Dyadic® International

Cost efficient production of fuels and chemicals based on lignocellulose

The Dyadic approach

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President and CEO

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
- 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

Dyadic Netherlands

- **Dyadic's Research & Development Subsidiary**
  - 18 employees – 6 with Ph.D.'s
  - Participation in a number of funded international projects

- **Core competencies**
  - Genome Mining
  - Fungal Molecular Biology
  - Fermentation technology
  - Enzymology

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

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

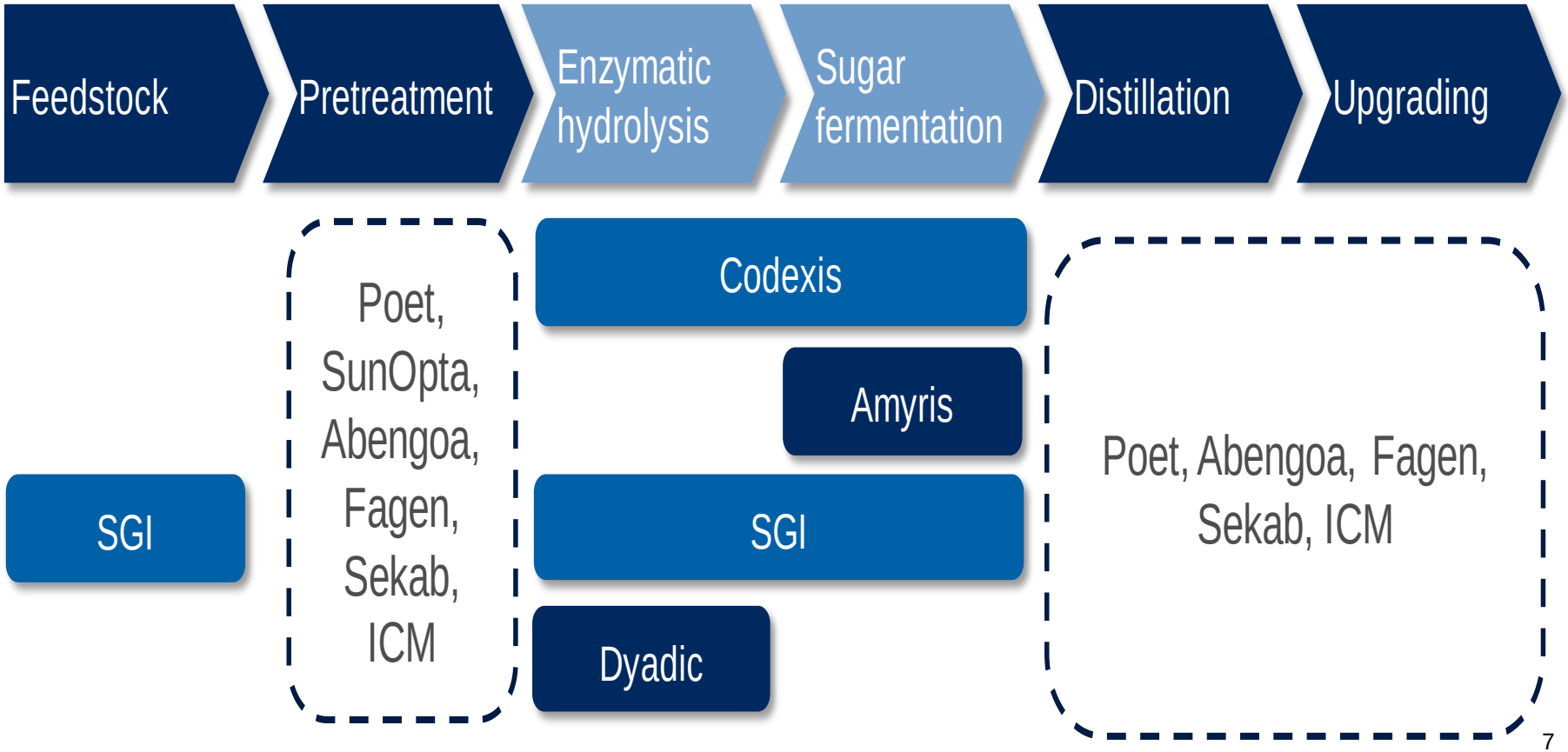
SRNL
SAVANNAH RIVER NATIONAL LABORATORY
We Put Science To Work
U.S. DEPARTMENT OF ENERGY

Savannah River National Laboratory

Kluyver CENTRE | Kluyver Centre for Genomics of Industrial Fermentation

2nd Generation Biofuels

Value Chain



Source: Bloomberg New Energy Finance

The Challenge

To make the production of ethanol/chemicals **economically viable** by **reducing** the contributing **cost of enzymes** to the overall costs of ethanol/chemicals production

The Dyadic Approach

Provide one strain and one process for the

on-site production

of the enzymes needed for

the efficient saccharification of lignocellulose

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 Dyadic Approach

Requirements:

- **Down Stream Processing should be avoided as much as possible**
- **Access to strains that**
  - **produce a wide range of (hemi)-cellulases**
  - **produce high amounts of (hemi)-cellulases**
  - **produce (hemi)-cellulases in a low cost, robust process**
- **Capability to modify the composition of the enzymes produced by the strains (tailor-made strains)**
- **(Hemi)-cellulases should be active in wide pH and temperature range to allow their use in a broad range of applications**

Dyadic's Production Platforms

Aspergillus niger

Trichoderma reesei

*Chrysosporium lucknowense (C1)**

**Agency Response Letter GRAS Notice No. GRN 000292, CFSAN/Office of Food Additive Safety: 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.*

C1 vs. *Trichoderma*

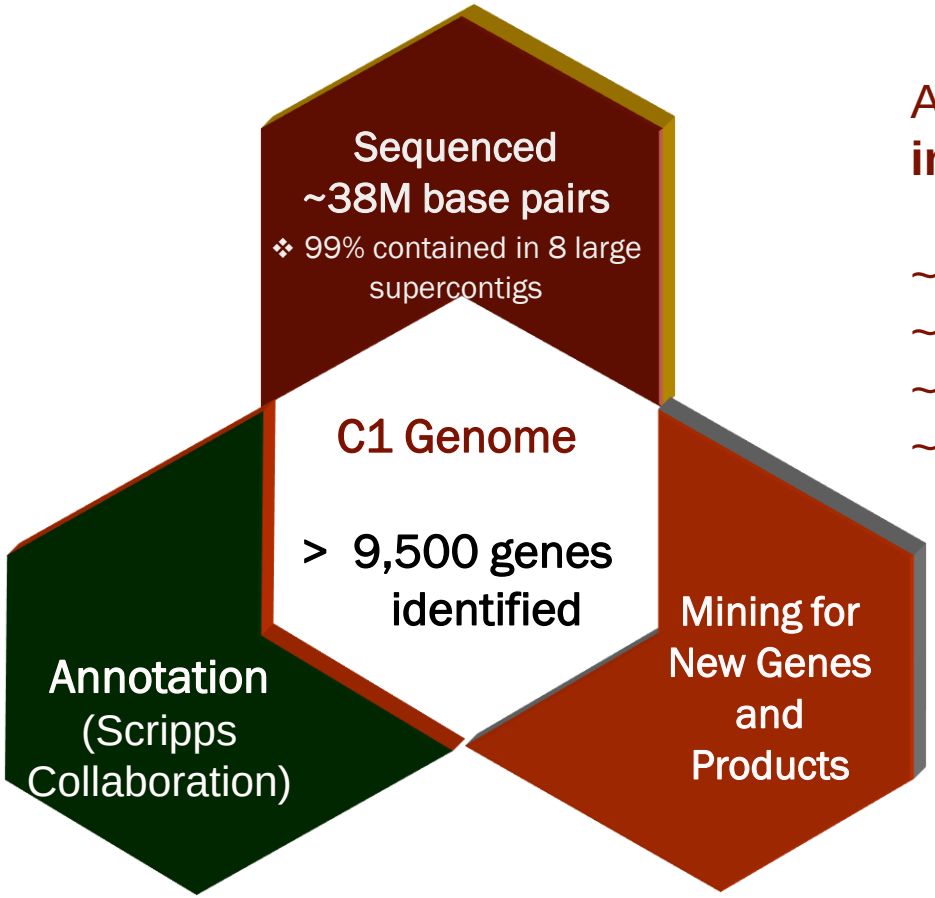
Lignocellulolytic Potential of C1 vs. *Trichoderma reesei*
(the main industrial source for biofuel enzymes, e.g. Accellerase™**)**

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#	

***From the JGI database*
 # Based on literature and JGI database searches

C1 is a Rich Source of Lignocellulolytic Enzymes!

C1: a rich source for (hemi)-cellulases

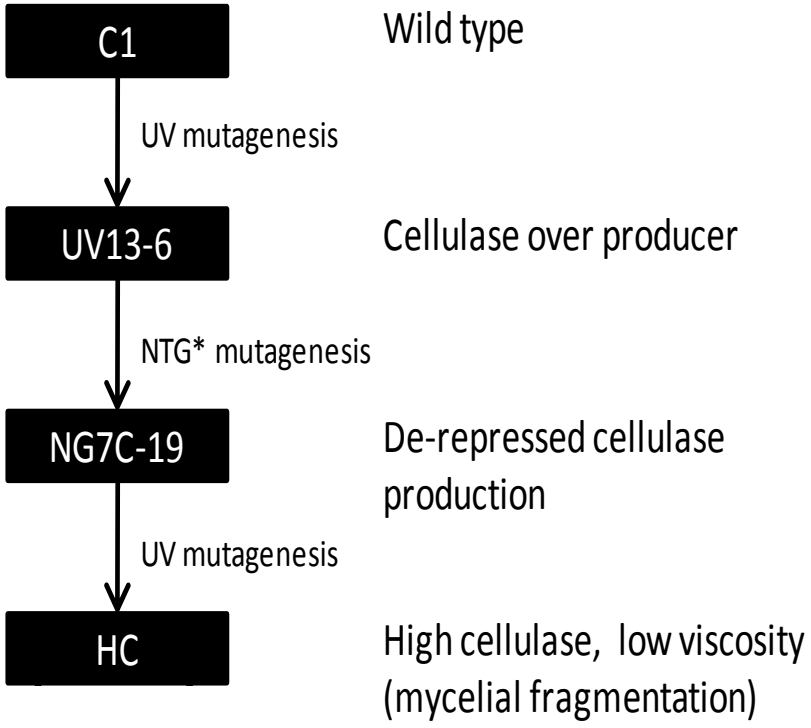


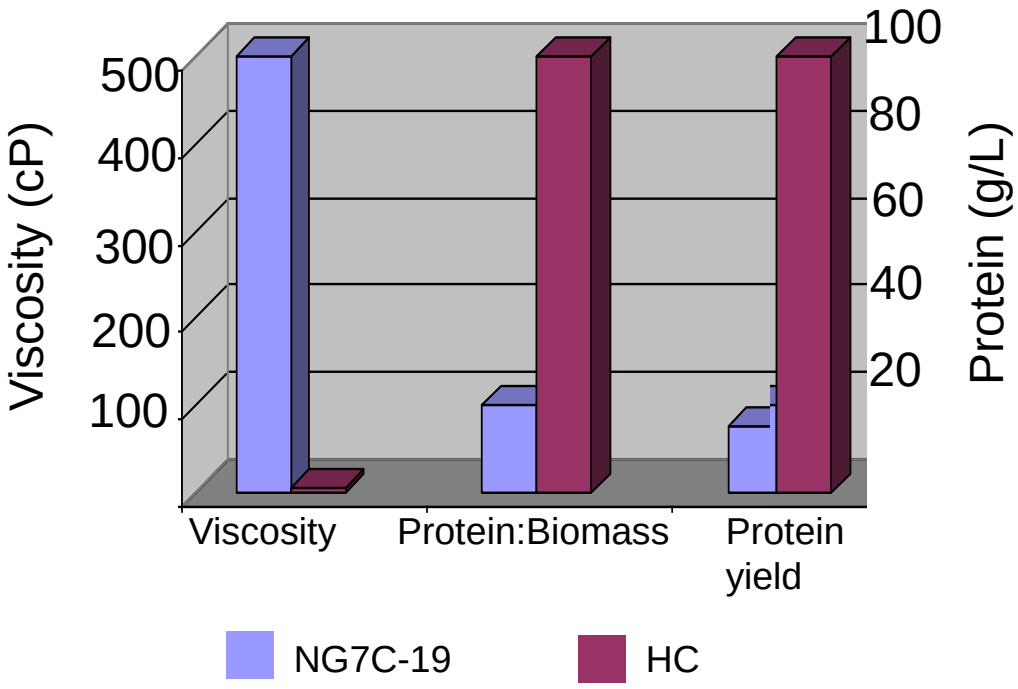
A large number of genes putatively encoding **industrially important** enzymes discovered:

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

The C1 Technology Platform

Development of protein hyper-producing strains





C1: a robust strain

Platform for enzyme and protein production

- **A strain that changes morphology during fermentation**
- **Low viscosity fermentations < 10 cP**
- **High yields (up to 100g/l)**
- **Robust fermentation characteristics (pH 4,5-9, T 25-48C)**

Excellent expression system

- **Can be used to produce homologous and heterologous proteins**

*

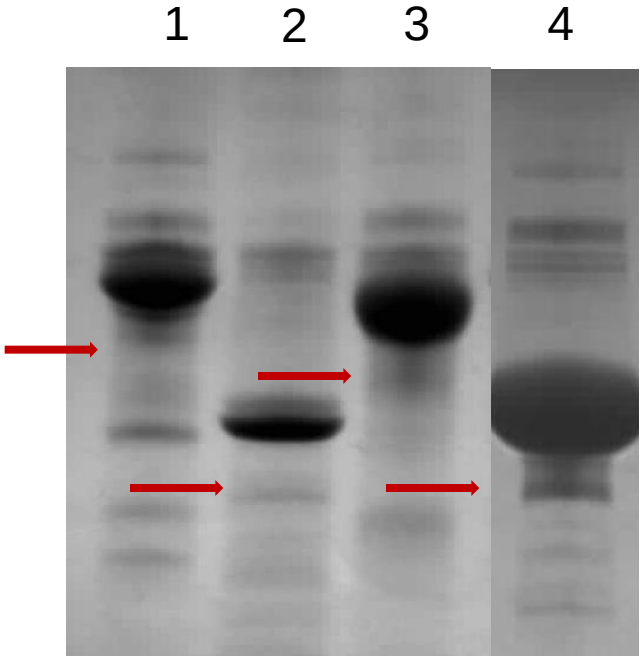
Construction of tailored C1 strains

1. Develop single enzymes in "clean" background strains (LC-strains)

2. Determine the "rate-limiting" enzyme by adding single enzymes to enzyme mix produced by one production strain

3. Overexpress gene(s) encoding the rate limiting enzyme(s) in production strain

Producing single (hemi)-cellulases

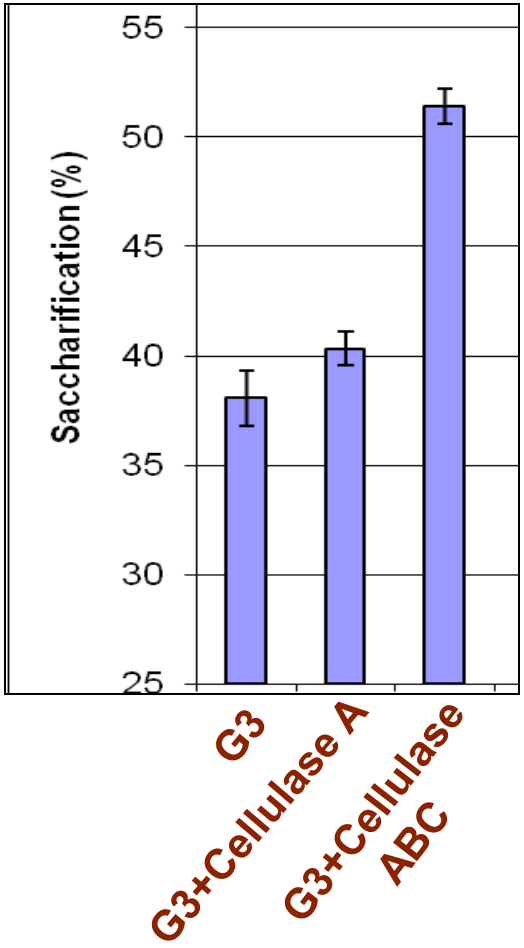


- 70 functional single (hemi-) cellulase expressing strains obtained
- Production levels: up to 40 g/L scale
- Up to 30 g/L of relatively pure target enzyme has been produced

→ Important for both research and commercial purposes

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

Eliminate limitations by mixing with single (hemi)-cellulases



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

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

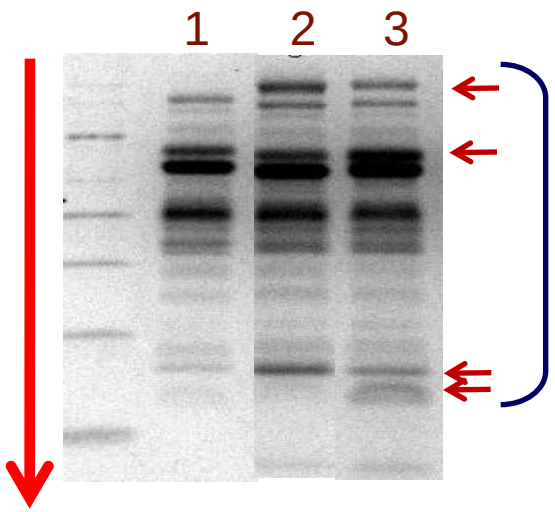
→ New C1-strains developed: **G5 and G7**

Dyadic Approach:

Single Strain to Produce Cost Effective Enzyme Mixtures

Enzyme Loading to Achieve >70% Saccharification

C1 Strain: Baseline Enzyme Mix (G1) 100%



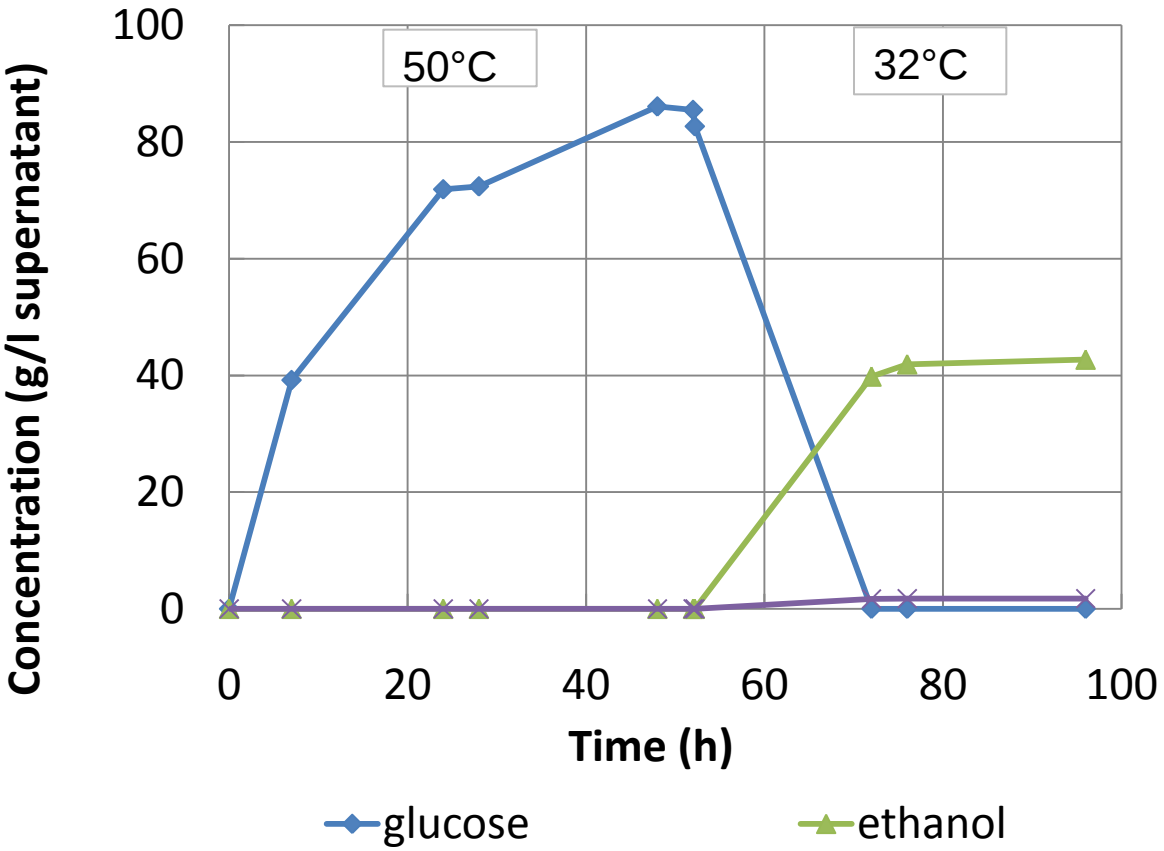
Example:
Specifically overexpressed cellulases

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

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

Hybrid SSF using C1 enzymes

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

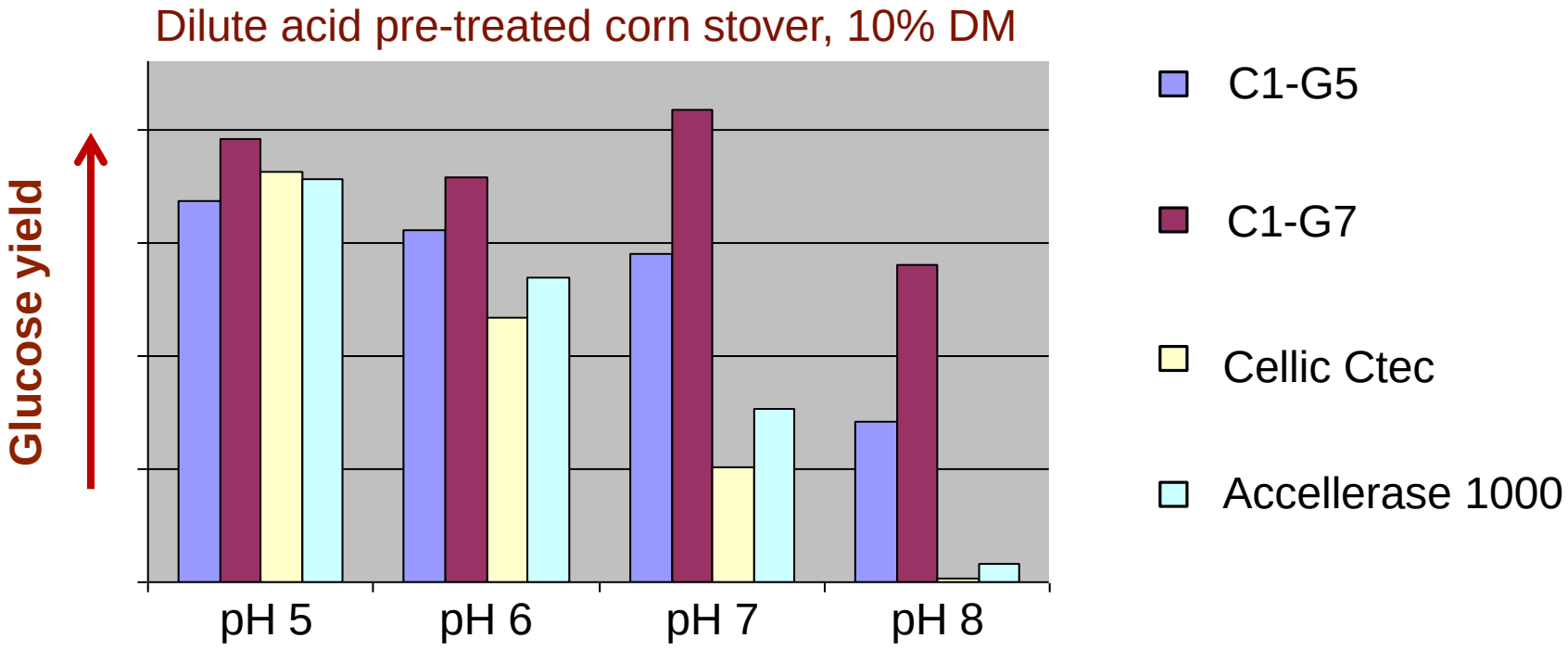


Conclusions:

- 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

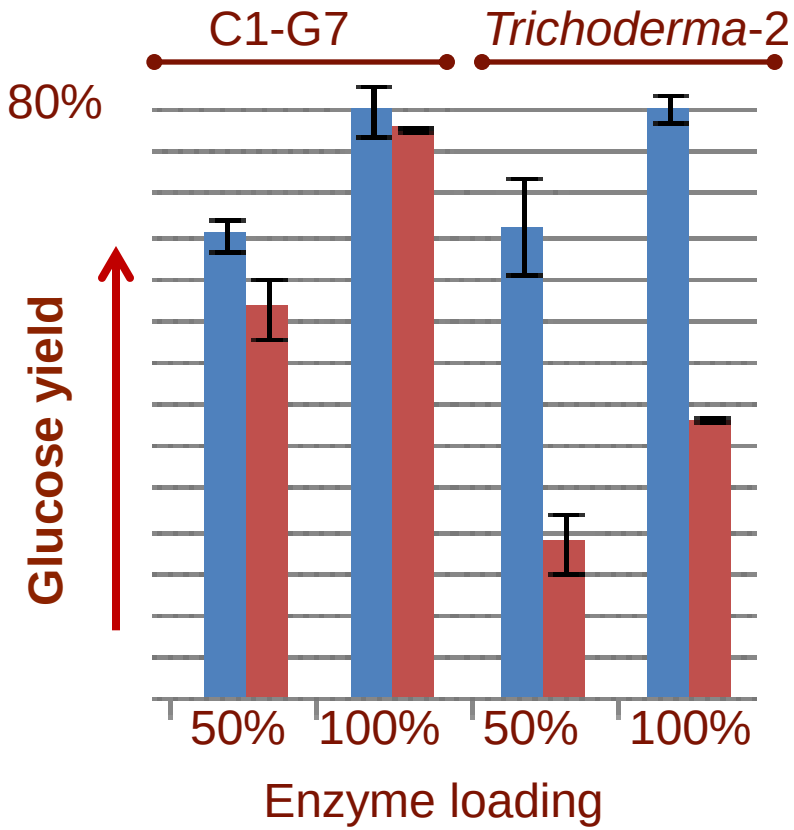
C1 Biofuel Enzymes: Broad Active pH Range

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



C1 Biofuel Enzymes: Broad Active pH Range

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

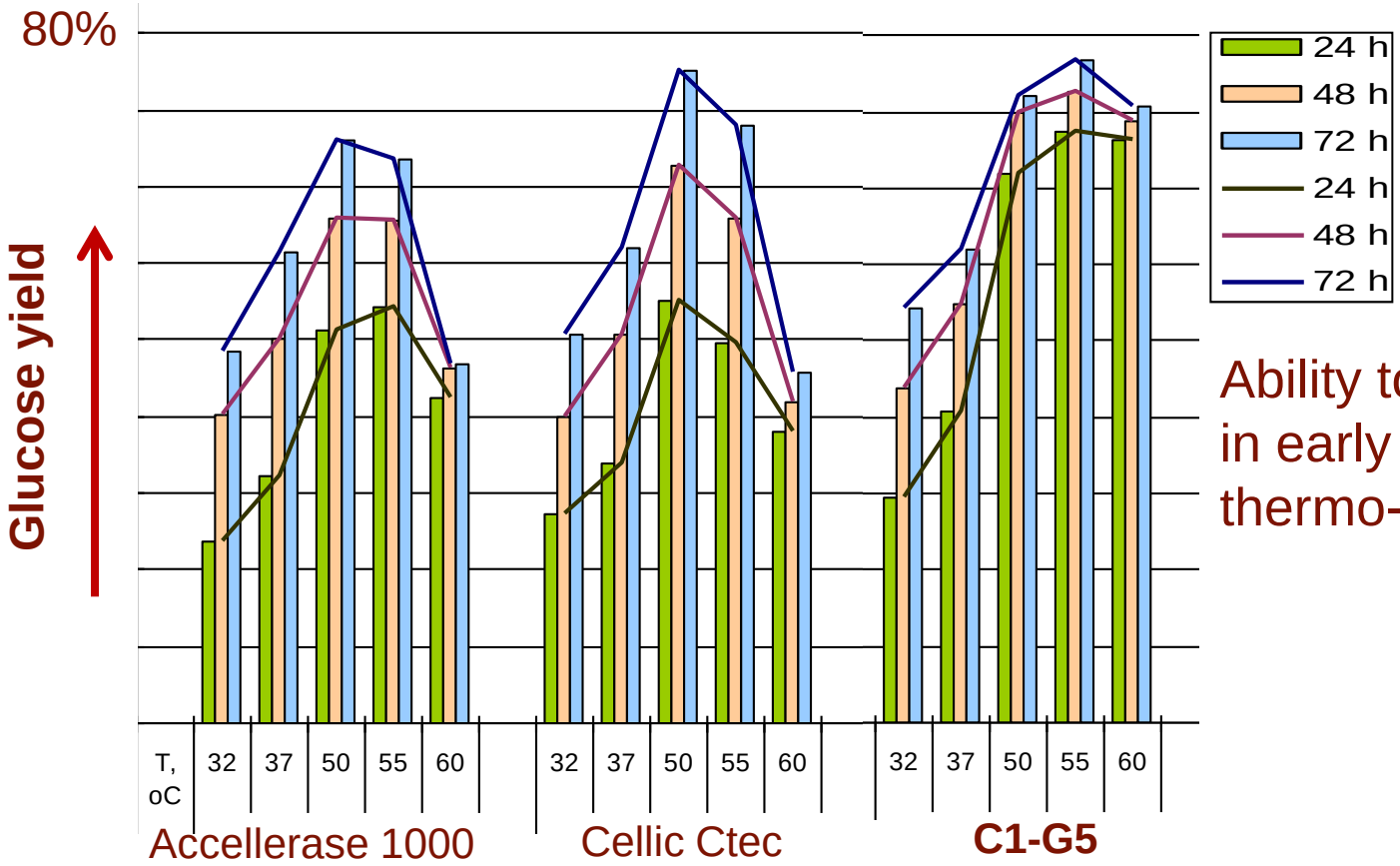


Conclusion:

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

C1 Biofuel Enzymes: Broad Active Temperature Range

Dilute acid pre-treated corn stover, 10% DM



Ability to add enzyme in early stage after thermo-pretreatment

Strong Intellectual Property

- **10 issued U.S. patents**
  - Broad claims blocking use of C1
- **10 pending U.S. patent applications**
- **74 issued foreign patents**
- **23 pending foreign applications**


The United States Of America

The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America for the term set forth below, subject to the payment of maintenance fees as provided by law.

If this application was filed prior to June 8, 1995, the term of this patent is the longer of seventeen years from the date of grant of this patent or twenty years from the earliest effective U.S. filing date of the application, subject to any statutory extension.

If this application was filed on or after June 8, 1995, the term of this patent is twenty years from the U.S filing date, subject to any statutory extension. If the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121 or 365(c), the term of the patent is twenty years from the date on which the earliest application was filed, subject to any statutory extensions.

Director of the United States Patent and Trademark Office

The Dyadic Approach

Requirements vs Achievements

- **DSP should be avoided as much as possible;**
  No DSP has to be used; whole fermentation broth can be applied

- **Access to strains that**
  - **produce a wide range of (hemi)-cellulases;**
    More than 285 identified
  - **produce high amounts of (hemi)-cellulases;**
    Yields of up to 100 g protein/l can be obtained
  - **produce (hemi)-cellulases in a low cost, robust process:**
    Low viscosity and low cost fermentation characteristics of C1

The Dyadic Approach

Requirements vs Achievements

- **Capability to modify the composition of the enzymes produced by the strains (tailor-made strains)**

  Extensive genetic toolbox is available; resulting strains do not contain foreign DNA

- **(Hemi)-cellulases should be active in wide pH and temperature range to allow their use in a broad range of applications.**

  C1 enzymes are active between 32°C and 60°C at a pH between 4 and 8

The C1 Technology Platform

Additional advantages

Large scale experiments have shown that

- **C1 enzymes work very fast in comparison to *Trichoderma* enzymes**
- **C1 enzyme activity results in a fast reduction of viscosity**

The C1 enzymes are active on a Variety of Biomass Substrates:
E.g., Corn Stover, Wheat Straw, Wheat bran, Sugar Cane Bagasse, Switch Grass, Sorghum, Wood

The improved C1 strains do not contain foreign DNA

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

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

Conclusion

C1 strains have been developed that produce in one fermentation run a mix of enzymes that allow for a cost efficient production of bioethanol or chemicals based on lignocellulose present in a wide variety of agricultural waste streams.

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

Dyadic's Biofuels Partners

ABENGOA

CODEXIS®

Shell

cosan

- Non-exclusive licensees

Thank You

For more information about Dyadic and its C1 Platform Technology, you are invited to visit our stand

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