Enzymes and biotechnology: could we overcome modern challenges?

2nd International Conference on Genomics & Pharmacogenomics

Dr. Junio Cota
VTT Brasil LTDA
SUMMARY

- What is VTT?
- The OMICS Era
- New Enzyme Discovery
- Protein Engineering
# VTT Group in brief


## Customer sectors
- Biotechnology, pharmaceutical and food industries
- Electronics
- Energy
- ICT
- Real estate and construction
- Machines and vehicles
- Services and logistics
- Forest industry
- Process industry and environment

## Focus areas of research
- Applied materials
- Bio- and chemical processes
- Energy
- Information and communication technologies
- Industrial systems management
- Microtechnologies and electronics
- Services and the built environment
- Business research

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### VTT’s operations
- Research and Development  ■  Strategic Research  ■  Business Solutions  ■  IP Business  ■  Group Services

### VTT’s companies
- VTT Expert Services Ltd (incl. Labtium Ltd, Enas Ltd)  ■  VTT Ventures Ltd  ■  VTT International Ltd  ■  VTT Memsfab Ltd
VTT Group on the map

Assessments of new international projects
BIOREFINERY

Brazilian biomass raw material

Process

Sector

Key service & offering

Key technologies

End products

Wood and fibre processing

Biological processing

Energy production

Pulp, paper & Packaging & Solid wood

Bio & Chemistry

Energy

Multi-technology solutions & pilot scale infrastructure

Biotechnical conversion technologies

Thermal fuel & waste conversion technologies

Fibre processing, Paper making, Coating, Modelling & Simulation

Biomass hydrolysis, Enzymes & Cell factories

Gasification, pyrolysis & combustion technologies

Novel fibre based products & Advanced wood products

Chemicals, Bio alcohols & Biomaterials

Biofuel, Electricity & Heat
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Human Genome Project

1990 - 2003

http://web.ornl.gov/sci/techresources/Human_Genome/project/whydoe.shtml
Evolution of Cost per Megabase

http://evomics.org/2014/01/sequencing-technology-wheres-my-minion/
Evolution of Whole-Genome Sequencing

Big Data, Genome Assembling, Gene Annotation, Computer Modeling
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New Enzymes for Biofuels: GH 10 Xylanase

Development and Biotechnological Application of a Novel Endoxylanase Family GH10 Identified from Sugarcane Soil Metagenome

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Enzymes for Biofuels: GH 10 Xylanase
Proteomics: Secretome of *Penicillium equinulatum*

The *Penicillium echinulatum* Secretome on Sugar Cane Bagasse

Daniela A. Ribeiro¹, Júnio Cota¹, Thabata M. Alvarez¹, Fernanda Brüchli¹, Juliano Bragato¹, Beatriz M. P. Pereira¹, Bianca A. Pauletti¹, George Jackson¹, Maria T. B. Pimenta¹, Mario T. Murakami², Marli Camassola³, Roberto Ruller¹, Aldo J. P. Dillon³, Jose G. C. Pradella¹, Adriana F. Paes Leme¹, Fabio M. Squina¹*

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Proteomics: Secretome of *Penicillium equinulatum*

SCB: Sugar Cane Bagasse  
HDT: Hydrothermal Treatment  
SET: Steam Explosion Treatment  
SAT: Sulfuric Acid Treatment  
MCL: Microcrystalline Cellulose

![Graph showing the composition of secretome with SCB, HDT, SET, SAT, and MCL categories]
Proteomics: Secretome of *Trichoderma harzianum*

Understanding the cellulolytic system of *Trichoderma harzianum* P49P11 and enhancing saccharification of pretreated sugarcane bagasse by supplementation with pectinase and α-L-arabinofuranosidase

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Proteomics: Secretome of *Trichoderma harzianum*
SUMMARY

- What is VTT?
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- New Enzyme Discovery
- Protein Engineering
Protein Engineering is a tailor-made process

THE SCIENCE OF WHAT'S POSSIBLE™

What's on your mind?
What's your need?
Protein Engineering: Rational or Non-rational Design?

Current Paradigms

Mechanism-based (Rational)
Detailed structural analysis

Empiricism-based (Non-rational)
Libraries based
Building a Xylanase – Lichenase Chimera

Biochimica et Biophysica Acta 1834 (2013) 1492–1500

Assembling a xylanase–lichenase chimera through all-atom molecular dynamics simulations

Junio Cota a,d,1, Leandro C. Oliveira a,b,1, André R.L. Damásio a, Ana P. Citadini a, Zaira B. Hoffmam a, Thabata M. Alvarez a, Carla A. Codima a, Vitor B.P. Leite b, Glauca Pastore c, Mario de Oliveira-Neto d, Mario T. Murakami e, Roberto Ruller a, Fabio M. Squina a,*

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CrossMark
Chimeras: Multidomain Proteins

- Multidomain/multifunctional proteins can reduce costs with enzyme load;
- End-to-end fusion between the N and C termini of the parental enzymes can result in nonfunctional chimeras.

S.Y. Hong et al., Biotechnology Letters, 29, 931-936 (2007)
Chimeras: Multidomain Proteins

✓ The selection of the linker sequence is particularly important for the construction of functional fusion proteins
Building Chimeras: Molecular Dynamics

- Energy Landscape Theory
- Structure Based Models
- The topology could drive the protein folding
- Save computational time

Structure Based Models (SB)

The unique Free Energy basin suggests a group of structures candidates: simulations are mainly driven by the entropy of the system.
Building Chimera

Overlap PCR

SDS-PAGE

A) Glycine linker

552 bp (22.8 kDa) 645 bp (26.7 kDa)

Xylanase  Lichenase

Fusion PCR

XylLich 1209 bp (47.2 kDa)

B) M

XylLich

BsLich

BsXyl
SAXS experimental and theoretical curves

Representative structure taken from the Free Energy basin with $\chi^2=2.80$, $R_g=26.0\text{Å}$ and $CM$ distance=$40.7\text{Å}$ inset

Theoretical scattering curve was generated in CRYSOL and the representation in VMD
Optimal pH

▲ Parental enzyme
■ Chimera
Optimal Temperature

▲ Parental enzyme  ■ Chimera
### Substrate Specificity

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Xylanase</th>
<th>Lichenase</th>
<th>XylLich</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birchwood Xylan</td>
<td>3.73 ± 0.29</td>
<td>ND</td>
<td>2.71 ± 0.13</td>
</tr>
<tr>
<td>Beechwood Xylan</td>
<td>3.17 ± 0.07</td>
<td>ND</td>
<td>2.87 ± 0.08</td>
</tr>
<tr>
<td>Rye Arabinoxylan</td>
<td>3.73 ± 0.14</td>
<td>ND</td>
<td>3.03 ± 0.15</td>
</tr>
<tr>
<td>Wheat Arabinoxylan</td>
<td>1.36 ± 0.12</td>
<td>ND</td>
<td>0.88 ± 0.07</td>
</tr>
<tr>
<td>Oat Spelt Xylan</td>
<td>3.28 ± 0.27</td>
<td>ND</td>
<td>2.15 ± 0.06</td>
</tr>
<tr>
<td>Lichenan</td>
<td>ND</td>
<td>3.65 ± 0.29</td>
<td>3.85 ± 0.16</td>
</tr>
<tr>
<td>β-Glucan</td>
<td>ND</td>
<td>5.03 ± 0.20</td>
<td>5.11 ± 0.07</td>
</tr>
<tr>
<td>Laminarin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Xyloglucan</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glucomannan (Konjac)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
Capillary Electrophoresis

Xylohexaose

Lichenan

Retention time (min)
Capillary Electrophoresis

Xylohexaose + Lichenan

Retention time (min)

3  4  5  6  7  8  9

XyLich

APTS X2 X3 X4/G4

Lichenase

APTS G4 X6

Xylanase

APTS X2 X3 X4  

Retention time (min)
Enzyme Yield

- Xylanase: 0.1 U/mg/g cell
- Lichenase: 0.2 U/mg/g cell
- XylLich (Xyl): 0.3 U/mg/g cell
- XylLich (Lich): 0.2 U/mg/g cell

- 6 times greater
- 20% greater
Conclusions

✓ This work presented a novelty way to predict the disposal of chimera domains in solution before experimental assays;

✓ A potential tool for screening and development of enzyme cocktails for second generation biofuels;

✓ The expansion of hydrolase activities in an unique protein could be a route for increase cost-effective of biomass saccharification;

✓ Enzyme production data suggests an advantage on producing the fused protein instead the wild type ones separated.
Protein Engineering: Typical Challenges

- Design proteins with certain function;
- Design proteins which bind novel ligands;
- Alter binding affinity and specificity of proteins;
  - Increase activity of enzymes;
- Change thermal tolerance, pH stability;
  - Alter allostERIC regulation;
- Decrease inhibition of enzymes;
- Increase protease resistance;
- Reactivity in nonaqueous solvents;
- Eliminate cofactor requirement.
Acknowledgements