

Project end date: August 2022

Prepared by: Professor Emma Master (Aalto University)

Summary of project scope:

The BIOSEMBL project is distinguished by its focus on the development of enzyme families with unique ability to upgrade hemicellulose fractions into materials of higher value, including bifunctional crosslinking agents and polymer precursors. BIOSEMBL brings together research teams at Aalto University and the Swedish University of Agricultural Sciences (SLU) in Uppsala. Together, these teams integrate expertise in industrial protein production, enzyme engineering, structural biology, and synthesis of bio-based materials. A key outcome of the BIOSEMBL project is the establishment of a new enzyme family with ability to non-destructively introduce new chemical functionality into the backbone of cellulose and hemicellulose structures. More specifically, we established a new biocatalytic pathway to aminated polysaccharides from lignocellulose resources, which can open up new application areas for the forest sector including antimicrobial packaging and bio-based polycationic materials for water treatment.

Hired Post-doctoral fellow: Dr. Leena Penttinen

Start date: December 1, 2018

Table 1. Specific project objectives and achievements:

Objective	Achievement
Deploy existing sequence databases to identify and produce novel ω -transaminase (ω -TAs) that target oxidized hemicelluloses	<ul style="list-style-type: none">• 10 ω-TAs were recombinantly expressed and tested for carbohydrate transaminase activity (Table 2)• 10 carbohydrate active ω-TAs were discovered• 4 were prioritized for protein engineering
Enhance ω -TAs activity on high molecular weight polysaccharides through enzyme engineering	<ul style="list-style-type: none">• A method was established for directed evolution of ω-TAs for increased activity on structural polysaccharides (Figure 1).• A method was established for structure-guided engineering of ω-TAs for increased activity on structural polysaccharides• The above engineering efforts required the development of new enzyme screens for increased action on solid substrates• A mutant ω-TAs was identified that displays higher activity on cellulosic and hemicellulose substrates compared to the wild-type enzyme. Additional rounds of mutation and selection are underway to investigate the potential to further enhance

	enzyme performance on high molecular weight polysaccharides.
Characterize the structure and function of ω -TAs shown to transform carbohydrates	<ul style="list-style-type: none">• Protein crystallization trials and structural analysis of four carbohydrate active ω-TAs were initiated at SLU in January, 2020 (see table 3)• Protein crystallization trials included efforts to co-crystallize the enzyme with different carbohydrates (ie., 50 mM Galactose, Lactose, oxidized Galactose, oxidized Lactose, oxidized Melibiose.• Co-crystallisation studies were performed in 15-well plates x12, >500 drops• >40 crystals were sent for X-ray data collection. Remote x-ray data collection were performed using the synchrotron beamline ESRF ID23-1; automated X-ray data collection were performed using the synchrotron beamline Diamond Light Source i03• More than 20 structures of CvTAw1, SpTA7 and ATA117 were solved and examined (Figure 2)• Enzyme-substrate co-crystals were successfully obtained for CvTAw1, SpTA7 and ATA117; however, so far no clear electron density were found for the bound sugar. This effort will continue using 6-amino-6-deoxy-glucose and o-xylene-diamine +/- sugars and the bound substrates
Enzymatic synthesis of aminated carbohydrates for detailed product characterization	<ul style="list-style-type: none">• Method established to use X-ray photoelectron spectroscopy to quantify the nitrogen content of cellulosic substrates after enzymatic amination• Method established to use mass spectroscopy to quantify the nitrogen content of cellulosic substrates after enzymatic amination

Table 2. ω-Transaminase selected for activity towards oxidized carbohydrates

Target #	origin	acronym	reference
1	<i>Silicibacter sp.</i> <i>TM1040</i>	3FCR wt	Weiss et al. 2016, Org. Biomol. Chem.
2		3FCR_ATA TM = WLAMA	Weiss et al. 2017, ChemBioChem
3		3FCR_ATA 4M	Pavlidis et al. 2016, Nat. Chem.
4		3FCR_ATA	Weiss et al. 2016, Org. Biomol. Chem.
5	<i>Silicibacter pomeroyi</i>	3HMU wt	
6		3HMU F92Y	
7		3HMU W63Y	
8	<i>Aspergillus fumigatus</i>	4CHI = AspFum wt	Skalden et al. 2015, FEBS J.
9	<i>Arthrobacter sp.</i> <i>KNK168</i>	ATA117 (Codexis) 3WWJ	Savile et al. 2010, Science
10	<i>Chromobacterium violaceum</i>	Cv-w-TA	Kaulman et al 2007, EMT; Humble et al 2012, FEBS J; Aumala et al 2019, ChemSusChem

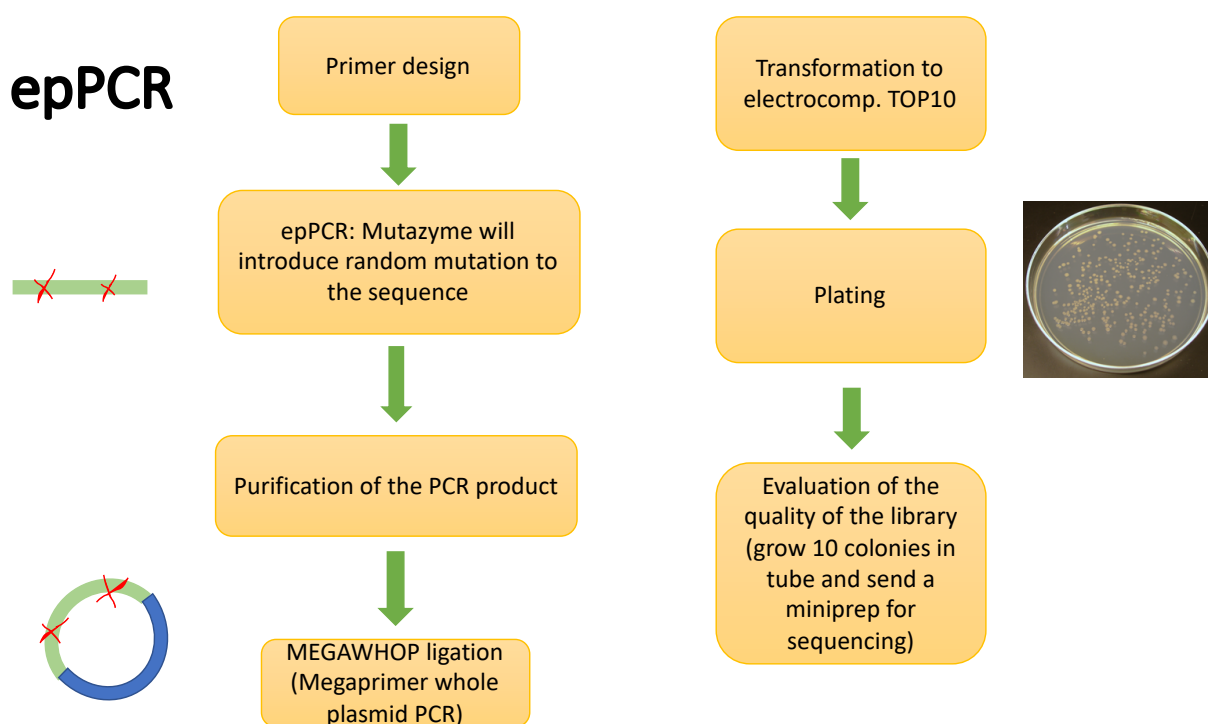
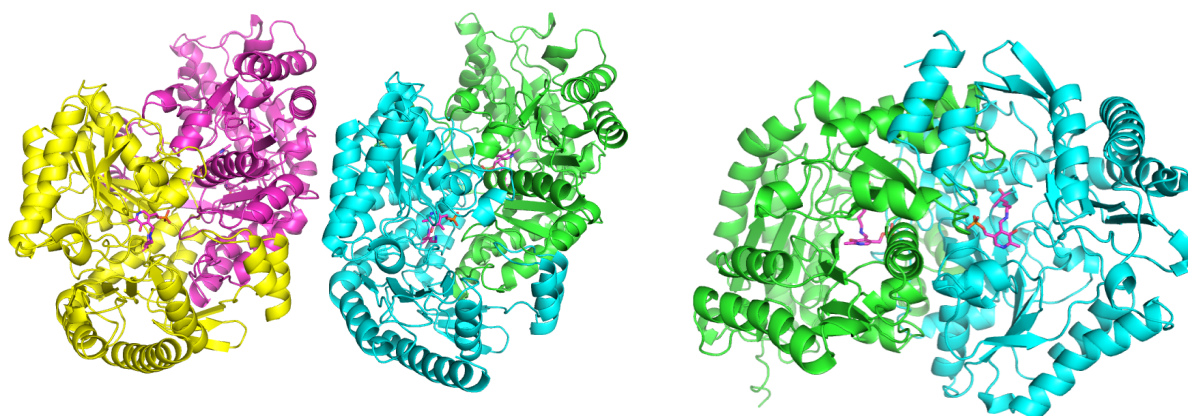


Figure 1. Work flow for directed evolution of carbohydrate acting transaminases

Table 3. Transaminases selected for structural characterization

Enzyme	PDB	Description	Status
CvTAw1	4A6T	<i>Chromobacterium violaceum</i> omega transaminase 1; (S)-selective	Structures >1.8 Å; PLP but no sugar
SpTA7	3HMU	<i>Silicibacter pomeroyi</i> Class III aminotransferase TA7	Structures >1.3 Å; PLP but no sugar
SpTA2	3FCR	<i>Silicibacter pomeroyi</i> aminotransferase TA2	No crystals (yet)
ATA117	3WWJ	<i>Arthrobacter sp. KNK168</i> engineered TA with 27 mutations (Codexis); (R)-selective	Structure >1.6 Å; no sugar

**CvTAw1**

- Improved crystallization (0.1 M Hepes pH 7.5, 0.1 M MgCl₂, 15-20% PEG 3350)
- 1.8-2.5 Å resolution
- Structure with PLP coenzyme
 - Pyridoxal phosphate is covalently bound to Lys-288

SpTA7

- Improved crystallization (0.1 M BisTris pH 7.0, 0.1 M MgCl₂, 15-18% PEG 3350)
- New space group P2₁
- High resolution, 1.3 Å
- Structure with PLP coenzyme
 - Pyridoxal phosphate is covalently bound to Lys-290

Figure 2. Solved structures of two carbohydrate-acting transaminases

Summary of BIOSEMBL research deliverables and next steps:

- We established a **new enzyme family for non-destructive chemical functionalization (i.e., amination) of cellulose and hemicellulose**
- We demonstrated the enzyme activity on high molecular weight polysaccharides from lignocellulose sources (e.g., cellulose, galactomannans)
- We **solved the structures** of the three best performing enzymes in our collection
- We initiated an **enzyme engineering** effort to improve the performance of carbohydrate acting transaminases
- We continue efforts to solve the structure of enzyme-substrate complexes, which will help uncover key amino acid residues contributing to transaminase action on lignocellulose-derived polysaccharides.
- We are optimizing methods to quantify the degree of amination of enzymatically treated celluloses and hemicelluloses.

Bilateral work performed through BIOSEMBL:

- Leena Penttinen visited project collaborators at SLU, Uppsala, two times over the course of the project: in January 2019 and between January and February 2020
- Additional planned research exchanges between Aalto University and SLU were cancelled due to travel restrictions caused by covid-19.
- Tight connections between the participating labs were maintained through videoconference meetings and email exchanges
- To achieve the project goals despite restrictions to travel, 200 000 SEK of the project budget will be invoiced directly by SLU, to cover costs associated with continued structural characterization of prioritized ω -transaminase.

Examples of dissemination:

- Poster presentation at the 13th Carbohydrate Bioengineering Meeting (May 19-22, 2019, Toulouse, France)
- Talk at the 2019 Course on Industrial Biotechnology for Lignocellulose Based Processes; Chalmers University, Gothenburg, Sweden
- Departmental seminar series, Aalto University, Espoo, Finland
- Manuscripts in preparation for publication
- Research publication:
Hameleers L, **Penttinen L**, Ikonen M, Jaillot L, Fauré R, Terrapon N, Deuss PJ, Hakulinen N, Master ER, Jurak E. Biotechnol Biofuels. 2021. Polysaccharide utilization loci-driven enzyme discovery reveals BD-FAE: a bifunctional feruloyl and acetyl xylan esterase active on complex natural xylans. 14(1):127. doi: 10.1186/s13068-021-01976-0.
(Leena Penttinen is co-first author on this article)

Research areas being started or strengthened at the departments in Finland and Sweden.

The following new collaborations emerged from the BIOSEMBL project:

- Hired a Ph.D. graduate from SLU as a post-doctoral fellow at Aalto University
- A post-doctoral fellow work with E. Master and holding a Finnish Academy fellowship initiated a collaboration with partners at SLU on the structural characterization of carbohydrate-active enzymes from plant sources
- A FET-Open project (Horizon 2020) awarded to E. Master at Aalto University includes partners at KTH, Stockholm to characterize the material properties of polysaccharides after enzymatic treatment

How the grant has contributed to strengthening the forest sector in Finland and in Sweden.

Today, enzymes used for lignocellulose processing largely deconstruct cellulose, hemicelluloses and lignin to mixed sugars and monolignols for subsequent microbial fermentation to commodity fuels and chemicals. For example, metabolic engineering (e.g., synthetic biology) approaches have been applied to convert plant derived sugars to biofuels (e.g. ethanol, butanol) and organic acid precursors (e.g. propionic acid, succinic acid, and adipic acid). While commodity biochemicals and fuels will remain an important part of capturing the full potential of renewable biomass, such products are not ideal as primary products from the boreal forest which yields slower growing and high quality fiber. To better serve Finland's and Sweden's forest sectors, BIOSEMBL concentrated on **enzyme technologies that upgrade (rather than degrade) cellulose and hemicelluloses** from wood fiber through the **non-destructive introduction of reactive chemical functionalities** (specifically, carbonyl and amine functionality that **impact strength and adsorptive properties of cellulosic materials**). We established a new protein system for fiber engineering and applied methods in structural biology and protein engineering to enhance the enzyme performance. The established bioprocess has potential to expand the range of high value bio-materials from wood fiber.

Financial accounting:

The table below summarizes the use of Tandem Forest Value funds through the BIOSEMBL project.

Of the 2,000,000 SEK award, 1,800,000 SEK were used at Aalto University; the remaining 200,000 SEK will be invoiced directly by partners at SLU.

Aalto University								
		Budget (EUR)	Expenses (EUR)	Left (EUR)		Budget (SEK)	Expenses (SEK)	Left (SEK)
	Salaries	201 105	225 452		Salaries	2 190 000	2 454 971	
	Materials	15 200	5 816		Materials	165 368	63 332	
	Travel costs	14 442	3 842		Travel costs	157 260	41 833	
	Outsourced services	5 400	1 100		Outsourced services	58 801	11 981	
	Total	236 147	236 210	-63	Total	2 571 429	2 572 116	-687
	Funding (EUR)				Funding (SEK)		Total funding (SEK)	
	KSLA 70%	165 302,90			KSLA 70%	1 800 000	2 000 000	
	Aalto 30%	70 844,10			Aalto 30%	771 429	Remaining funding (SEK)	
	Total	236 147,00			Total	2 571 429	200 000	