Exploring fungal diversity of Vietnam for novel lignocellulolytic enzymes

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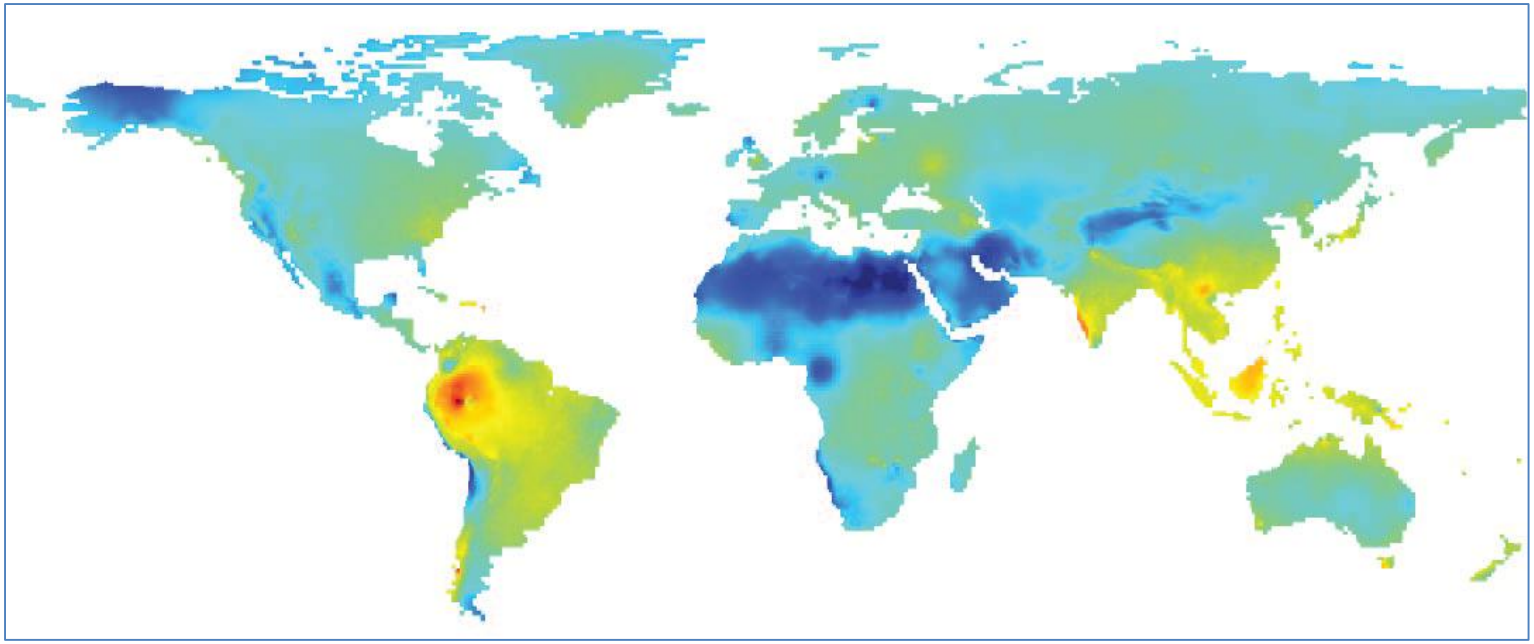
Global taxonomic richness of fungi



Tedersoo et al., 2014

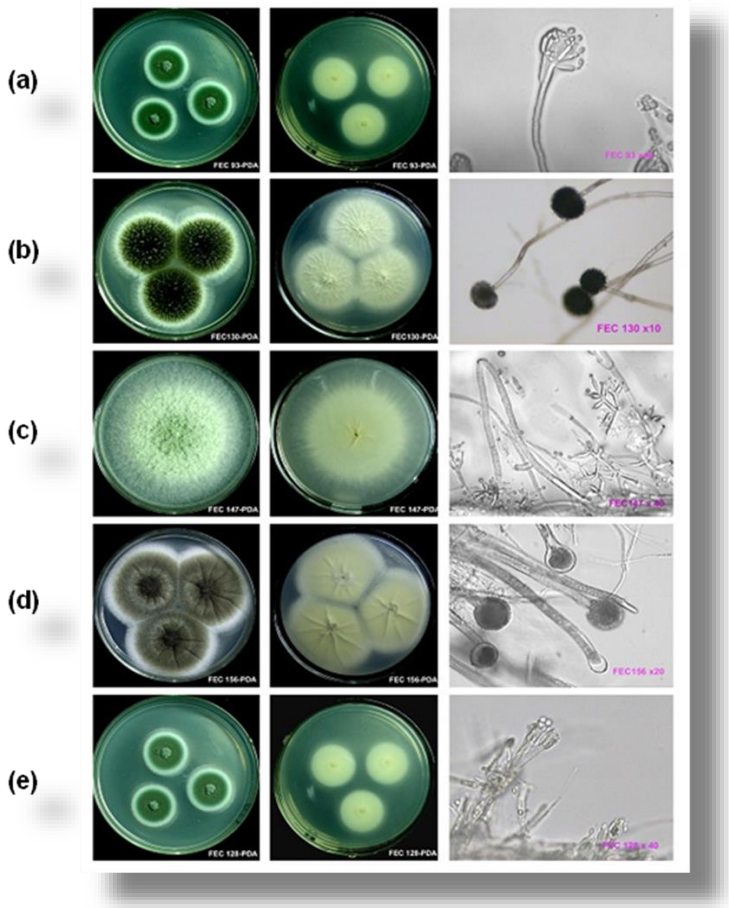
1.5 million species (Hawksworth, based on ratio 6:1 of fungi to plants in the British Isles).
3.5 to 5.1 million species (O' Brien 2005, based on large number of ITS clones from soil)
0.6 to 1.0 million spcies (Tedersoo *et al.,* 2014, based on large scale pyrosequencing of soil samples collected across the globe)

Currently 99,000 species are known
Most diverse in the tropics

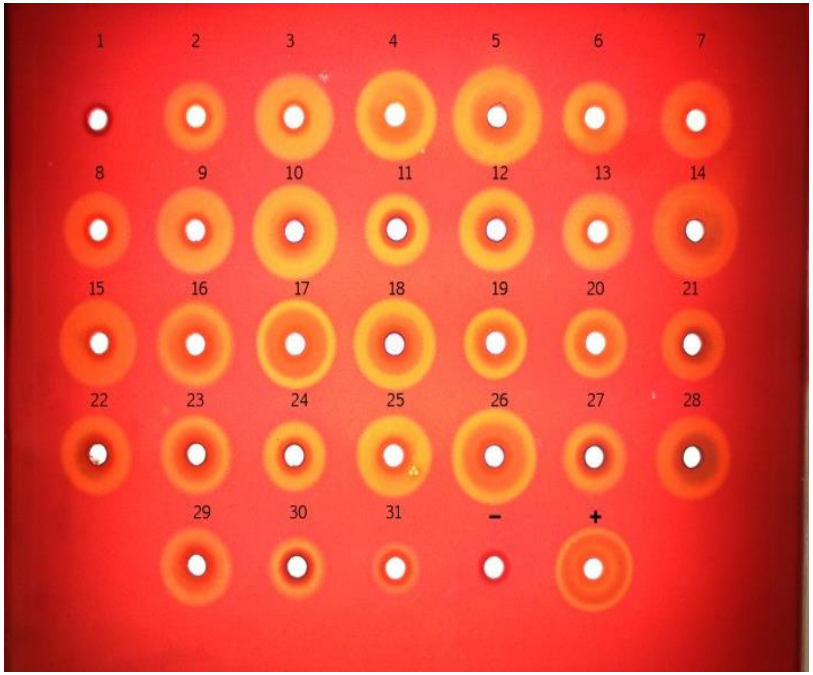
Aims

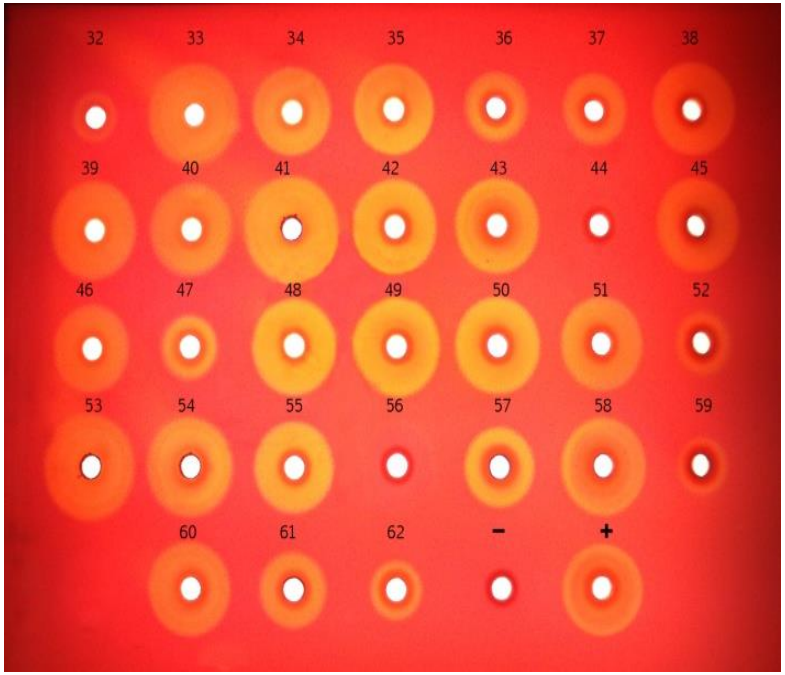
- ✓ Discovery of new lignocellulolytic enzymes and enzyme mixtures from tropical fungi
- ✓ Obtaining enzymes in large quantities for application in feed industry and for other processes

Mesophilic lignocellulolytic fungi

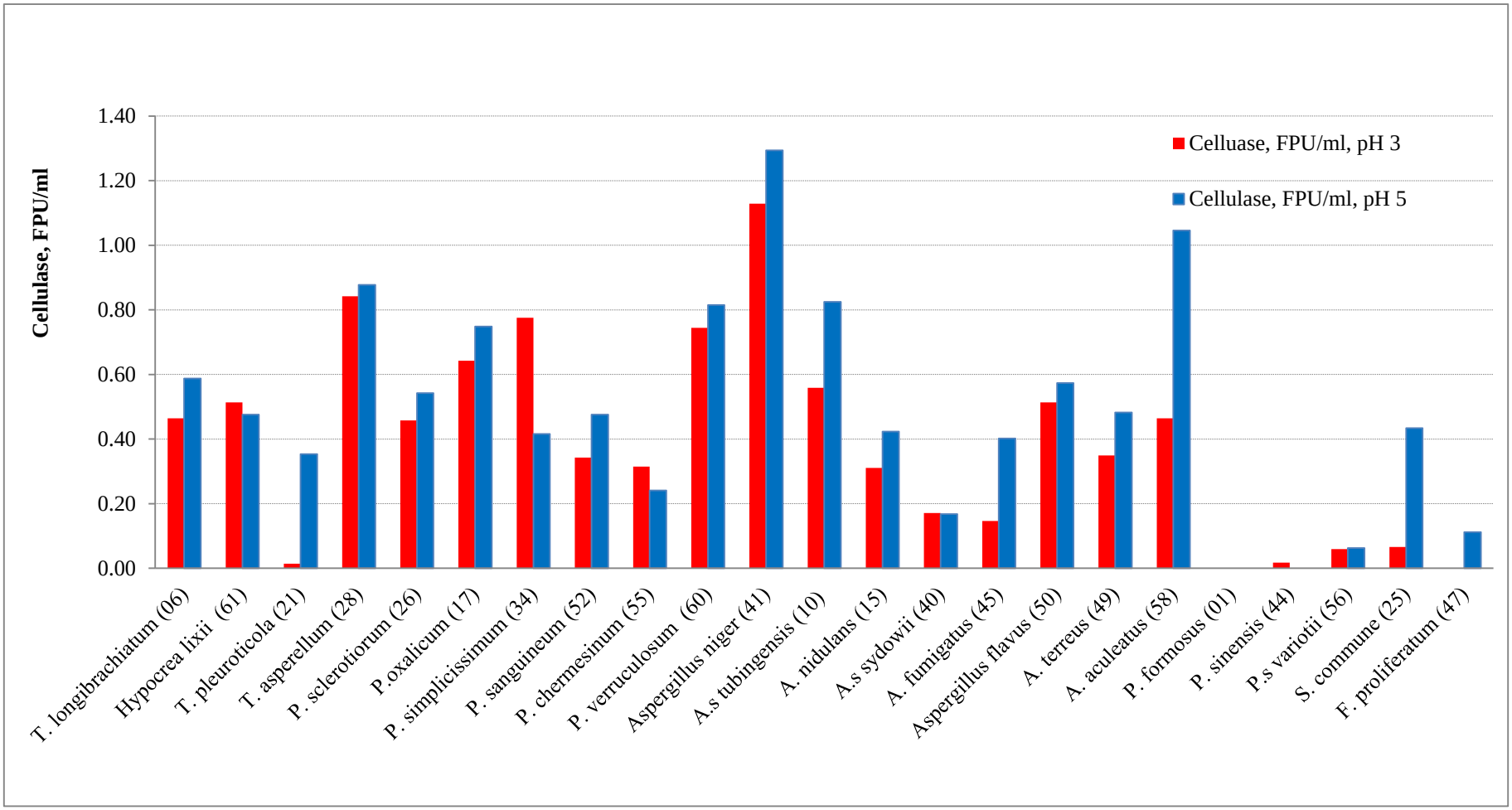


CMCase activity of mesophilic strains

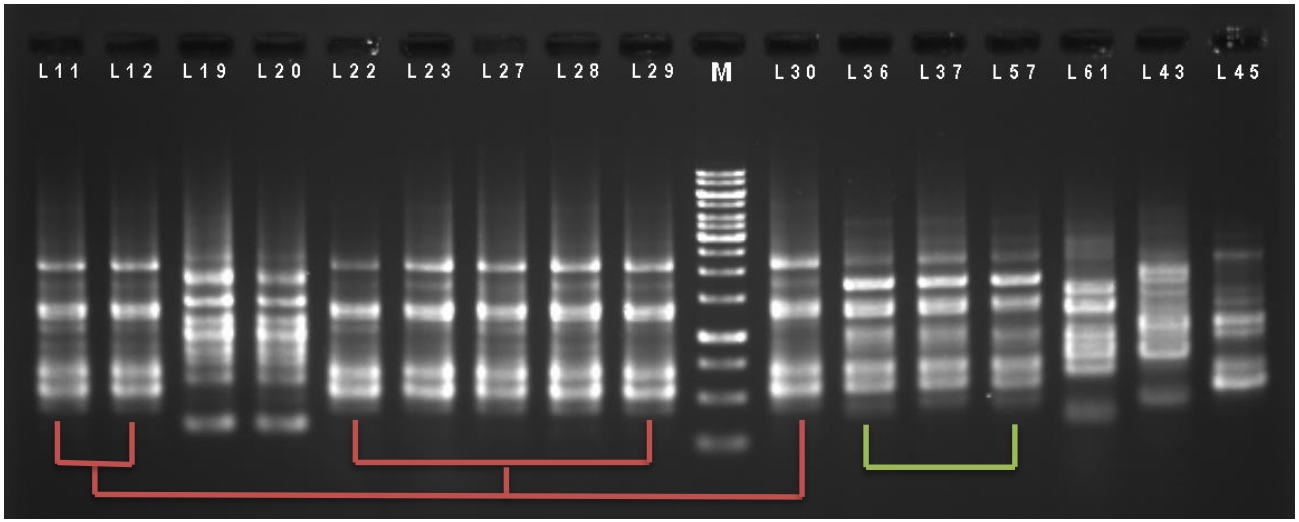




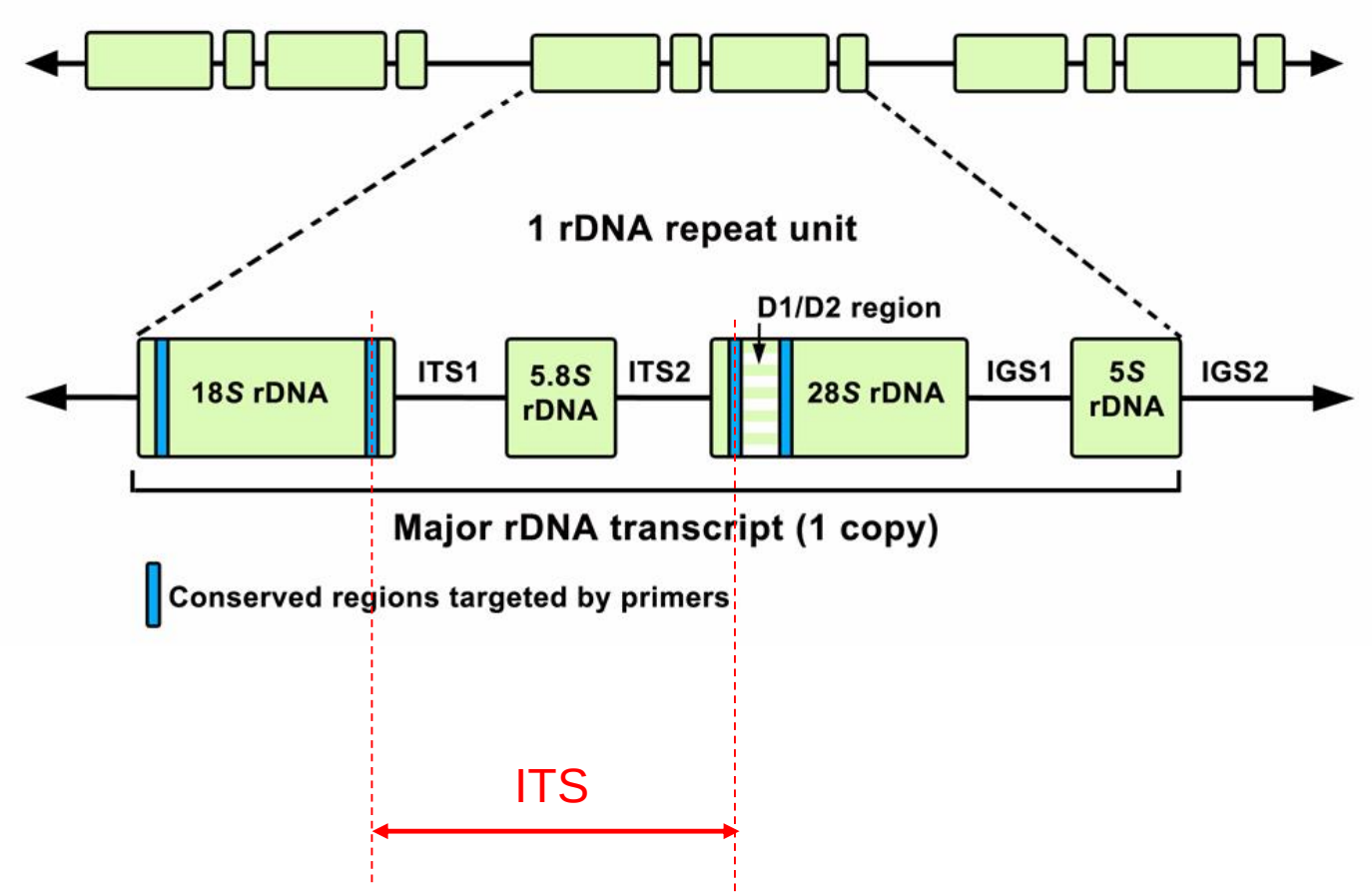
Cellulase activity at different pH



Microsatellite PCR fingerprinting for differentiating strains



Species identification
ITS as universal DNA barcode for fungi



Mesophilic lignocellulolytic strains

- ✓ 1100 fungal strains were isolated and screened
- ✓ 36 strains were assessed for the rice straw saccharification potential
- ✓ Enzyme mixture of several strains outperformed *Trichoderma reesei* RUT C-30. Some demonstrated high specific lignocellulolytic activity.
- ✓ Biomass degrading capacity is strain specific
- ✓ *Aspergillus brunneoviolaceus* FEC 156 was chosen for novel enzyme discovery

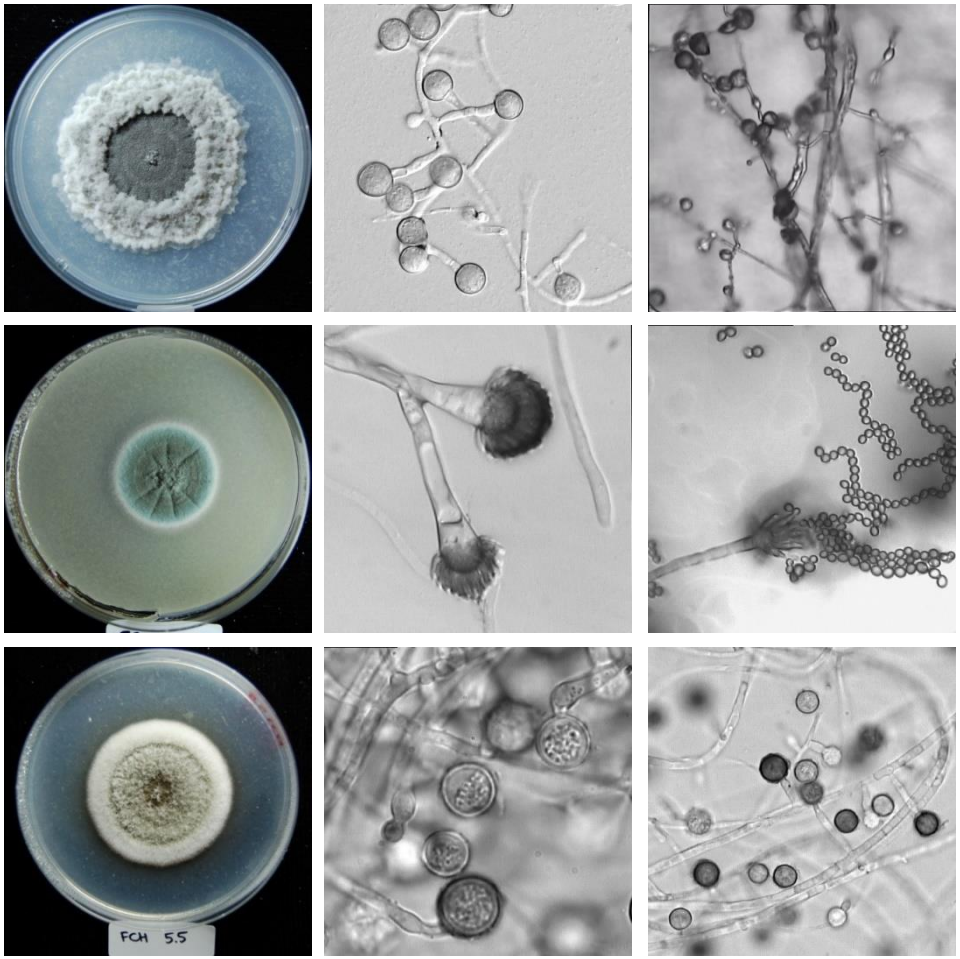
Thermophilic lignocellulolytic fungi

(growth at 50 ℃ and above)

Humicola insolens FCH 5.3

Aspergillus fumigatus FCH 5.1

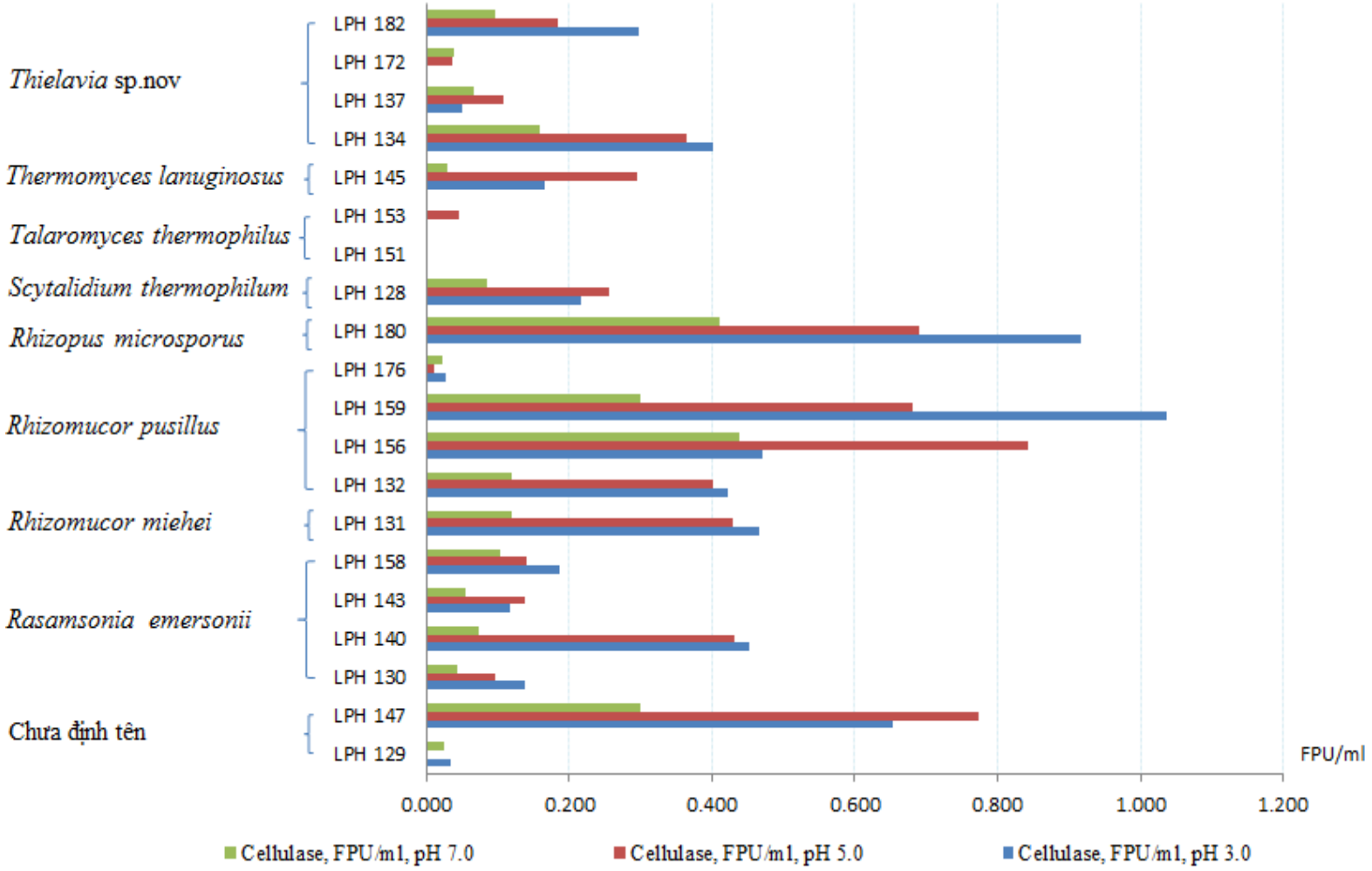
Thermomyces lanuginosus FCH 5.5



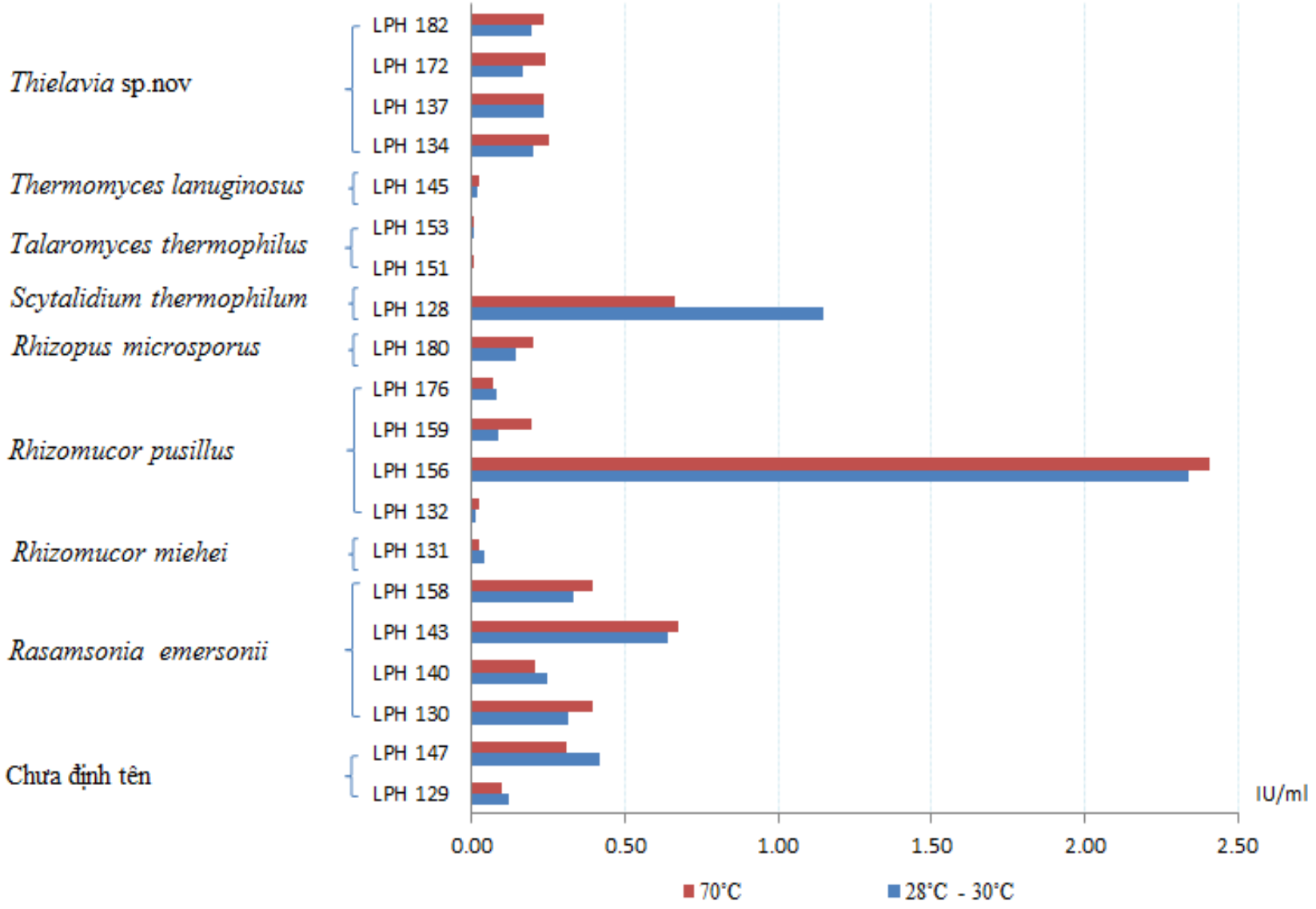
High xylanase activity in thermophilic lignocellulolytic fungi

			48h			96h			144h			192h		
Cultivation on Wheat bran at 50°C , 150 rpm			Cellulase	Xylanase	Protein	Cellulase	Xylanase	Protein	Cellulase	Xylanase	Protein	Cellulase	Xylanase	Protein
No.	Strains	Species	IU/ml	IU/ml	mg/ml	IU/ml	IU/ml	mg/ml	IU/ml	IU/ml	mg/ml	IU/ml	IU/ml	mg/ml
1	FCH 5.2	Rhizomucor miehei	0.01	0.03	0.076	-0.02	0.02	0.048	0.01	0.01	0.058	0.00	0.04	0.051
2	FCH 6.3	Rhizomucor miehei	-0.07	-0.01	0.083	-0.01	0.00	0.156	-0.03	0.03	0.106	-0.03	0.01	0.109
3	FCH 6.4	Rhizomucor pusillus	-0.02	0.01	0.048	0.00	0.00	0.041	-0.01	0.01	0.128	0.00	0.01	0.125
10	FCH 5.7	Rhizomucor pusillus	-0.03	0.01	0.133	0.00	-0.01	0.093	0.00	0.01	0.111	-0.01	0.00	0.077
4	FCH 5.3	Humicola insolens	-0.01	**0.33**	0.098	0.07	**3.95**	0.135	0.15	**3.10**	0.100	0.17	**1.86**	0.148
5	FCH 6.2	Humicola insolens	0.10	**0.22**	0.098	0.03	**3.53**	0.211	0.07	**8.31**	0.295	0.15	**5.97**	0.250
6	FCH 5.5	Thermomyces lanuginosus	0.01	**85.33**	0.108	0.06	**105.26**	0.161	0.00	**92.95**	0.151	0.02	**85.33**	0.110
7	FCH 6.5	Thermomyces lanuginosus	-0.01	**0.02**	0.073	-0.01	**0.03**	0.165	0.00	**0.03**	0.169	0.01	**-0.01**	0.134
8	FCH 8.1	Thermomyces lanuginosus	-0.09	**29.03**	0.095	0.08	**69.73**	0.172	0.03	**61.06**	0.143	-0.06	**56.29**	0.123
9	FCH 10.6	Thermomyces lanuginosus	0.14	**66.57**	0.090	0.02	**79.43**	0.194	-0.02	**69.22**	0.186	-0.08	**63.05**	0.144
17	FCH 10.4	Talaromyces thermophilus	0.17	**12.12**	0.578	-0.07	**20.64**	0.239	-0.10	**18.88**	0.255	0.00	**15.38**	0.259

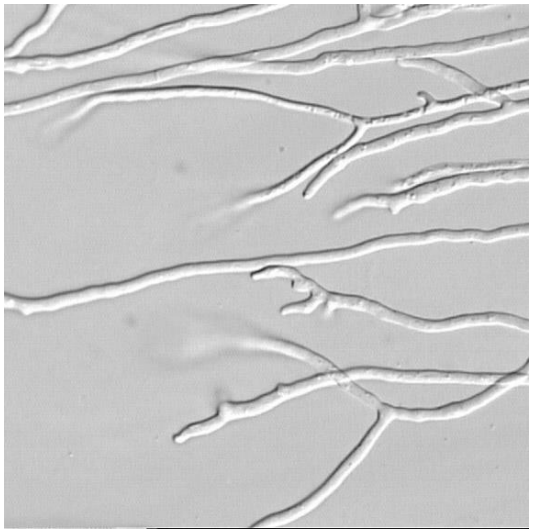
Cellulase activity of new isolates at different pH



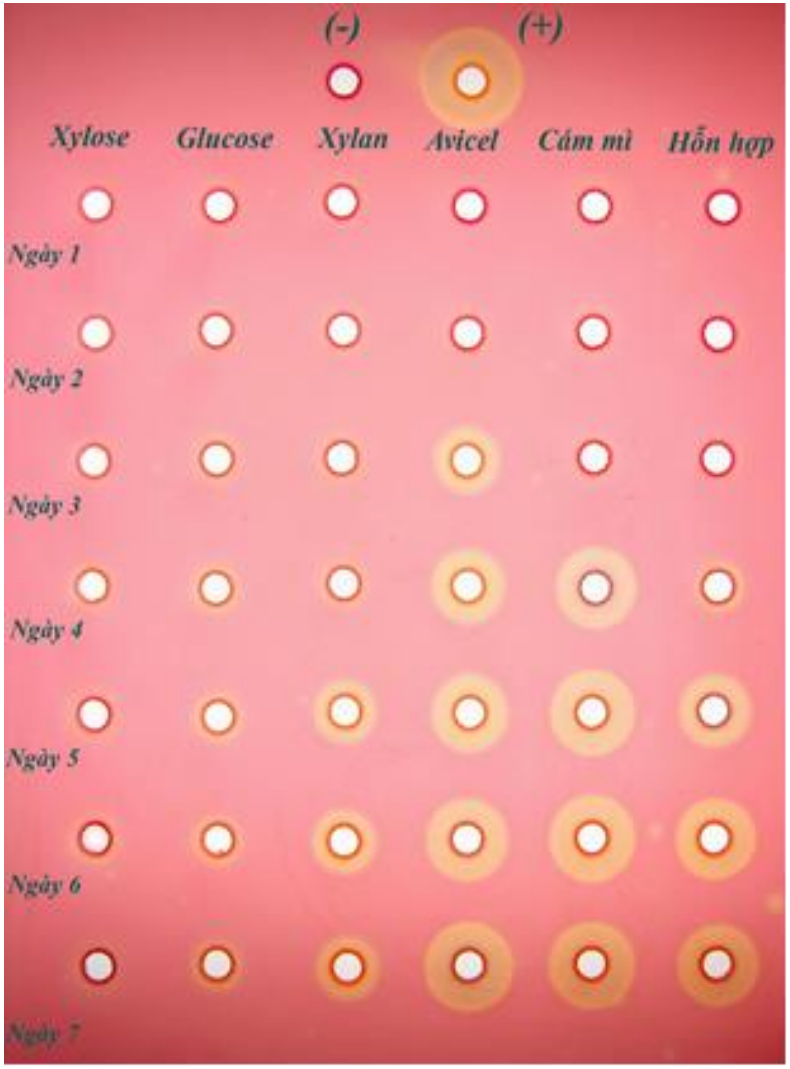
Thermo-stability of CMCase of the new isolates



Purification of enzymes from *Thielavia* sp. FCH 9.3

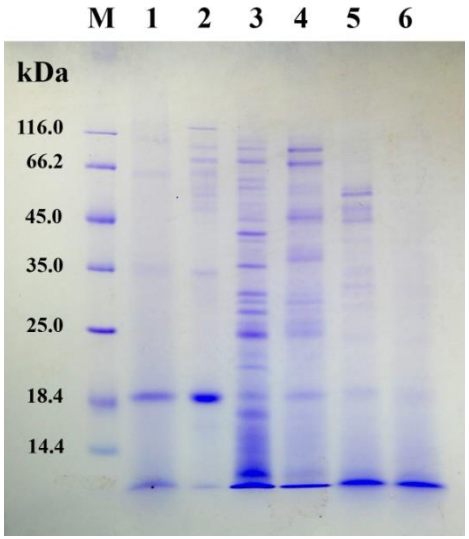


CMCase of *Thielavia* sp. FCH 9.3 grown on 6 different substrates

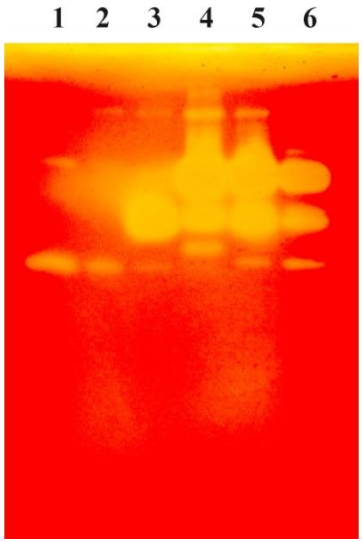


SDS-PAGE and zymogram CMC,xylan photos of FCH 9.3 on 6 substrates.

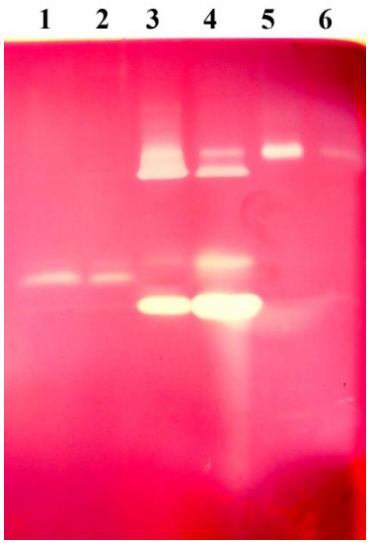
1: Xylose, 2: Glucose, 3: Xylan, 4: Avicel, 5: Wheat bran, 6: Mixtures



SDS-PAGE



Zymogram CMC



Zymogram xylan

Purification of CMCase 1

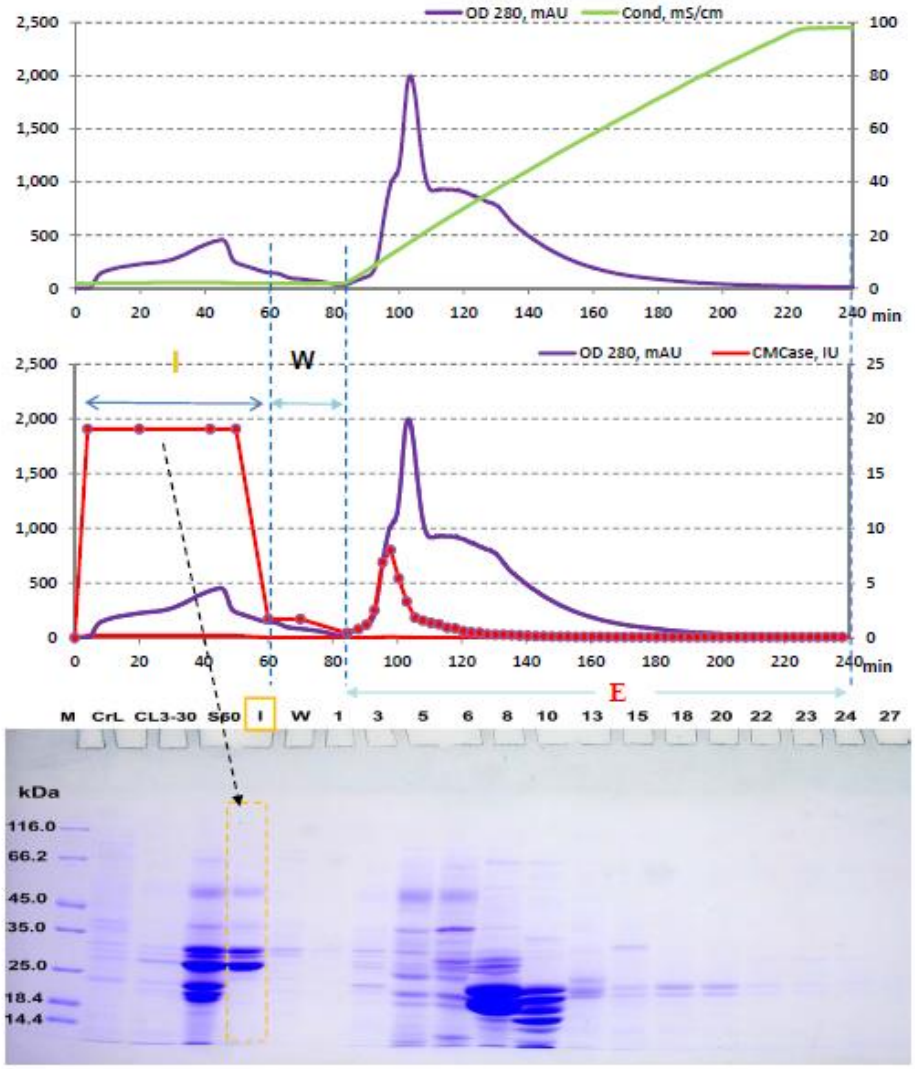


Fig 10. Chromatogram and SDS-PAGE of 60% AP fraction after DEAE Sepharose FF IEC.
M: protein marker, CrL: crude liquid, CL: concentrated liquid 3 <30, S60: 60% AP fraction, I: Unbound (inject); W: washed; E1-E27: eluted factions.

Purification of CMCase 1

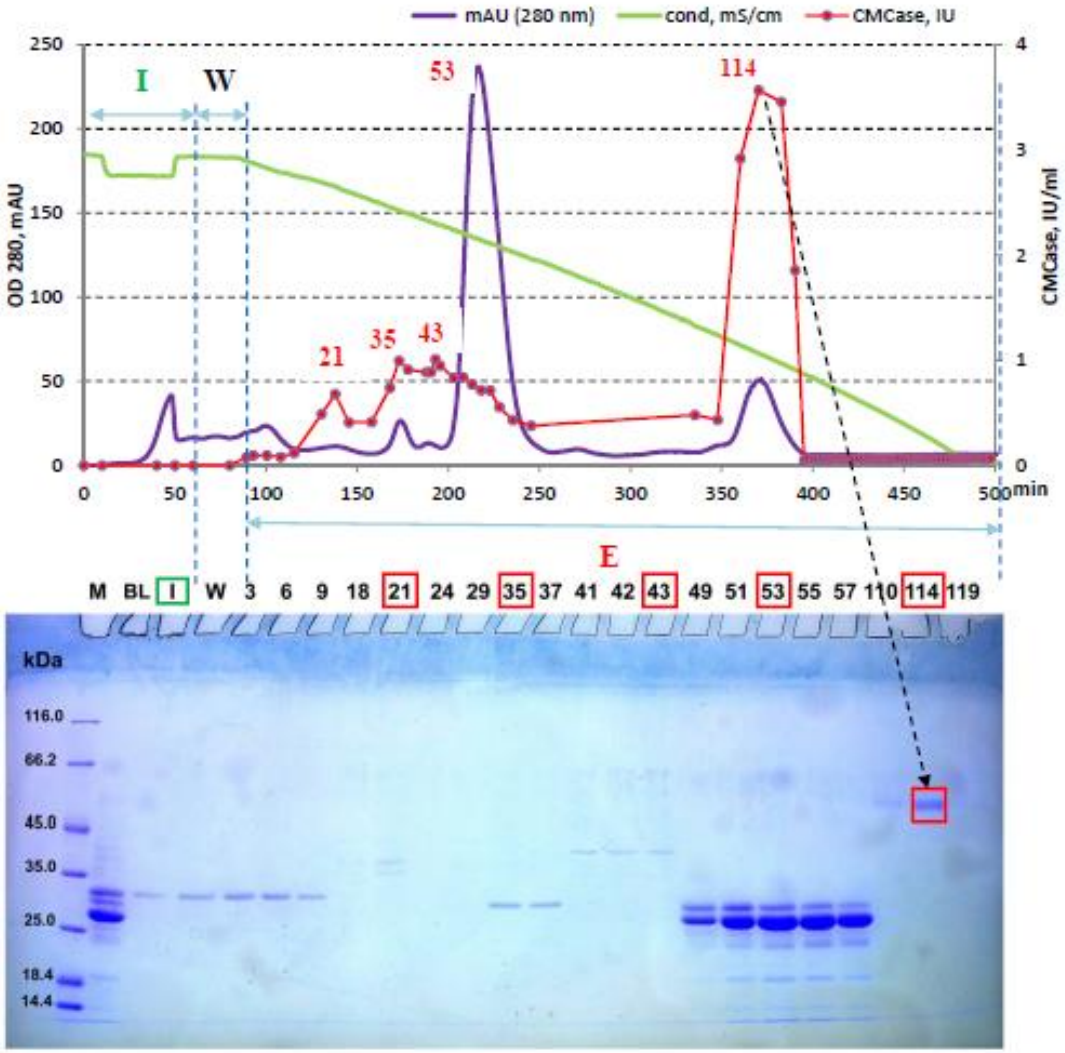
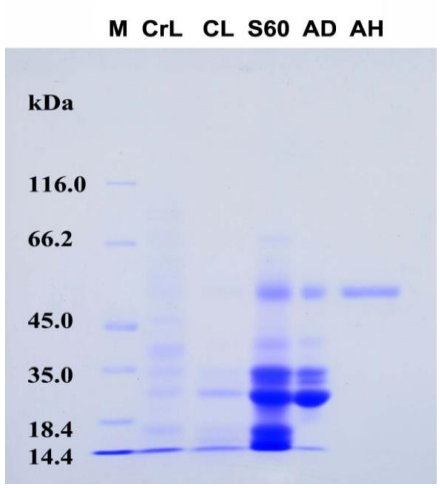
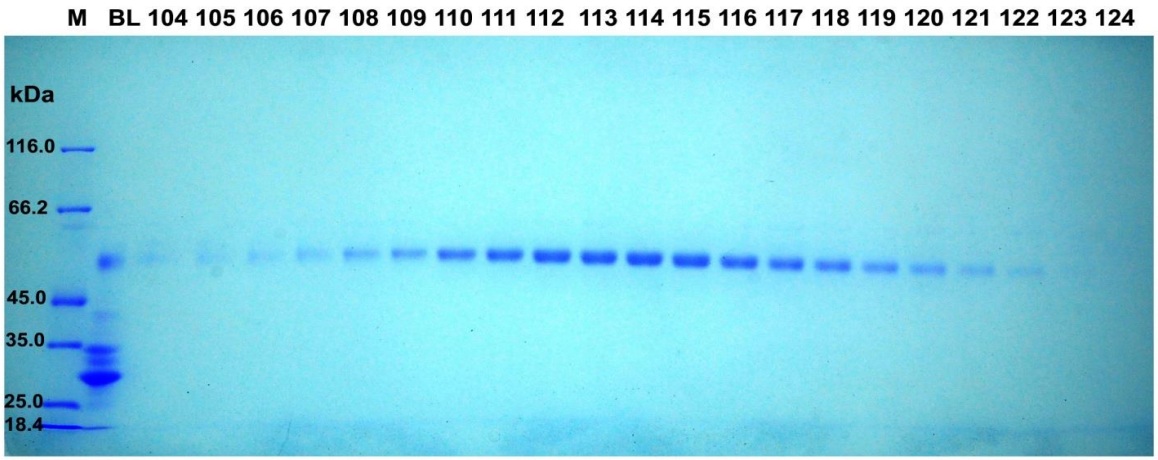


Figure 11. Chromatogram and SDS-PAGE photo of DEAE unbound fraction after Butyl Sepharose FF column. Note: M: protein marker, BL: Sample before loading; I: unbound (inject); W: washed; E1-E127: eluted factions.

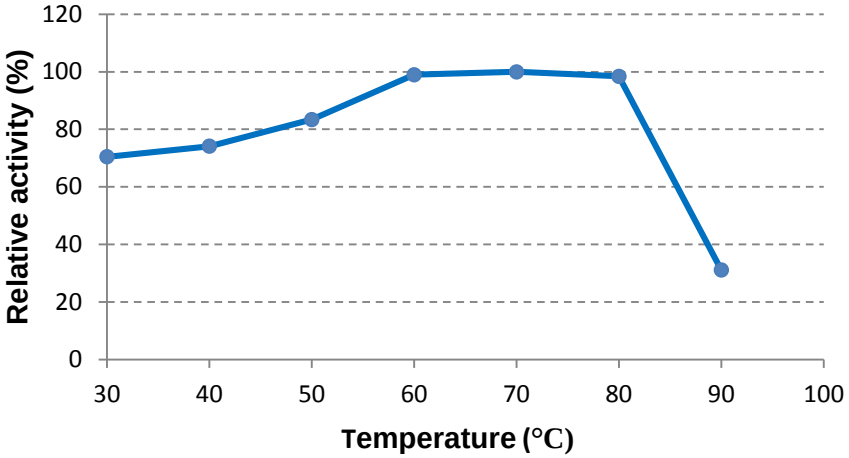
Purification of CMCase 1



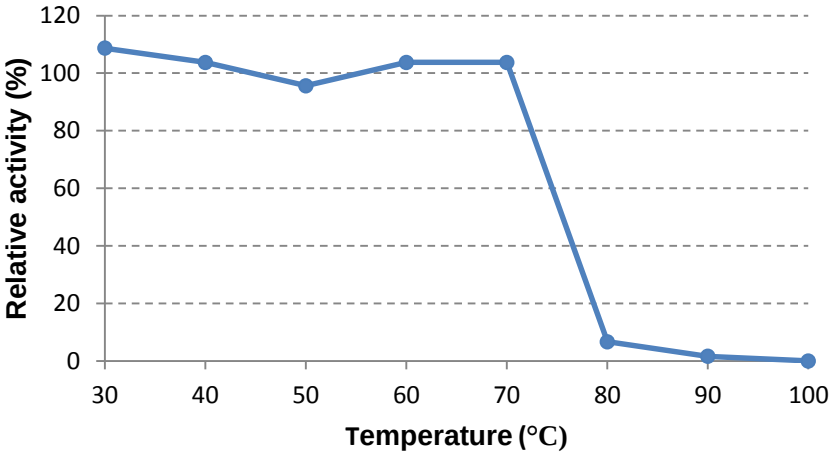


Characteristics of CMCase 1

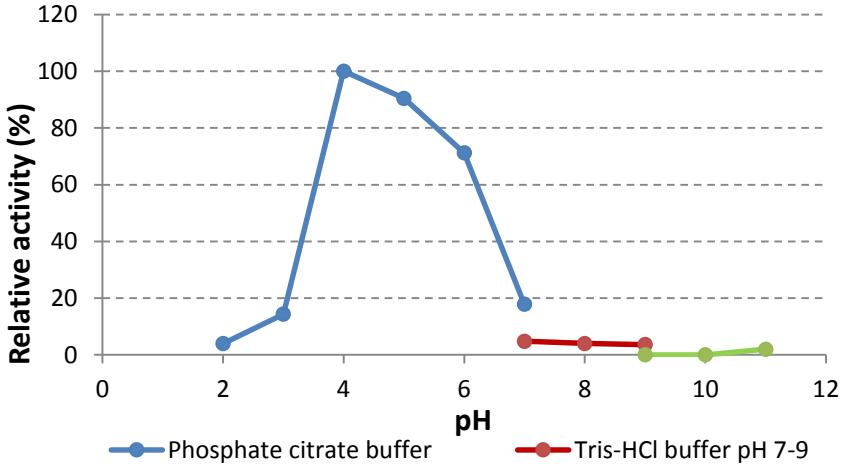
Temperature optimum



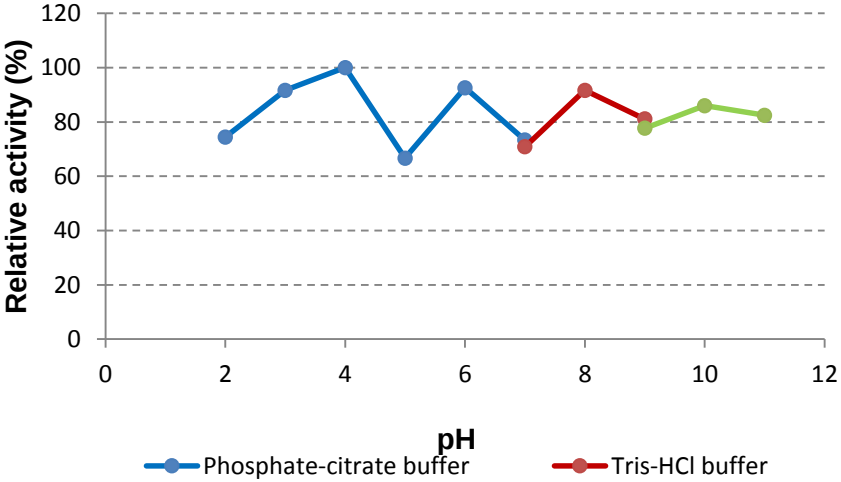
Thermal stability



Optimal pH



pH stability



Purification of CMCase 2

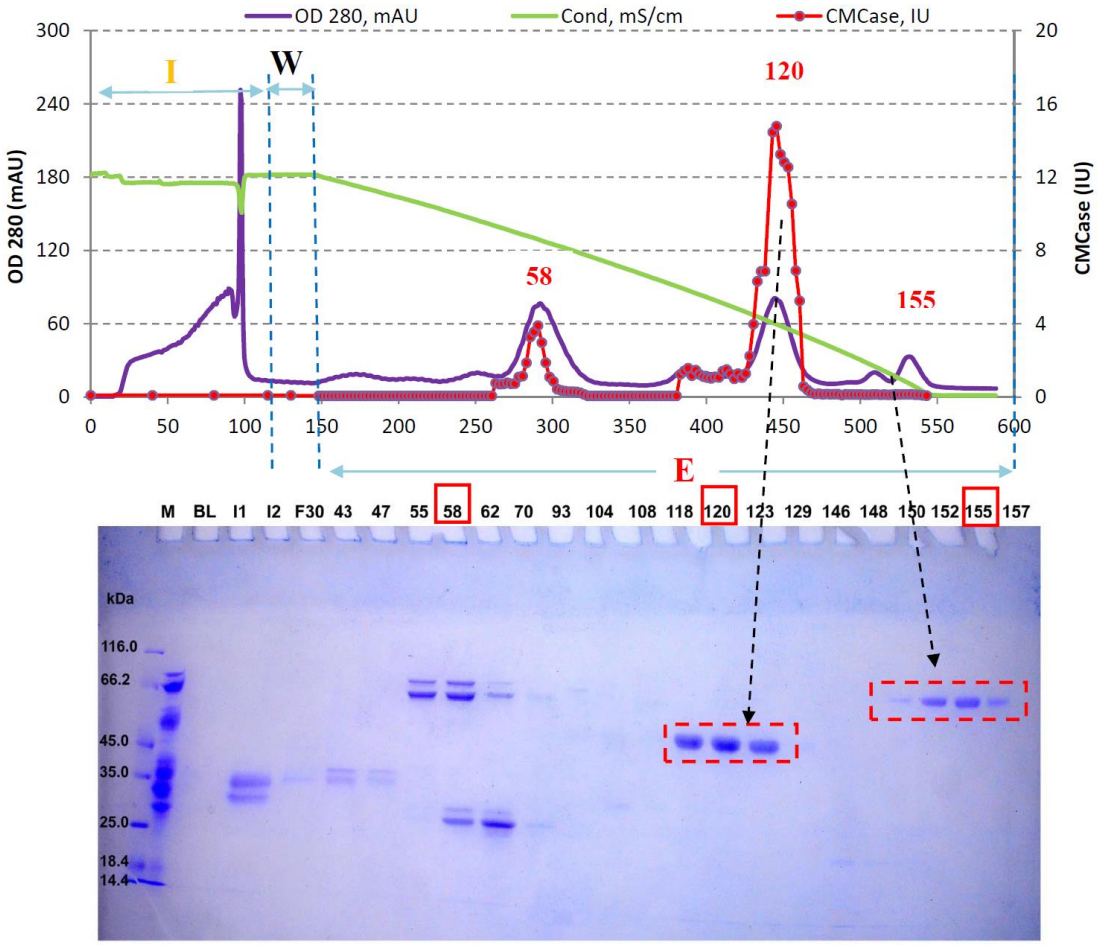
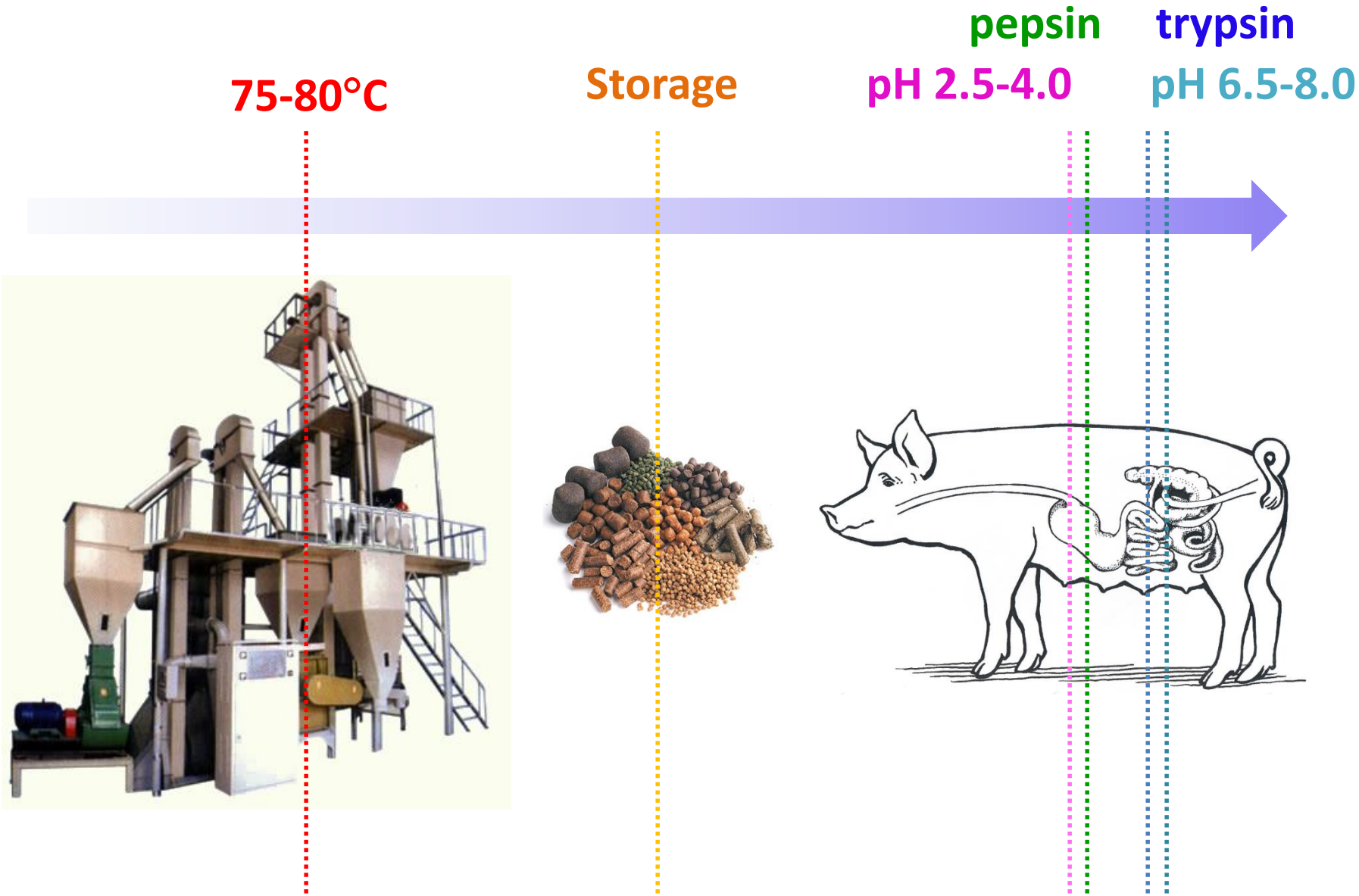


Figure 20. Chromatogram and SDS-PAGE photo of DEAE unbound fraction after Butyl Sepharose FF column. M: protein marker, BL: Sample before loading; I: unbound (inject); W: washed; E30-E157: eluted factions.

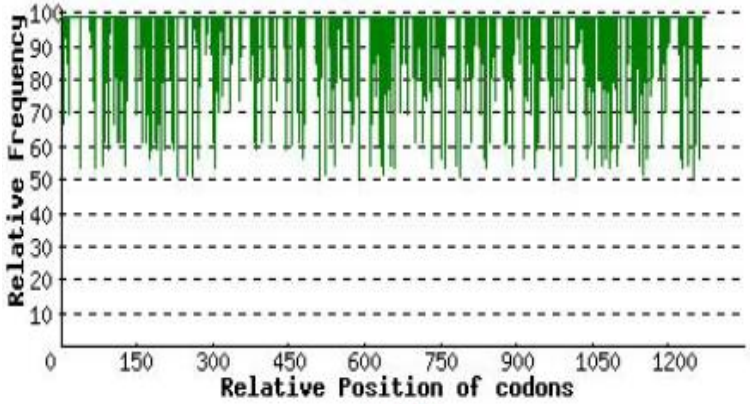
Note:

- *E120 (top of peak 2 E104-E126 fraction) CMCase 1 that has been purified.*
- *Fractions 150-157 also only 1 protein band however CMCase activity was very low (CMCase 2).*

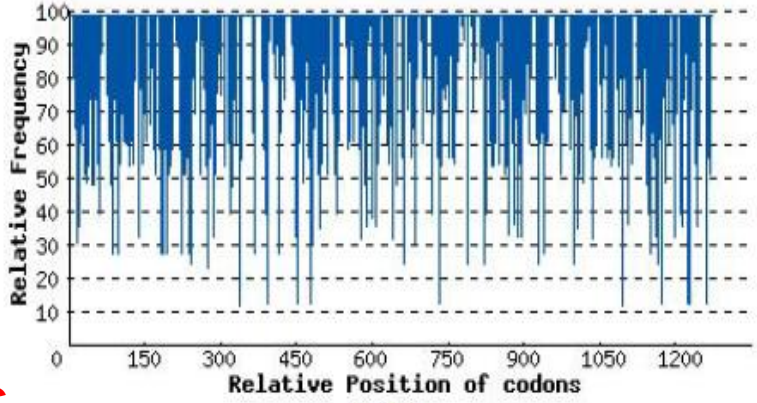
Enzyme production


pepsin
trypsin
75-80°C
Storage
pH 2.5-4.0
pH 6.5-8.0

Synthetic sequences encoding Xyl10C and Xyl11B optimized codon usage for *Pichia pastoris*

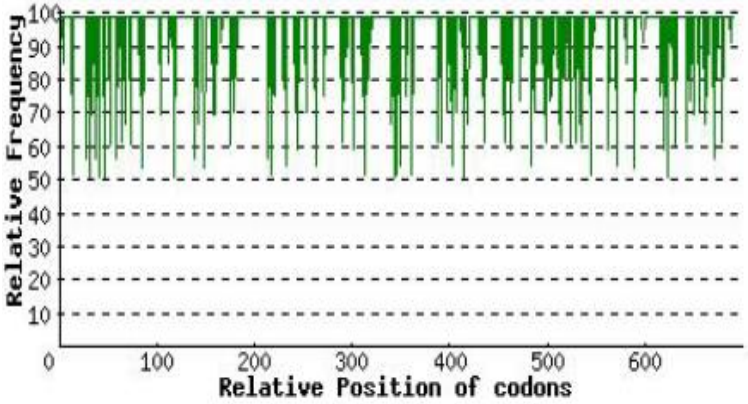


0.84

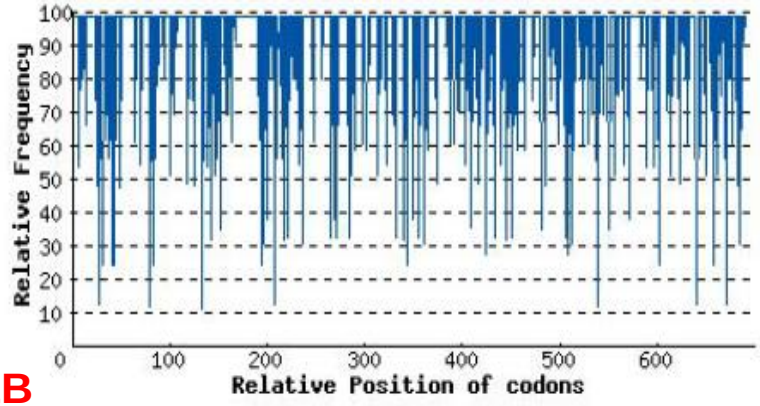


0.67

Xyl10C



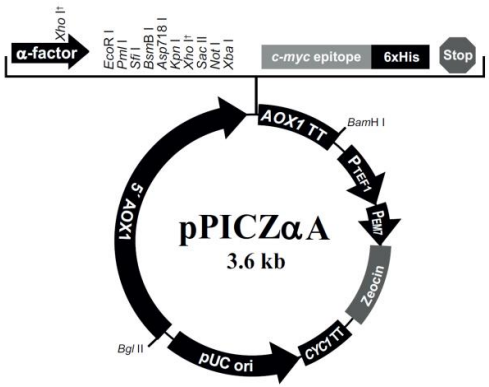
0.83

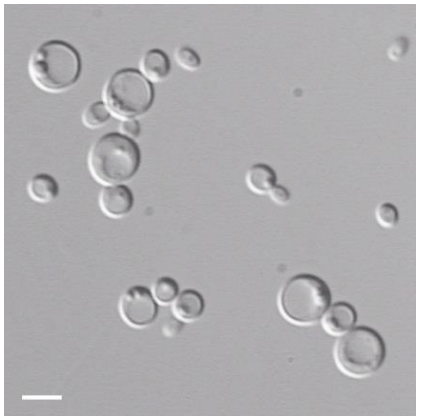


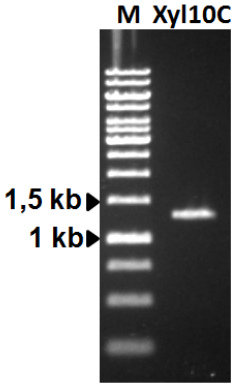
0.64

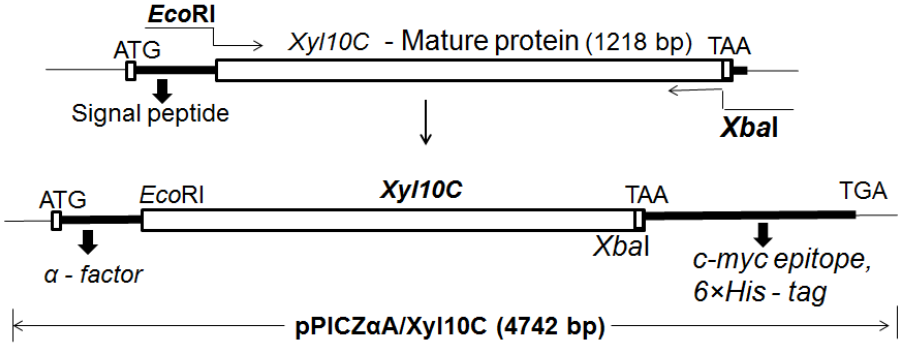
Xyl11B

Pichia pastoris expression system

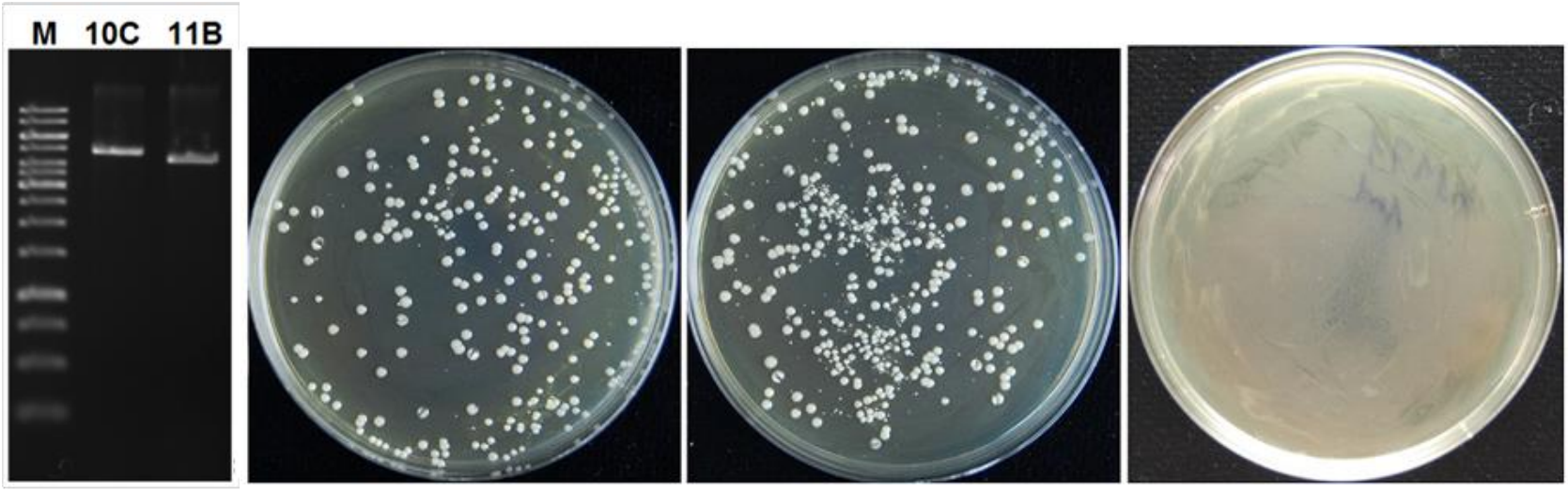




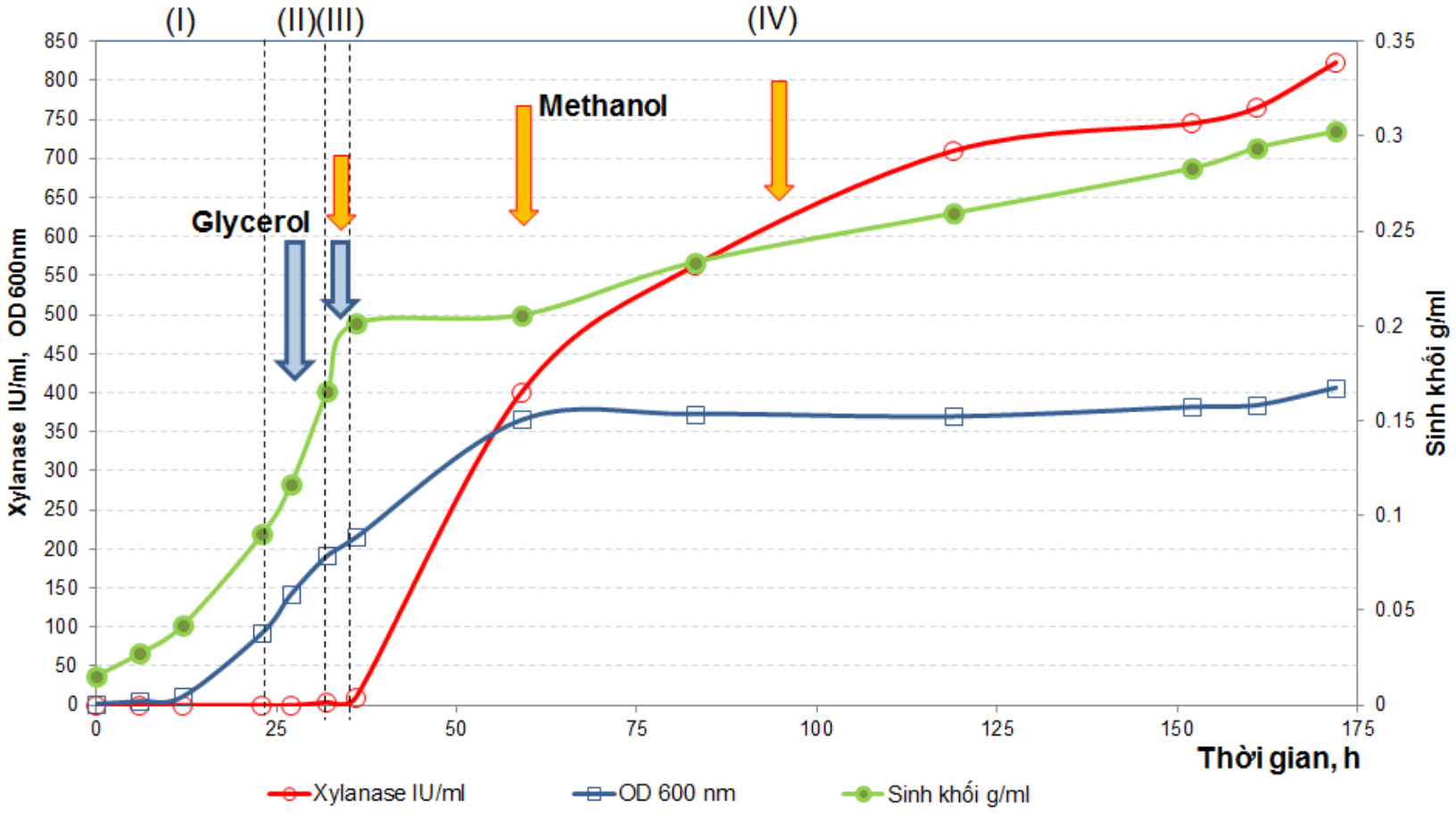




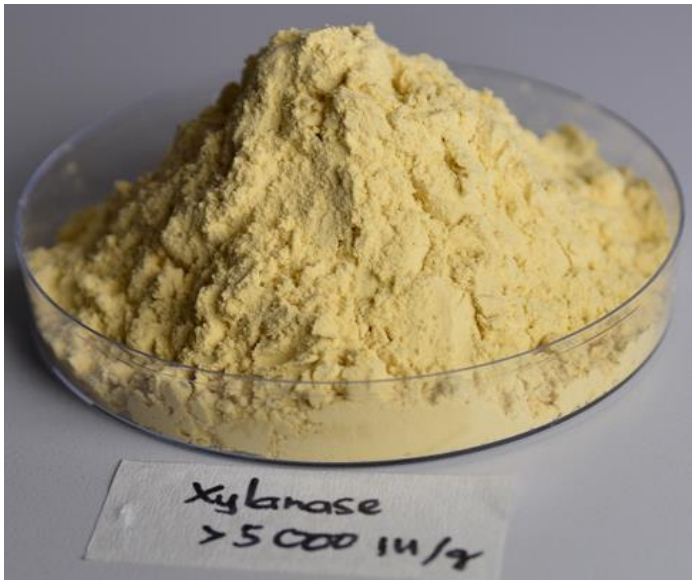
Transformation of pPICZαA-Xyl10C and pPICZαA-Xyl11B to *Pichia pastoris*



Production of recombinant xylanase in 300L fermentor



Pilot production of xylanase



Future aspects

Mining of the transcriptome data for new enzymes

Isolation and characterization of fungi from extreme environments

Optimization of expression conditions for high enzyme yield