GERMAN
VIETNAMESE
SCIENCE
DAY
NGÀY KHOA HỌC ĐỨC VIỆT
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ĐÀ NẴNG

Investigation and exploitation of some enzymes from biodiversity of Vietnamese fungi for efficient conversion of lignocellulose by applying an integrated genomic and secretomic approach

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KHOA HỌC VÀ CÔNG NGHỆ

Fungal resources applied in biochemical/biotechnological terms are widely unused and hardly investigated in Vietnam

So far, more than **1.400 species of higher fungi have been described for Vietnam**[Kiet *et al.* 2011, Dörfelt *et al.* 2004] but this is surely just “*the tip of the iceberg*” (estimated **~72.000 species** in nature). There is huge biodiversity of fungal organisms in the country due to diverse habitats, which let expect a promising potential of species and **biocatalysts** (**enzymes**)

Degradation of the lignocellulose complex (LCC) by wood-rotting fungi

Brown rot

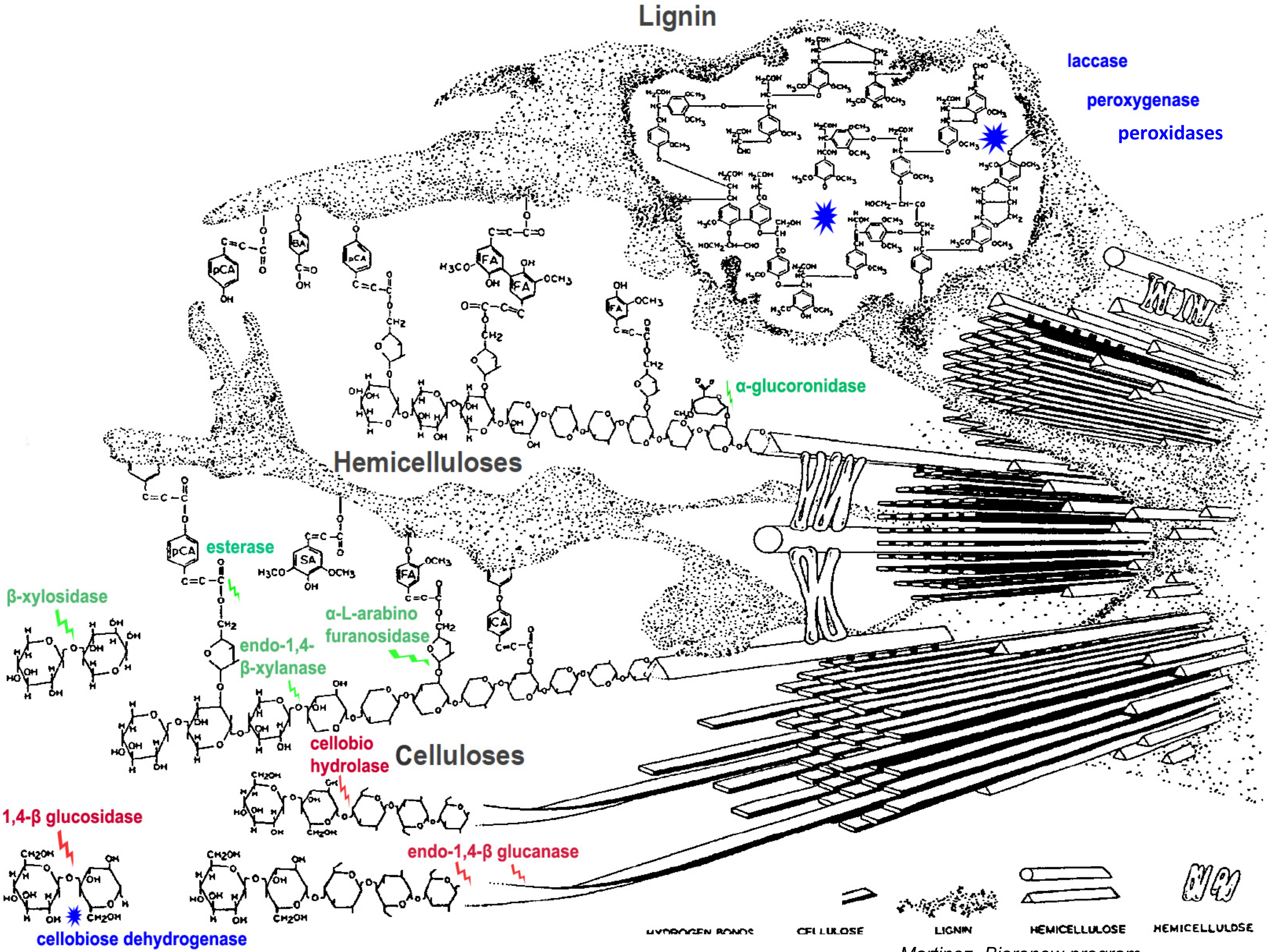
Fomitopsis pinicola

White rot

Trametes versicolor

Soft rot

Xylaria polymorpha



Lignin

laccase

peroxygenase

peroxidases

α-glucoronidase

Hemicelluloses

esterase

β-xylosidase

α-L-arabino furanosidase

endo-1,4-β-xylanase

cellobio hydrolase

Celluloses

1,4-β glucosidase

endo-1,4-β glucanase

cellobiose dehydrogenase

Martinez, Biorenew program

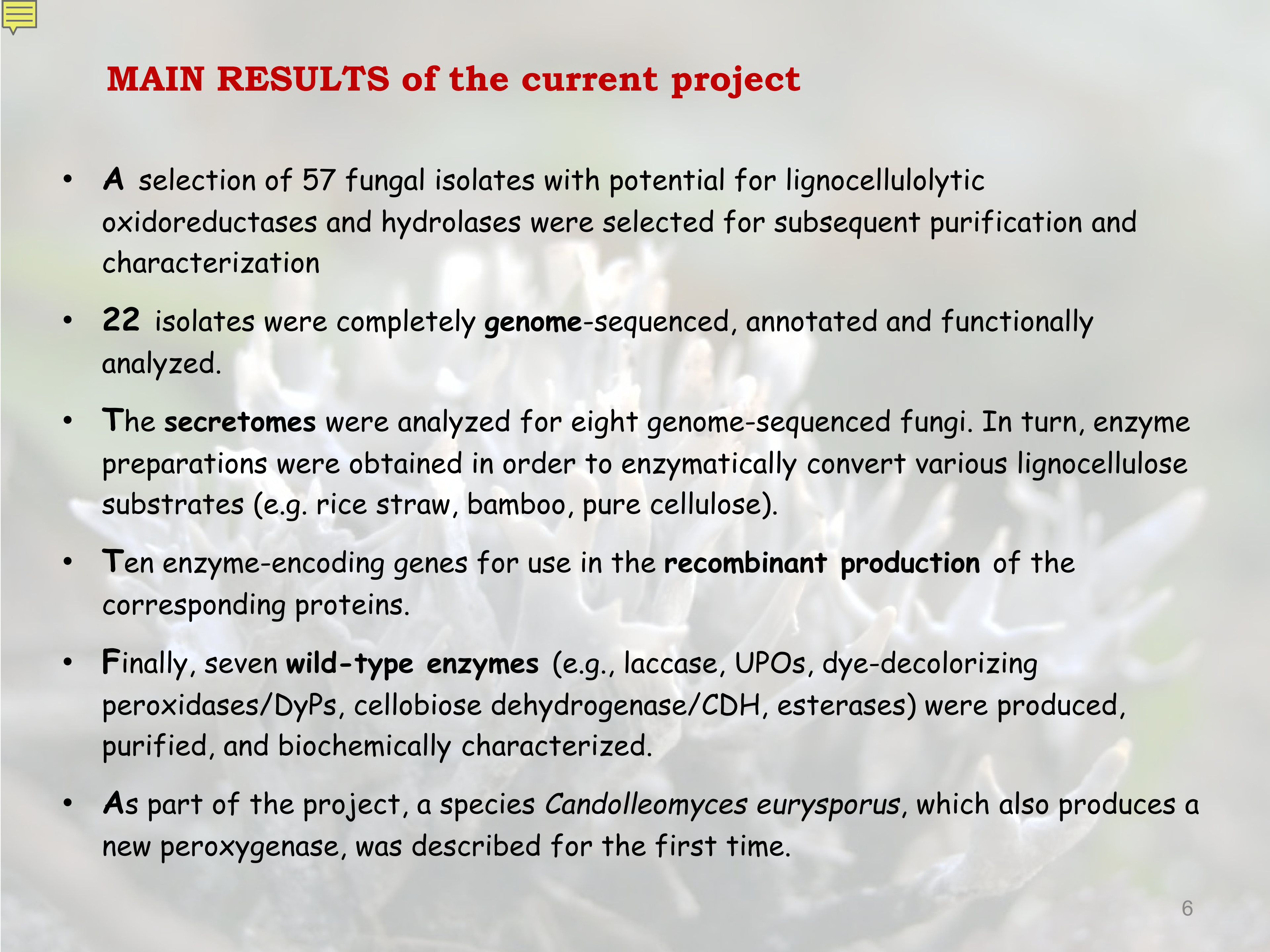
MAIN OBJECTIVES

Overall goal of the project is to obtain whole **genomes of some Vietnamese fungi** for the novel **enzymes**, which would facilitate the conversion of **lignocellulosic biomass** into biobased products required by the chemical and pharmaceutical industries.

The second goal is to identify the most relevant enzymes by a **secrectomic** approach and followed by **recombinant study** to produce the promising biocatalysts. Genes of interest could be also identified by *in silico* screening.

The project fostered the exchange of mycological and biochemical knowledge to enhance the academic education from both collaborating countries, i.e., in the fields of innovative analytical, molecular techniques and exploitation of bioinformatic data.

The project helped to build a bridge between *White*, industrial-relevant *biotechnology* and ecological research by focussing on functional key aspects of fungal biodiversity.



MAIN RESULTS of the current project

- **A** selection of 57 fungal isolates with potential for lignocellulolytic oxidoreductases and hydrolases were selected for subsequent purification and characterization
- **22** isolates were completely **genome**-sequenced, annotated and functionally analyzed.
- The **secretomes** were analyzed for eight genome-sequenced fungi. In turn, enzyme preparations were obtained in order to enzymatically convert various lignocellulose substrates (e.g. rice straw, bamboo, pure cellulose).
- Ten enzyme-encoding genes for use in the **recombinant production** of the corresponding proteins.
- Finally, seven **wild-type enzymes** (e.g., laccase, UPOs, dye-decolorizing peroxidases/DyPs, cellobiose dehydrogenase/CDH, esterases) were produced, purified, and biochemically characterized.
- As part of the project, a species *Candolleomyces eurysporus*, which also produces a new peroxygenase, was described for the first time.


NextSeq 550
Welcome to NextSeq
illumina
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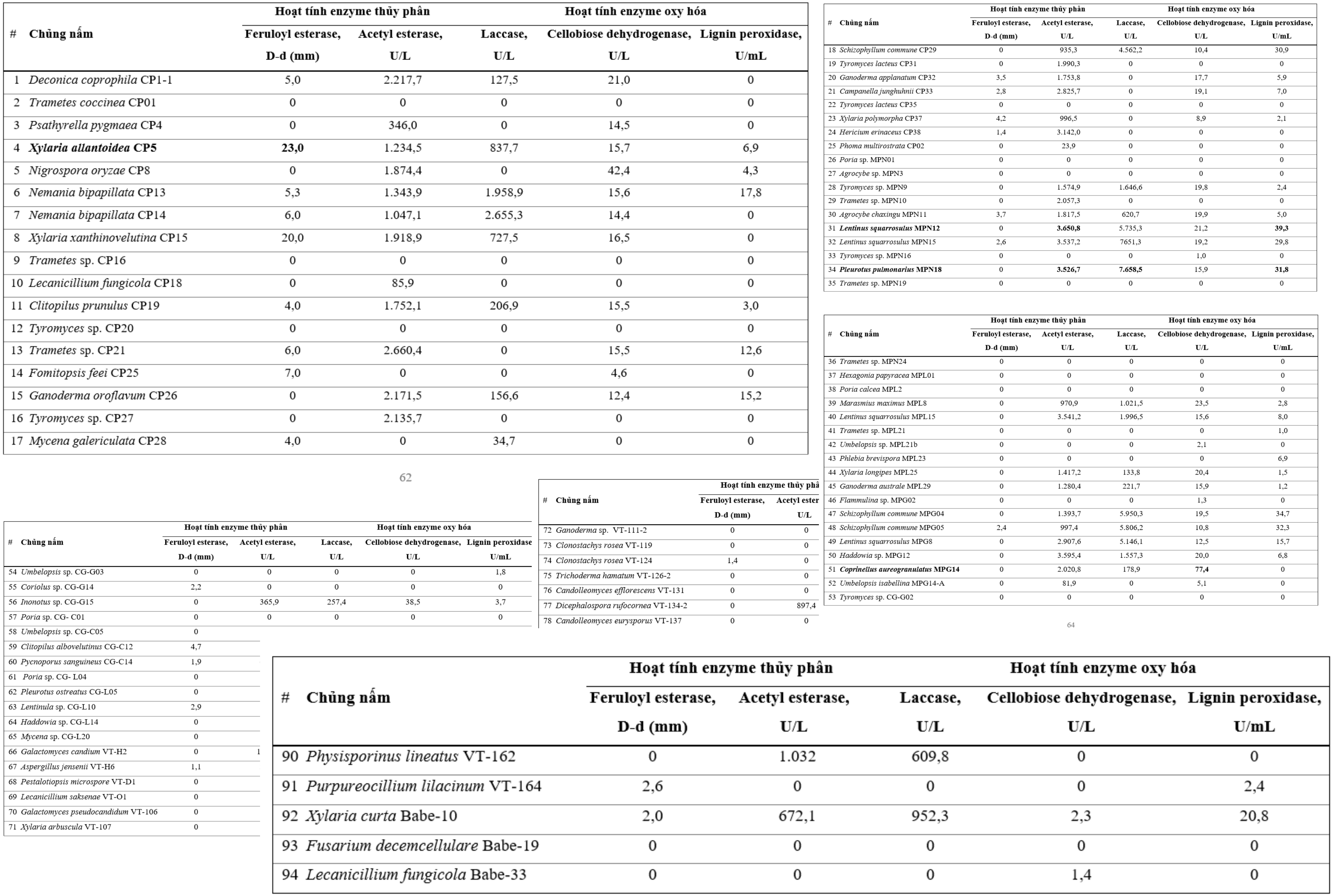
S.E.M

Bảng 3.1. Hoạt tính enzyme chuyển hóa lignocellulose của các chủng nấm

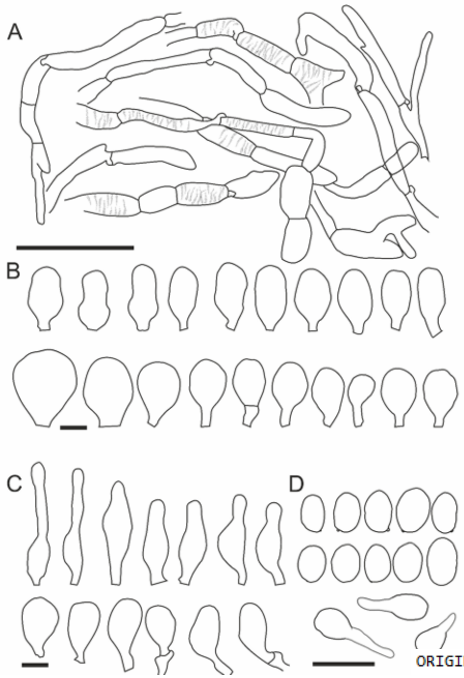


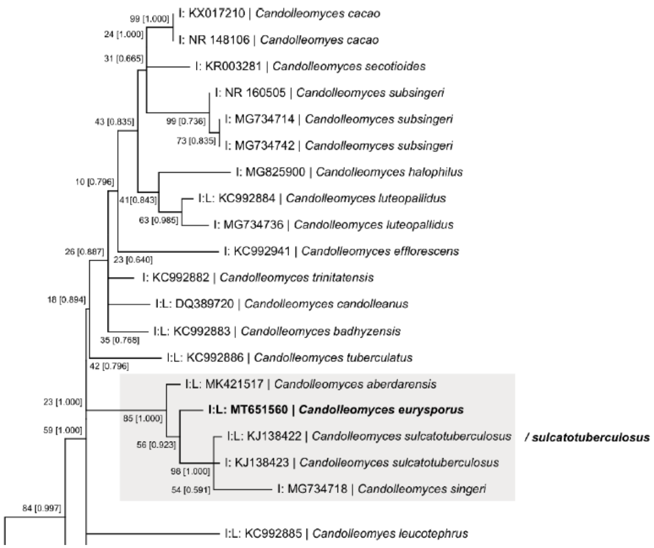
#	Chủng nấm	Hoạt tính enzyme thủy phân		Hoạt tính enzyme oxy hóa		
		Feruloyl esterase, D-d (mm)	Acetyl esterase, U/L	Laccase, U/L	Cellobiose dehydrogenase, U/L	Lignin peroxidase, U/mL
1	*Deconica coprophila* CP1-1	5,0	2.217,7	127,5	21,0	0
2	*Trametes coccinea* CP01	0	0	0	0	0
3	*Psathyrella pygmaea* CP4	0	346,0	0	14,5	0
4	***Xylaria allantoidea* CP5**	**23,0**	1.234,5	837,7	15,7	6,9
5	*Nigrospora oryzae* CP8	0	1.874,4	0	42,4	4,3
6	*Nemania bipapillata* CP13	5,3	1.343,9	1.958,9	15,6	17,8
7	*Nemania bipapillata* CP14	6,0	1.047,1	2.655,3	14,4	0
8	*Xylaria xanthinovelutina* CP15	20,0	1.918,9	727,5	16,5	0
9	*Trametes* sp. CP16	0	0	0	0	0
10	*Lecanicillium fungicola* CP18	0	85,9	0	0	0
11	*Clitopilus prunulus* CP19	4,0	1.752,1	206,9	15,5	3,0
12	*Tyromyces* sp. CP20	0	0	0	0	0
13	*Trametes* sp. CP21	6,0	2.660,4	0	15,5	12,6
14	*Fomitopsis feei* CP25	7,0	0	0	4,6	0
15	*Ganoderma oroflavum* CP26	0	2.171,5	156,6	12,4	15,2
16	*Tyromyces* sp. CP27	0	2.135,7	0	0	0
17	*Mycena galericulata* CP28	4,0	0	34,7	0	0
18	*Schizophyllum commune* CP29	0	935,3	4.562,2	10,4	30,9
19	*Tyromyces lacteus* CP31	0	1.990,3	0	0	0
20	*Ganoderma applanatum* CP32	3,5	1.753,8	0	17,7	5,9
21	*Campanella junghuhnii* CP33	2,8	2.825,7	0	19,1	7,0
22	*Tyromyces lacteus* CP35	0	0	0	0	0
23	*Xylaria polymorpha* CP37	4,2	996,5	0	8,9	2,1
24	*Hericium erinaceus* CP38	1,4	3.142,0	0	0	0
25	*Phoma multirostrata* CP02	0	23,9	0	0	0
26	*Poria* sp. MPN01	0	0	0	0	0
27	*Agrocybe* sp. MPN3	0	0	0	0	0
28	*Tyromyces* sp. MPN9	0	1.574,9	1.646,6	19,8	2,4
29	*Trametes* sp. MPN10	0	2.057,3	0	0	0
30	*Agrocybe chaxingu* MPN11	3,7	1.817,5	620,7	19,9	5,0
31	***Lentinus squarrosulus* MPN12**	0	**3.650,8**	5.735,3	21,2	**39,3**
32	*Lentinus squarrosulus* MPN15	2,6	3.537,2	7651,3	19,2	29,8
33	*Tyromyces* sp. MPN16	0	0	0	1,0	0
34	***Pleurotus pulmonarius* MPN18**	0	**3.526,7**	**7.658,5**	15,9	**31,8**
35	*Trametes* sp. MPN19	0	0	0	0	0
36	*Trametes* sp. MPN24	0	0	0	0	0
37	*Hexagonia papyracea* MPL01	0	0	0	0	0
38	*Poria calcea* MPL2	0	0	0	0	0
39	*Marasmius maximus* MPL8	0	970,9	1.021,5	23,5	2,8
40	*Lentinus squarrosulus* MPL15	0	3.541,2	1.996,5	15,6	8,0
41	*Trametes* sp. MPL21	0	0	0	0	1,0
42	*Umbelopsis* sp. MPL21b	0	0	0	2,1	0
43	*Phlebia brevispora* MPL23	0	0	0	0	6,9
44	*Xylaria longipes* MPL25	0	1.417,2	133,8	20,4	1,5
45	*Ganoderma australe* MPL29	0	1.280,4	221,7	15,9	1,2
46	*Flammulina* sp. MPG02	0	0	0	1,3	0
47	*Schizophyllum commune* MPG04	0	1.393,7	5.950,3	19,5	34,7
48	*Schizophyllum commune* MPG05	2,4	997,4	5.806,2	10,8	32,3
49	*Lentinus squarrosulus* MPG8	0	2.907,6	5.146,1	12,5	15,7
50	*Haddowia* sp. MPG12	0	3.595,4	1.557,3	20,0	6,8
51	***Coprinellus aureogranulatus* MPG14**	0	2.020,8	178,9	**77,4**	0
52	*Umbelopsis isabellina* MPG14-A	0	81,9	0	5,1	0
53	*Tyromyces* sp. CG-G02	0	0	0	0	0
54	*Umbelopsis* sp. CG-G03	0	0	0	0	1,8
55	*Coriolus* sp. CG-G14	2,2	0	0	0	0
56	*Inonotus* sp. CG-G15	0	365,9	257,4	38,5	3,7
57	*Poria* sp. CG- C01	0	0	0	0	0
58	*Umbelopsis* sp. CG-C05	0				
59	*Clitopilus albovelutinus* CG-C12	4,7				
60	*Pycnoporus sanguineus* CG-C14	1,9				
61	*Poria* sp. CG- L04	0				
62	*Pleurotus ostreatus* CG-L05	0				
63	*Lentinula* sp. CG-L10	2,9				
64	*Haddowia* sp. CG-L14	0				
65	*Mycena* sp. CG-L20	0				
66	*Galactomyces candium* VT-H2	0	1			
67	*Aspergillus jensenii* VT-H6	1,1				
68	*Pestalotiopsis microspore* VT-D1	0				
69	*Lecanicillium saksenae* VT-O1	0				
70	*Galactomyces pseudocandidum* VT-106	0				
71	*Xylaria arbuscula* VT-107	0				
72	*Ganoderma* sp. VT-111-2	0	0			
73	*Clonostachys rosea* VT-119	0	0			
74	*Clonostachys rosea* VT-124	1,4	0			
75	*Trichoderma hamatum* VT-126-2	0	0			
76	*Candolleomyces efflorescens* VT-131	0	0			
77	*Dicephalospora rufocornea* VT-134-2	0	897,4			
78	*Candolleomyces eurysporus* VT-137	0	0			
90	*Physisporinus lineatus* VT-162	0	1.032	609,8	0	0
91	*Purpureocillium lilacinum* VT-164	2,6	0	0	0	2,4
92	*Xylaria curta* Babe-10	2,0	672,1	952,3	2,3	20,8
93	*Fusarium decemcellulare* Babe-19	0	0	0	0	0
94	*Lecanicillium fungicola* Babe-33	0	0	0	1,4	0

Chú thích: - *CP = Cúc Phương, MP = Mường Phăng, CG = Cần Giờ; Babe = Ba Bể, VT = chủng CHLB Đức cung cấp.*
- *D-d = đường kính vòng thủy phân cơ chất.*

Basidiomycota	Tag Stamm	**ABTS**			**Naphtol**			**Tannin**			**Mn^{2+}**			**Orange G**			**AX**			**Casein**			**Cellulose**			**GM**			**Xylan**		
		10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21
Candolleomyces efflorescens	VT131	++	++	++				+	+	+																					
Candolleomyces eurysporus	VT138	+	++	++																											
Fomitopsis feei	VT142		(+)	(+)					(+)																						
Physisporinus lineatus	VT162	+++	+++	+++	(+)	+	+	+	+	+			+	+	+	+++															
Physisporinus sp.	VT165	+++	+++	+++	(+)	+	+	(+)	(+)	+			+	+++	+++	+++															
Ascomycota	Tag Stamm	**ABTS**			**Naphtol**			**Tannin**			**Mn^{2+}**			**Orange G**			**AX**			**Casein**			**Cellulose**			**GM**			**Xylan**		
		10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21
Alternaria alternata	VT145																						++	++	++	+	+	+			
Alternaria alternata	VT-H1	+++	+++	+++	+	+	+	++	++	++							+	++	++										++	++	+++
Alternaria alternata	VT-H2							+	+	+							(+)	+					(+)	+	+		(+)	(+)	++	+++	+++
Aspergillus terreus	VT148									++							+	++	+++		(+)	+		+	++				+	++	+++
Aspergillus versicolor	VT-H6							+	+	+																					
Clonostachys rosea	VT118							+	+	++							(+)	(+)	(+)		+	+						(+)		(+)	(+)
Clonostachys sp.	VT124							+	++	++																					
Clonostachys sp.	VT139																(+)	+													
Cylindrocladium sp.	VT153							+	+	+							+	+	+										(+)	(+)	
Dicephalospora rufocornea	VT134-2		+	+				++	++	++								+													
Fusarium acuminatum	VT150							+	+	+							+	++	++				+	+	+				++	+++	+++
Fusarium oxysporum	VT151							+	+	++																			(+)	(+)	
Fusarium oxysporum	VT151-1							+	+	+							+	++											+	++	
Fusarium oxysporum	VT111	++	++	++				++															(+)	(+)	(+)						
Fusarium proliferatum	VT147							+	+	++																					
Galactomyces pseudocandida	VT106																														
Gliocephalotrichum humicola	VT158-1	++	++	++				(+)																							
Lasiodiplodia mahajangana	VT137																														
Lecanicillium saksenae	VT-O1	++	++	++																											
Leptobacillium symbioticum	VT124-01																														
Metarhizium robertsii	VT158-2							++	+	+																					
Neocosmospora solani	VT105							++	++	++																					
Neopestalotiopsis microspora	VT-D1	+	++	++				++	++	++															+						
Purpureocillium lilacinum	VT164																														
Schizophyllum commune	VT143	++	++	++													++	++	++										++	++	+++
Trichoderma hamatum	VT126-2	++	++	++				++	++	++							++	++	++	(+)	(+)	(+)	+	++	+++	(+)	(+)	(+)	++	+++	+++
Trichoderma harzianum	VT125																++	++	++	+	+	++	+	++	+++	(+)	(+)	(+)	+++	+++	+++
Xylaria anisopleura	VT137-2	++	++	++				++	++	++																					
Mucormycota	Tag Stamm	**ABTS**			**Naphtol**			**Tannin**			**Mn^{2+}**			**Orange G**			**AX**			**Casein**			**Cellulose**			**GM**			**Xylan**		
		10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21	10	14	21
Mucor irregularis	VT111-2							++	++	+++					(+)	(+)							(+)	+	++						

ABTS, naphtol, tannin, Mn2+, Orange G, arabinoxylan (AX), casein, cellulose, galactomannan (GM), và xylan.
(+) Hoạt tính yếu, (++) trung bình và (+++) hoạt tính mạnh.





Basidiomycete *Candolleomyces eurysporus*, a new species isolated from Cúc Phương. Bar = 50 μm (A), 10 μm (B-D).

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GenBank NCBI:
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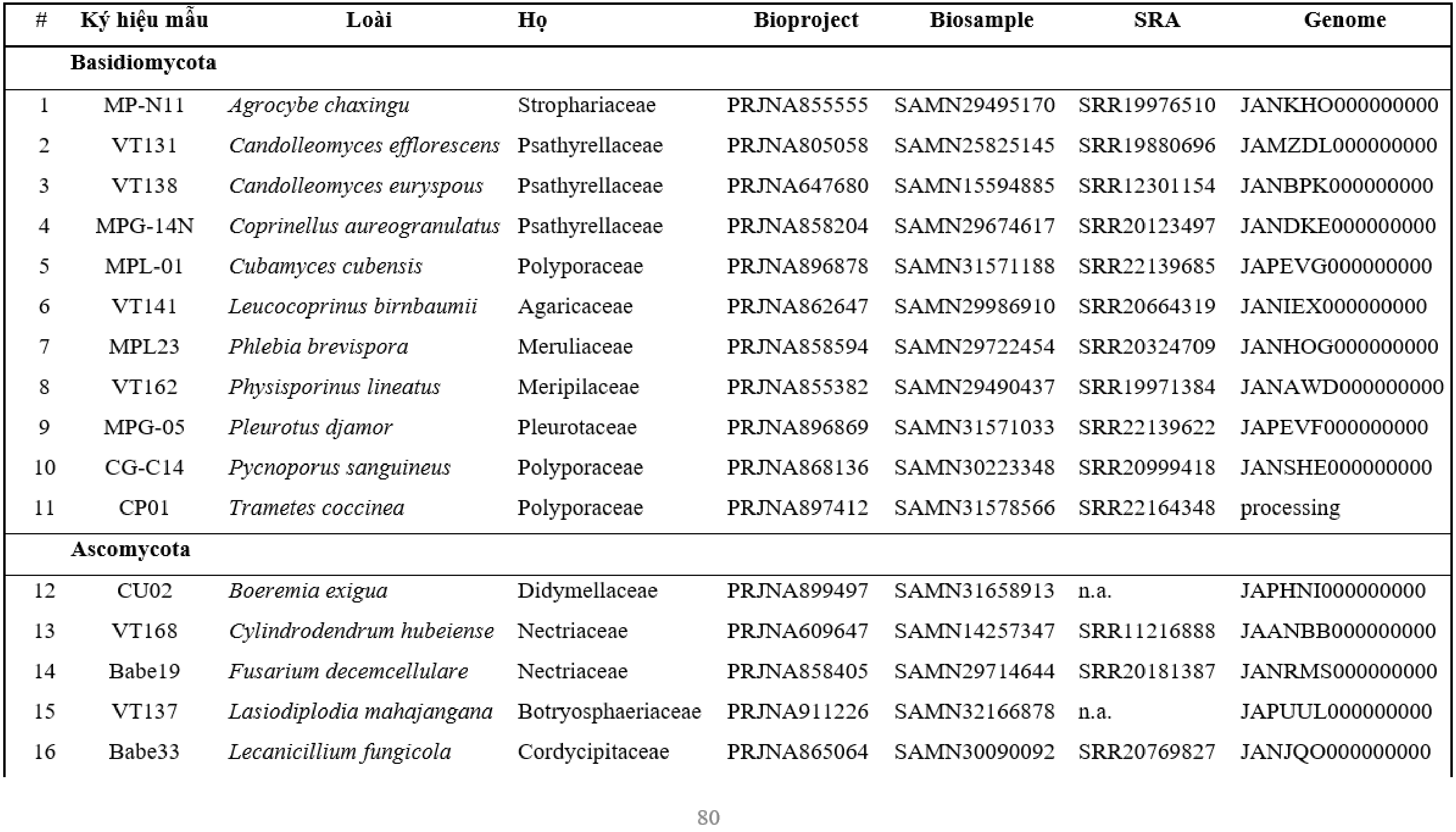
```

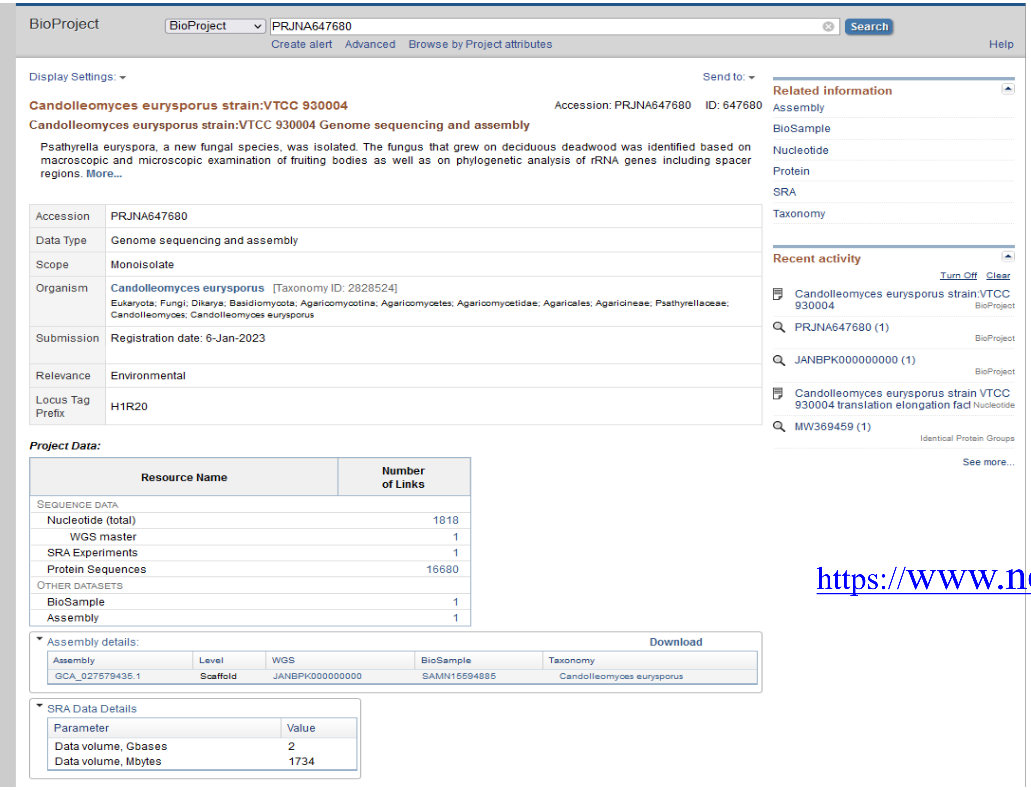
Kết quả phân tích genome nấm và các tham số đăng ký trên cơ sở dữ liệu NCBI

Übersicht über die genomsequenzierten vietnamesischen Pilze einschl. der zugehörigen NCBI-Referenznummern



#	Ký hiệu mẫu	Loài	Họ	Bioproject	Biosample	SRA	Genome
	Basidiomycota						
1	MP-N11	*Agrocybe chaxingu*	Strophariaceae	PRJNA855555	SAMN29495170	SRR19976510	JANKHO000000000
2	VT131	*Candolleomyces efflorescens*	Psathyrellaceae	PRJNA805058	SAMN25825145	SRR19880696	JAMZDL000000000
3	VT138	*Candolleomyces euryspous*	Psathyrellaceae	PRJNA647680	SAMN15594885	SRR12301154	JANBPK000000000
4	MPG-14N	*Coprinellus aureogranulatus*	Psathyrellaceae	PRJNA858204	SAMN29674617	SRR20123497	JANDKE000000000
5	MPL-01	*Cubamyces cubensis*	Polyporaceae	PRJNA896878	SAMN31571188	SRR22139685	JAPEVG000000000
6	VT141	*Leucocoprinus birnbaumii*	Agaricaceae	PRJNA862647	SAMN29986910	SRR20664319	JANIEX000000000
7	MPL23	*Phlebia brevispora*	Meruliaceae	PRJNA858594	SAMN29722454	SRR20324709	JANHOG000000000
8	VT162	*Physisporinus lineatus*	Meripilaceae	PRJNA855382	SAMN29490437	SRR19971384	JANAWD000000000
9	MPG-05	*Pleurotus djamor*	Pleurotaceae	PRJNA896869	SAMN31571033	SRR22139622	JAPEVF000000000
10	CG-C14	*Pycnoporus sanguineus*	Polyporaceae	PRJNA868136	SAMN30223348	SRR20999418	JANSHE000000000
11	CP01	*Trametes coccinea*	Polyporaceae	PRJNA897412	SAMN31578566	SRR22164348	processing
	Ascomycota						
12	CU02	*Boeremia exigua*	Didymellaceae	PRJNA899497	SAMN31658913	n.a.	JAPHNI000000000
13	VT168	*Cylindrodendrum hubeiense*	Nectriaceae	PRJNA609647	SAMN14257347	SRR11216888	JAANBB000000000
14	Babe19	*Fusarium decemcellulare*	Nectriaceae	PRJNA858405	SAMN29714644	SRR20181387	JANRMS000000000
15	VT137	*Lasiodiplodia mahajangana*	Botryosphaeriaceae	PRJNA911226	SAMN32166878	n.a.	JAPUUL000000000
16	Babe33	*Lecanicillium fungicola*	Cordycipitaceae	PRJNA865064	SAMN30090092	SRR20769827	JANJQO000000000

#	Ký hiệu mẫu	Loài	Họ	Bioproject	Biosample	SRA	Genome
17	VT-O1	*Lecanicillium saksenae*	Cordycipitaceae	PRJNA855341	SAMN29489342	SRR19970639	JANAKD000000000
18	CP14	***Nemania bipapillata***	Xylariaceae	PRJNA896792	SAMN31566648	n.a.	JAPESX000000000
19	VT107	*Xylaria arbuscula*	Xylariaceae	PRJNA862450	SAMN29977060	SRR20649208	JANPWZ000000000
20	Babe10	*Xylaria curta*	Xylariaceae	PRJNA868144	SAMN30224423	SRR20999430	JAPDGR000000000
	Mucormycota						
21	MPG-14A	*Umbelopsis isabellina*	Umbelopsidaceae	PRJNA864657	SAMN30077657	SRR20746300	JANJFK000000000
	Oomycota						
22	VT-H3	*Phytophthora cinnamomi*	Peronosporaceae	PRJNA896933	SAMN31572647	SRR22164408	processing



Candolleomyces eurysporus strain:VTCC 930004

Accession: PRJNA647680 ID: 647680

Candolleomyces eurysporus strain:VTCC 930004 Genome sequencing and assembly

Psathyrella euryspora, a new fungal species, was isolated. The fungus that grew on deciduous deadwood was identified based on macroscopic and microscopic examination of fruiting bodies as well as on phylogenetic analysis of rRNA genes including spacer regions. More...

Accession	PRJNA647680
Data Type	Genome sequencing and assembly
Scope	Monoisolate
Organism	Candolleomyces eurysporus [Taxonomy ID: 2828524] Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Agaricomycetidae; Agaricales; Agaricineae; Psathyrellaceae; Candolleomyces; Candolleomyces eurysporus
Submission	Registration date: 6-Jan-2023
Relevance	Environmental
Locus Tag Prefix	H1R20

Project Data:

Resource Name	Number of Links
SEQUENCE DATA	
Nucleotide (total)	1818
WGS master	1
SRA Experiments	1
Protein Sequences	16680
OTHER DATASETS	
BioSample	1
Assembly	1

Assembly details: Download

Assembly	Level	WGS	BioSample	Taxonomy
GCA_027579435.1	Scaffold	JANBPK000000000	SAMN15594885	Candolleomyces eurysporus

SRA Data Details

Parameter	Value
Data volume, Gbases	2
Data volume, Mbytes	1734

Related information
- Assembly
- BioSample
- Nucleotide
- Protein
- SRA
- Taxonomy

Recent activity

Turn Off Clear

- Candolleomyces eurysporus strain:VTCC 930004 BioProject
- PRJNA647680 (1) BioProject
- JANBPK000000000 (1) BioProject
- Candolleomyces eurysporus strain VTCC 930004 translation elongation facl Nucleotide
- MW369459 (1) Identical Protein Groups

See more...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NIH National Library of Medicine

National Center for Biotechnology Information

https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA647680

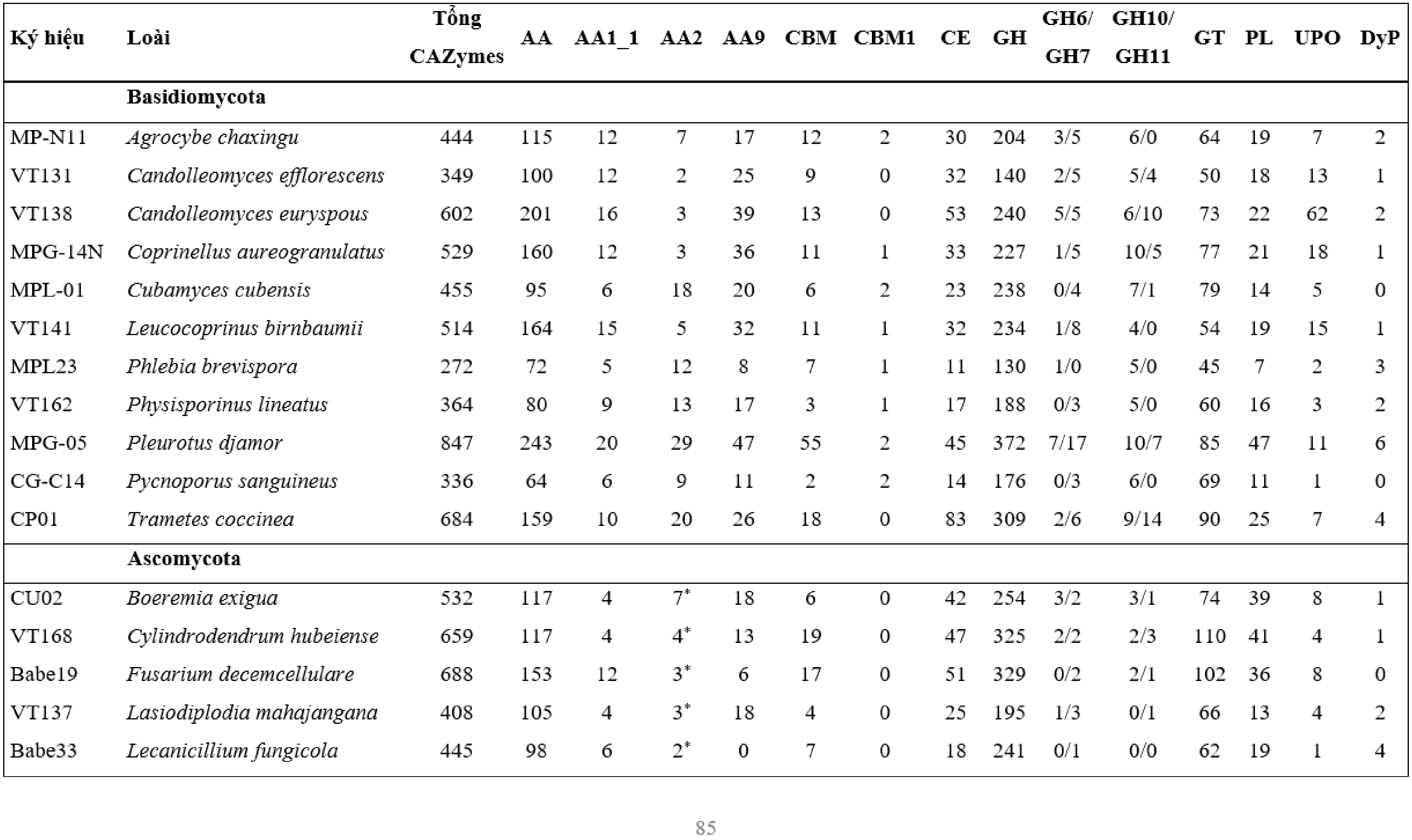
Các thông số bộ gene của các loài nấm nghiên cứu giải trình tự

Sequenzierte Pilzisolate und deren Genomparameter und weitere Kenngrößen

Ký hiệu	**Loài nấm**	**Kích thước (Mb)**	**# *Contigs***	**# Protein**	**G+C (%)**	**CDS (ø bp)**	**N_{50}**	**Predictor**
	Basidiomycota							
MP-N11	*Agrocybe (Cyclocybe) chaxingu*	40,4	4467	12440	50,9	220	18131	*Coprinus cinereus*
VT131	*Candolleomyces efflorescens*	33,6	565	9128	51,5	261	157606	*Coprinus cinereus*
VT138	*Candolleomyces euryspous*	70,0	1966	16680	50,0	252	78579	*Coprinus cinereus*
MPG-14N	*Coprinellus aureogranulatus*	58,2	4667	20330	52,7	271	16685	*Laccaria bicolor*
MPL-01	*Cubamyces cubensis*	42,0	1364	13614	55,6	232	72235	*Coprinus cinereus*
VT141	*Leucocoprinus birnbaumii*	45,7	2238	13114	47,9	230	32048	*Laccaria bicolor*
MPL23	*Phlebia brevispora*	28,6	2987	9457	50,5	200	12551	*Laccaria bicolor*
VT162	*Physisporinus lineatus*	46,9	2700	13430	48,4	257	51059	*Cryptococcus neoformans* var. *gattii*
MPG-05	*Pleurotus djamor*	62,5	2603	19729	49,9	224	52533	*Coprinus cinereus*
CG-C14	*Pycnoporus sanguineus*	35,2	7979	14788	55,1	206	6341	*Laccaria bicolor*
CP01	*Trametes coccinea*	71,8	3690	22555	51,3	261	44973	*Laccaria bicolor*
	Ascomycota							
CU02	*Boeremia exigua*	29,6	2028	10759	52,3	497	27149	*Botrytis cinerea*
VT168	*Cylindrodendrum hubeiense*	48,8	1038	13747	51,8	492	85271	*Neurospora crassa*
Babe19	*Fusarium decemcellulare*	50,5	6066	17261	50,1	443	19398	*Aspergillus oryzae*
VT137	*Lasiodiplodia mahajangana*	40,1	4499	11238	48,1	420	12129	*Botrytis cinerea*

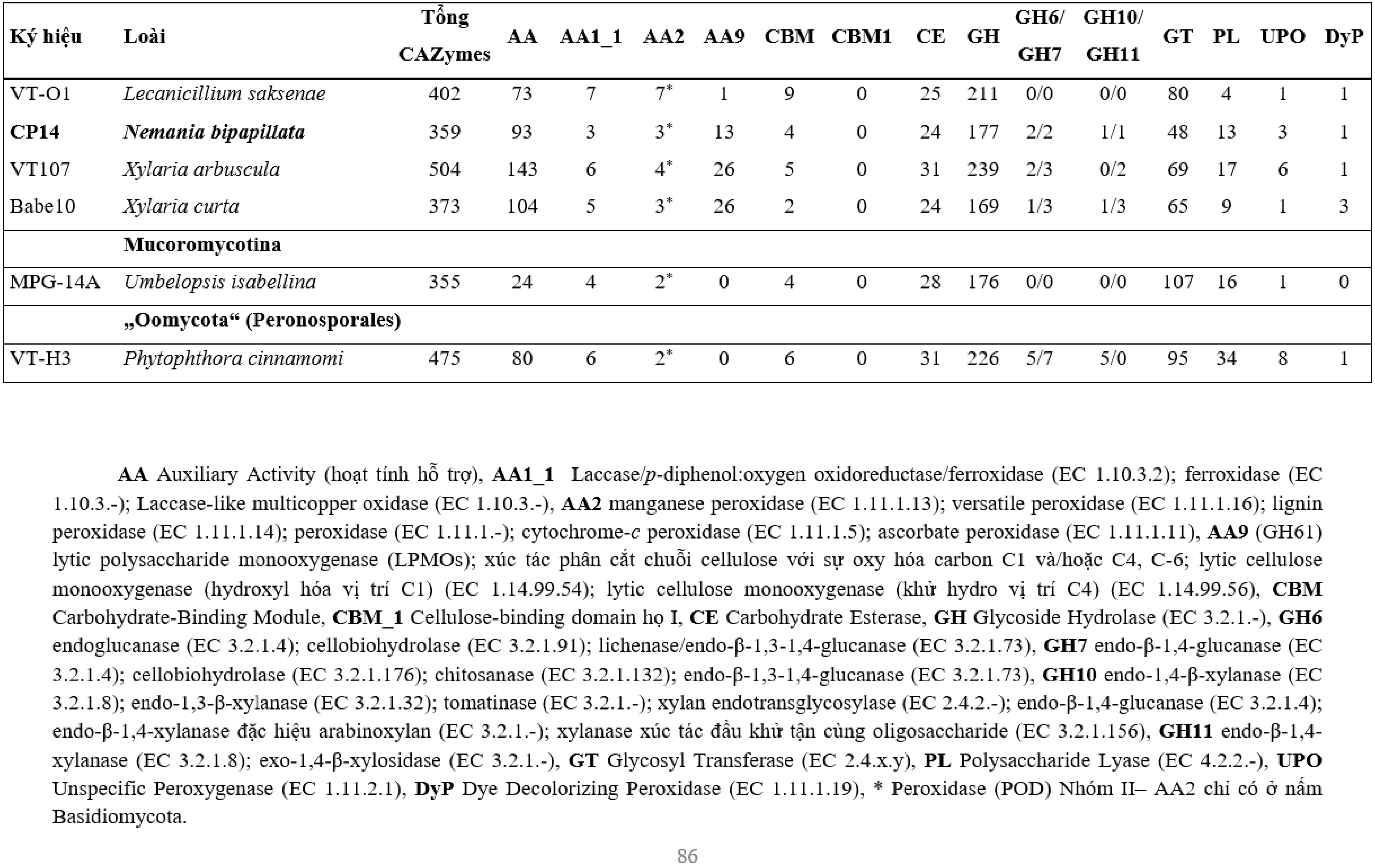
Chú thích chức năng gene

CAZymes (carbohydrate active enzymes, kohlenhydrataktive Enzyme)
und andere biotechnologisch relevante Zielenzyme der im Projekt genomsequenzierten Pilze.



Ký hiệu	**Loài**	**Tổng CAZymes**	**AA**	**AA1_1**	**AA2**	**AA9**	**CBM**	**CBM1**	**CE**	**GH**	**GH6/ GH7**	**GH10/ GH11**	**GT**	**PL**	**UPO**	**DyP**
	Basidiomycota															
MP-N11	*Agrocybe chaxingu*	444	115	12	7	17	12	2	30	204	3/5	6/0	64	19	7	2
VT131	*Candolleomyces efflorescens*	349	100	12	2	25	9	0	32	140	2/5	5/4	50	18	13	1
VT138	*Candolleomyces euryspous*	602	201	16	3	39	13	0	53	240	5/5	6/10	73	22	62	2
MPG-14N	*Coprinellus aureogranulatus*	529	160	12	3	36	11	1	33	227	1/5	10/5	77	21	18	1
MPL-01	*Cubamyces cubensis*	455	95	6	18	20	6	2	23	238	0/4	7/1	79	14	5	0
VT141	*Leucocoprinus birnbaumii*	514	164	15	5	32	11	1	32	234	1/8	4/0	54	19	15	1
MPL23	*Phlebia brevispora*	272	72	5	12	8	7	1	11	130	1/0	5/0	45	7	2	3
VT162	*Physisporinus lineatus*	364	80	9	13	17	3	1	17	188	0/3	5/0	60	16	3	2
MPG-05	*Pleurotus djamor*	847	243	20	29	47	55	2	45	372	7/17	10/7	85	47	11	6
CG-C14	*Pycnoporus sanguineus*	336	64	6	9	11	2	2	14	176	0/3	6/0	69	11	1	0
CP01	*Trametes coccinea*	684	159	10	20	26	18	0	83	309	2/6	9/14	90	25	7	4
	Ascomycota															
CU02	*Boeremia exigua*	532	117	4	7*	18	6	0	42	254	3/2	3/1	74	39	8	1
VT168	*Cylindrodendrum hubeiense*	659	117	4	4*	13	19	0	47	325	2/2	2/3	110	41	4	1
Babe19	*Fusarium decemcellulare*	688	153	12	3*	6	17	0	51	329	0/2	2/1	102	36	8	0
VT137	*Lasiodiplodia mahajangana*	408	105	4	3*	18	4	0	25	195	1/3	0/1	66	13	4	2
Babe33	*Lecanicillium fungicola*	445	98	6	2*	0	7	0	18	241	0/1	0/0	62	19	1	4

85

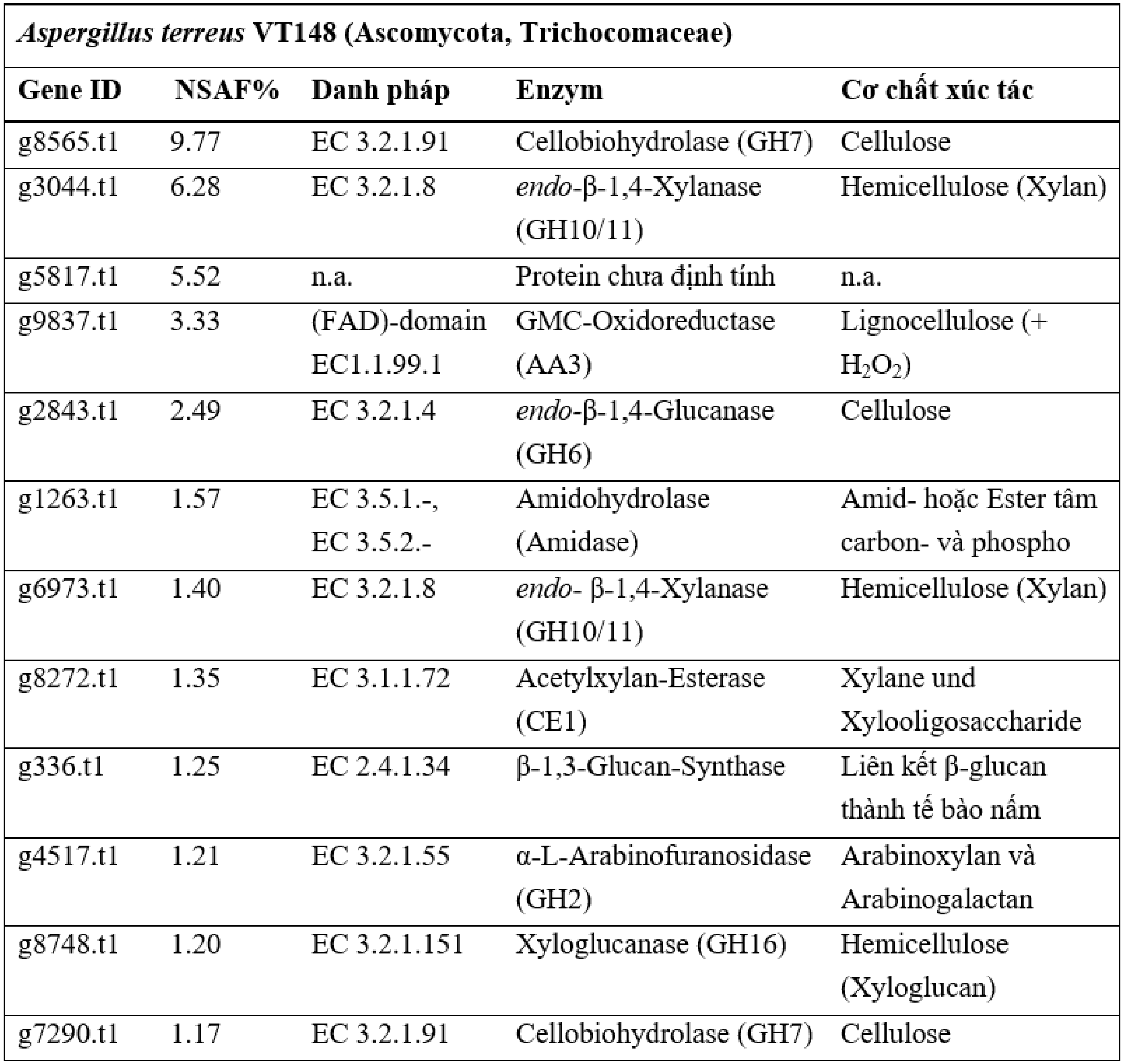


Ký hiệu	Loài	Tổng CAZymes	AA	AA1_1	AA2	AA9	CBM	CBM1	CE	GH	GH6/ GH7	GH10/ GH11	GT	PL	UPO	DyP
VT-O1	*Lecanicillium saksenae*	402	73	7	7^{*}	1	9	0	25	211	0/0	0/0	80	4	1	1
CP14	***Nemania bipapillata***	359	93	3	3^{*}	13	4	0	24	177	2/2	1/1	48	13	3	1
VT107	*Xylaria arbuscula*	504	143	6	4^{*}	26	5	0	31	239	2/3	0/2	69	17	6	1
Babe10	*Xylaria curta*	373	104	5	3^{*}	26	2	0	24	169	1/3	1/3	65	9	1	3
	Mucoromycotina															
MPG-14A	*Umbelopsis isabellina*	355	24	4	2^{*}	0	4	0	28	176	0/0	0/0	107	16	1	0
	„Oomycota“ (Peronosporales)															
VT-H3	*Phytophthora cinnamomi*	475	80	6	2^{*}	0	6	0	31	226	5/7	5/0	95	34	8	1

AA Auxiliary Activity (hoạt tính hỗ trợ), **AA1_1** Laccase/*p*-diphenol:oxygen oxidoreductase/ferroxidase (EC 1.10.3.2); ferroxidase (EC 1.10.3.-); Laccase-like multicopper oxidase (EC 1.10.3.-), **AA2** manganese peroxidase (EC 1.11.1.13); versatile peroxidase (EC 1.11.1.16); lignin peroxidase (EC 1.11.1.14); peroxidase (EC 1.11.1.-); cytochrome-*c* peroxidase (EC 1.11.1.5); ascorbate peroxidase (EC 1.11.1.11), **AA9** (GH61) lytic polysaccharide monooxygenase (LPMOs); xúc tác phân cắt chuỗi cellulose với sự oxy hóa carbon C1 và/hoặc C4, C-6; lytic cellulose monooxygenase (hydroxyl hóa vị trí C1) (EC 1.14.99.54); lytic cellulose monooxygenase (khử hydro vị trí C4) (EC 1.14.99.56), **CBM** Carbohydrate-Binding Module, **CBM_1** Cellulose-binding domain họ I, **CE** Carbohydrate Esterase, **GH** Glycoside Hydrolase (EC 3.2.1.-), **GH6** endoglucanase (EC 3.2.1.4); cellobiohydrolase (EC 3.2.1.91); lichenase/endo-β-1,3-1,4-glucanase (EC 3.2.1.73), **GH7** endo-β-1,4-glucanase (EC 3.2.1.4); cellobiohydrolase (EC 3.2.1.176); chitosanase (EC 3.2.1.132); endo-β-1,3-1,4-glucanase (EC 3.2.1.73), **GH10** endo-1,4-β-xylanase (EC 3.2.1.8); endo-1,3-β-xylanase (EC 3.2.1.32); tomatinase (EC 3.2.1.-); xylan endotransglycosylase (EC 2.4.2.-); endo-β-1,4-glucanase (EC 3.2.1.4); endo-β-1,4-xylanase đặc hiệu arabinoxylan (EC 3.2.1.-); xylanase xúc tác đầu khử tận cùng oligosaccharide (EC 3.2.1.156), **GH11** endo-β-1,4-xylanase (EC 3.2.1.8); exo-1,4-β-xylosidase (EC 3.2.1.-), **GT** Glycosyl Transferase (EC 2.4.x.y), **PL** Polysaccharide Lyase (EC 4.2.2.-), **UPO** Unspecific Peroxygenase (EC 1.11.2.1), **DyP** Dye Decolorizing Peroxidase (EC 1.11.1.19), * Peroxidase (POD) Nhóm II– AA2 chỉ có ở nấm Basidiomycota.

Kết quả nghiên cứu phân tích secretome

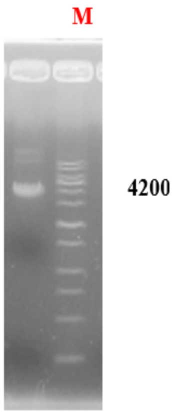
Proteomisch nachgewiesene, sekretierte Enzyme und deren Substrat-Aktivitäten

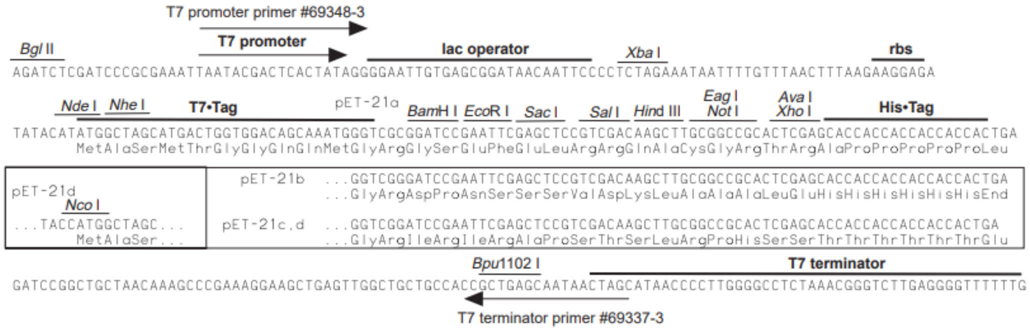


Aspergillus terreus **VT148 (Ascomycota, Trichocomaceae)**

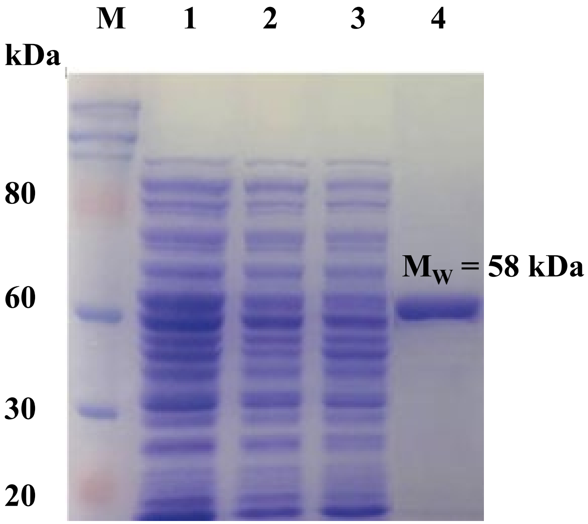
Gene ID	NSAF%	Danh pháp	Enzym	Cơ chất xúc tác
g8565.t1	9.77	EC 3.2.1.91	Cellobiohydrolase (GH7)	Cellulose
g3044.t1	6.28	EC 3.2.1.8	*endo*-β-1,4-Xylanase (GH10/11)	Hemicellulose (Xylan)
g5817.t1	5.52	n.a.	Protein chưa định tính	n.a.
g9837.t1	3.33	(FAD)-domain EC1.1.99.1	GMC-Oxidoreductase (AA3)	Lignocellulose (+ H_2O_2)
g2843.t1	2.49	EC 3.2.1.4	*endo*-β-1,4-Glucanase (GH6)	Cellulose
g1263.t1	1.57	EC 3.5.1.-, EC 3.5.2.-	Amidohydrolase (Amidase)	Amid- hoặc Ester tâm carbon- và phospho
g6973.t1	1.40	EC 3.2.1.8	*endo*- β-1,4-Xylanase (GH10/11)	Hemicellulose (Xylan)
g8272.t1	1.35	EC 3.1.1.72	Acetylxylan-Esterase (CE1)	Xylane und Xylooligosaccharide
g336.t1	1.25	EC 2.4.1.34	β-1,3-Glucan-Synthase	Liên kết β-glucan thành tế bào nấm
g4517.t1	1.21	EC 3.2.1.55	α-L-Arabinofuranosidase (GH2)	Arabinoxylan và Arabinogalactan
g8748.t1	1.20	EC 3.2.1.151	Xyloglucanase (GH16)	Hemicellulose (Xyloglucan)
g7290.t1	1.17	EC 3.2.1.91	Cellobiohydrolase (GH7)	Cellulose

Expression of acetyl esterase (*Pp*AE) from *Pleurotus pulmonarius* MPN18) in *E. coli* BL21





pET-21a-d(+) cloning/expression region



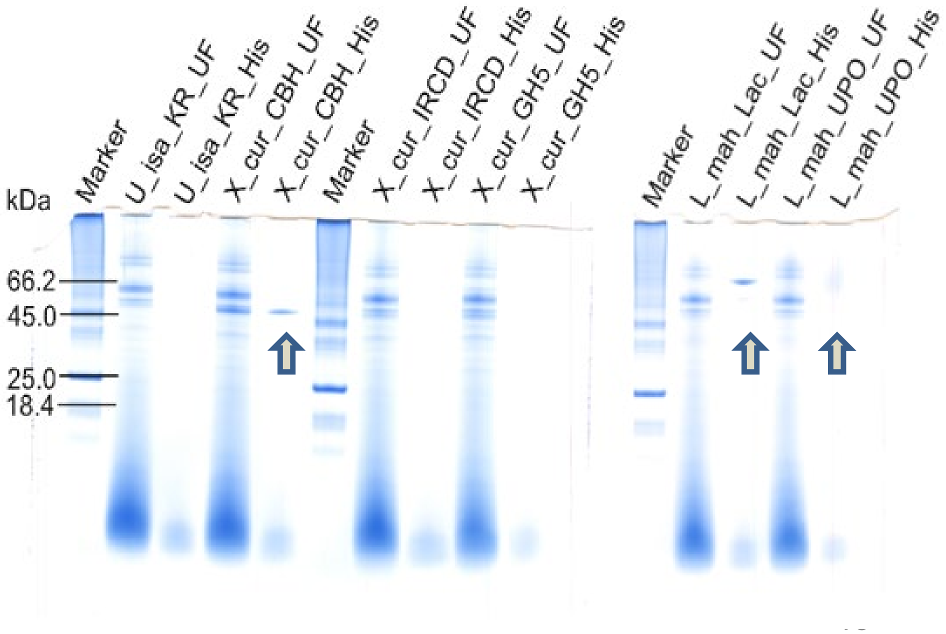
Các bước tinh sạch	Protein tổng (mg)	Hoạt tính tổng (U)	Hoạt độ riêng (U mg^{-1})	Hiệu suất (%)	Độ tinh sạch (lần)
Dịch chiết thô	2.232,4	12.940,6	5,8	100	1
HisTrap^{TM} sp	207,9	10.000,5	48,1	77	8,3
10 kDa cut-off	106,6	8.581,3	80,5	66,3	13,8

Bảng 3.12. Các enzyme (nguồn, cấu trúc gen liên quan, số EC) cho biểu hiện tái tổ hợp trong *Saccharomyces cerevisiae* sử dụng plasmid biểu hiện pYES2.1/V5-His-TOPO.

Loài	Ký hiệu	ID gene, kích thước (bp)	Enzyme đích	Danh pháp
Lasiodiplodia mahajangana	VT137	20ADUHLP, 1710	Laccase	EC 1.10.3.2
Lasiodiplodia mahajangana	VT137	20ADW4WP, 768	Unspecific Peroxygenase	EC 1.11.2.1
Lecanicillium fungicola	Babe33	20ADW4LP, 1290	Chitinase/ Chitobiase GH18	EC 3.2.1.-
Lecanicillium fungicola	Babe33	20ADW4TP, 684	Peroxiredoxin	EC 1.11.1.15
Nemania bipapillata	CP14	20ADW4QP, 1329	classIIPOD, Peroxidase	EC 1.11.1.7
Nemania bipapillata	CP14	20ADW4NP, 693	Xylanase GH11	EC 3.2.1.-
Umbelopsis isabellina	MPG-14A	20ADW4MP, 942	Ketoreductase	
Xylaria curta	Babe10	20ADW4OP, 1356	Cellobiohydrolase	
Xylaria curta	Babe10	20ADW4PP, 1728	Glycoside hydrolase GH5	
Xylaria curta	Babe10	20ADW4RP, 1296	IRDC, Protocatechuate-3 4-dioxygenase	

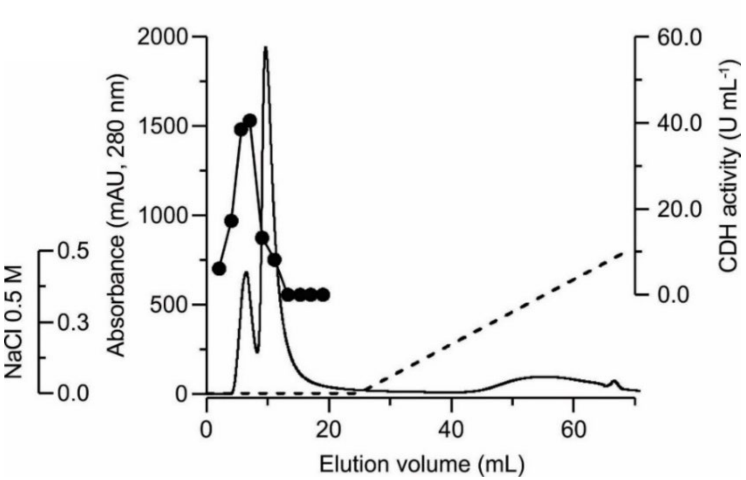
03 enzyme tái tổ hợp được biểu hiện trong *S. cerevisiae đảm bảo độ tinh sạch*:

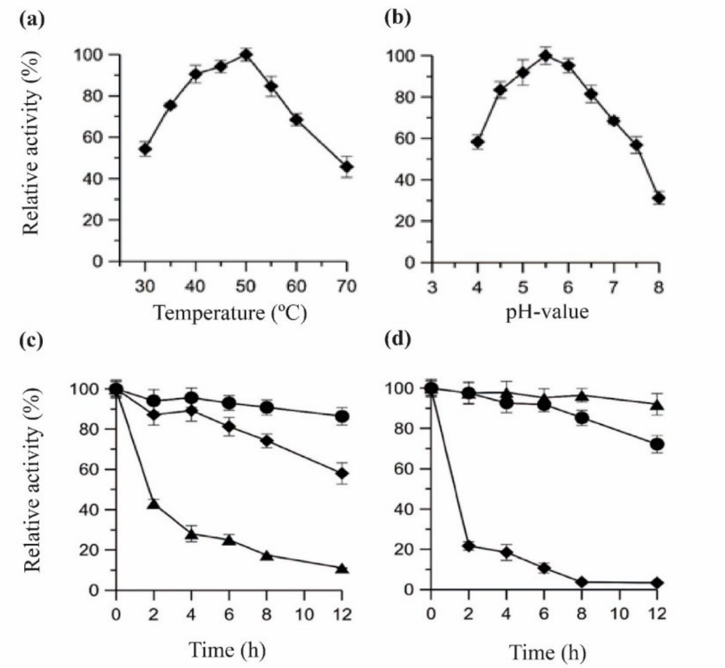
- Laccase (Lac) từ *Lasiodiplodia mahajagana*
- Unspecific peroxygenase (UPO) từ *Lasiodiplodia mahajangana*
- Cellobiohydrolase (CBH) từ *Xylaria curta*.

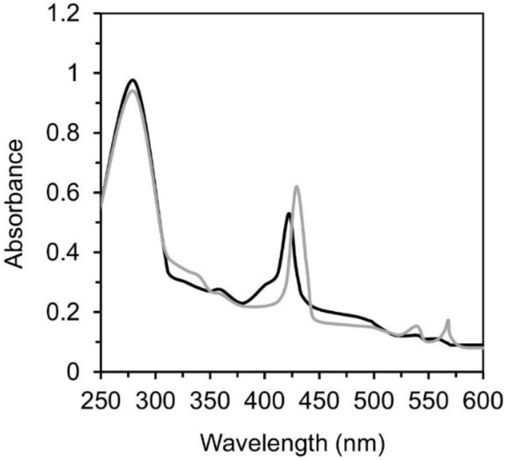


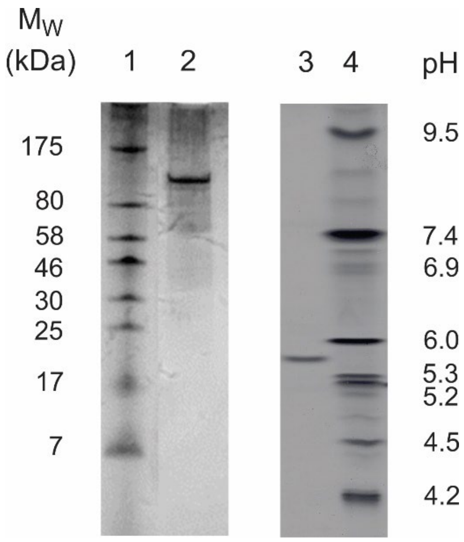
Cellobiose dehydrogenase (CauCDH) from *Coprinellus aureogranulatus MPG14*

Chất nhận điện tử [a]	Số e^- [b]	K_m (µM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1} s^{-1}$)
Cyt *c*	2	1.3	30.1	23.2×10^6
DCIP	2	1.6	30.0	18.4×10^6
$ABTS^{\bullet +}$	1	15.6	47.4	30.4×10^5
Potassium ferricyanide	2	18.5	37.7	20.4×10^5
1,4-Benzoquinone	1	37.1	25.2	67.9×10^4
2,6-Dimethyl-1,4-benzoquinone	1	292.2	19.3	66.1×10^3
2,6-Dimethoxy-1,4-benzoquinone	1	325.5	11.4	35.0×10^3







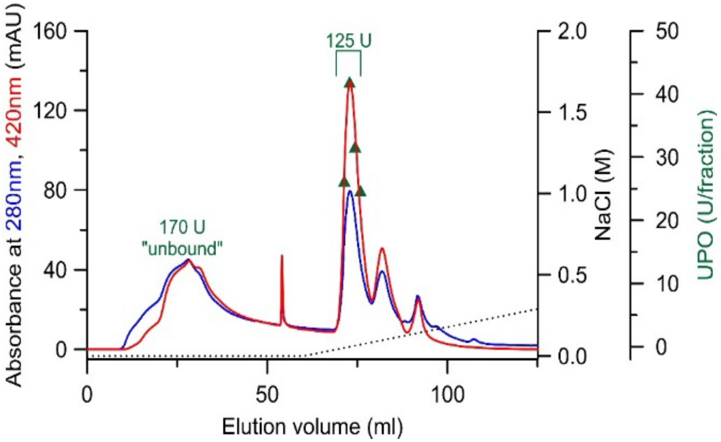


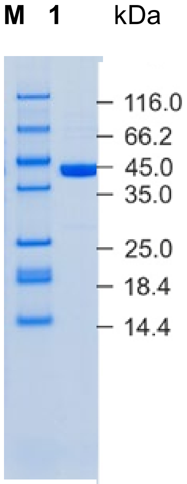
M_W = 109 kDa & ***p*I = 5.4**

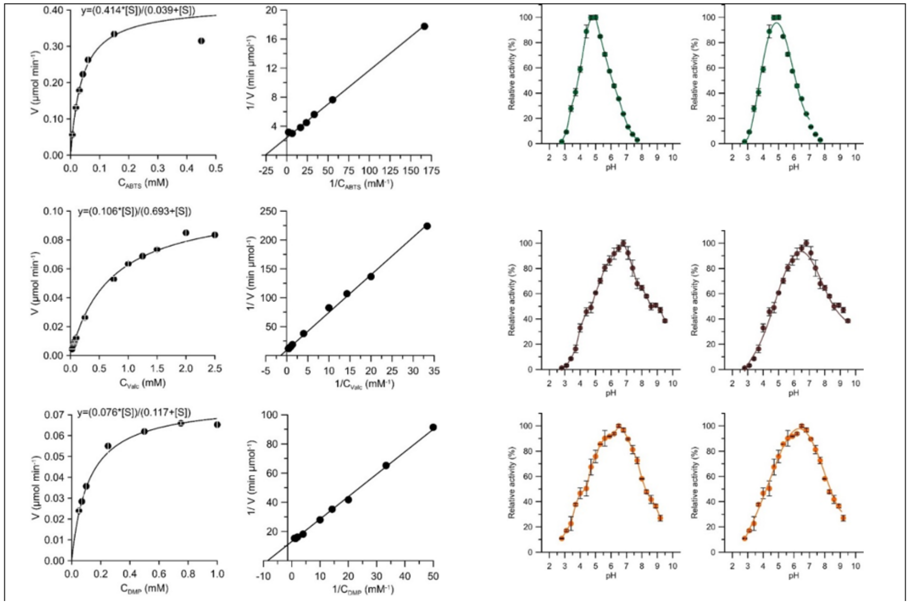
Unspecific peroxygenase from *Candolleomyces euryspous* (*Ceu*UPO)

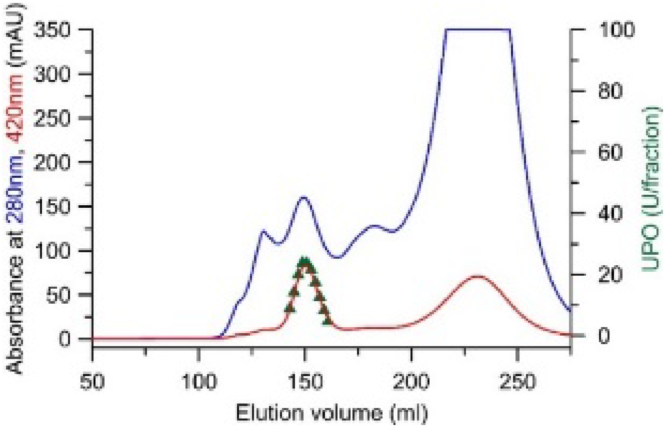
Cơ chất*	**K_m (µM)**	**V_{max} (µmol min^{-1})**	**k_{cat} (s^{-1})**	**k_{cat}/K_m ($s^{-1}mM^{-1}$)**	**pH**
ABTS	39	0,414	270	7,01 x 10^6	5,0
VA	693	0,106	69	9,98 x 10^4	6,5
DMP	117	0,076	50	4,23 x 10^5	6,5

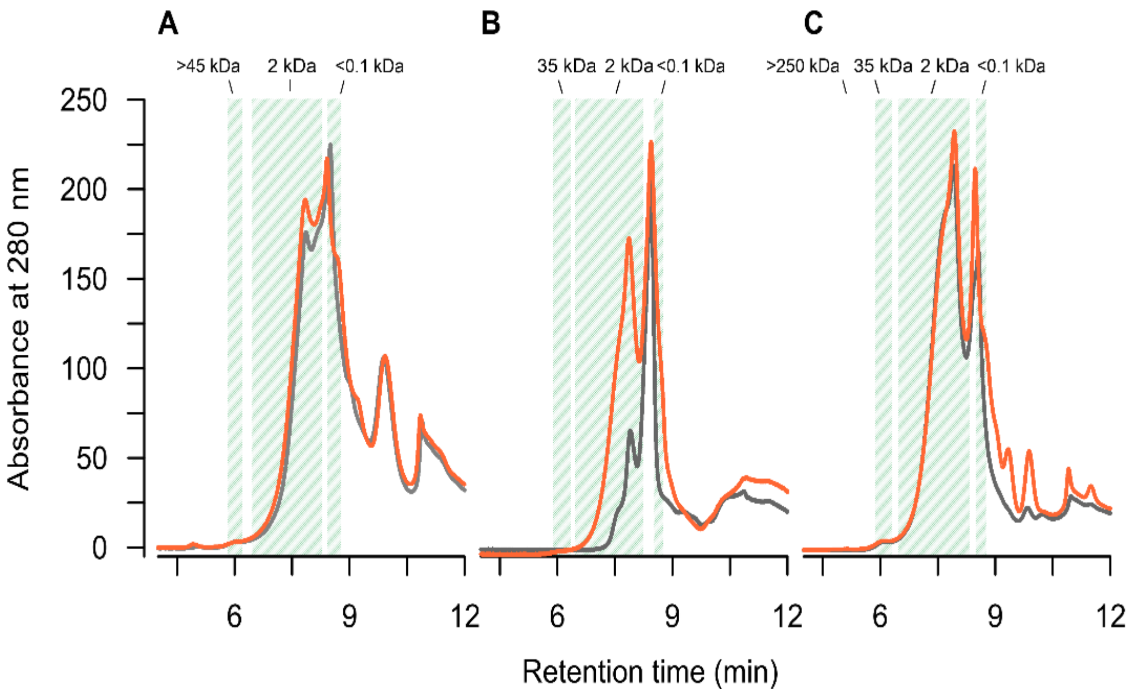
*ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonate)]; VA: veratryl alcohol; DMP: 2,6-dimethoxyphenol.











”Enzyme cocktail”

- 0,5% (w/v) LLC substrate
- 5 mg Σ protein
- Na-acetate 500 mM, pH 4,8
- Ǔ 24 ^{o}C, 24-168 h

HPSEC-profile of lignocellulosic fragments

after treatment with fungal culture of Xylaria curta

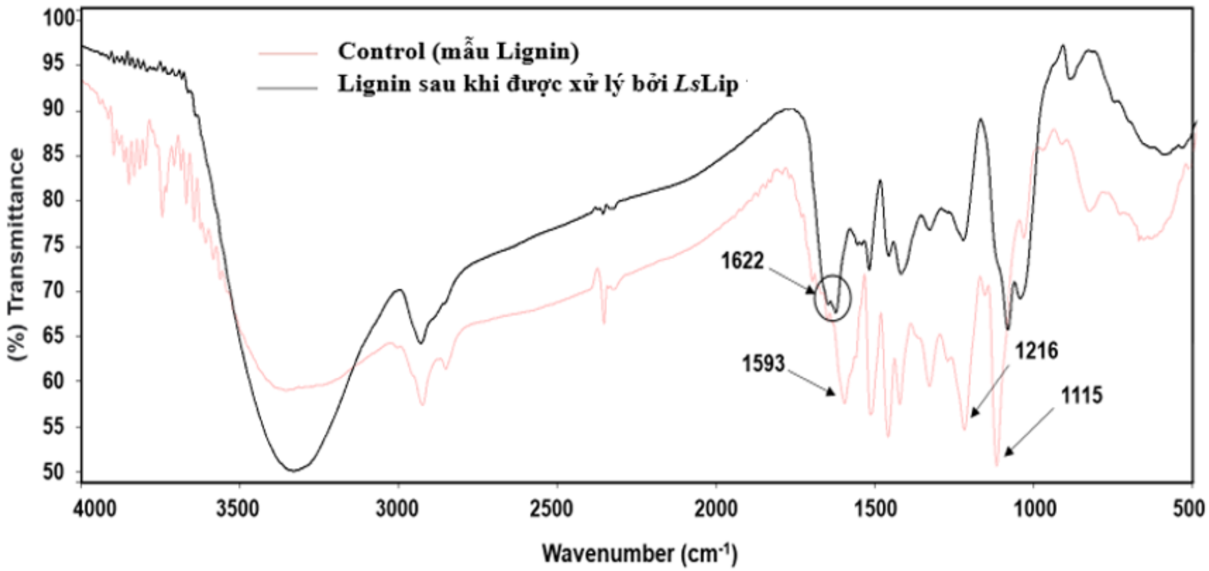
Substrates: - Cellulose (*middle*, B);

- Lignocellulosic biomass (A – bamboo, C – rice straw);

Black- control without enzyme, Read – incubation with enzyme;

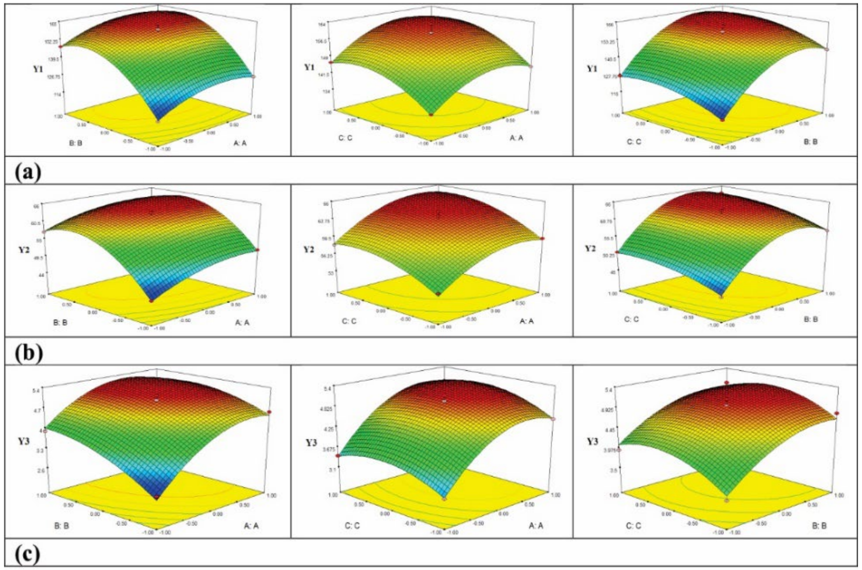
Green – molecular mass region (kDa)

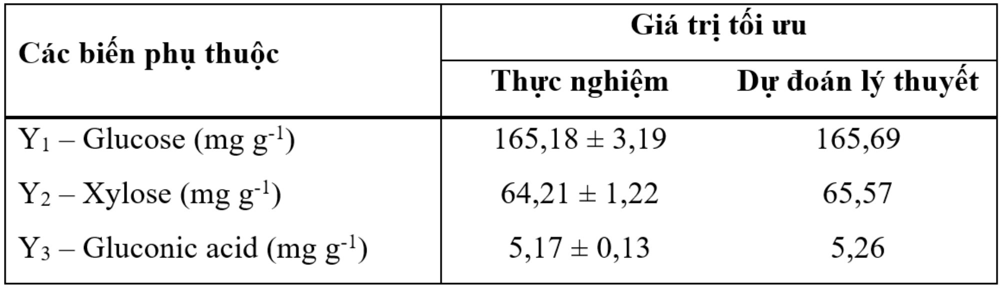
Oxidization of lignin by Lignin peroxidase (*Ls*LiP) from *Lentinus squarrosulus*



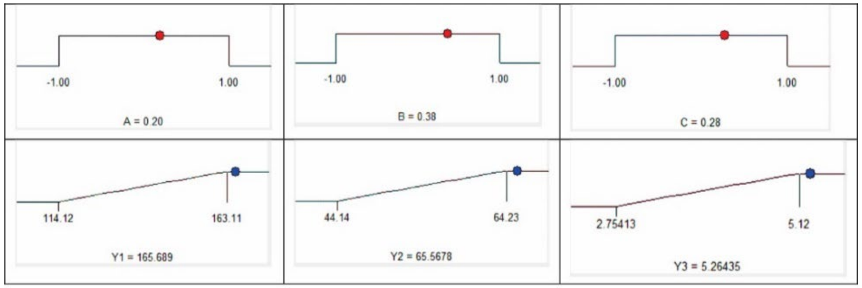
Synergetic conversion of biomass by “enzyme cocktail” and optimization

- **Lignolcellulose (rice straw 3%, w/v)**
- **“Enzyme cocktail”:**
  - **Cellobiose dehydrogenase (*Cau*CDH)**
  - **CMC/glucuronoxylanase (Cell/Xyl)**
  - **Acetyl esterase (AE)**
- **Products: carbohydrate (glucose, xylose) & gluconic acid**



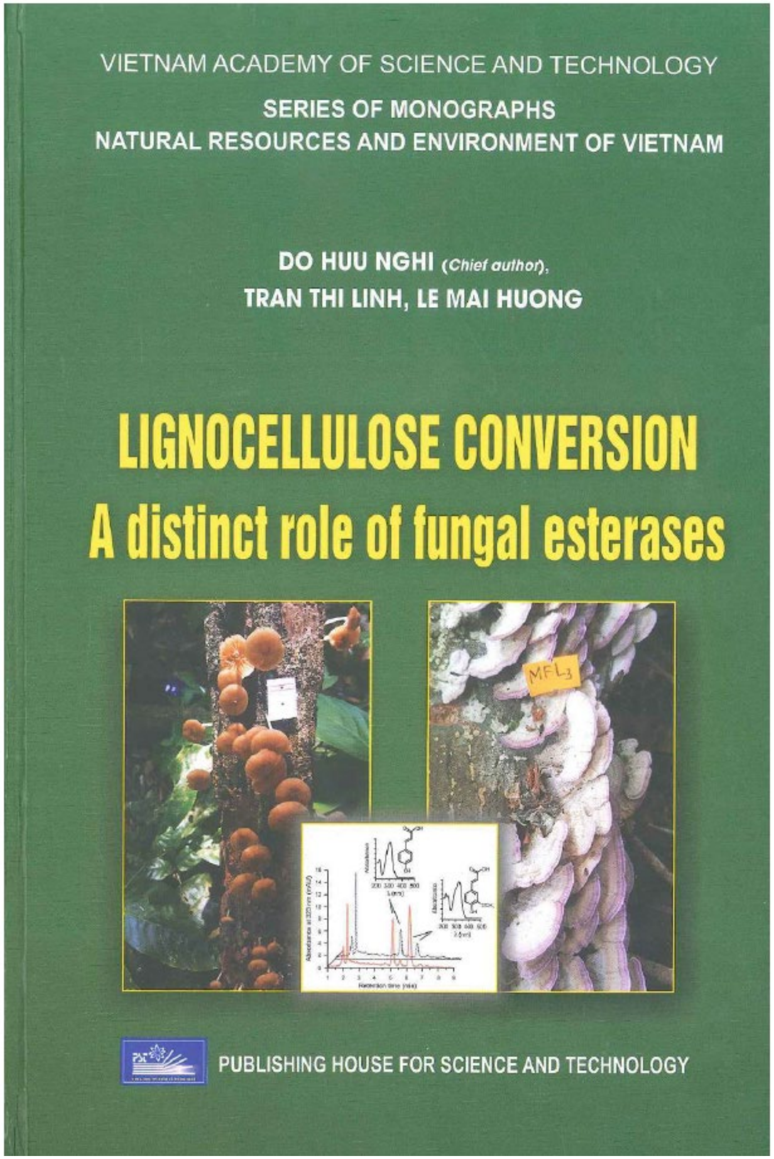


Các biến phụ thuộc	Giá trị tối ưu	
	Thực nghiệm	Dự đoán lý thuyết
Y_1 – Glucose (mg g^{-1})	165,18 ± 3,19	165,69
Y_2 – Xylose (mg g^{-1})	64,21 ± 1,22	65,57
Y_3 – Gluconic acid (mg g^{-1})	5,17 ± 0,13	5,26



Cellulase/xylanase: Acetyl-esterase: CauCDH:

Cell/Xyl:AE: CauCDH = 18,9:26,8: 49,5 (U/g) t_{opt} = 45^{o}C, pH_{opt} 5.0, for 48 hrs.



VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

SERIES OF MONOGRAPHS
NATURAL RESOURCES AND ENVIRONMENT OF VIETNAM

DO HUU NGHI (*Chief author*),
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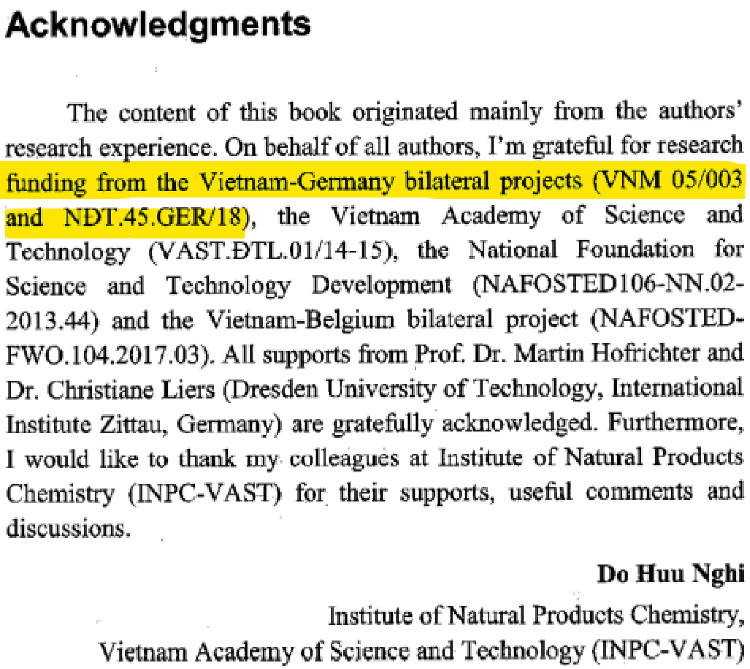
LIGNOCELLULOSE CONVERSION
A distinct role of fungal esterases

PUBLISHING HOUSE FOR SCIENCE AND TECHNOLOGY



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PLANNED UTILIZATION OF RESULTS

1. **G**enerating the first set of complete genomes of “Vietnam-specific” macro-fungi as data-mining basis for diverse biotechnological research approaches and applications.
2. **E**nabling Vietnamese scientists to make use of their local mycological and functional diversity with the perspective to exploit them in the future, e.g. in the fields of *Green Chemistry* and *Biorefinery* (biomass conversion).
3. **D**eveloping and transferring advanced analytical and molecular “Omics” knowledge with/to Vietnam to facilitate continuation and extension of this kind of research.
4. **I**nvestigation and demonstration of applicability of enzyme-based methods to convert lignocelluloses and plant biomass into valuable and usable platform chemicals for industrial purposes.

ADDED VALUE OF THE INTERNATIONAL COOPERATION

The partners - TU Dresden (IHI Zittau) and VAST (INPC Hanoi) - intend to continue the over twenty-years collaboration in the fields of **Biotechnology** and want to further contribute to the exchange of scientific knowledge (young scientists, Ph.D., MSc. students, postdocs). Since 2003, 20 Vietnamese (INPC-VAST) and 15 German (IHI Zittau) researchers/scientists have been visiting the partners’ institutes and some of them stayed several times for several months there.

Generally, the bilateral relations between Germany and Vietnam are of a particular quality. The successful cooperation between both partners has become manifest in the identification of new fungi, biocatalysts, and metabolites. Altogether, the partners have published hundreds of SCI/E articles, most of which dealt with fungal enzymes and their applications.

Thanks for your attention!