенко: https://orcid.org/0000-0002-5022-143X