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#### A novel biocatalyst for Cellulosic Ethanol Production

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#### First Annual TIMBR Conference on Cellulosic Biofuels

University of Massachusetts Amherst ~ September 19, 2008

# A novel biocatalyst for cellulosic ethanol production

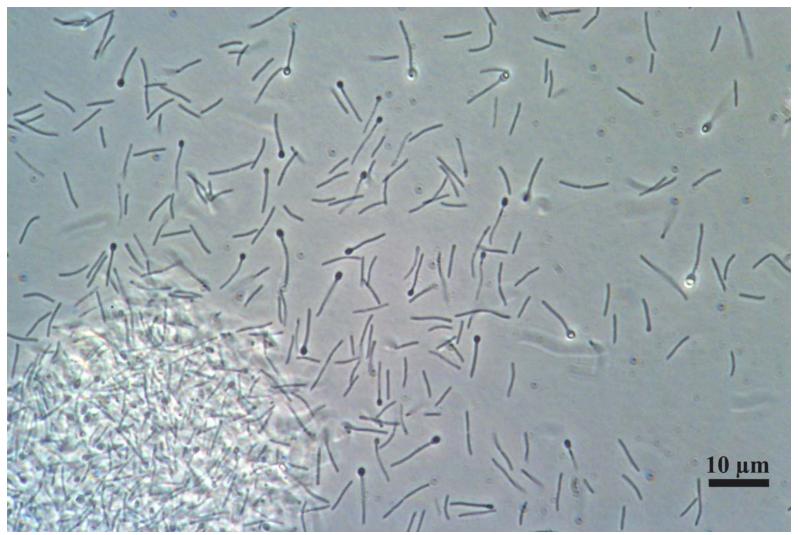
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**University of Massachusetts Amherst** 



## A novel biocatalyst for cellulosic ethanol production



#### Clostridium phytofermentans ~ Q Microbe™

## A novel biocatalyst for cellulosic ethanol production

- Cellulosic Ethanol Technologies
  - ✓ Key impediments
  - Complete Cellulosic Conversion (C3) Technology
  - Clostridium phytofermentans (Q) Characteristics
    - ✓ Naturally occurring bacterium
    - ✓ C3 biocatalyst

Clostridium phytofermentans ~ Q Microbe™

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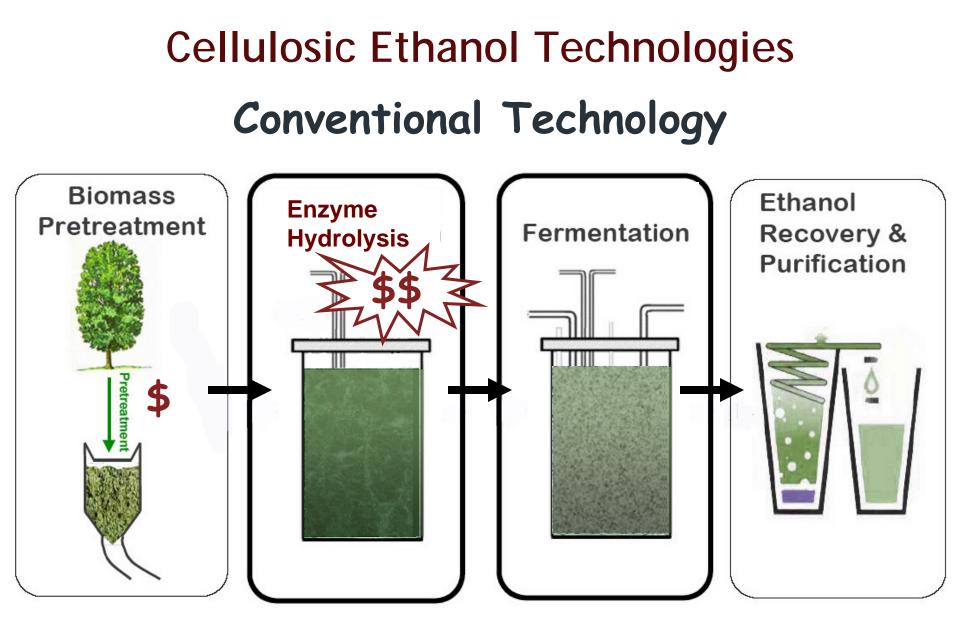
### **Cellulosic Ethanol**

Fossil fuel combustion has led to global warming.

As we meet our energy negreenhouse gas emissions.

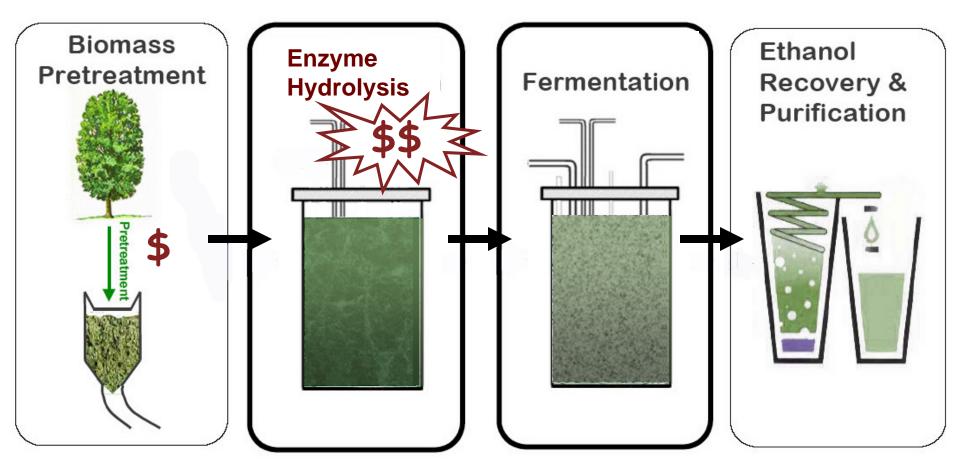
Key Impediment!

 The only form of energy that contribute substantially to meeting transportation fuel needs at costs competitive with fossil fuel is solar energy captured by photosynthesis and stored in biomass.



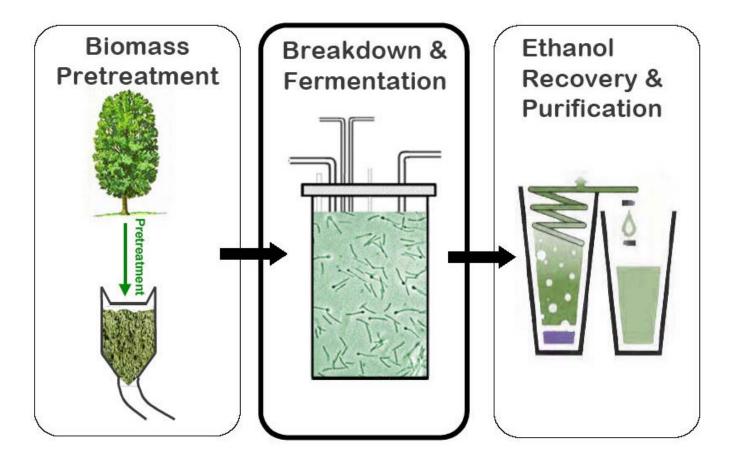
Current cellulosic ethanol processes require enzymes \$\$

## Cellulosic Ethanol Technologies Q Microbe - C3 Technology



Enzyme production, cellulose breakdown, fermentation are consolidated in a single step in a bioreactor

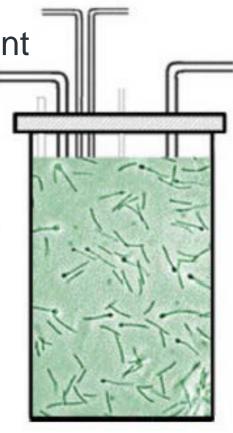
## Cellulosic Ethanol Technologies Q Microbe - C3 Technology



DOE Biofuels Roadmap: *"The ultimate low-cost configuration"* for cellulosic ethanol production.

## Properties of a C3 Microbe

- simultaneously ferment multiple different components of biomass
- ✓ ferment high concentrations of biomass
- produce ethanol as primary product of fermentation
- ✓ exhibit ethanol tolerance



## Q fits the bill!

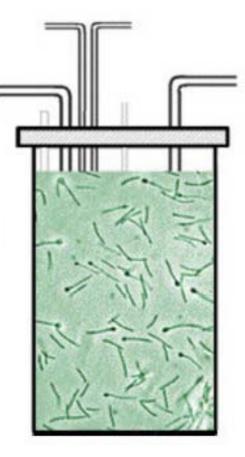


## SunEthanol, Inc.

✓ biofuels technology company

✓ headquartered in Amherst, MA

✓ commercializing the
*Q-Microbe*<sup>™</sup>- C3 technology
for cellulosic ethanol production



## Q fits the bill!

## *Q Microbe* is a novel, naturally occurring C3 microbe



- Isolated from forest soil near Quabbin Reservoir
- Part of a study: Diversity of anaerobic cellulosedecomposing microbes
- Role in the global carbon cycle





## Cellulose-fermenting microbes are at the base of the anaerobic food chain



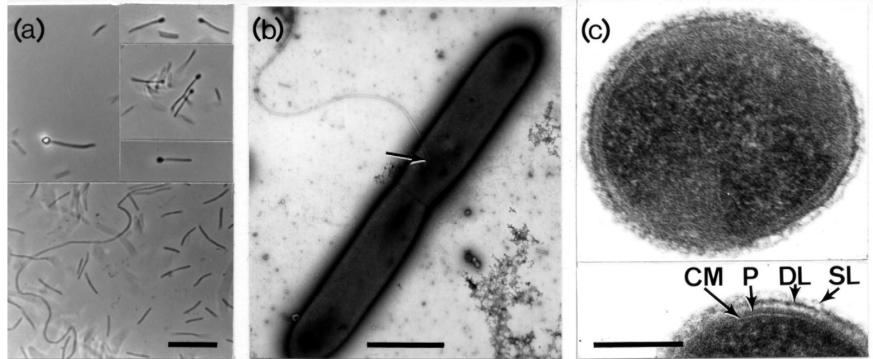
#### Cedar Swamp, Woods Hole

## Diversity of cellulose-fermenting bacteria from soils and sediments

#### Most Isolates:

- morphologically & physiologically similar
- closely related, members of "Cluster III" of clostridia

#### Q is a novel microbe "Cluster XIVa"



## Uncommon properties of Q make C3 technology possible

- Directly converts all fermentable components of biomass (cellulose, xylan, pectin, & starch) to ethanol at >90% theoretical efficiency
- Ethanol is its primary product
- Ferments broad range of feedstocks - e.g., paper sludge, corn stover, grass clippings *very versatile!*
- Efficiently decomposes plant cell walls

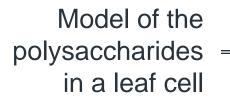


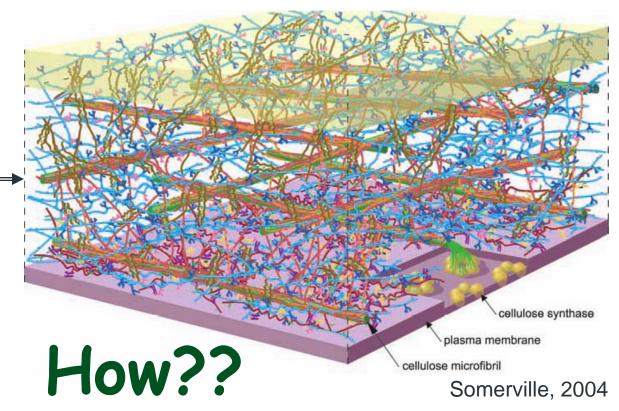
#### Q digests paper



## Plant Cell Walls are Tough & Complex!

- Composed mostly of high molecular weight polysaccharides
- Cellulose is the primary structural component
- Provides strength and a barrier to infection by pathogens

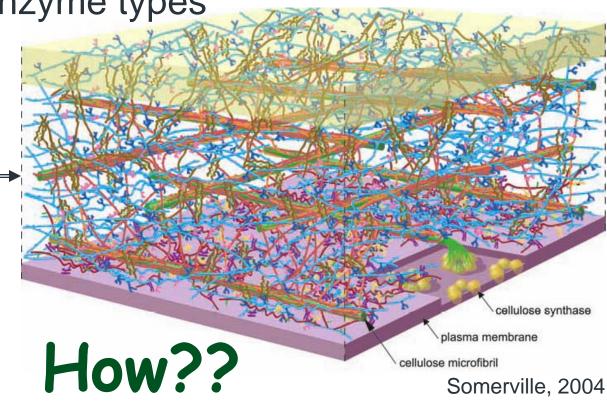




## Common Theme for Efficient Degradation of Plant Cell Walls

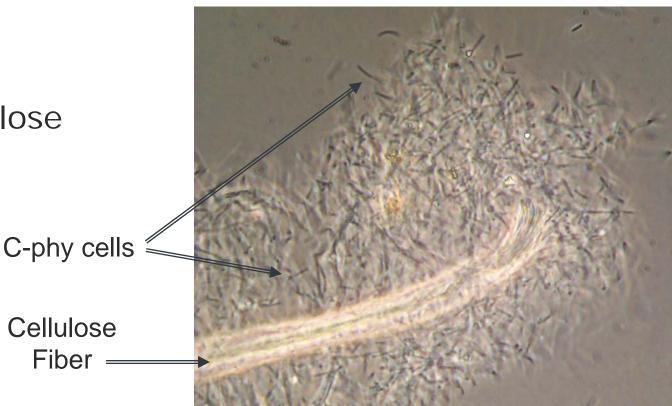
- Efficient binding of enzymes to substrate
- Synergy between enzymes & microbe
- Multiplicity of enzyme types

Model of the polysaccharides in a leaf cell

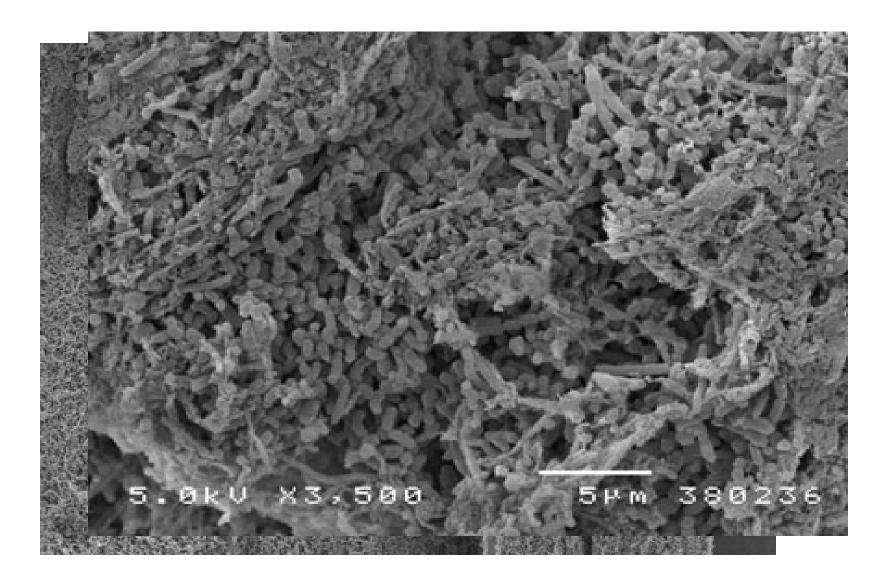


## "Enzyme-Microbe Synergy" Cells form cellulose-enzyme-microbe complexes

- Specific cellulose hydrolysis rates by cells > purified cellulase preparations (Lu, Zhang, Lynd, 2006)
- Suggests an advantage of C3
- Q grows as a biofilm on cellulose



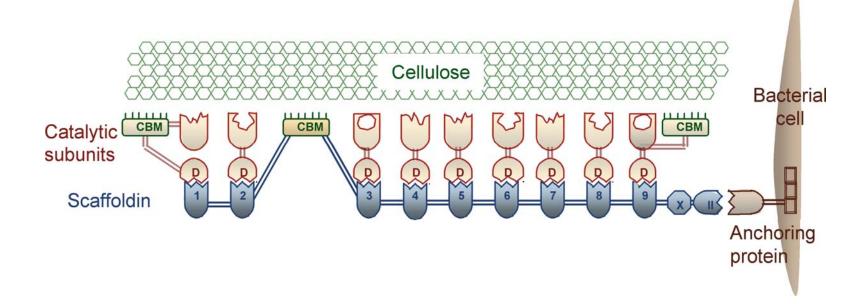
### Q grows as a biofilm on cellulose



## What is the nature of Q's enzyme system?

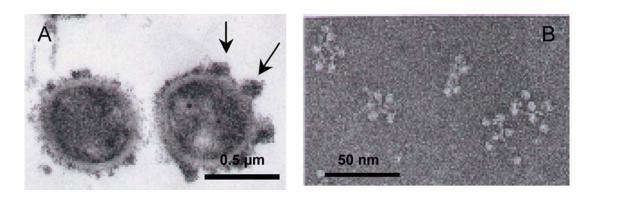
- Cellulolytic bacteria produce extracellular enzymes
- Multiple enzymes digest insoluble biomass

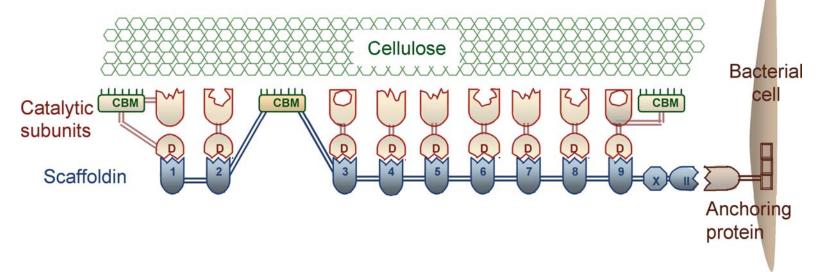
Most cellulolytic anaerobes produce multiprotein enzyme complexes: **Cellulosomes** 



## Cellulosomes

## Multi-protein enzyme complexes that degrade cellulose



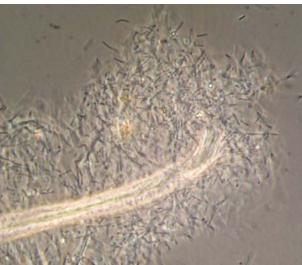


## Q produces a multi-component <u>non-complexed</u> cellulase system (non-cellulosomal)

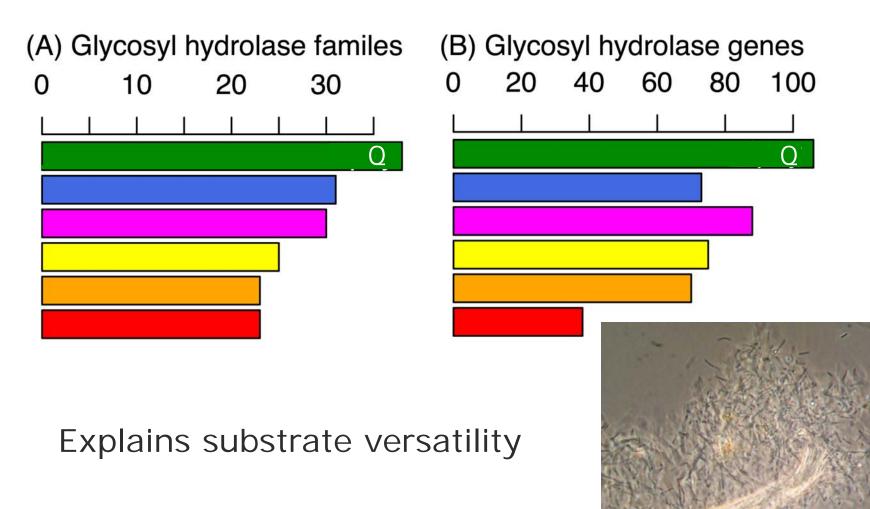
Based on analyses of Q's genome sequence:

- No evidence for scaffoldin-like proteins
- No evidence for dockerin-containing carbohydrateactive enzymes (e.g., hydrolases)

No evidence for cellulosomes!



## The genome of Q contains a great diversity & abundance of plant-degrading enzymes



### What's next for *Q*?

## Analyses of the Q genome & gene expression UMass TIMBR Team

- Q adjusts its metabolism & production of enzymes in response to feedstock.
- Q produces cellular machinery & metabolic pathways
  - ✓ to process sugar products of feedstock degradation
  - ✓ to generate energy for growth
- Q naturally integrates complex cellular processes



### Clostridium phytofermentans ~ Q Microbe™

A voracious appetite for plant polysaccharides

Effective metabolic pathways

An efficient microbial catalyst & model system for biofuel production

Microbial catalyst in C3 technology

SunEthanol

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Thank you!

