

USDA Seminar Series: Agricultural Science & Technology, and Sustainability

January 23, 2008

“A novel microbial catalyst for cellulosic ethanol production”

Susan Leschine

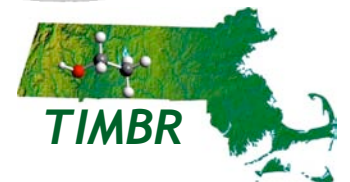
The Institute for Massachusetts Biofuels Research
University of Massachusetts Amherst



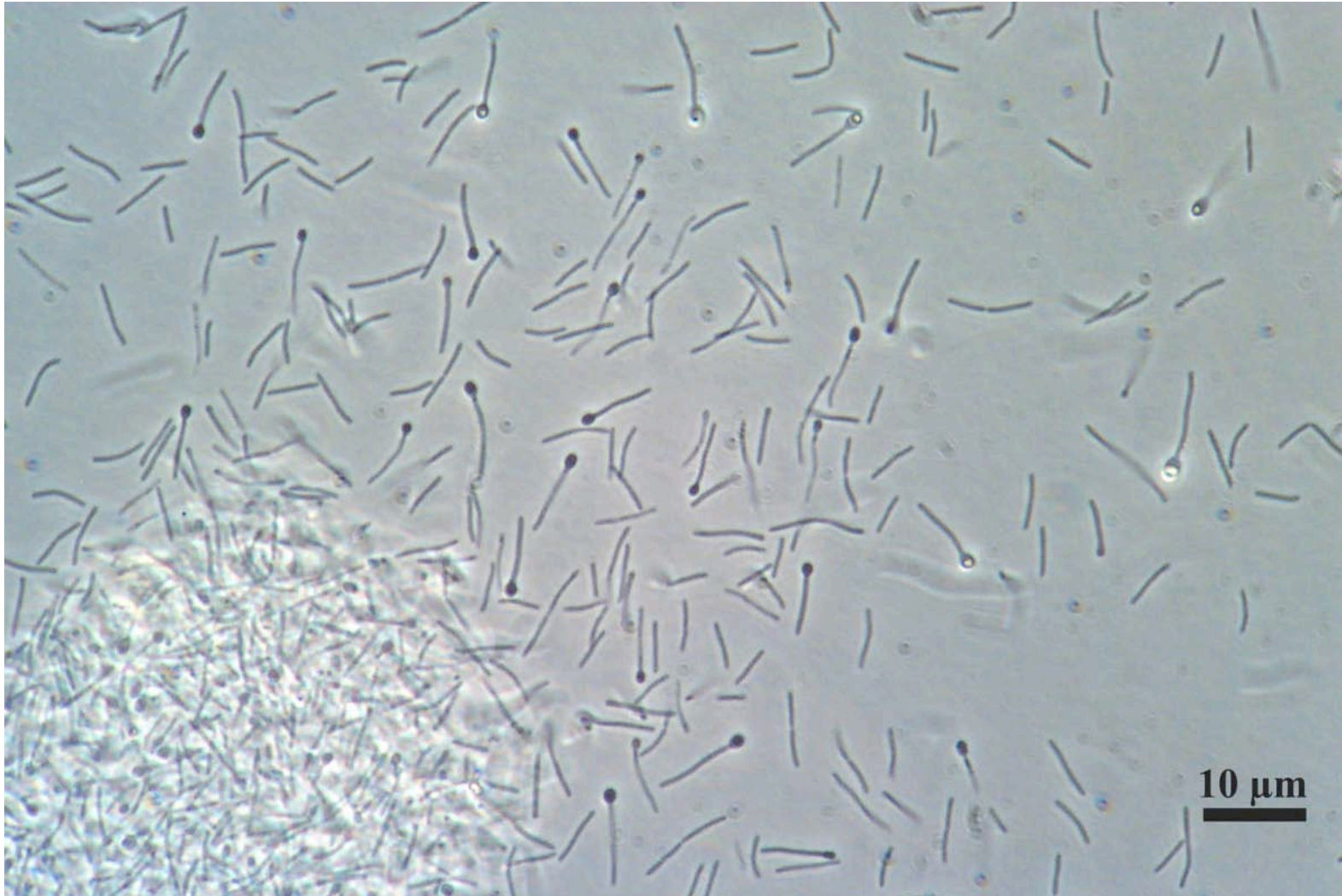
SunEthanol

and

SunEthanol, Inc.



A novel microbial catalyst for cellulosic ethanol production



Clostridium phytofermentans (The Q Microbe)

A novel microbial catalyst for cellulosic ethanol production

- Cellulosic Ethanol Technologies

- ✓ Key impediments

- ✓ **C**onsolidated **BioP**rocessing

- The Q Microbe

- ✓ The Q microbe is a naturally occurring CBP organism

- ✓ Characteristics

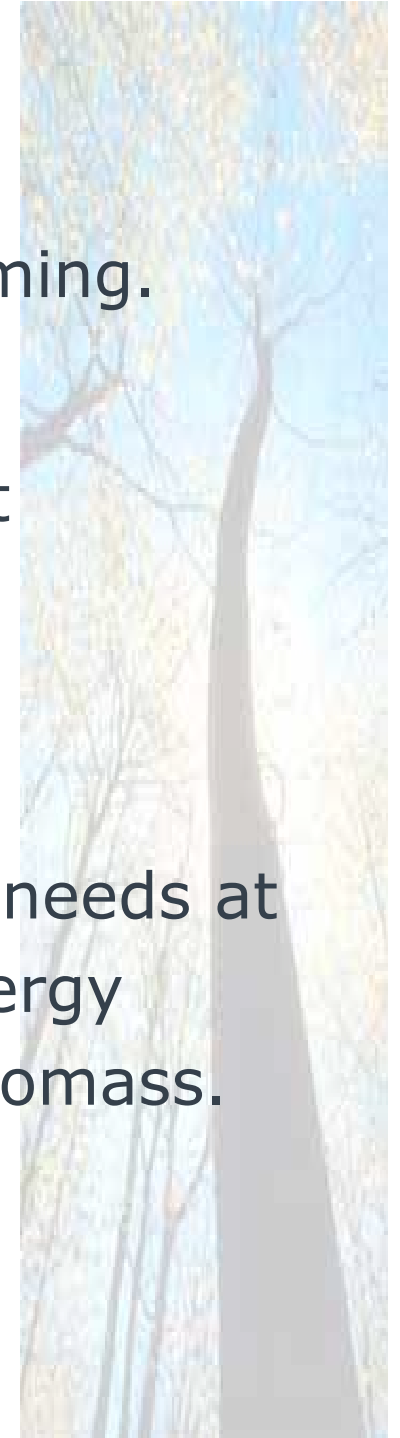
The Energy Independence and Security Act of 2007

- Signed into law December 19, 2007
- Amends Renewable Fuels Standard (RFS)
- Total RFS: 36 B gallons/yr by 2022
- Cellulosic Biofuel: 16 B gallons/yr by 2022



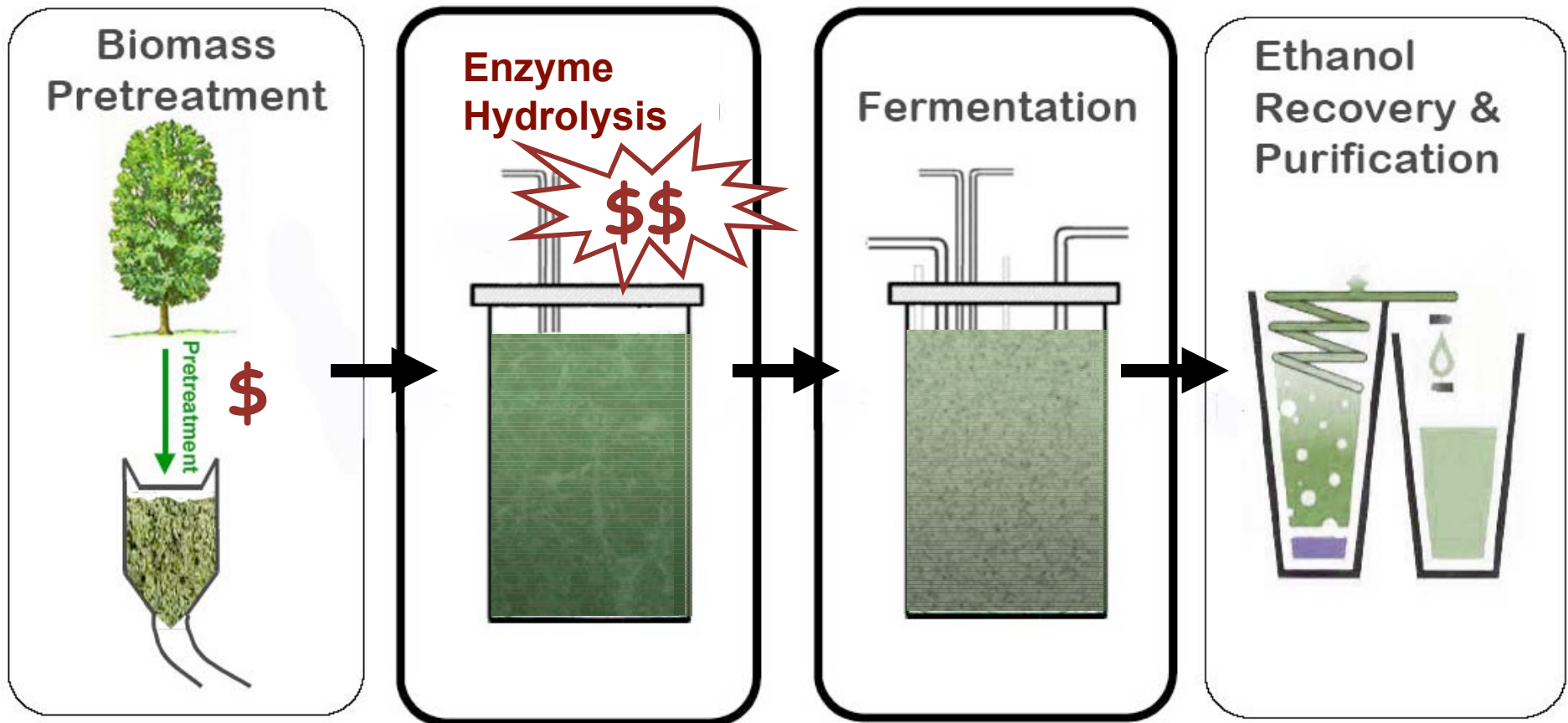
Cellulosic Ethanol

- Fossil fuel combustion has led to global warming.
- As we meet our energy needs, we must limit greenhouse gas emissions.
- The only form of energy that can contribute substantially to meeting transportation fuel needs at costs competitive with fossil fuel is solar energy captured by photosynthesis and stored in biomass.



Cellulosic Ethanol Technologies

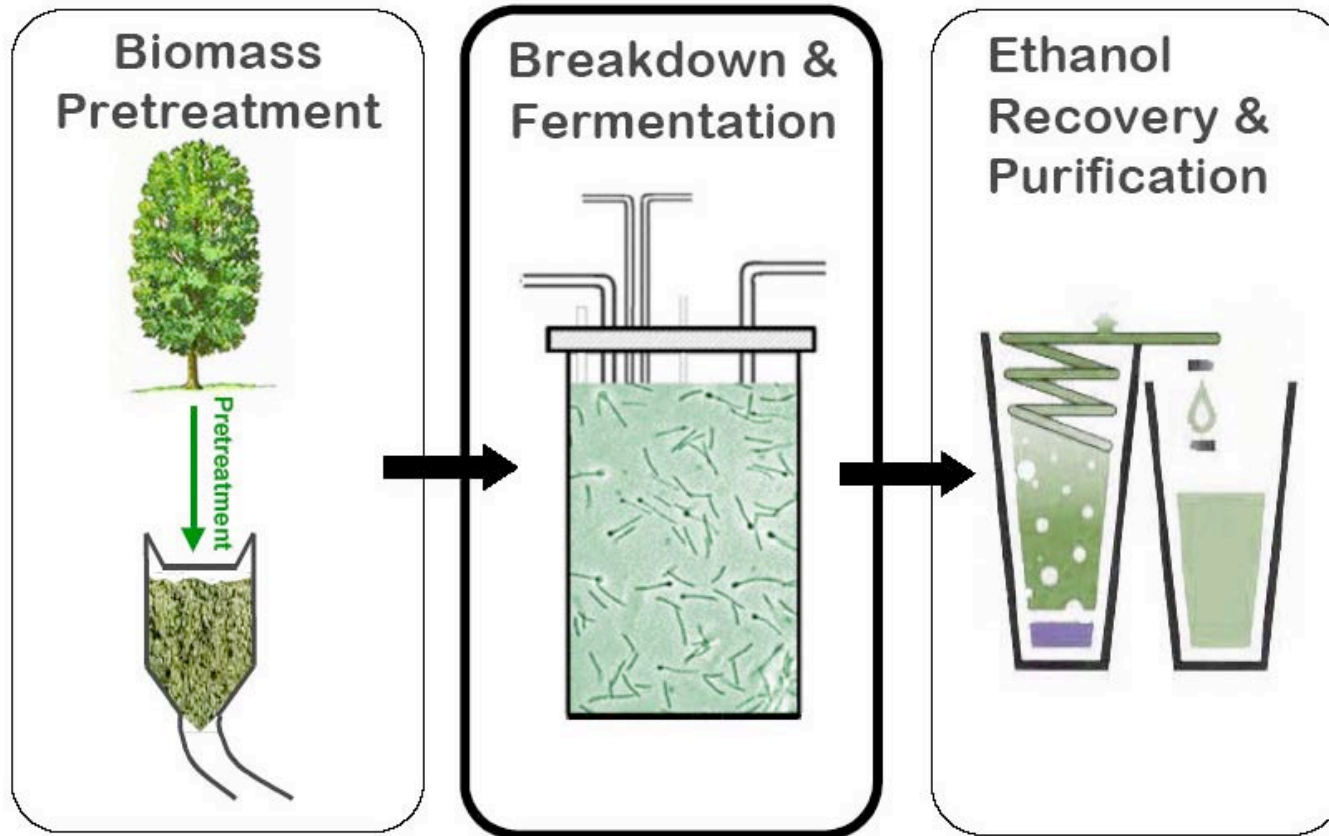
Existing Technologies



Current cellulosic ethanol processes require
enzymes \$\$

Cellulosic Ethanol Technologies

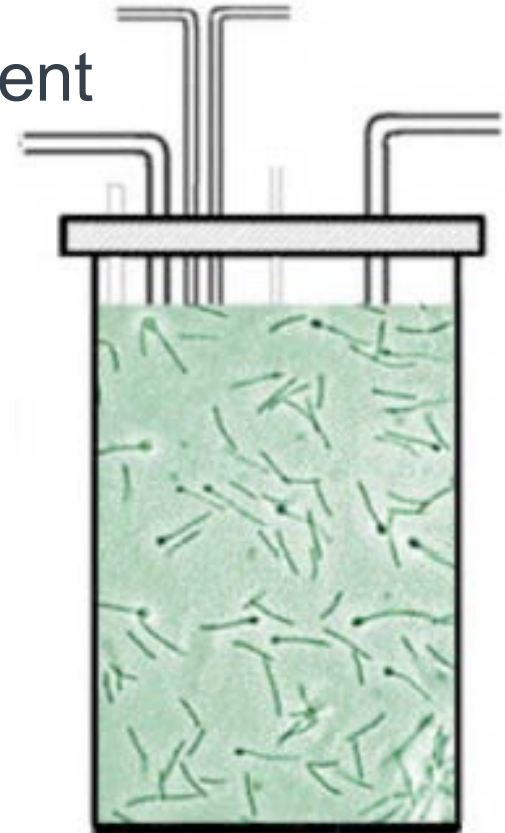
Consolidated BioProcessing



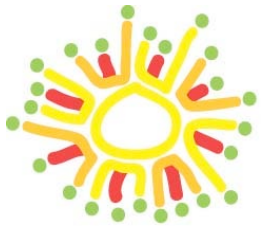
Enzyme production, cellulose breakdown, fermentation are consolidated in a single step in a bioreactor

Properties of a CBP Microbe

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

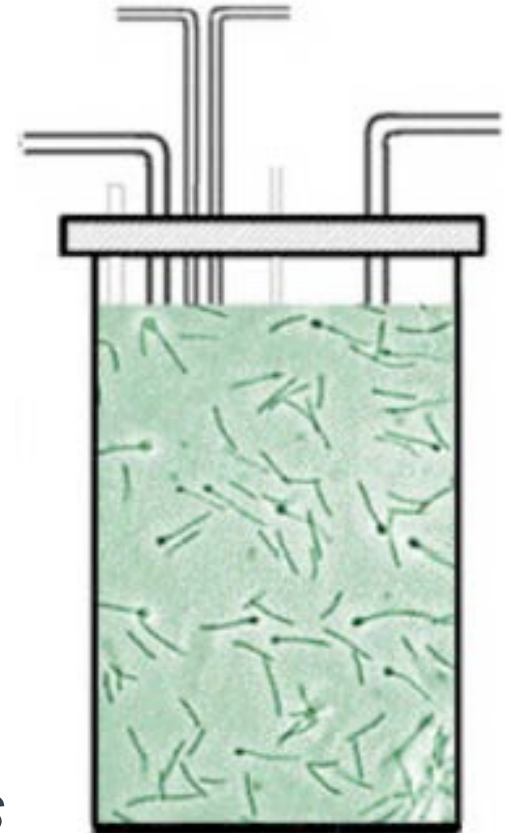


The Q Microbe exhibits the properties of a CBP microbe



SunEthanol, Inc.

- ✓ biofuels technology company
- ✓ headquartered in Amherst, MA
- ✓ commercializing bioethanol CBP technology
- ✓ **Q microbe**
strain of *Clostridium phytofermentans*



The Q Microbe exhibits the properties of a CBP microbe

Q *microbe* is a naturally occurring CBP *microbe*



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



Tom Warnick collecting forest
soil samples

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



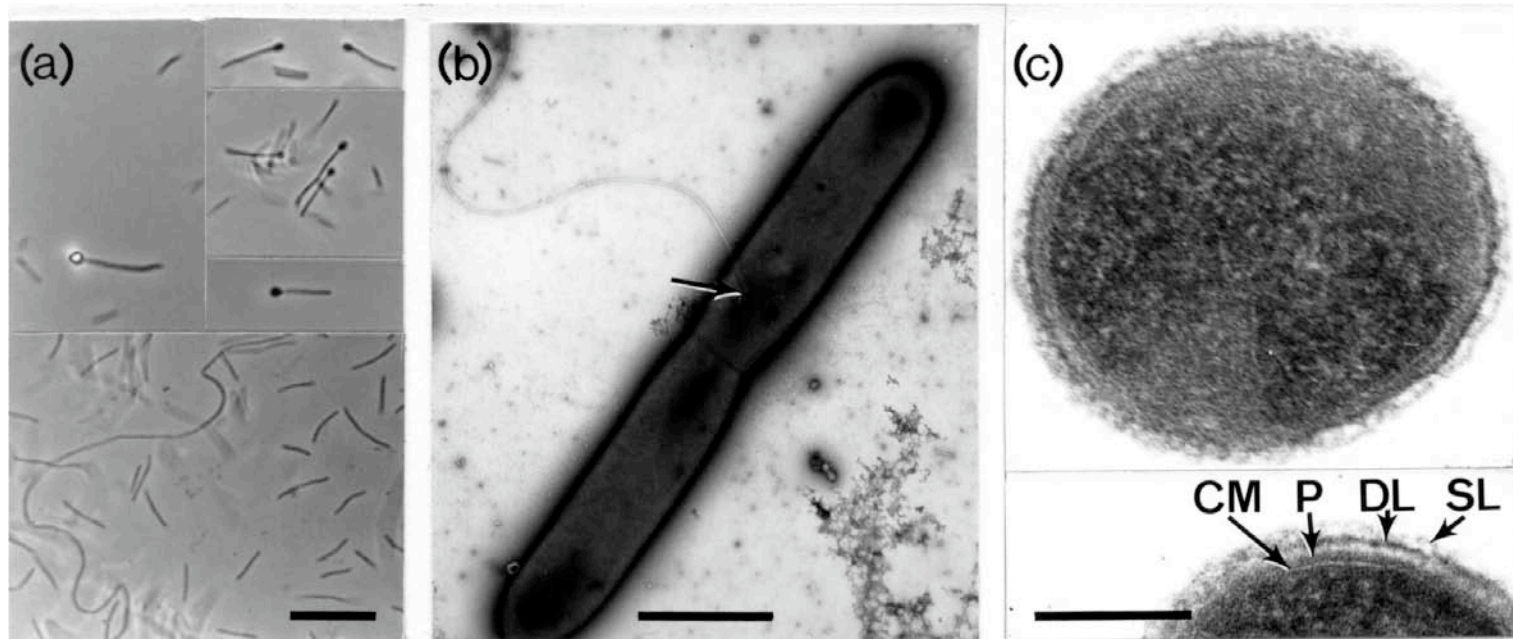
Cedar Swamp, Woods Hole

Diversity of cellulose-fermenting bacteria from soils and sediments

Most Isolates:

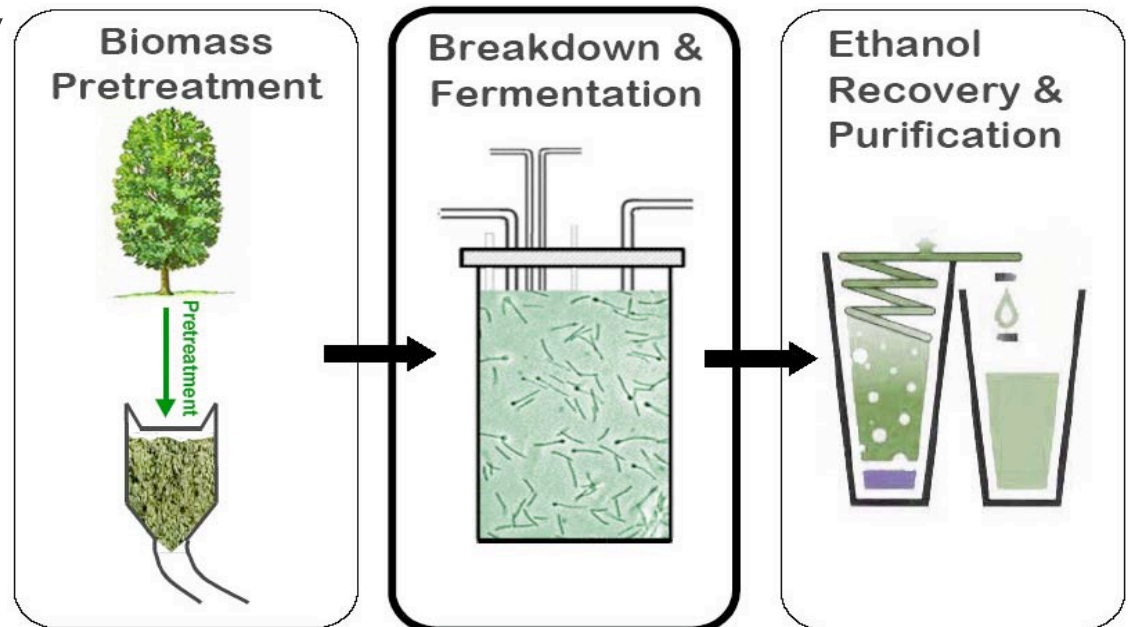
- morphologically & physiologically similar
- closely related, members of “Gram+ Cluster III”

Q **microbe** is a novel microbe “Gram+ Cluster XIVa”



Uncommon properties of the *Q* *microbe* make CBP technology possible

- Directly converts all fermentable components of biomass (cellulose, xylan, pectin, & starch) to ethanol
- Ferments broad range of feedstocks -- very versatile
- Ethanol is its primary product
- Ferments unusually high concentrations of cellulose



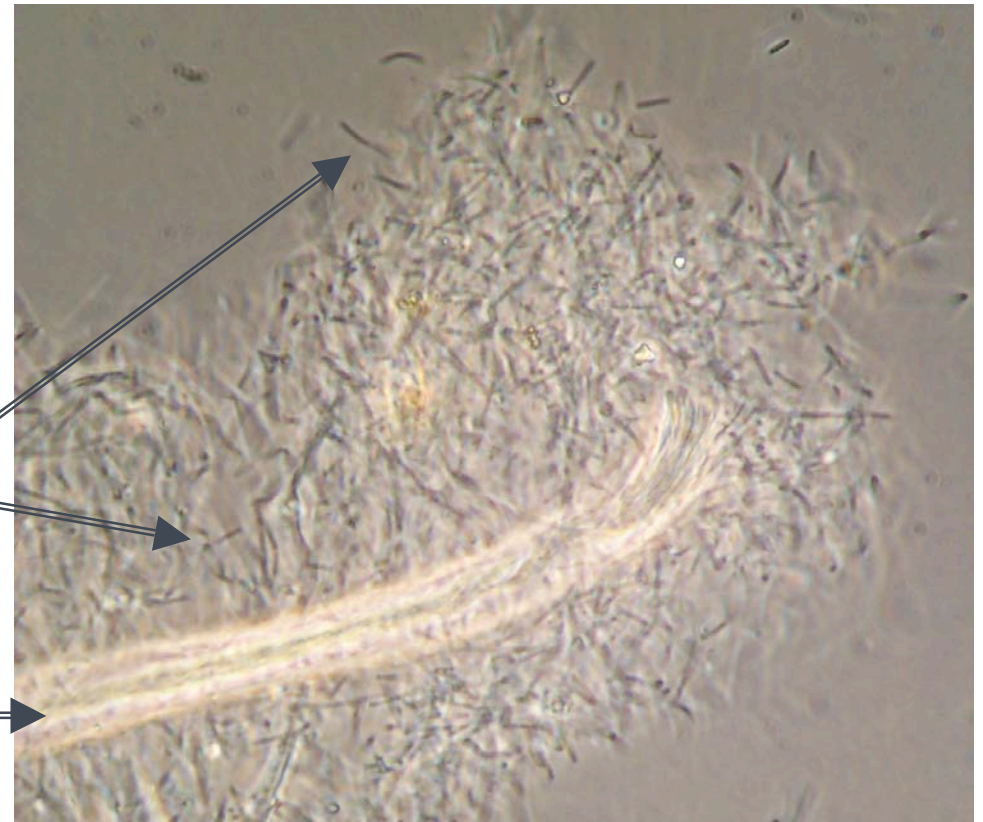
“Enzyme-Microbe Synergy”

Q cells form cellulose-enzyme-microbe complexes

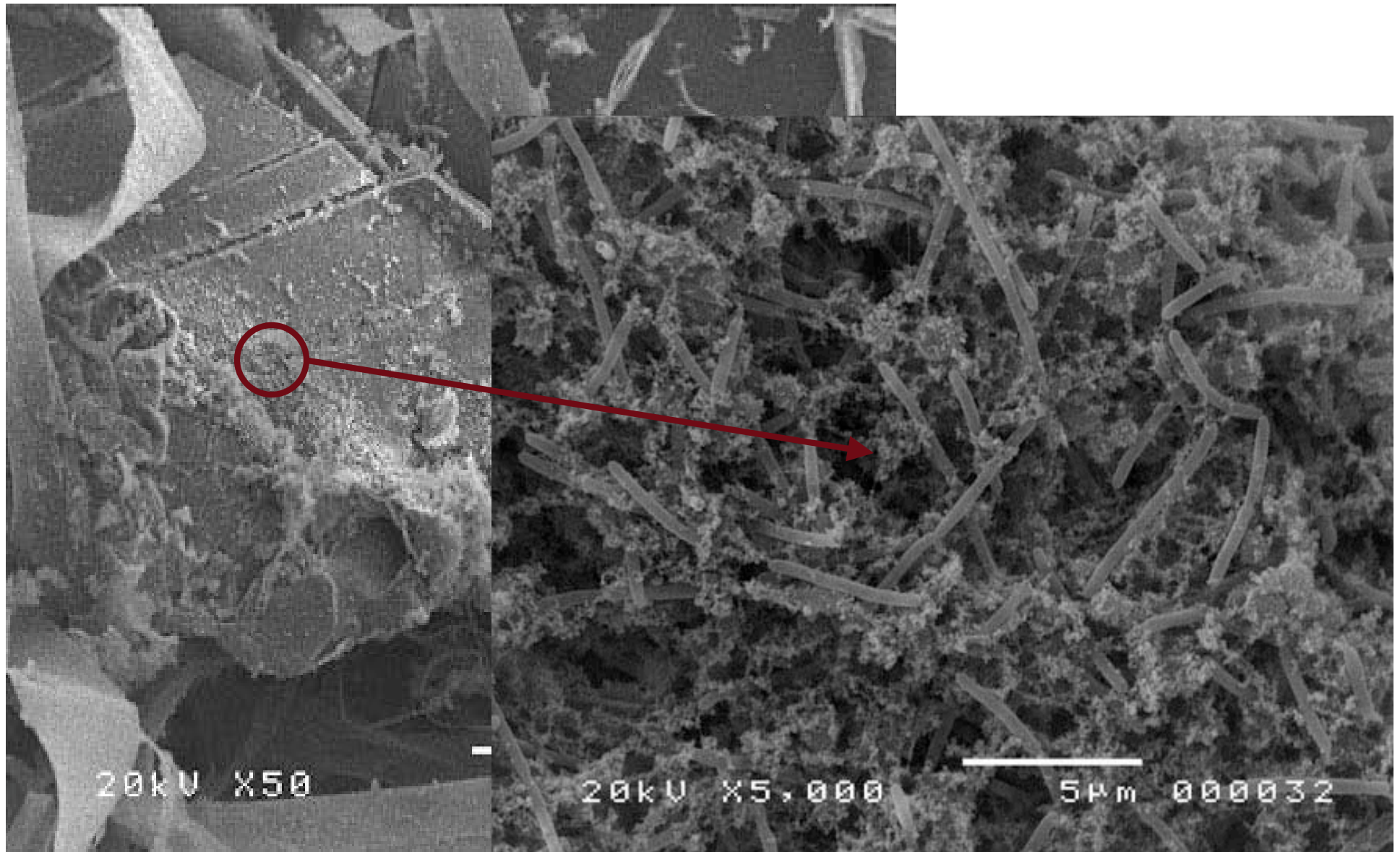
- Specific cellulose hydrolysis rates by cells > purified cellulase preparations (Lu, Zhang, Lynd, 2006)
- Suggests an advantage of CBP
- Q Microbe associates closely with cellulose and forms biofilms on cellulose

C-phy cells

Cellulose
Fiber



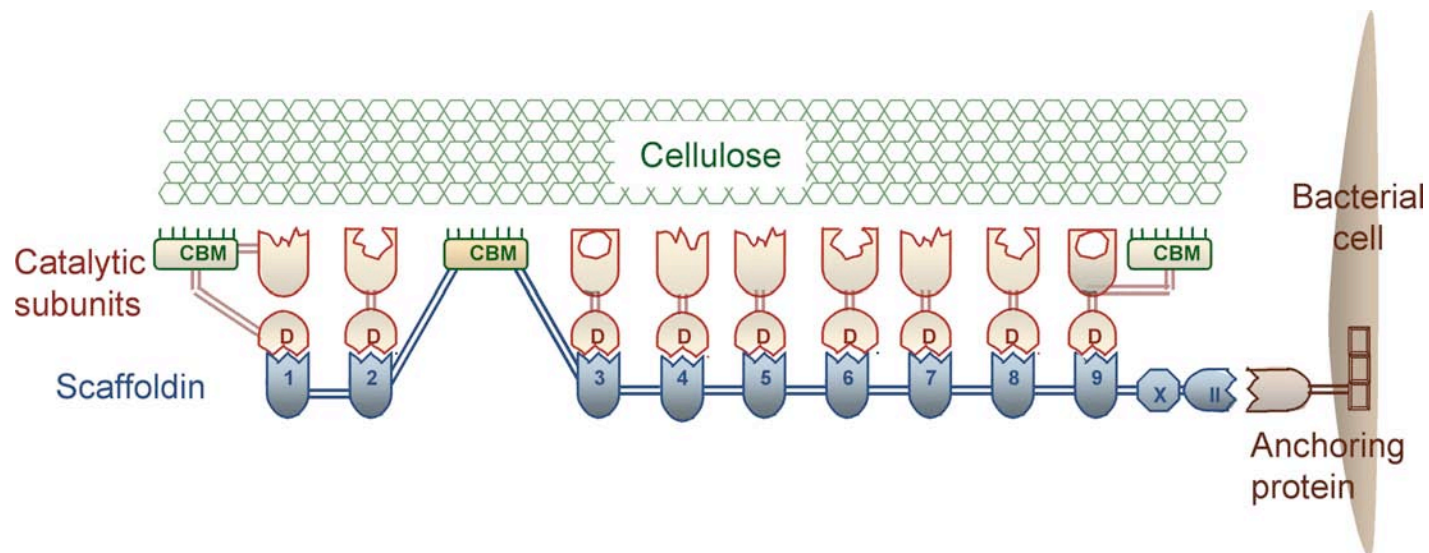
Q microbe grows as a biofilm on cellulose



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

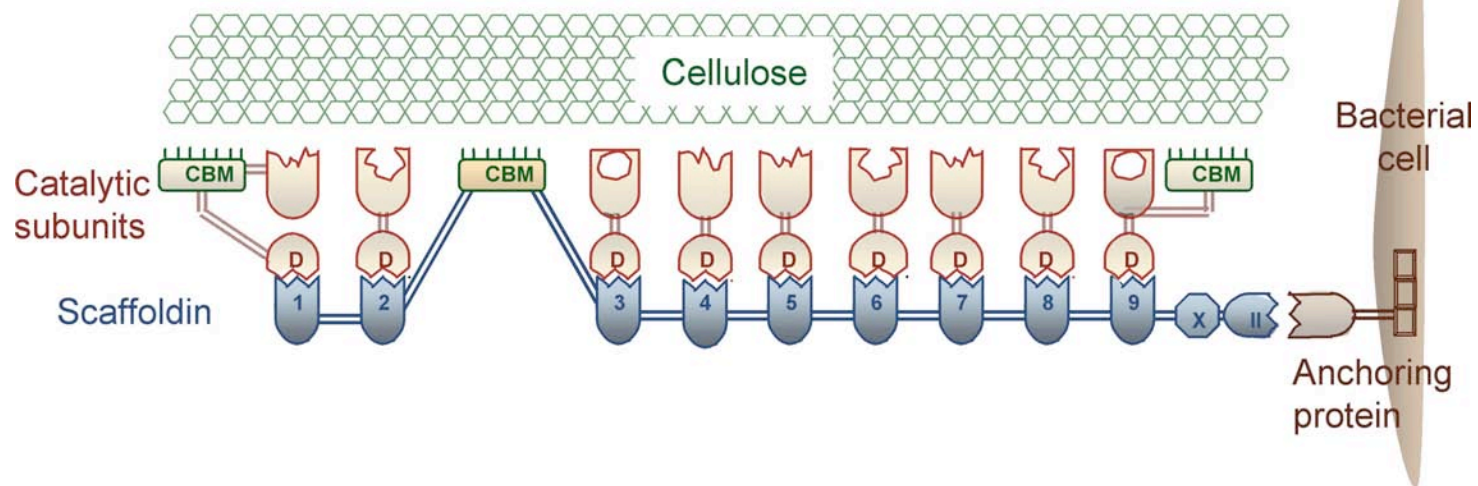
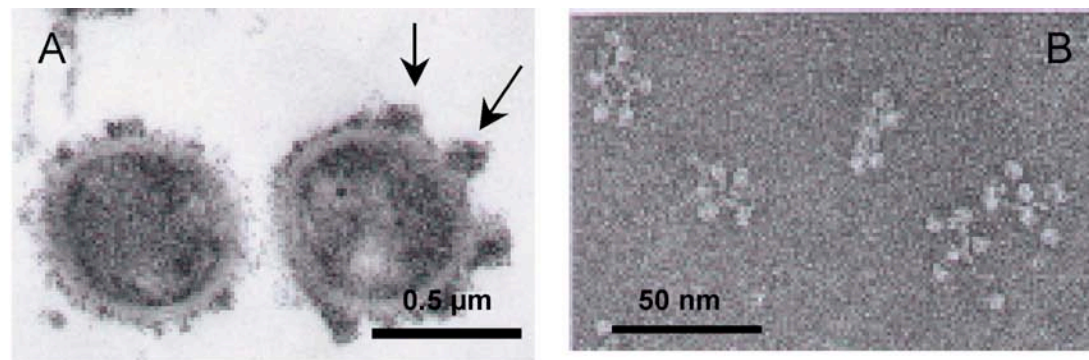
- Cellulolytic bacteria produce extracellular enzyme systems
 - Multiple enzymes digest insoluble components of biomass
-

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



Cellulosomes

Multi-protein enzyme complexes that degrade cellulose



Q microbe produces a
multi-component non-complexed
cellulase system
(non-cellulosomal)

Based on analyses of Q Microbe genome sequence:

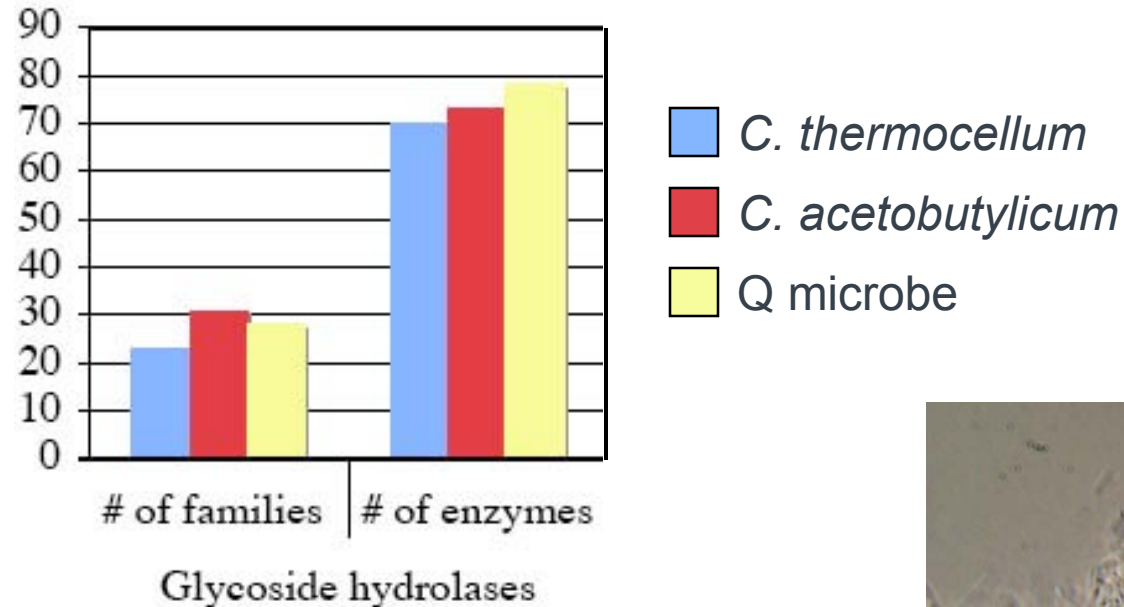
- No evidence for scaffoldin-like proteins
- No evidence for dockerin-containing carbohydrate-active enzymes (e.g., hydrolases)

No evidence for cellulosomes!

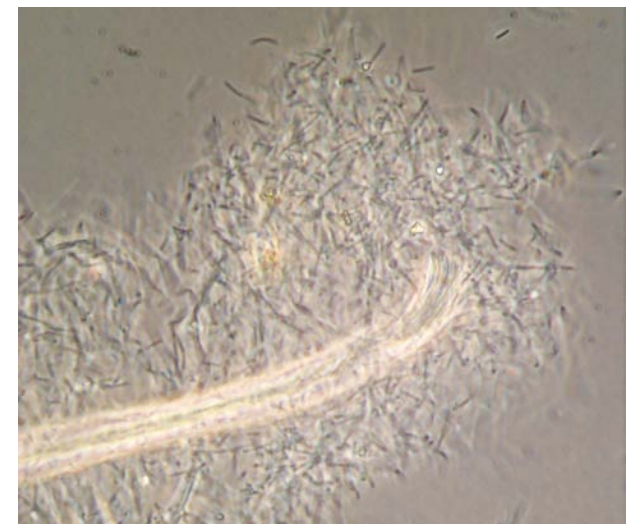


The Q Microbe Genome

- Size: 4.53 MB - Largest among the clostridia!
- Number of protein-coding genes: 3,671
- Versatile carbohydrate use



- >100 ABC transporters
~53 independent ABC sugar/
carbohydrate transport systems



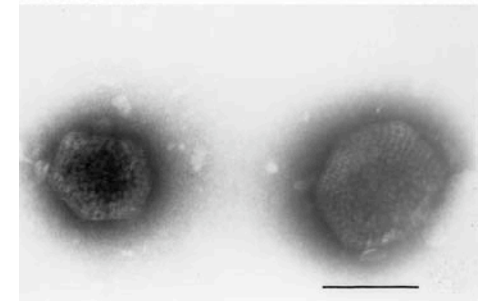
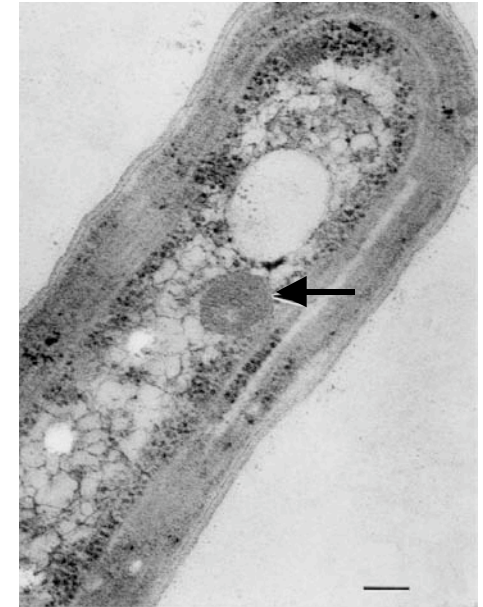
Genomic analyses also revealed the presence of shell protein genes

Shell proteins discovered in cyanobacteria

- Protein shell of carboxysomes
- Associated with CO₂ fixation

Carboxysomes

- Bacterial organelles or micro-compartments, ~100nm diameter
- Assembled entirely from protein subunits
- Found in all cyanobacteria



Synechococcus
(Cannon et al. 2001)

Carboxysomes enhance the efficiency of carbon fixation

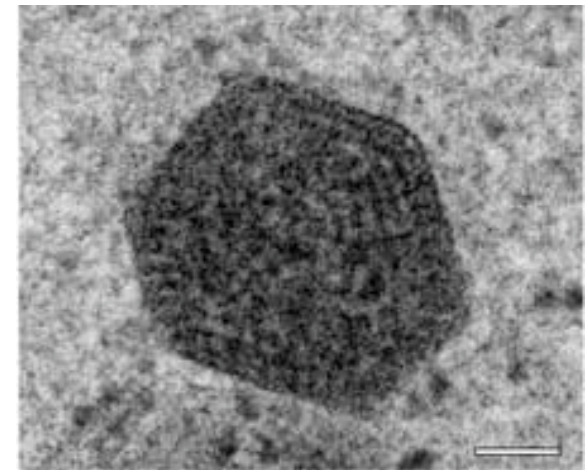
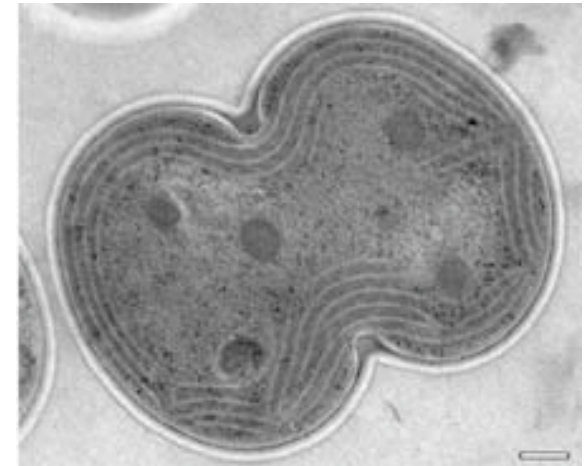
Macromolecular protein shell

- 2-3 nm thick
- Porous shell
- Directed flow of metabolites

Organelle lumen

- Enzyme: RuBisCO
- Substrate: CO₂

Specialized compartments for concentrating RuBisCO and CO₂

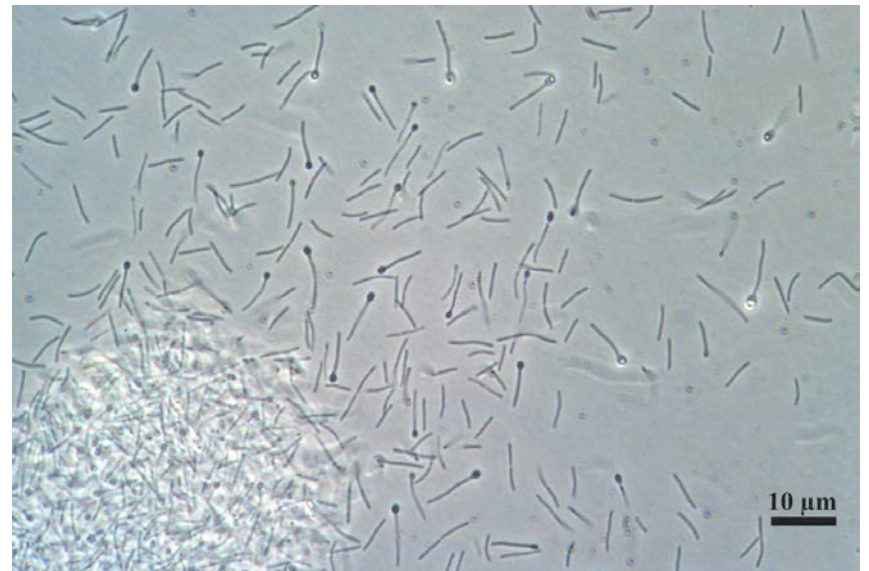


Synechocystis
(Kerfeld et al. 2005)

Bacterial MicroCompartments - BMCs

Q microbe has 23 genes with highly conserved BMC domains

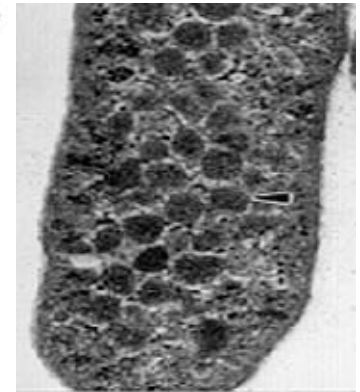
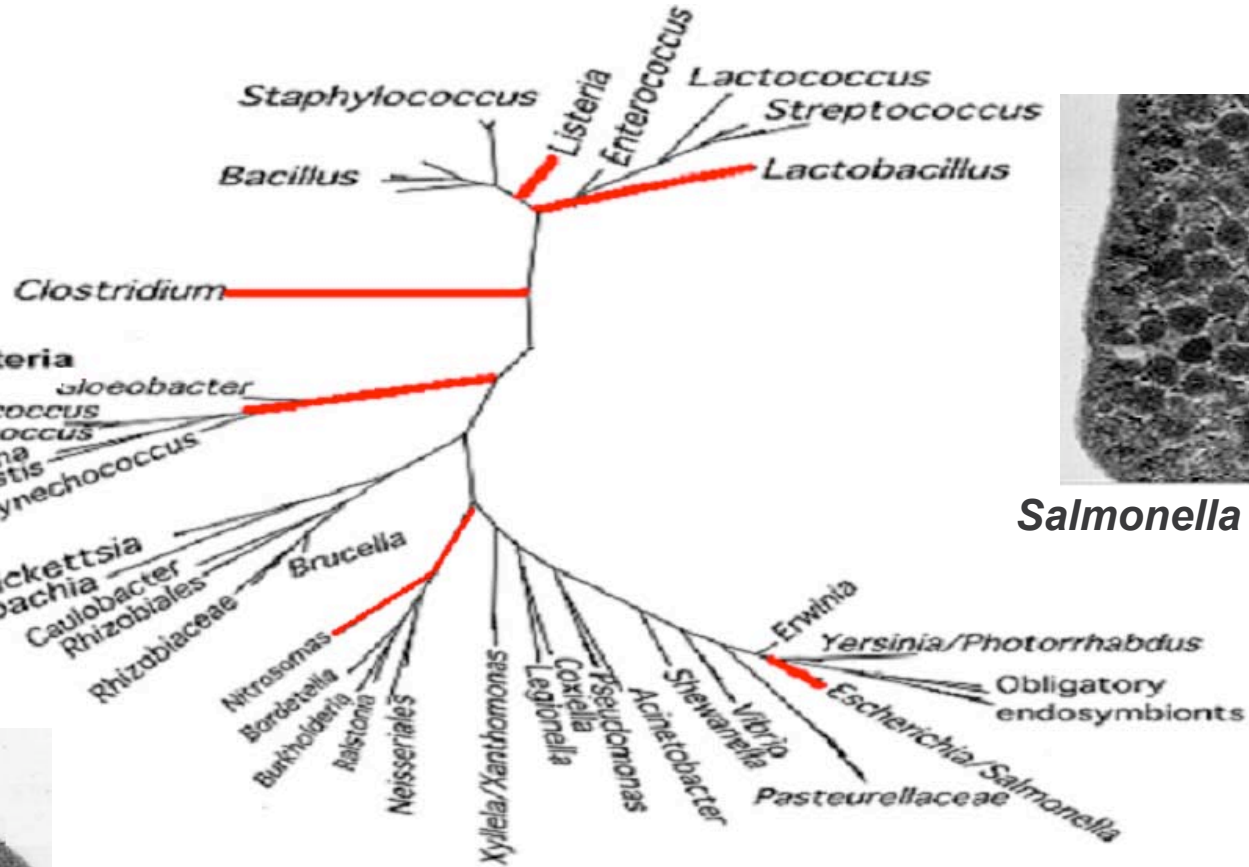
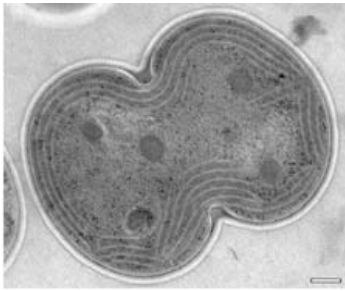
- 23 putative shell proteins
- 3 distinct loci each with 7-9 shell proteins
- No RuBisCO genes



Other than for CO₂ fixation, in what other processes are microcompartments involved?

Shell protein sequences are dispersed throughout the bacterial domain

Synechocystis sp.



Salmonella enterica



Nitrosomonas europaea

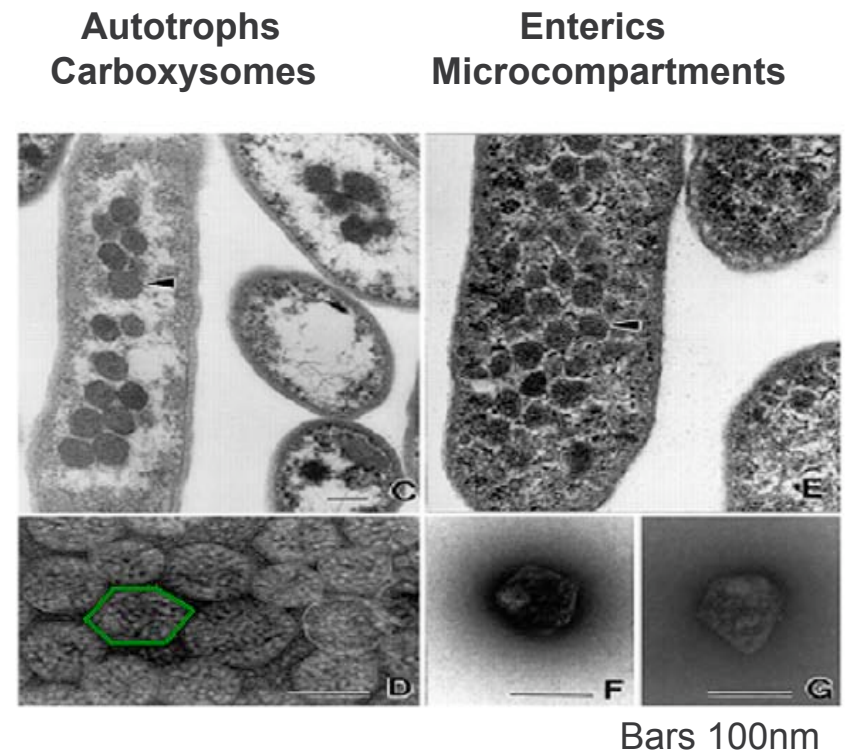
Microcompartments concentrate enzymes and volatile substrates

Processes:

- CO₂ fixation in autotrophs
- Ethanolamine or 1,2-propanediol fermentation in enterics

Purpose: Concentrate volatile substrates, enhance reaction efficiency

- CO₂
- Acetaldehyde
- Propionaldehyde



Cannon et al. 2001

What is the role of Proteinaceous Microcompartments in Q microbe ?

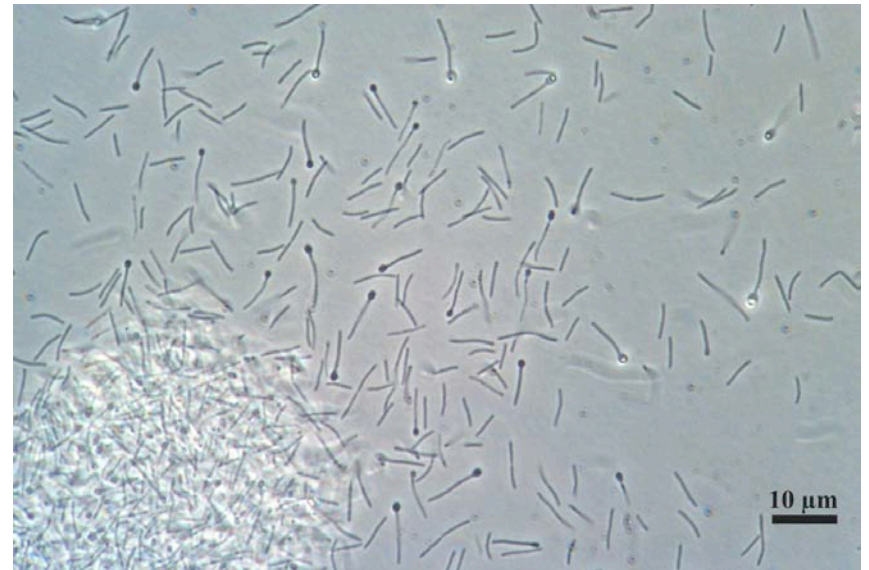
Q Microbe has 3 shell protein loci

I) Ethanolamine (eut)

II) 1,2-Propanediol (pdu)

III) Similarities to both eut & pdu microcompartment loci

We focused on Locus II



Locus II encodes shell proteins and six related enzymes

Enzymes:

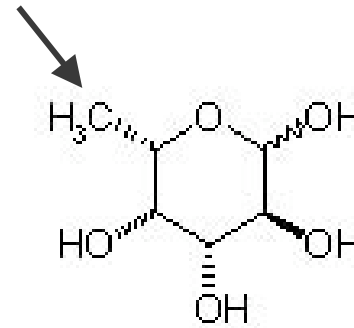
- Alcohol dehydrogenase
- Aldehyde dehydrogenase
- Diol dehydratase and Activase
- Oxidoreductase
- **Fucose aldolase** (*fucA*)
(69% identity to fucose aldolase of *R. inulinivorans*)

Link to hemicellulose



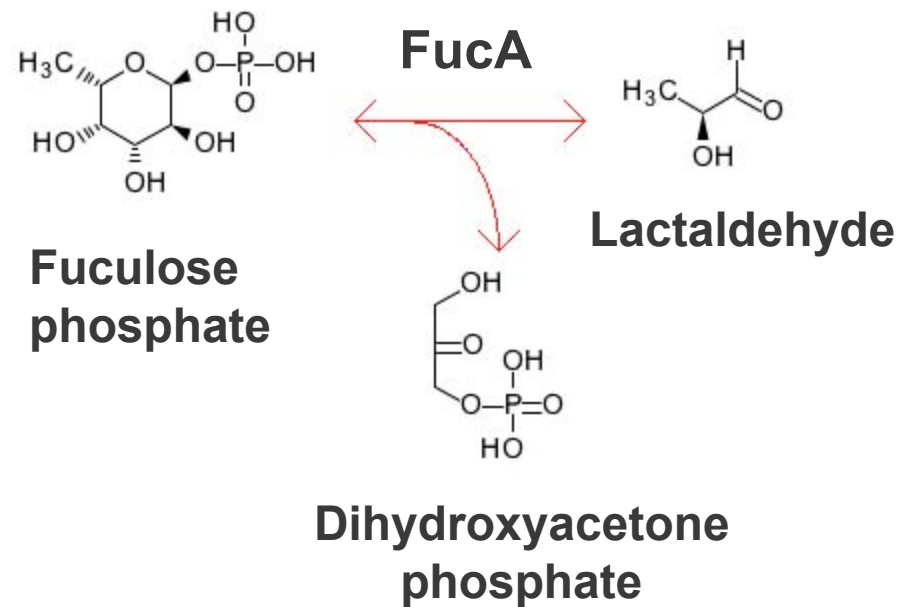
Fucose is a component of hemicellulose

- 6-Deoxy-L-galactose

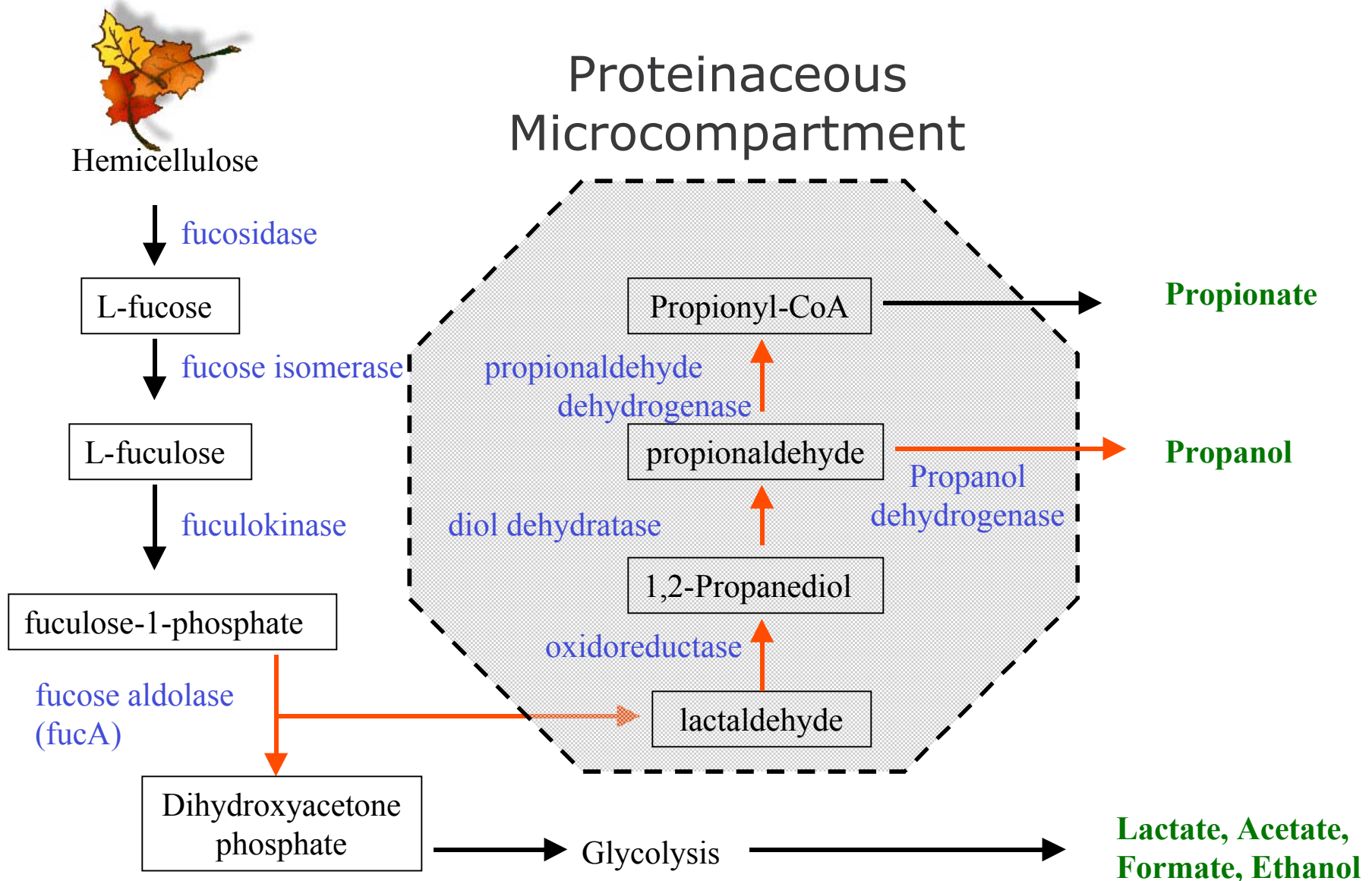


Fucose

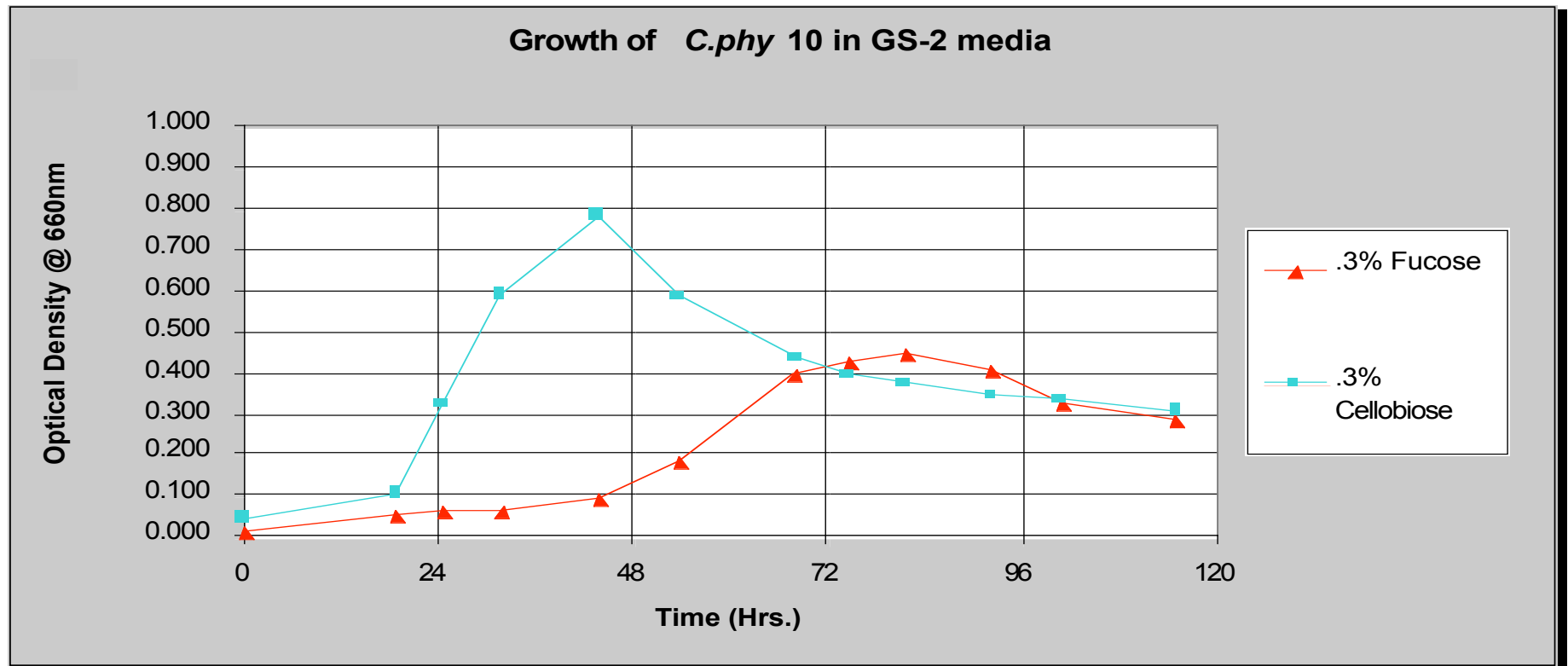
- Fucose aldolase (FucA) - Key enzyme



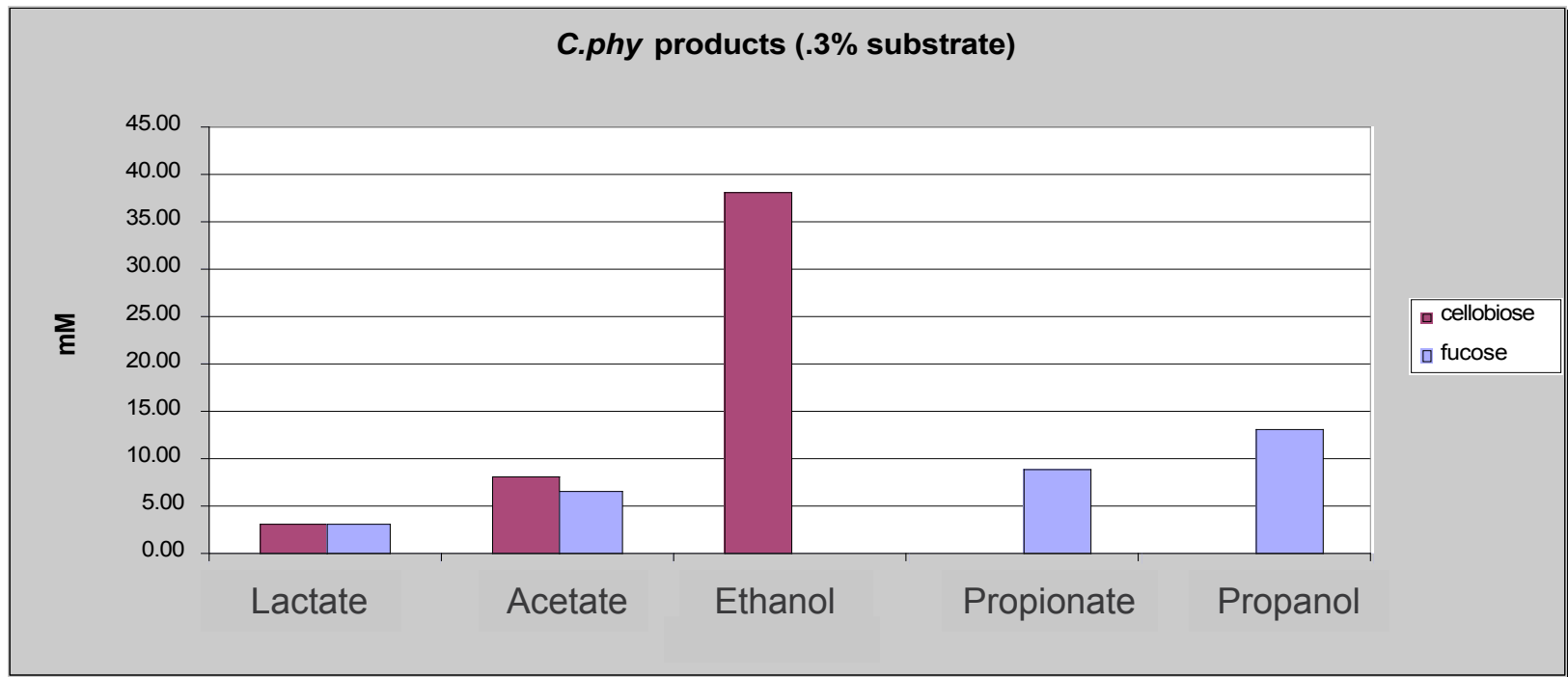
Fucose Metabolism Model



Q microbe is able to grow on Fucose

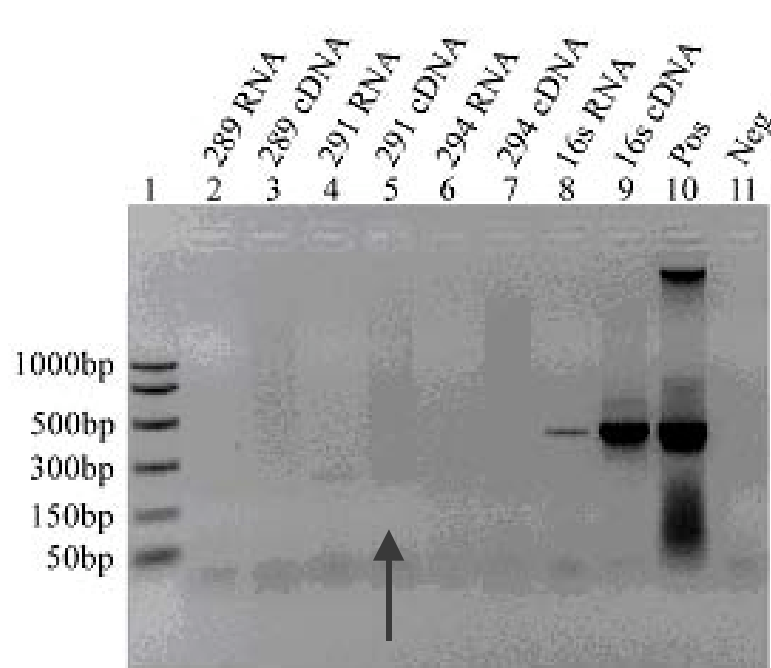


Fermentation products include propanol, propionate, acetate, and lactate

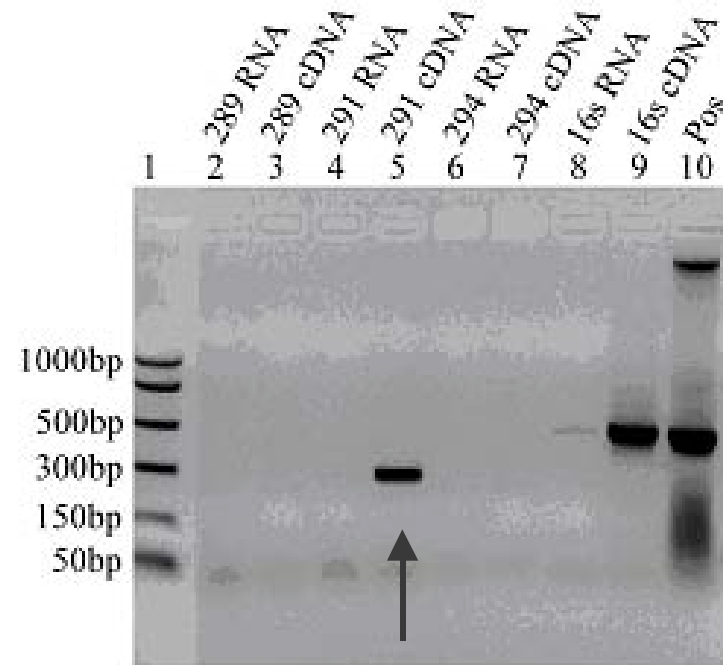


Shell protein genes are expressed during growth on fucose

- Target *ccmL*
 - Shell protein
 - Present in single copy at each locus
- Detect via Reverse Transcriptase-PCR

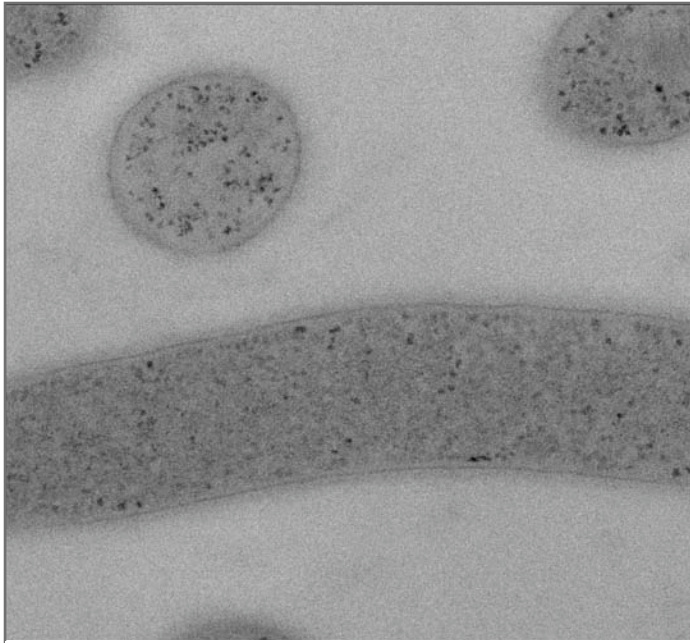


Cellobiose

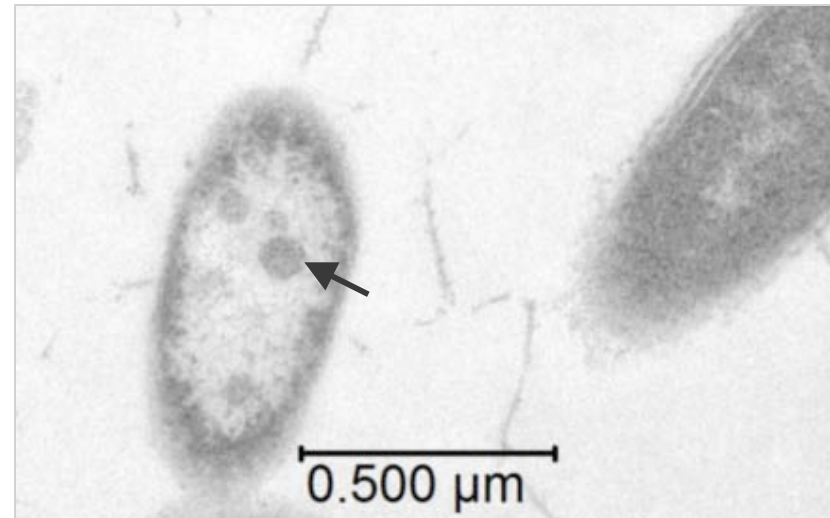
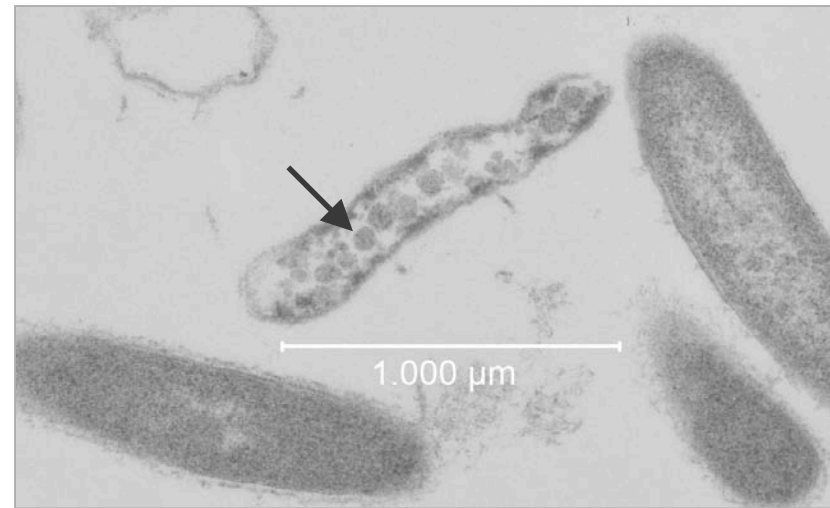


Fucose

Thin sectioned cells provide the first images of microcompartments in the clostridia



Cellobiose Culture



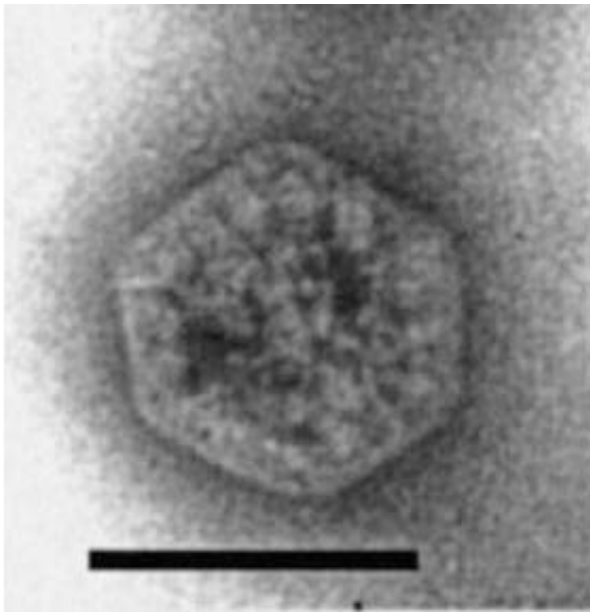
Fucose Culture

D. Callaham

Microcompartments were purified from *Q*

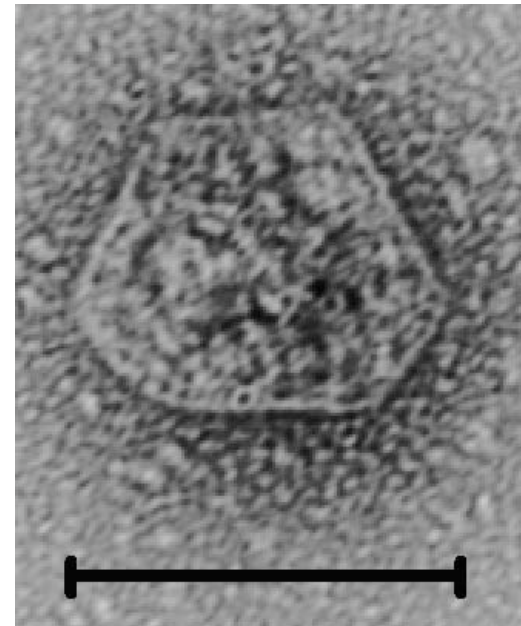
TEM of negatively stained microcompartments

Carboxysome
H. neapolitanus



Cannon et al. 2001

Microcompartment
Q *microbe*



Bars 100nm

Proposed function: to enhance enzyme activity in alcohol production

Microcompartments may play a role in converting biomass to fuel

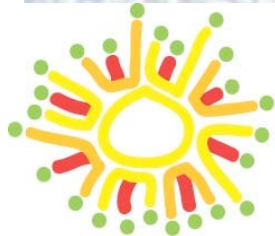
Q microbe

A voracious appetite for plant polysaccharides

Effective metabolic pathways

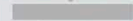


An efficient microbial catalyst for biofuel production



SunEthanol

10 μm



January 23, 2008

Acknowledgements

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SunEthanol

Thank you!

