Dyadic® International

Presentation For

RENEWABLE FUELS ASSOCIATION

Continuing Enzymatic Advancements

February 22, 2011

Safe Harbor Statement

Certain statements contained in this presentation are forward-looking statements. These forward-looking statements involve risks and uncertainties that could cause Dyadic's actual results, performance or achievements to be materially different from any future results, performance or achievements expressed or implied by such forward-looking statements. Except as required by law, Dyadic expressly disclaims any intent or obligation to update any forward-looking statements.

Dyadic International

- A global biotechnology company
- Founded in 1979 by Mark A. Emalfarb
- Unique provider of licensed patented and proprietary technologies for on-site manufacturing of enzymes
- Applications in bioenergy, biopharmaceutical and industrial enzyme markets
- Manufacturing/selling enzymes since 1994
- Publicly traded since 2004 (DYAI)
- Headquartered in Jupiter, Florida, USA
- R&D arm located in the Netherlands

Dyadic International

Biofuels

Provides technology to enable the development and manufacture of fuels & chemicals from agricultural feedstocks

Ethanol

Chemicals

Biopharmaceuticals

Provides technology to enable the development and manufacture of antibodies and other therapeutic proteins

Pharmaceutical

Biotech

Enzymes

Develops, manufactures and markets enzymes and other biological products for a variety of industrial uses

Textiles

Food

Animal Feed

Pulp & Paper

Scientific Collaborations

The Scripps Research Institute

Scripps Florida
The Scripps Research Institute

TNO

Ride the Wave of the Future with BTR!
BTR
Bio-Technical Resources

Moscow State University

NREL National Renewable Energy Laboratory
Innovation for Our Energy Future
U.S. Department of Energy

Wageningen Universiteit
Wageningen UR

SRNL Savannah River National Laboratory
We Put Science To Work
U.S. Department of Energy

Savannah River National Laboratory

Kluyver Centre
Kluyver Centre for Genomics of Industrial Fermentation

Scripps Collaboration

- **One of the world's largest and most reputable biomedical research organizations**
  - Dr. Richard Lerner, President of Scripps and Chairman of Dyadic's Scientific Advisory Board
- **Annotated sequenced C1 genome (2005-2010)**
- **Re-annotated re-sequenced C1 genome (2009-2010)**
  - Expanding knowledge of C1 genetics
  - Provides information and knowledge to improve C1 Technology Platform – to do more for less at higher yields.
  - Provides new product candidates and enzyme catalysts to improve manufacturing processes
  - Enter new markets

Dyadic Netherlands

- **Dyadic's Research & Development Subsidiary**
  - 18 employees – 6 with Ph.D.'s
  - Participation in a number of funded international projects
  - Member of the Industrial Platform of the Kluyver Centre for Genomics of Industrial Fermentation
  - Partner in The Eurofung Project (European scientific and industrial network on fungal research)

- **Management Team**
  - Wim van der Wilden, Ph.D (ETH Zurich Switzerland) – General Manager
    - Former Director of R&D, Gist-brocades/DSM
    - Former Director Biotechnology Division, TNO Quality of Life
  - Jan Wery, Ph.D. – Science Director
    - Former Senior Scientist, Netherlands Organization for Applied Scientific Research (TNO)

- **Located in Wageningen, the Netherlands**
  - Wageningen University and Research Institutes
  - Centre of excellence for Life Sciences research

Dyadic Netherlands

- **Core competencies in:**
  - Molecular Biology
  - Fermentation Technology
  - Enzymology
  - Gene Expression

- **Collaborates with Strategic Partners to:**
  - Provide on-site research and development capabilities
  - Assist in producing customized C1 fungal strains for the manufacture of large quantities of diverse enzymes and proteins at higher yields and lower costs

Dyadic's International Initiative

The Abraham Group LLC

The Abraham Group LLC

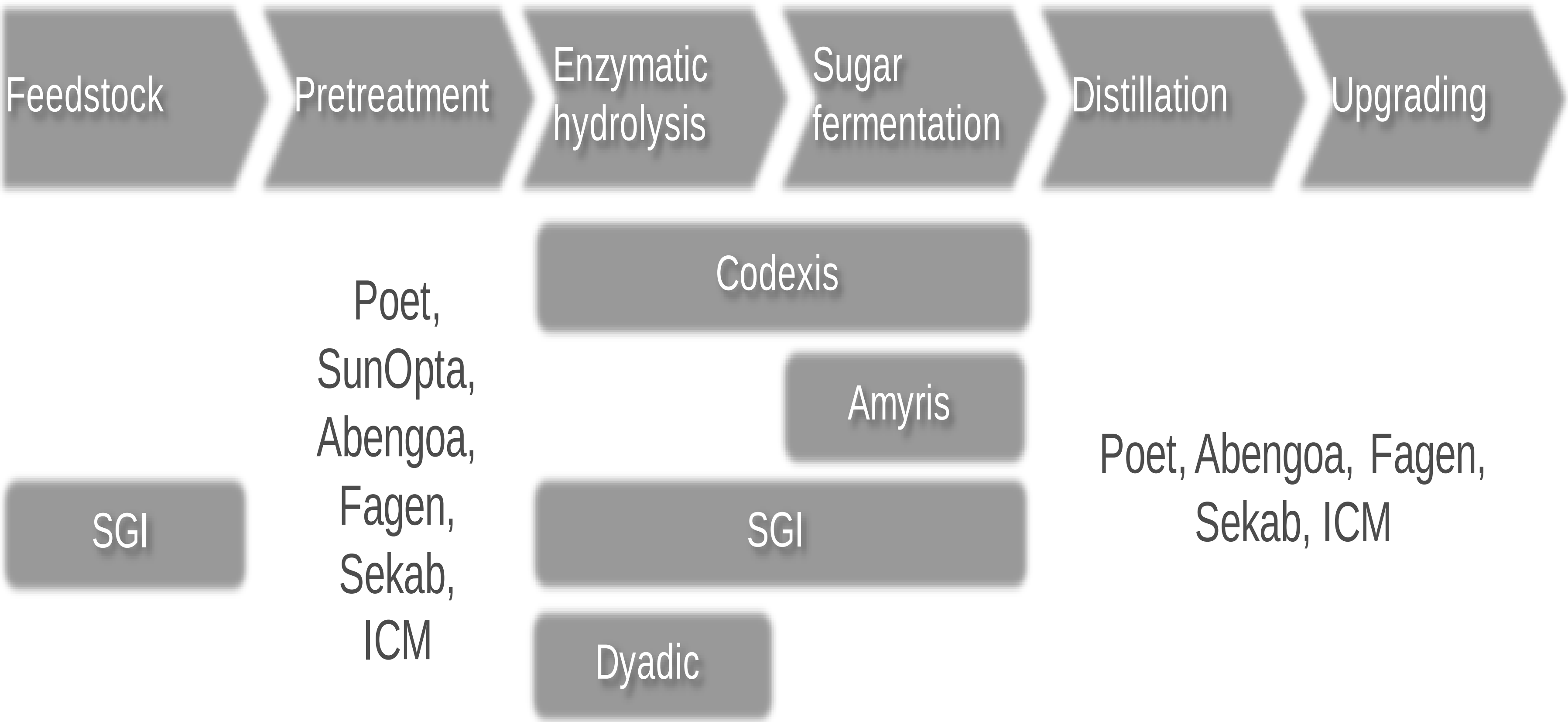
- Influential consulting firm led by former U.S. Secretary of Energy, Spencer Abraham

- Secretary Abraham also serves as non-executive Chairman of AREVA, Inc. and as a member of the Board of Directors of Occidental Petroleum

- Pursuing a global strategy to communicate advantages of Dyadic's C1 platform technology and its R&D capabilities to major international energy groups committed to cellulosic ethanol and other forms of sustainable energy

2nd Generation Biofuels

Value Chain



Source: Bloomberg New Energy Finance

Biofuels Partners

ABENGOA

- One of the largest ethanol producers in the world
- R&D program led to non-exclusive Dyadic C1 license agreement
- Covers use of C1 expression system for large-scale production of enzymes for use in manufacturing of biofuels, power and chemicals
- Currently focused on enzymes for lignocellulosic bioethanol production
- Biomass Pilot Plant (US) in 2007 - 0.02 Mgal/yr capacity
    - Objective: competitive process with grain ethanol culture-based systems
- Biomass Demonstration Plant (Spain) in 2008 – 1.3 Mgal/yr capacity
    - Objective: demonstrate commercial-scale process systems
- Established the Enzyme Development Technological Program
- Commercial Plant (US) in 2012
    - Objective: production at a cost line competitive

***Source: Abengoa Bioenergy PowerPoint presentation at the Cellulosic Biofuels Summit 2010, Washington DC**

Biofuels Partners (Cont.)

- 2008 - Non-exclusive Dyadic C1 license agreement
- Biofuels partner of Royal Dutch Shell/Cosan

Strong Intellectual Property

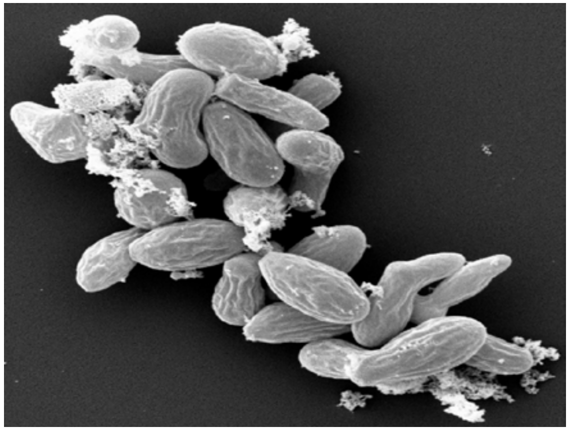
- 7 issued U.S. patents
  - Broad claims blocking use of C1
- 10 pending U.S. patent applications
- 60 issued foreign patents
- 34 pending foreign applications

The C1 Technology Platform

*Chrysosporium lucknowense** (C1)

System for gene discovery, expression and protein production

- A fungus isolated from alkaline soil in Russia
- Sequenced and annotated (large enzymatic potential)
- Versatile genetic tools developed (programmable)
- Mature platform for enzyme and protein production
  - Favorable fermentation characteristics
  - High yields, low costs
- Highly versatile
  - Can be used to produce a growing number of enzymes or proteins
- Broad platform capabilities in biofuels and biochemicals



*The C1 strain was initially deposited with the International Depository of the All Russian Collection of Microorganisms of the Russian Academy of Sciences, and was assigned Accession Number VKM-3500D and classified as *Chrysosporium luckowense* based on morphological characteristics and subsequently reclassified as *M. thermophilia* based on genetic tests

Dyadic’s Licensing Model vs. Commercial Enzyme Sale Model

- Proprietary Ownership
- Customized C1 Fungal Strains
  - Feedstock
  - Pre-treatment
  - Fermentation agents and process
  - Broad operating conditions (pH and temperature)
  - *Trichoderma*-based enzymes in state of patent/legal conflict
- Tax and accounting flexibility allows for treatment of licensing fee as capital expense or operating expense
- Elimination of commercial enzyme production and transportation costs

On-Site vs. Off-Site Production

Enzyme Production Costs/Benefits	Purchasing Enzymes Offsite	Producing Enzymes Onsite
Fermentation process	•30 -50% of the cost of production	**•No markup of fermentation costs**
Cell separation process	•Cost of equipment and additives •Loss of approximately between 5-10% of enzyme activity •Loss of approximately 5-15% of total enzyme quantity	**•No cell separation process required •No loss of enzyme activity/quality •No loss of enzyme quantity**
Ultra-filtration (concentration) process	•Cost of equipment and replacement filter cartridges	**•No ultra-filtration required •No stabilizers or other additives required**
Transportation	•Shipping costs and delivery time •Potential loss of activity from heat	**•No shipping costs or delivery time**
Forecasting/Inventory	•Longer lead times •Higher inventory levels and warehousing costs	**•Reduced lead times •Lower inventory levels and warehouse costs**
•Ownership •Customization •Improvements	•Customer owns the product but not the process •Customer reliant on supplier to customize and improve product and lower cost of goods	**•Proprietary process and product •Programmable system •Control your own destiny**

The C1 Technology Platform

A mature enzyme production system

1. Development of protein hyper-producing strains
2. Development of versatile genetic tools
3. Exploration of the enzymatic potential by genomics
4. Construction of tailored strains for desired enzymes and enzyme mixtures

The C1 Technology Platform

Construction of tailored strains

Result of constructing more than 1000 modified C1 strains:

1. **Strains with high level expression of specific enzymes (HC-strains)**
   - Successfully have over expressed 7 different genes simultaneously

2. **Construction of “clean” background strains (LC-strains)**
   - Suitable for pure single enzyme expression

3. **Strains exhibiting low protease profile**
   - Suitable for heterologous enzyme expression

The C1 Technology Platform

Development of protein hyper-producing strains

Wild type

UV mutagenesis

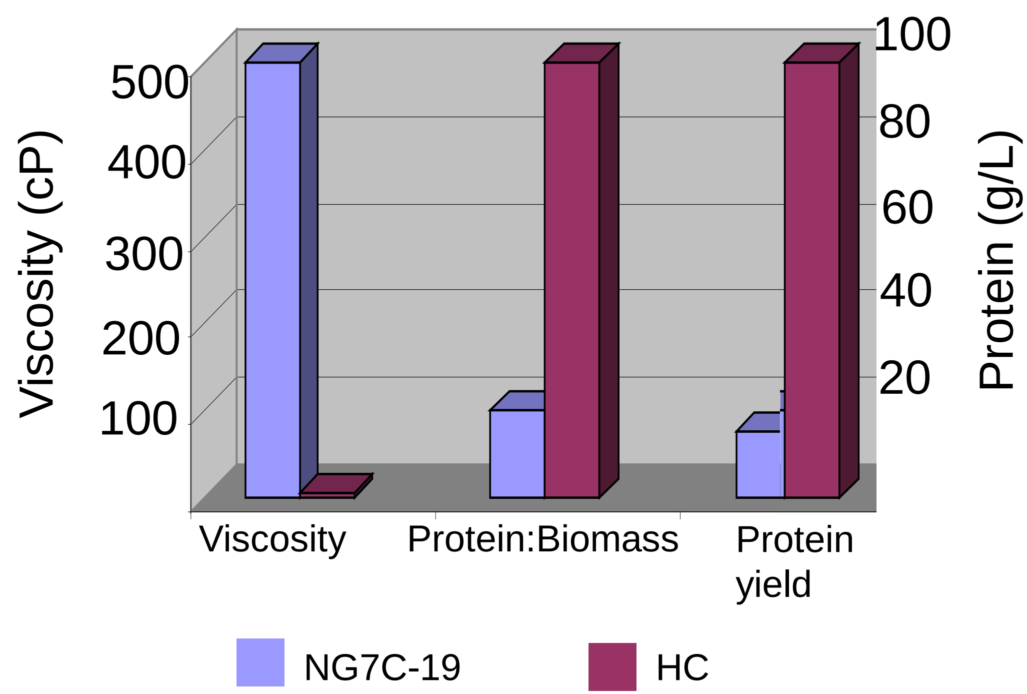
Cellulase over producer

NTG* mutagenesis

De-repressed cellulase production

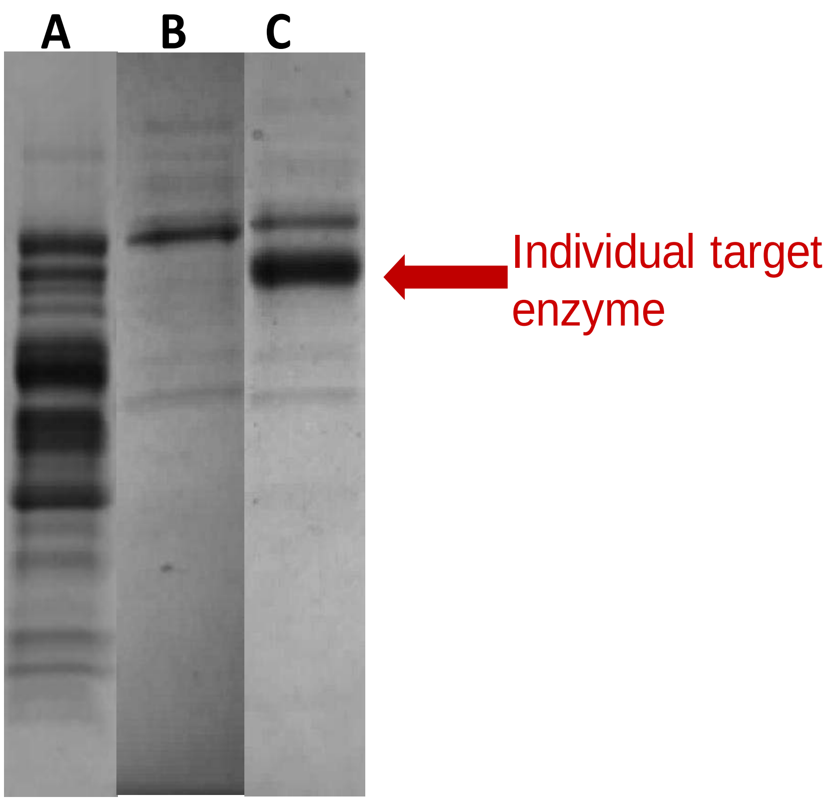
UV mutagenesis

High cellulase, low viscosity (mycelial fragmentation)



The C1 Technology Platform

Construction of “clean” background strains (LC-strains).



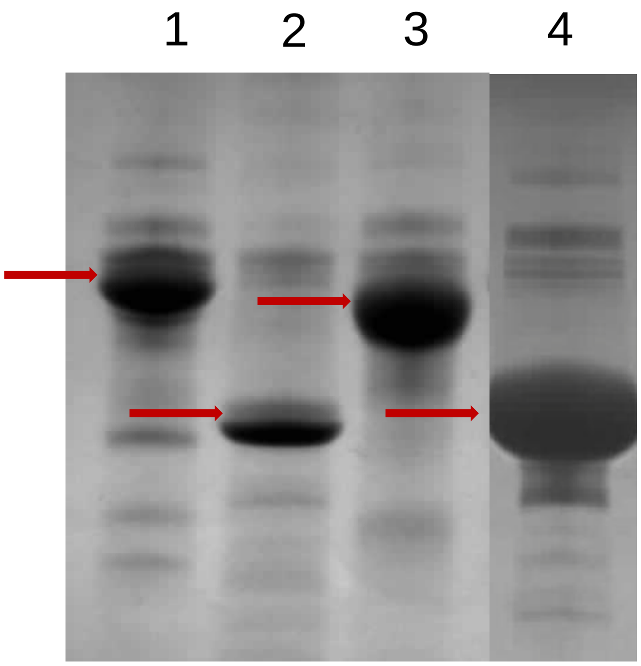
(A) Baseline C1-strain:
High cellulolytic activities
Diverse enzyme mixture
Up to 100 g/L total protein

(B) Low cellulase background strain (LC):
Almost no cellulolytic activities
Very few endogenous secreted
Suited for enzyme characterization

(C) ~ Up to 80% target protein

High Performance C1 Production Platform

Dyadic's library of LC-strains



SDS-Page analysis of end of fermentation broth of single enzyme producing LC-strains

- 70 functional single (hemi-) cellulase expressing strains obtained
- Production levels: g/L scale
- Up to15 g/L of relatively pure target enzyme has been produced

→ Important for both research and commercial purposes

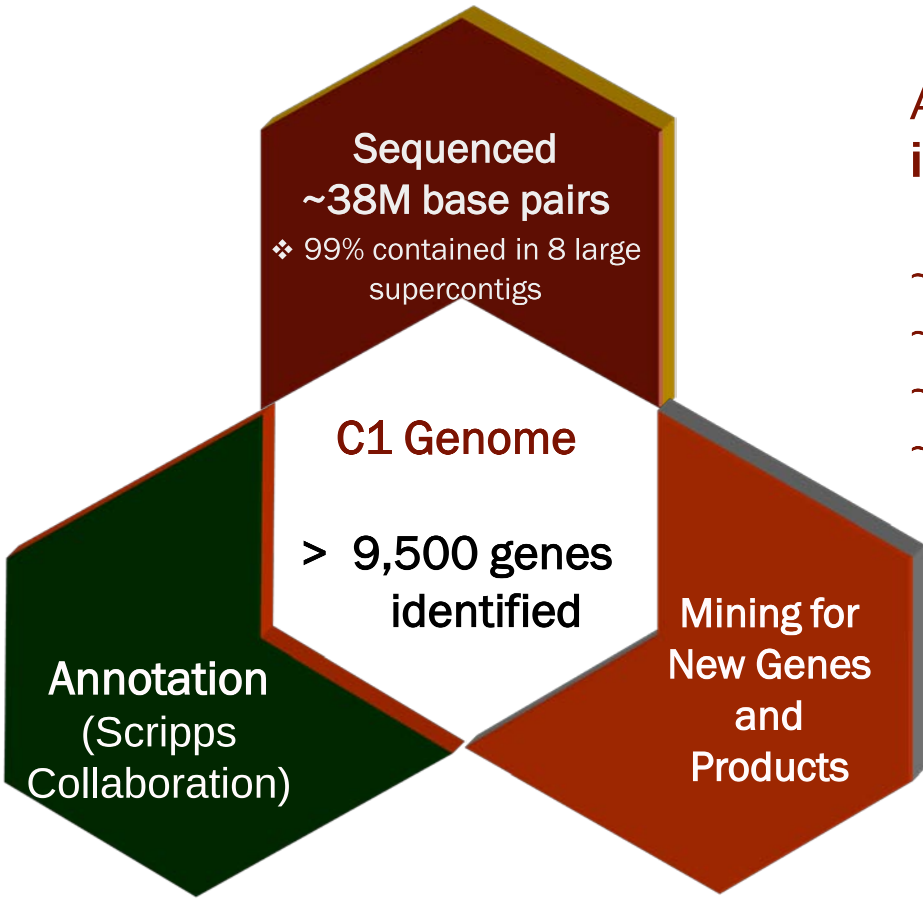
The C1 Technology Platform

Development of versatile genetic tools

- Transformation system: High efficiency, stable integration
- Several genetic markers available: Auxotrophic and dominant
  - Allows for multiple rounds of transformation
- Gene expression: Variety of expression signals.
  - Constitutive, inducible at various strengths
- Protein production: Efficient secretion signals
- Targeted gene disruption: Efficiency up to 90%
- Variety of optimized C1-hosts
- Based on self cloning: No foreign DNA needed

The C1 Technology Platform

Exploration Enzymatic Potential: Genome Mining



A large number of genes putatively encoding **industrially important** enzymes discovered:

~**250** Carbohydrate-active Enzymes (CAZy)
~**150** proteases
~**700** oxido-reductases
~**75** lipases / esterases.

Sugar-based Fuels and Chemicals

Replacing chemicals now derived from fossil oil with **sugar-based** fuels and chemicals

Main Sugars from Biomass	Microbial Conversion	Fuels and Chemicals (or building blocks)
Glucose (C6) Fructose (C6) Xylose (C5) Arabinose (C5)	C2	**Ethanol**
	C3	Glycerol, lactic acid, 1,3-propandediol, (OH-) propionic acid
	C4	Butanol, 1,4 succinic, fumaric and malic acids
	C5	Itaconic acid, xylitol, levunic acid
	C6	Citric acid , lysin, gluconic acid, 2,5 furan-dicarboxylic acid

Adapted from US Dep. Of Energy. Top Value Added Chemicals from Biomass, Vol. 1, 2004

Sustainable Fuels and Chemicals

Production from (hemi-) Cellulosic Sugars

Three important processes need to be integrated:

1. Thermo/Chemical Pretreatment | **2. Enzyme treatment** | **3. Microbial conversion**

Biomass

--Corn stover
--Wheat straw
--Bagasse
--Energy crops

Interaction ↔

Dyadic

Liberate C6 and C5 sugars from (hemi-) cellulose fibers

Interaction ↔

Fuels and Chemicals

--Ethanol
--Butanol
--Chemicals
(Building blocks)

Matching Enzyme Activity to Substrates

DDG's

Corn Fiber

Corn Stover

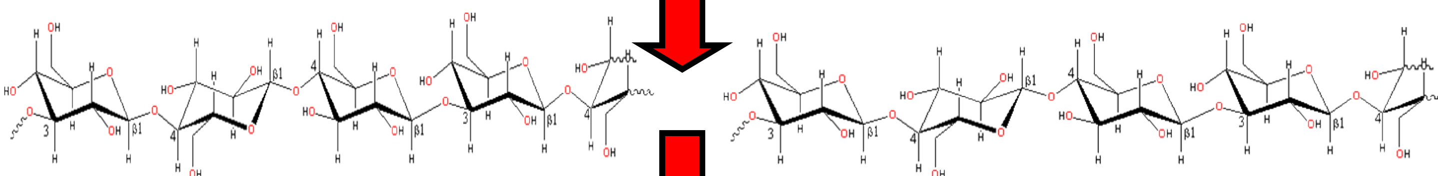
Wheat Straw

Wood Pulp

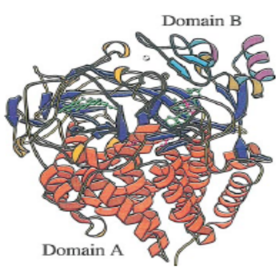
Sugar Cane Bagasse

Agricultural Residues

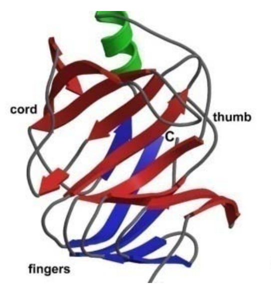
Alternative Pretreatments



Proprietary Enzyme Library

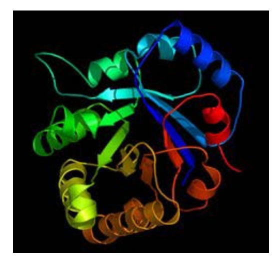


EG's

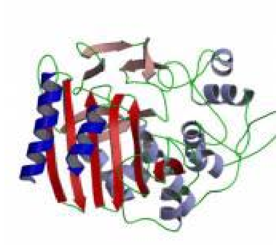


CBH's

βG



Xylanases



Esterase + Accessory Enzymes

High Yield of Fermentable C5's and C6's

C1 vs. *Trichoderma*

Lignocellulolytic Potential of C1 vs. *Trichoderma reesei*
(the main industrial source for biofuel enzymes, e.g. Accellerase™**)**

Genes encoding	***Number in C1***	***Number in T.reesei****	***Biomass Fiber***
Endo-glucanases, Cellobiohydrolases, β-glucosidases	~ 55	~ 35	Cellulose
Cellulose binding domains (CBM1-type)	~ 46	~11#	
Xylanases/Xylosidases	~ 13	~ 5	Hemi-cellulose
Arabinofuranosidases/arabinases	~ 14	~ 3	
Esterases (Axe, Fae)	~ 13	~2#	

Based on literature and JGI database searches

C1 is a Rich Source of Lignocellulolytic Enzymes!

Biomass Degrading Potential of C1

Carbohydrate active enzymes (CAZy) as revealed from automated annotation

CAZy Enzymes in C1	Number in C1
Glycoside hydrolases (GH)	175
Carbohydrate esterases (CE)	18
Polysaccharide Lyases (PL)	7
Glycosyl Transferases (GT)	55
Carbohydrate Binding Domains (CBM)	75 (72 part of GH-enzymes)
Total (no-overlap)	**258**

Exploitation of C1's (Hemi-) Cellulolytic Potential

- ❖Collection of about 70 functional single (hemi-) cellulase expressing strains have been obtained

- ❖Detailed biochemical and mechanistic studies performed*
  - ❖Endo/exo-glucanases, Cellobiohydrolases, β-glucosidases, endo/exo-xylanases, acetyl-esterases, ferulic acid esterases, arabinofuranosidases, β-xylosidases

- ❖Great diversity of activities discovered--Important for synergy and versatility

- ❖Ongoing activity in collaborative projects

This information can be used to develop highly efficient biofuel enzyme mixtures

* Hinz, S.W.A., Pouvreau, L., Joosten, R., Bartels, J., Jonathan, M.C., Wery, J., Schols, H.A. (2009). J. Cereal Sci., 50: 318-323.

Kühnel, S., Hinz , S.W.A., Pouvreau, L., Wery, J., Schols, H.A. Gruppen, H. (2010) J. Biores. Technol., DOI: 10.1016/j.biortech.2010.05.070

Experimental C1 Biofuel Enzymes in Industrial Conditions

In specially designed reactors:

- ❖High biomass loadings possible (>20% DM)
- ❖Controlled pH, Temperatures
- ❖Combined (hybrid) saccharification and yeast fermentation (SSF) possible
- ❖Online ethanol measurements

0.5h **1h** **2h** **5h** **48h**

20% solids
100 g/L glucan

Fast viscosity reduction and formation of glucose →

~ 90 g/L glucose

Enzyme Development for (Hemi-) Cellulosic Biofuels

Enzyme Mixtures for Cellulosic (C6) Substrates

Pretreated Wheat Straw

Cellulose: 50-55%
Hemicellulose: 5%

Buffer →

Pretreated Wheat Straw
(20% TS w/w)

Insoluble Cellulose (≈100g/kg)

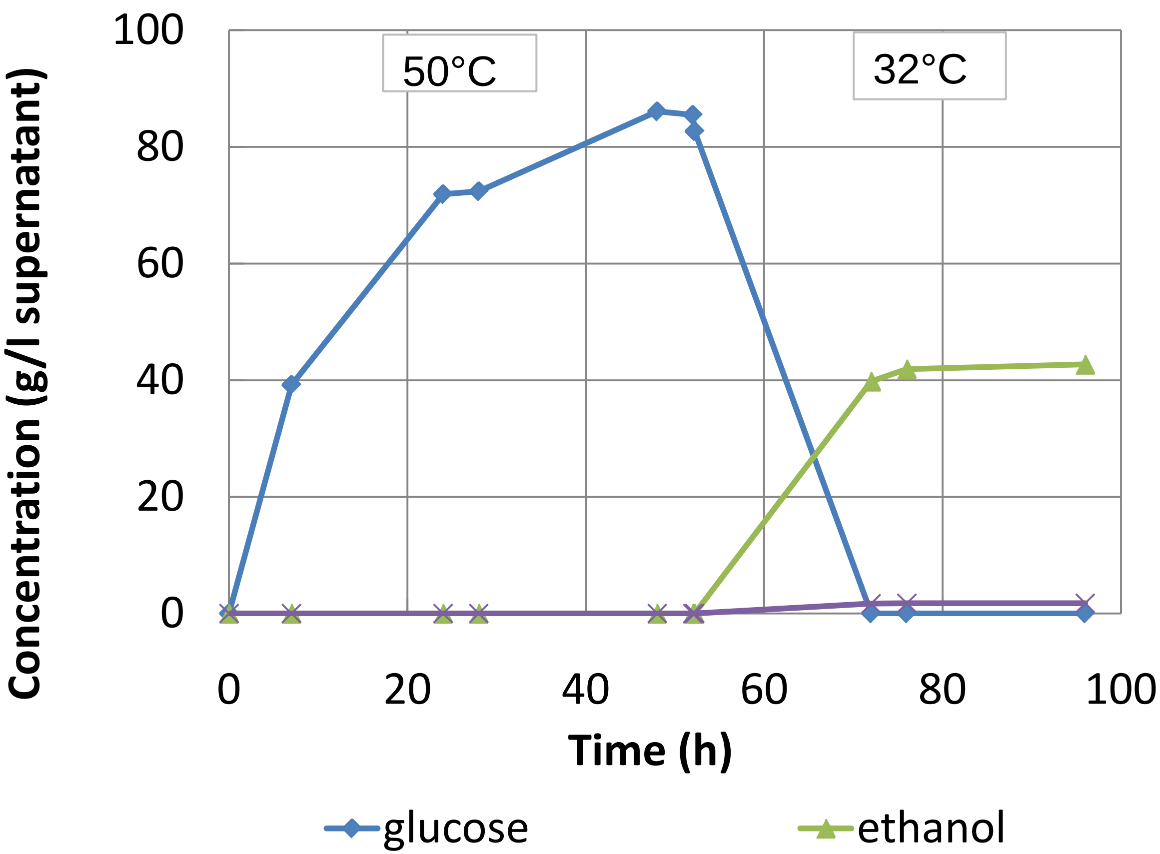
C1 Enzyme Mixture →

Hydrolysed Wheat Straw

Glucose (≈100g/kg)

Hybrid Saccharification and Fermentation by G3

Example: Dilute acid pre-treated wheat straw, 20% DM



Conclusions:

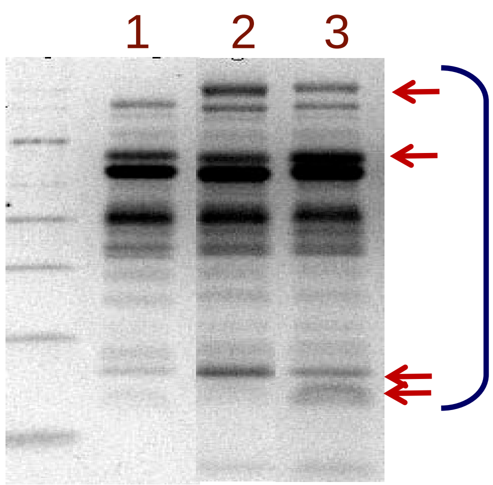
- Efficient conversion of glucan to ethanol enabled by G3 (80%) in **3** days
- Both clarified enzyme and crude fungal broth lead to efficient ethanol production

Dyadic Approach:

Single Strain to Produce Cost Effective Enzyme Mixtures

Enzyme Loading to Achieve >70% Saccharification

C1 Strain: Baseline Enzyme Mix (G1) 100%

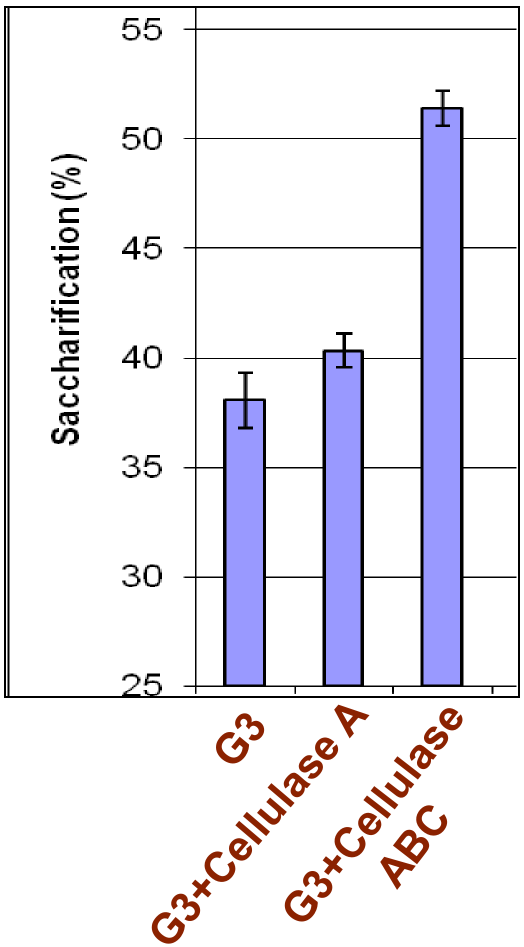


Example:
Specifically overexpressed cellulases

Next Generation Optimized Strain: Improved Enzyme Mix (GX) 20%

Addition of Specific Single (hemi-) Cellulases 20-X %

Eliminate G3 Limitations by Mixing with Single Enzymes



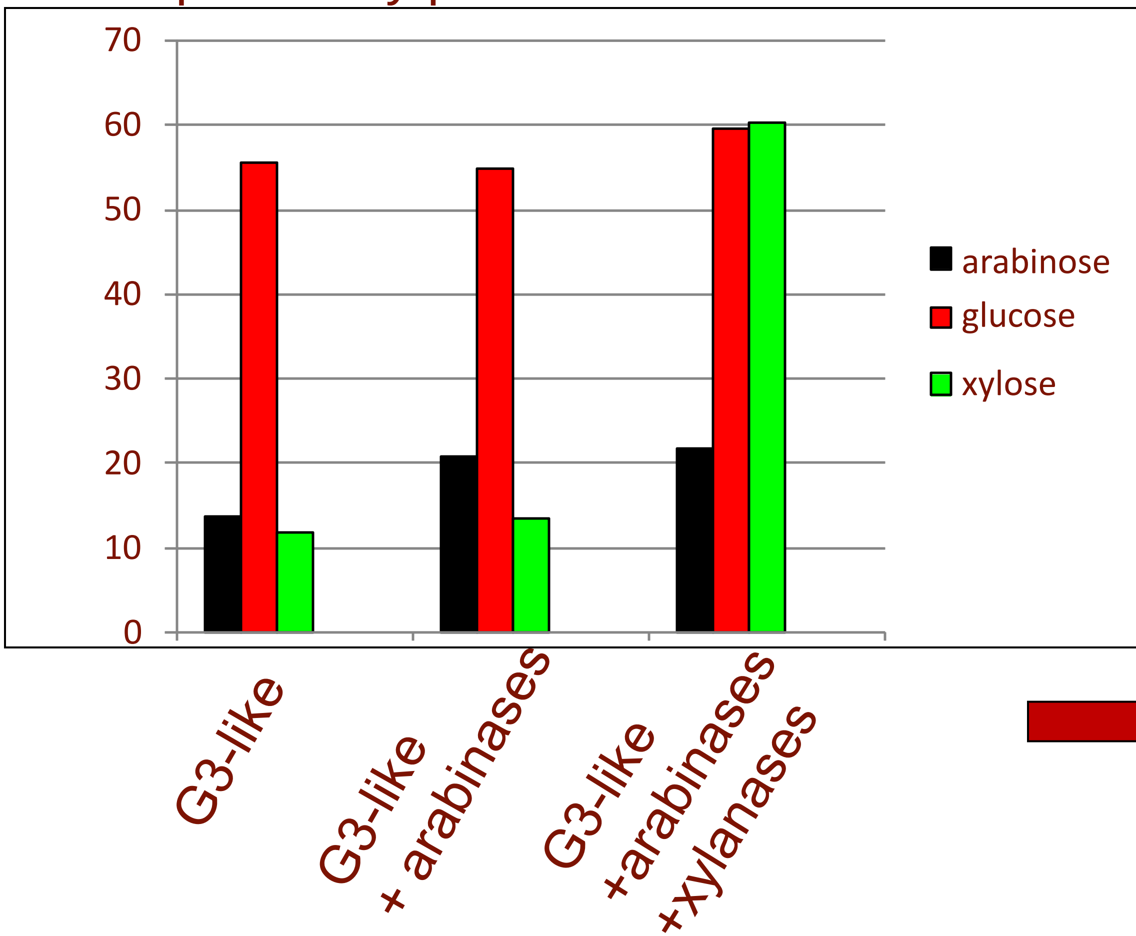
- Substrate: Acid pretreated corn stover
- Low enzyme loading G3 used

➡ Addition of distinct single cellulases to G3 yielded a tremendous increase in efficiency

➡ New C1-strains developed: **G5 and G7**

Enzyme Development for Hemicellulosic Substrates

Example: mildly pretreated wheat bran

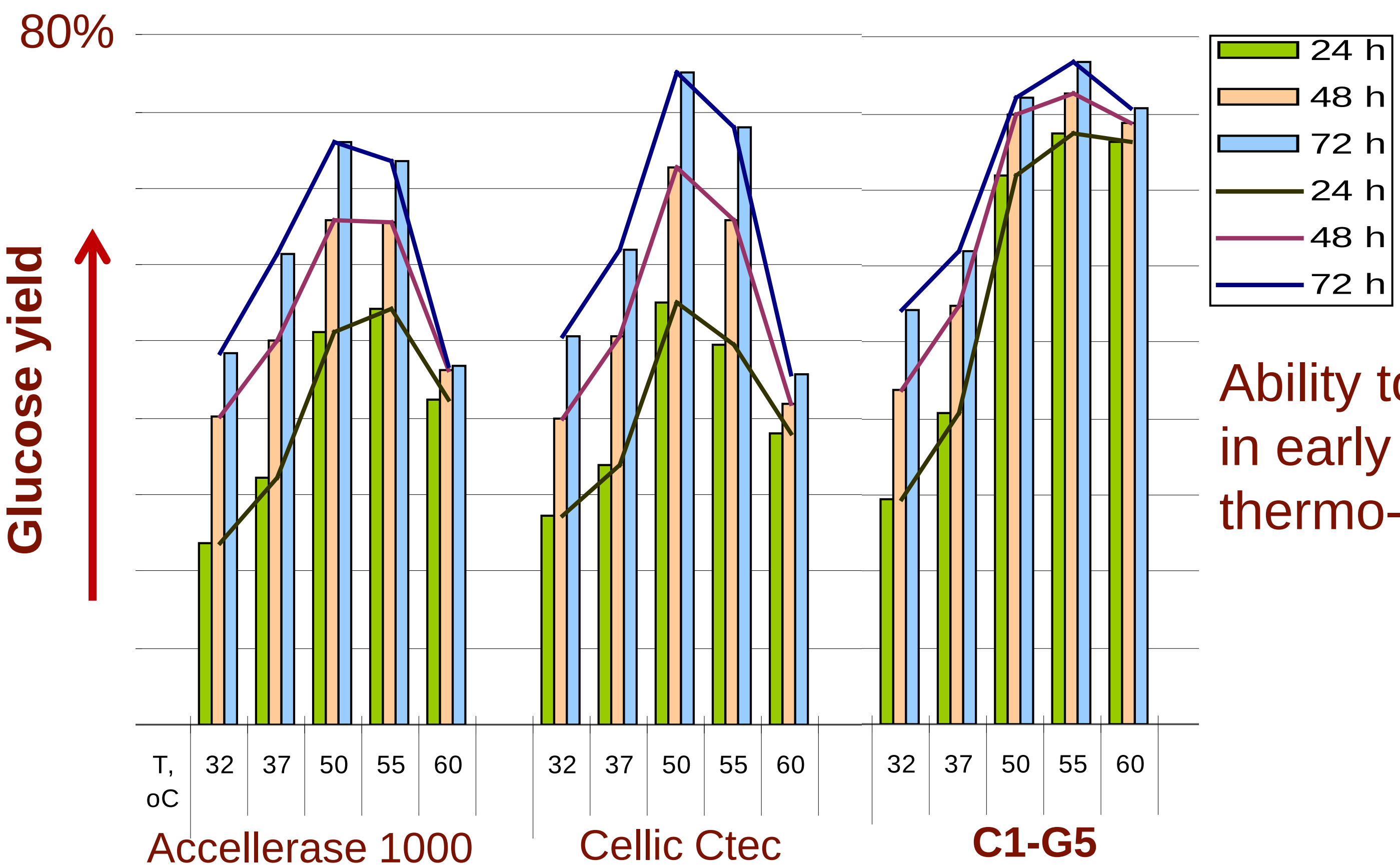


- High hemicellulose content (20%)
- Low enzyme loading G3 used.
- Addition of distinct single hemicellulases to G3 yielded a tremendous increase in efficiency.

New C1-strains developed for ligno-hemi-cellulosic biomass: G4, G6, G8

C1 Biofuel Enzymes:
Broad Active Temperature Range

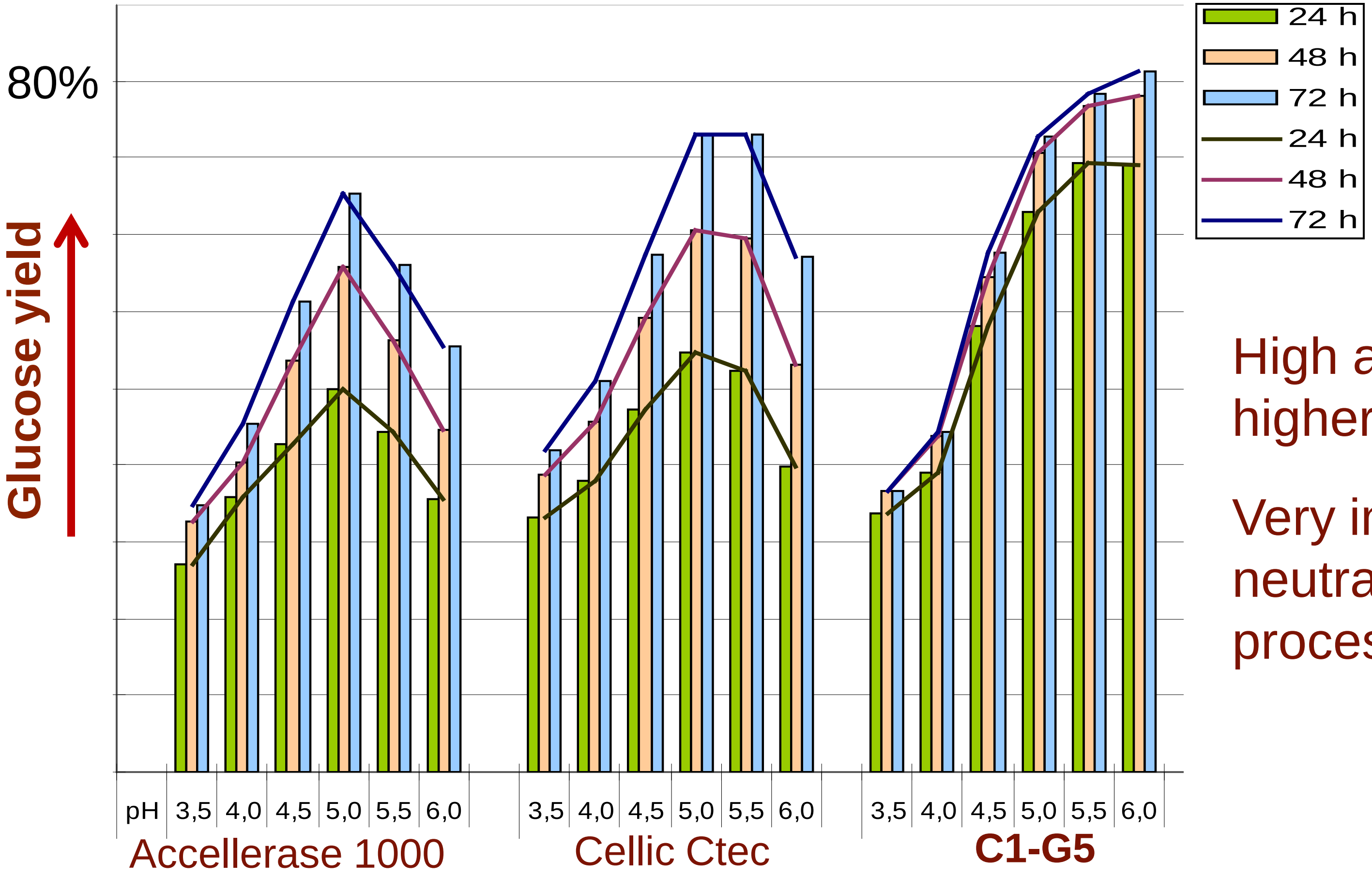
Dilute acid pre-treated corn stover, 10% DM



Ability to add enzyme in early stage after thermo-pretreatment

C1 Biofuel Enzymes: Broad Active pH Range

Dilute acid pre-treated corn stover, 10% DM



High activity at higher pH's:

Very important for neutral (bacterial) processes

Matching Enzyme Activity to Microbial Conversion (SSF conditions)

Fuel/chemical	Micro-organism	T (°C)	pH-range	Selection of Companies
Ethanol	Yeast	32-37	4-5	Nedalco, DSM, Mascoma (CBP)
Ethanol	*Z. mobilis*	30	7	Dupont/Genencor
Ethanol	*E. coli*	37	6-7	
Ethanol	*T. saccharolyticum*	50-60	5-6	Mascoma
Ethanol	*T. mathranii*	50-80	6.5-7.5	Biogasol
Ethanol	*C. phytofermentans*	37	6 - 9	Qteros
Butanol	*C. acetobutilicum, E.coli, yeast*	30-37	4-7	BP/Dupont, Butalco, Gevo, Tetravitae
1,3-Propanediol	*E. coli*	37	6-7	Dupont/Genencor
Succinic acid	*E. coli* and other	37	6-7	DSM/Roquette, Myriant
Fatty acids (diesel)	*E. coli*	37	6-7	LS9/JBEI (DOE)
Farnesene (building blocks, biodiesel)	Yeast	32-37	4-5	Amyris
Isoprene (building block chemical)	*E. coli*	37	6-7	Genencor/Goodyear
Lactic acid	Bacteria and Fungi	30-60	6-6.5	Purac, Myriant

Critical process variables: pH and T

Algae: A Route to Numerous Biobased Compounds

Deployed by e.g. Martek Biosciences Corp, Solazyme Inc.

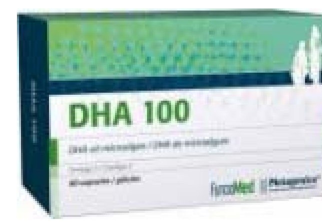
Cellulosic Feedstock

Heterotrophic Algae

pH 6-9
T=25-30°C

C1-enzymes

Products

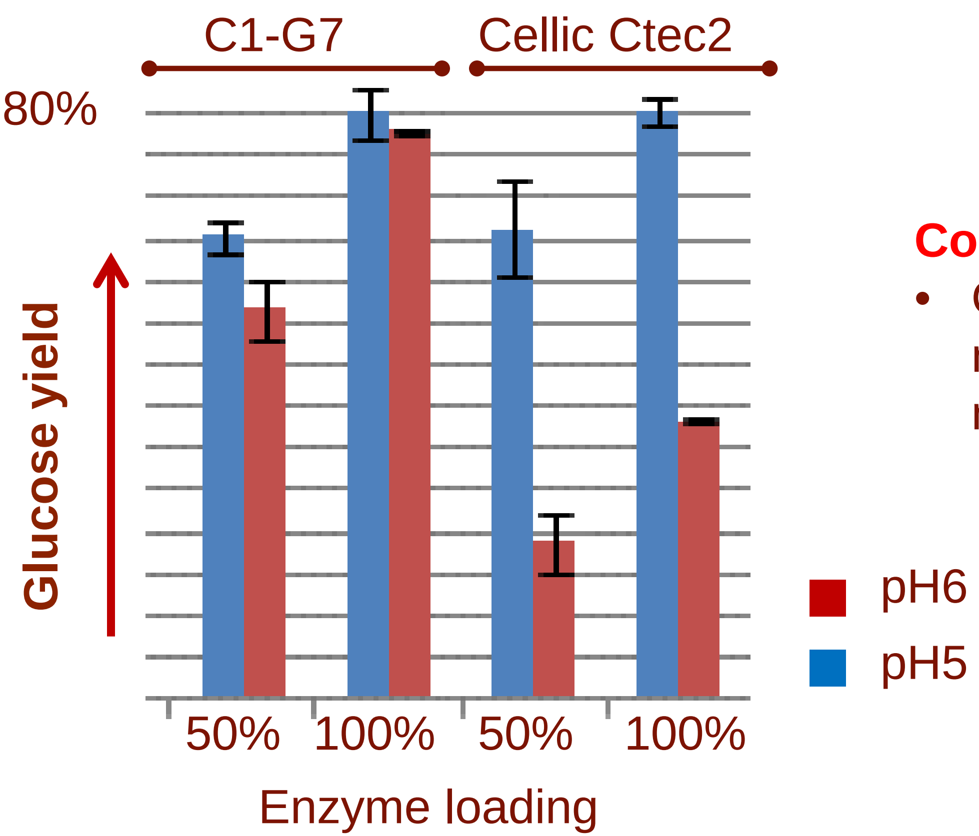


Health supplements

Food ingredients
Transport fuel
Lubricants, polymers

C1 Biofuel Enzymes: Broad Active pH Range

Dilute acid pre-treated corn stover, 10% DM, **24h** saccharification time

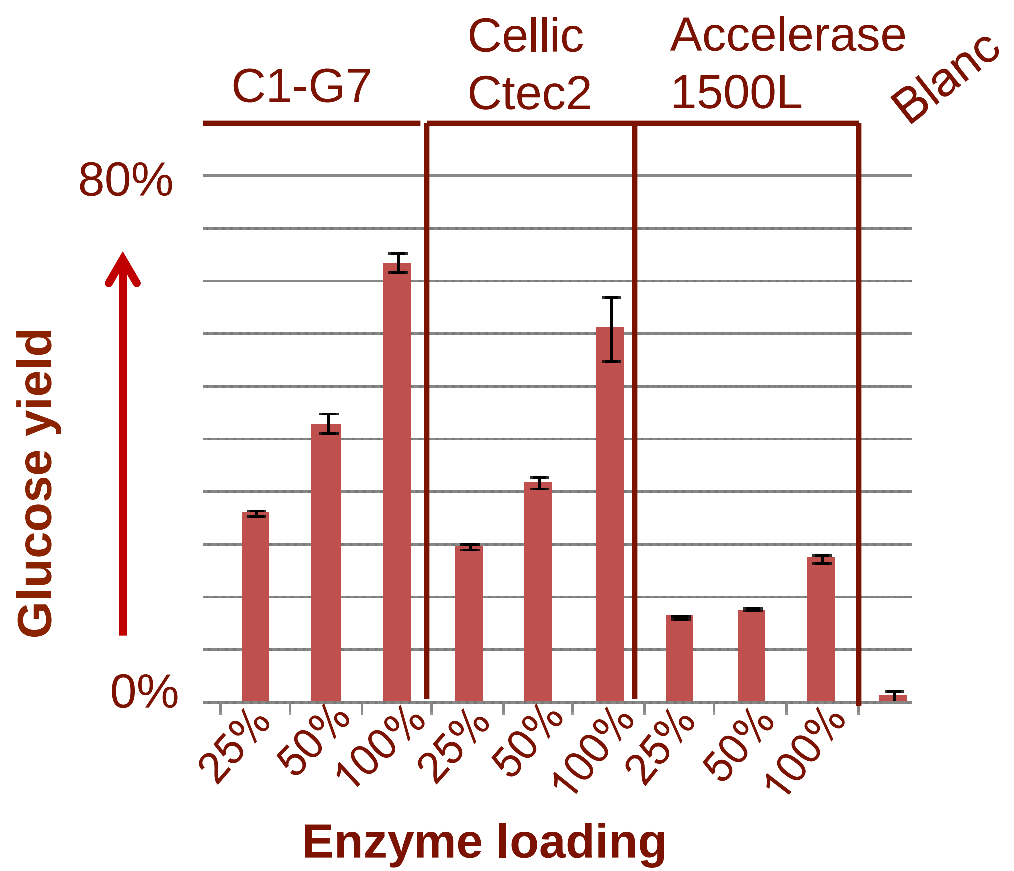


Conclusion:

- G7 makes an excellent enzyme mixture for **BOTH** acidic and more neutral processes

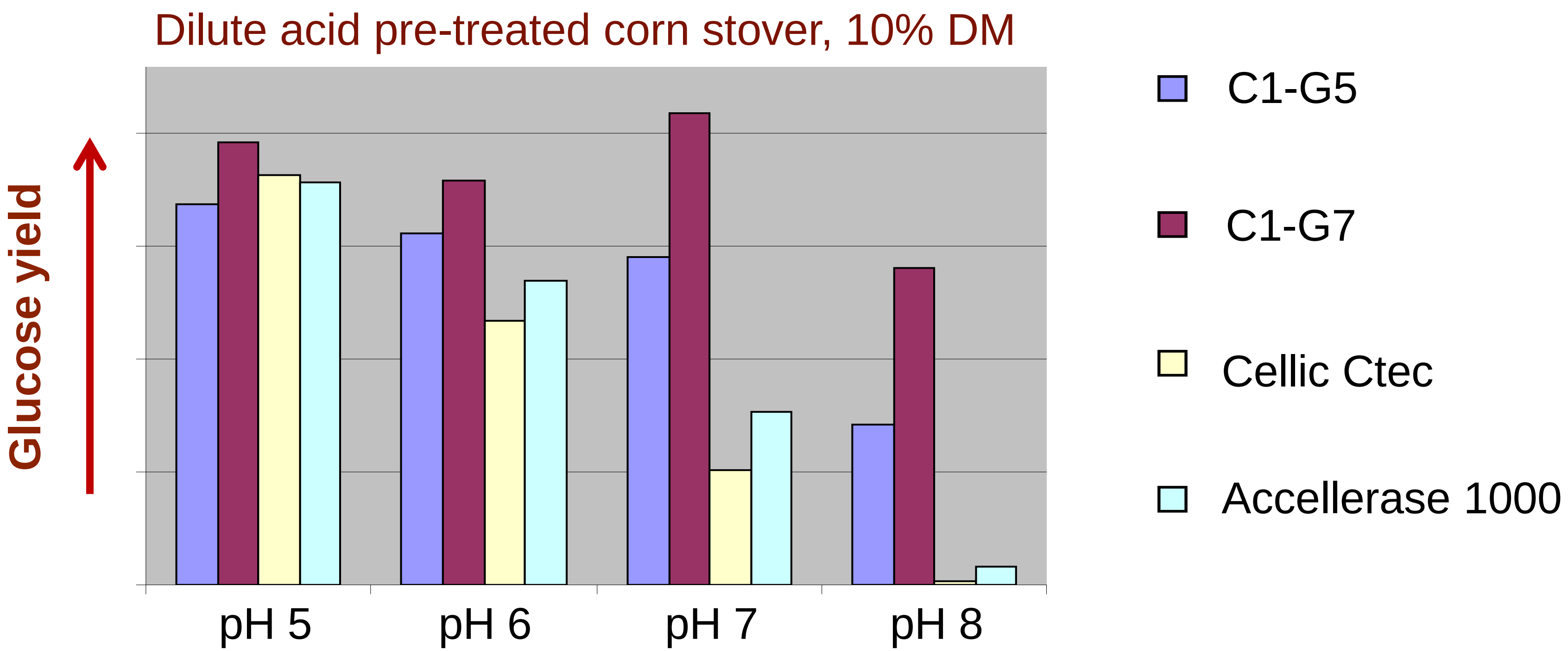
C1 Biofuel Enzymes: Broad Active pH Range

Pre-treated Empty Fruit Bunch, 10% DM, **pH6**

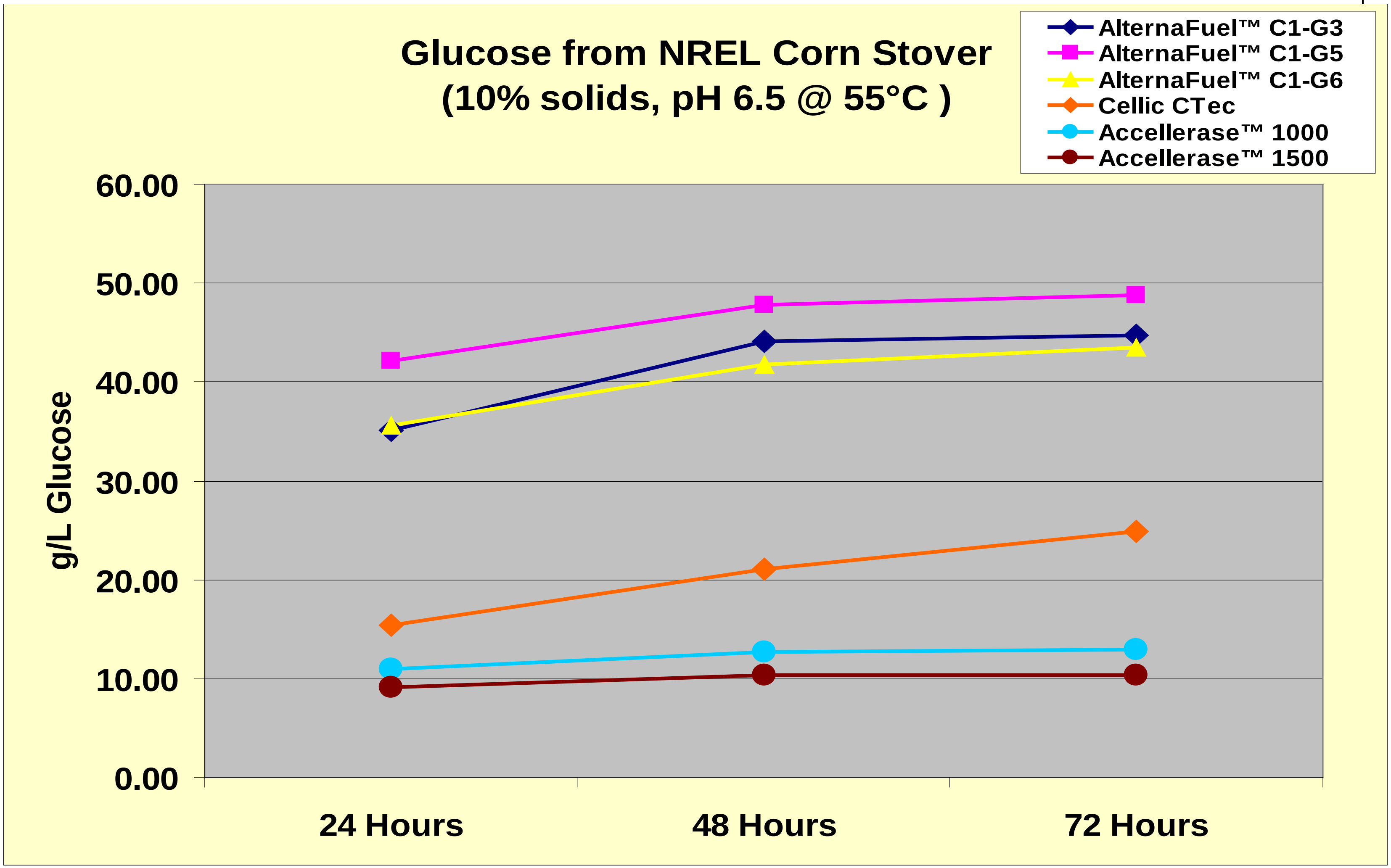


C1 Biofuel Enzymes: Broad Active pH Range

Testing the upper limits of relevant SSF pH's: pH5, pH6, pH7 and pH8



C1 vs. Genencor/Novozymes at pH 6.5

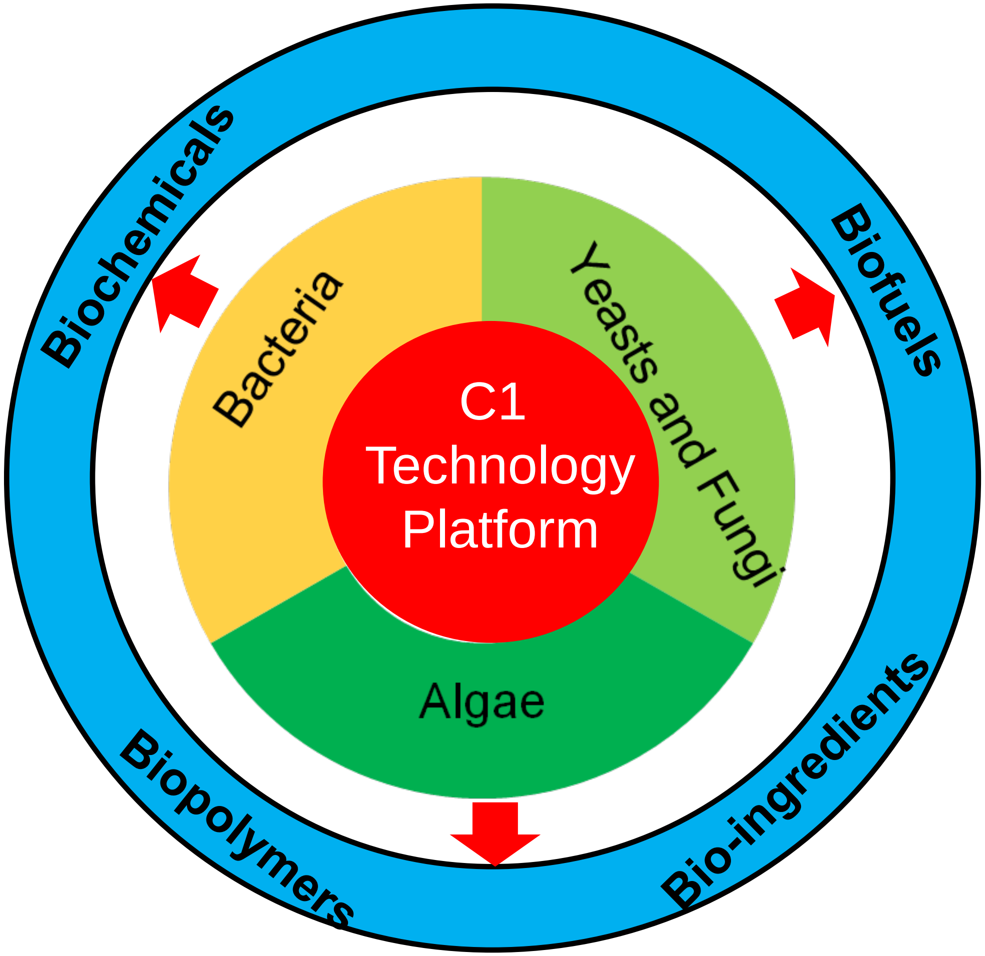


C1--G Series Improvements

Versatile Cellulosic Enzyme Mixtures from a Single C1 Strain

- **Fast Saccharification**: Ethanol process completed in **72** hours
- **Broad Temperature Range**: Active between **32°C** and **60°C**
- **Broad pH Range**: High activity between **pH 4** and **pH 8**
- **Active on a Variety of Biomass Substrates**: E.g., Corn Stover, Wheat Straw, Wheat bran, Sugar Cane Bagasse, Switch Grass, Sorghum, Wood

C1 Technology: High Compatibility with Biobased Processes



U.S. Management Team

Name	Title	Experience
Mark A. Emalfarb	President and CEO Chmn. of the Board/Founder	Dyadic International, Inc.
Anne E. Whitehead, Esq.	Executive Director Strategic Alliances	Shearman & Sterling Skadden Arps
Adam J. Morgan, Esq.	VP General Counsel & Bus. Dev. / Secretary	Advance Publishers, L.C. Rexall Sundown, Inc.
Michael J. Faby, CPA	VP Finance	Perry Slingsby Systems PricewaterhouseCoopers
Richard H. Jundzil	VP Operations	Genzyme Corporation
Thomas M. O’Shaughnessy	VP Sales & Marketing	Hexion Specialty Chemicals Occidental Chemical/GE

Scientific Advisory Board

Name	Title	Experience
Richard Lerner, MD	Chairman	President of the Scripps Research Institute
Carlos Barbas, Ph.D	Advisor	Scripps Research Institute, Chair in Molecular Biology and Chemistry
Arnold Demain, Ph.D	Advisor	Fellow at Charles A. Dana Institute for Scientists Emeriti/MIT /Merck
Arkady Sinitsyn, Ph.D	Advisor	Head of Dept. of Enzymology, Moscow State University (Russia)
Cees van den Hondel, Ph.D	Advisor	Professor of Fungal Genetics, Leiden University

Recent Publications

- Mode of action of *Chrysosporium lucknowense* C1 a-L-arabinohydrolases. Stefan Kühnel, Yvonne Westphal, Sandra W.A. Hinz, Henk A. Schols, Harry Gruppen. **Bioresource Technology 102: 1636-1643 (2011)**
- Branched arabino-oligosaccharides isolated from sugar beet arabinan. Yvonne Westphal, Stefan Kühnel, Pieter de Waard, Sandra W. A. Hinz, Henk A. Schols, Alphons G. J. Voragen, Harry Gruppen. **Carbohydrate Research 345:1180-1189 (2010)**
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Summary—Key Points

- **Broad variety of single (hemi-) cellulase enzymes in C1 is much higher than in the traditional *Trichoderma* host**
  - Better positioned to develop tailor-made enzyme mixtures for a variety of second generation feedstocks

- **Broad operating conditions (pH, temperature) allow applications in different process set-ups**

- **C1 strains have been developed that already produce very efficient and versatile (hemi-) cellulosic enzyme mixtures**

- **Further immediate optimization of C1 is ongoing**

- **Excellent outlook for additional significant enzyme cost reductions in the short term**

Thank You

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