SAND2015-5580C

# Quest of Synthetic Biology in the Production of Terpene as Natural Bioactive Products and Next Generation Fuel Compounds

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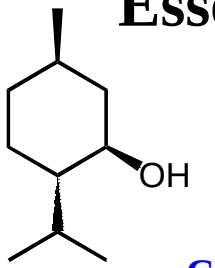
# Terpenoids

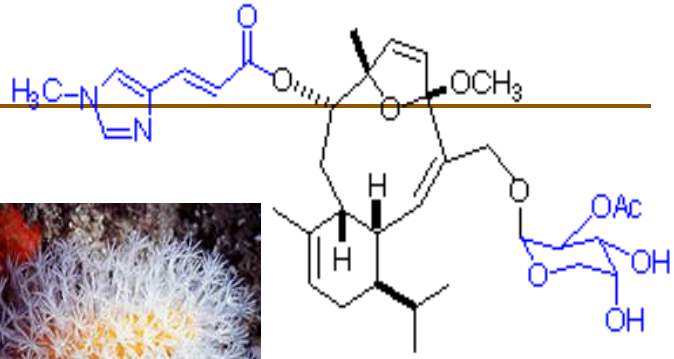
- > 55,000 known molecules

## Essential oils



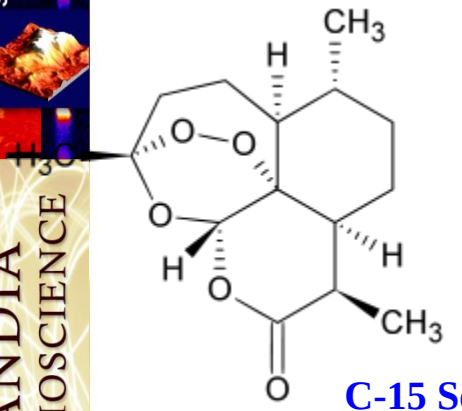

**Menthol**
**C-10 Monoterpene**

## Chemotherapeutics

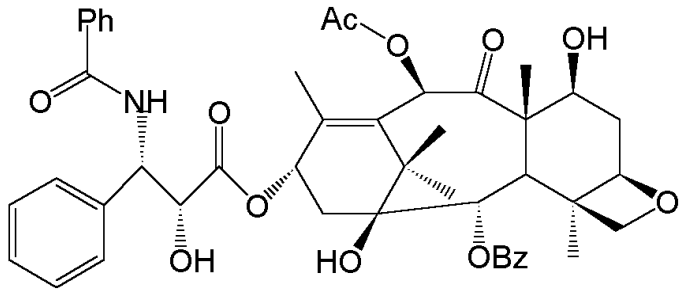



**Eleutherobin**
**C-20 Diterpene**

## Artemisinin




**C-15 Sesquiterpene Lactone**




**Taxol**
**C-20 diterpene**

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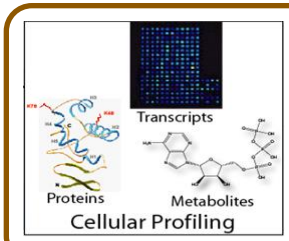
# Endophytic Terpene Synthases Discovery

Endophyte Isolation & Culture

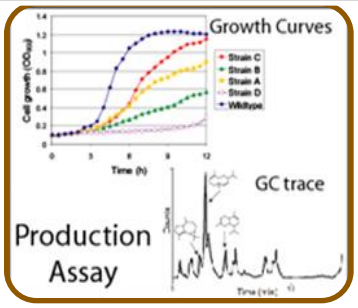
Cellular Profiling
Enzyme Screening




Enzyme Profile
**Activity Screens**

**Genomics**

Screen for Terpene Compounds



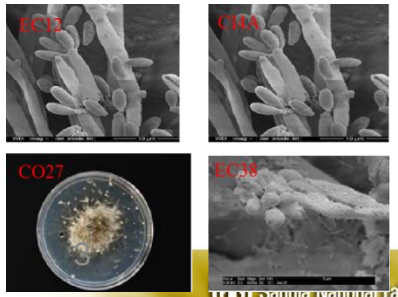

Genome Sequencing
Annotation
Transcriptomics
**Pathway Reconstruction**

JGI DOE JOINT GENOME INSTITUTE US DEPARTMENT OF ENERGY OFFICE OF SCIENCE

**Goals:**

**Novel TSs discovery**

**Targeted terpene biosynthesis (synthetic biology)**




**Volatile Organic Compound(VOC)**

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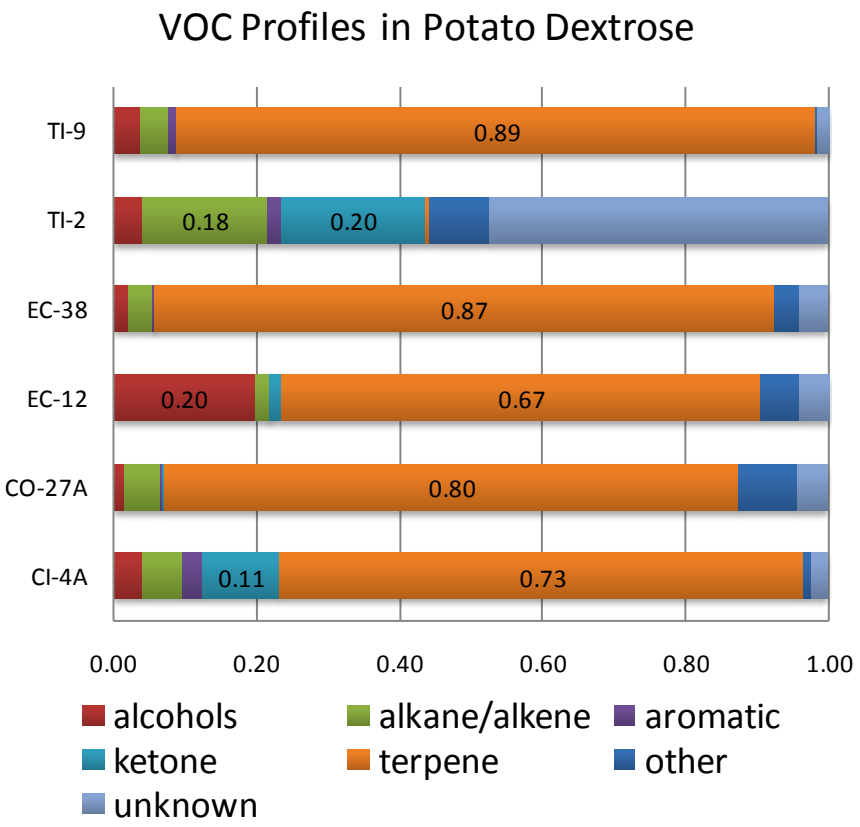
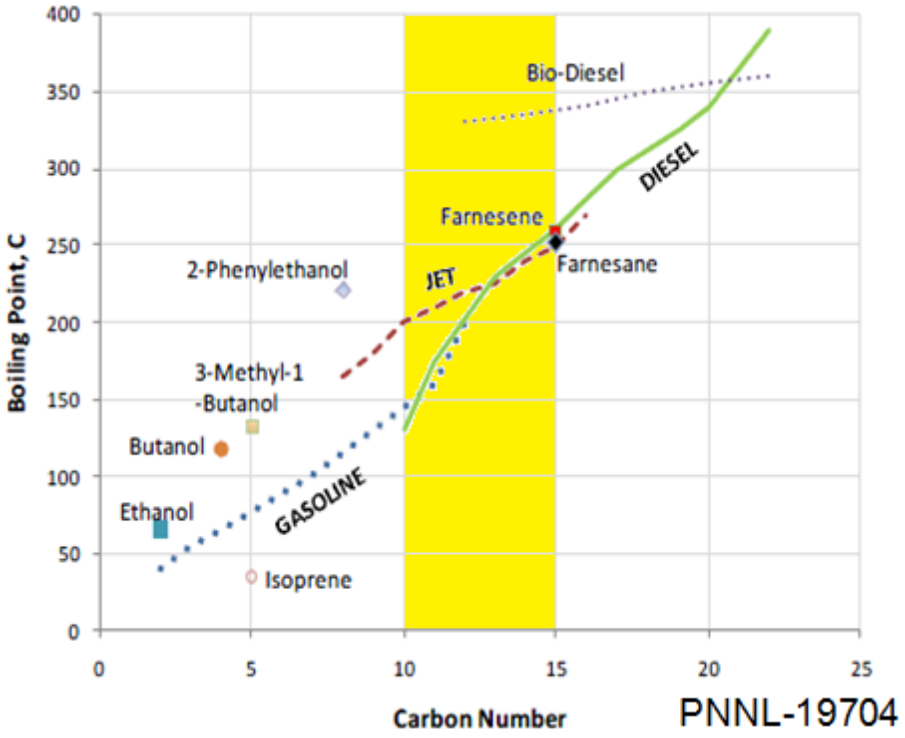
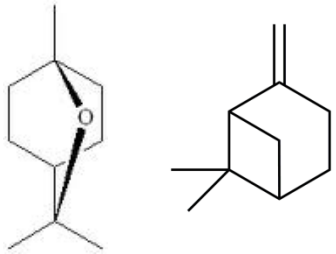
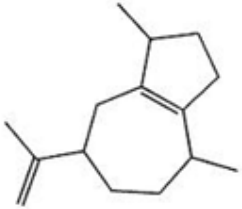
# Endophyte VOCs are Rich in Terpene Compounds



C10



C15

# Several Endophyte VOCs have Pharmaceutical Functions

| CI4A | | | |
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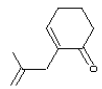
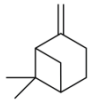
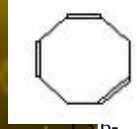
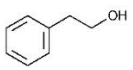
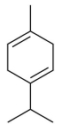
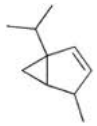
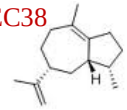
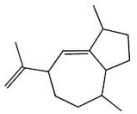
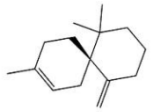
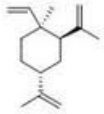
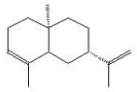
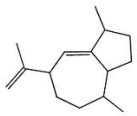
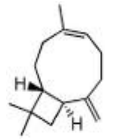
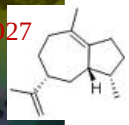
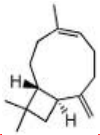
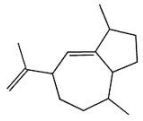
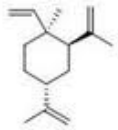
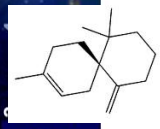
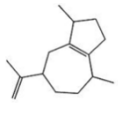
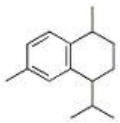
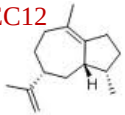
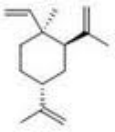
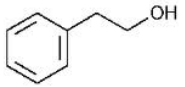
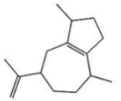
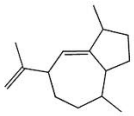
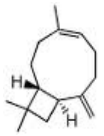
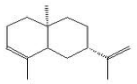
|  |  |  |  |
| **Eucalyptol (47.4%)** | **2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)- (19.2%)** | Cyclohexane, 1,2,4-tris(methylene)- **(3.3%)** | β-Pinene |
|  |  |  |  |
| 1,3,6-Cyclooctatriene | Phenylethyl Alcohol | γ-Terpinene | β-Thujene |

| EC38 | | | |
|---|---|---|---|
|  |  |  |  |
| Guaia-1(10),11-diene**(35%)** | γ-Gurjunene **(13.3%)** | **Eucalyptol (11.6%)** | β-Chamigrene **(9.2%)** |
|  |  |  |  |
| β-Elemene **(4.2%)** | 2-Isopropenyl-4a,8-dimethyl 1,2,3,4,4a,5,6,8-octahydronaphthalene (**3.5%**) | α-Guaiene | Caryophyllene |

| CO27 | | | |
|---|---|---|---|
|  |  |  |  |
| Guaia-1(10),11-diene (**31.5%**) | **Caryophyllene (13.8%)** | γ-Gurjunene**(12.6%)** | β-Elemene **(7.3%)** |
|  |  |  |  |
| Chamigrene (**4.8%**) | **Eucalyptol *(3.6%)*** | α-Guaiene | Calamenene |

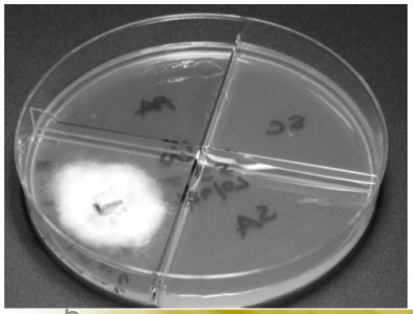
| EC12 | | | | |
|---|---|---|---|---|
|  |  |  |  | |
| Guaia-1(10),11diene (**24.1%**) | **β-Elemene** (16.3%) | Phenylethyl Alcohol | α-Guaiene | |
|  |  |  |  | |
| γ-Gurjunene | Caryophyllene | 2-Isopropenyl-4a,8-dimethyl 1,2,3,4,4a,5,6,8-octahydronaphthalene | **Eucalyptol (1%)** | |

Biological activities: **essential oil and pharmaceutical precursors**

Cineole: anti-inflammatory and anti-pain;

**Caryophyllene**: anti-inflammatory, anticancer

***beta*-Elemene**: anticancer

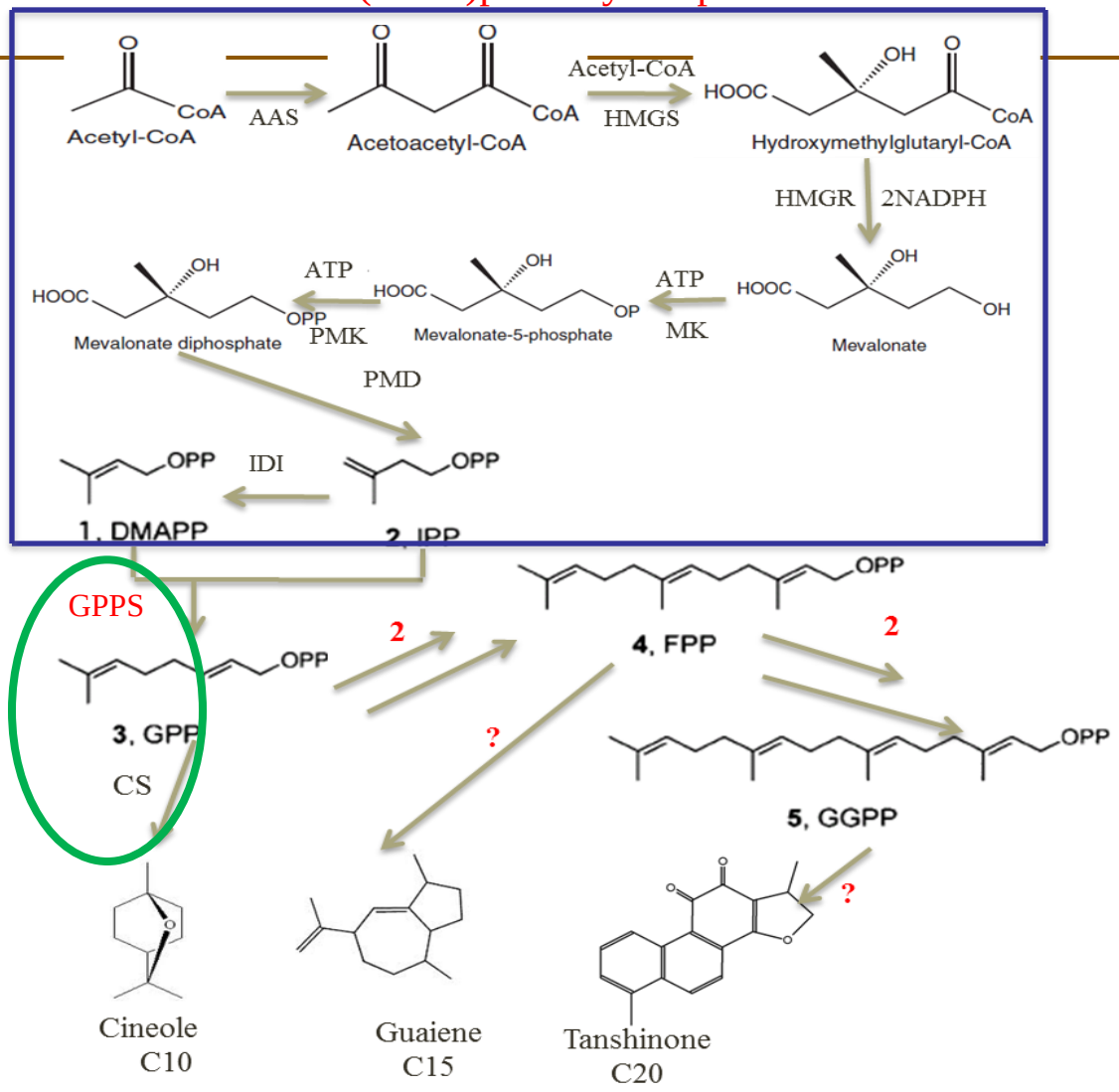


**Anti-Human bacterial Pathogens**

Angella et al. Microbiology(2010), 156:270-7

# Terpene Biosynthetic Pathway Presents in Endophytes

- Enzymes from Mevalonate (MEV)pathway are present!



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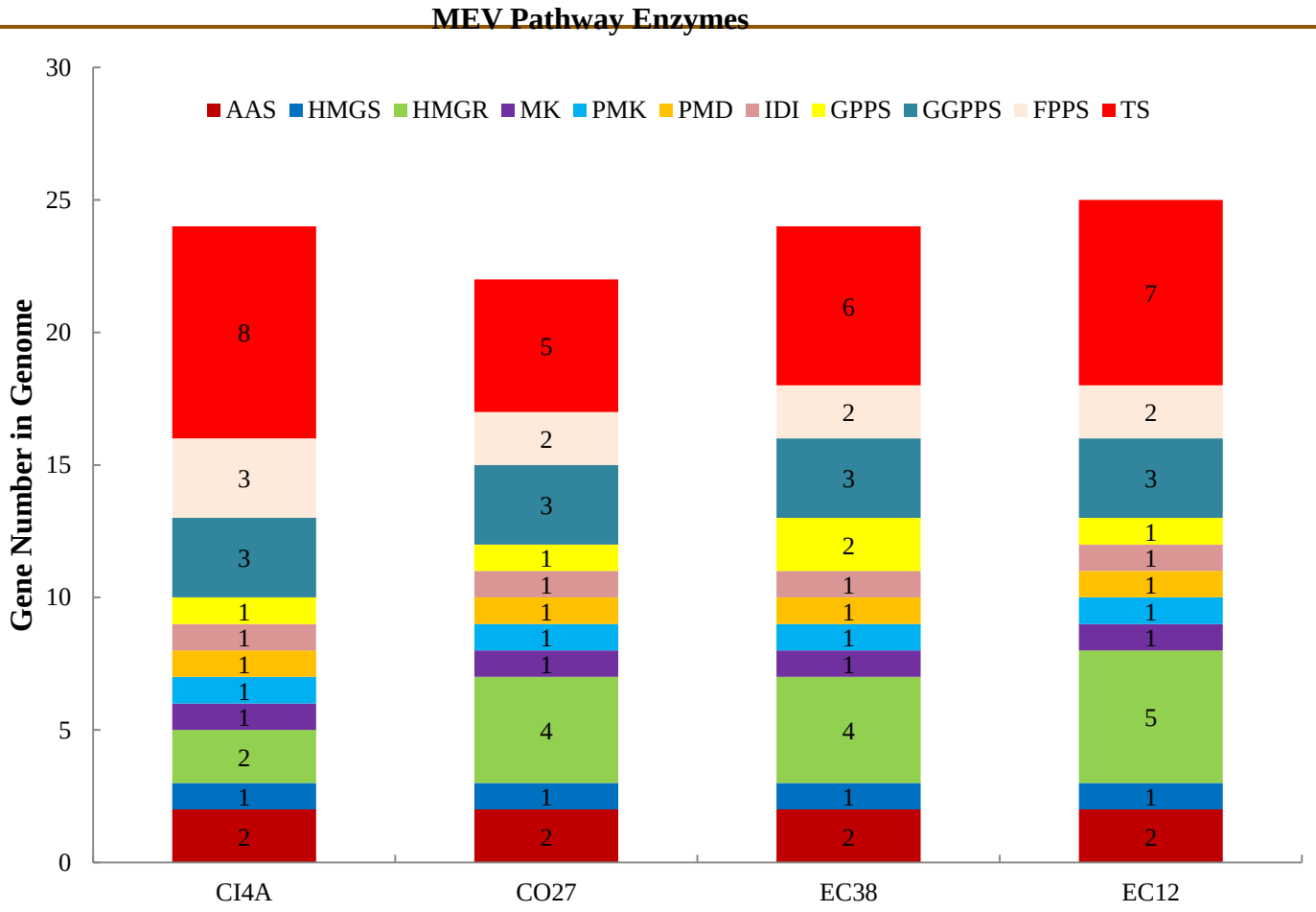
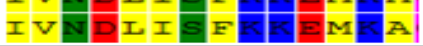
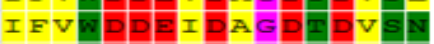
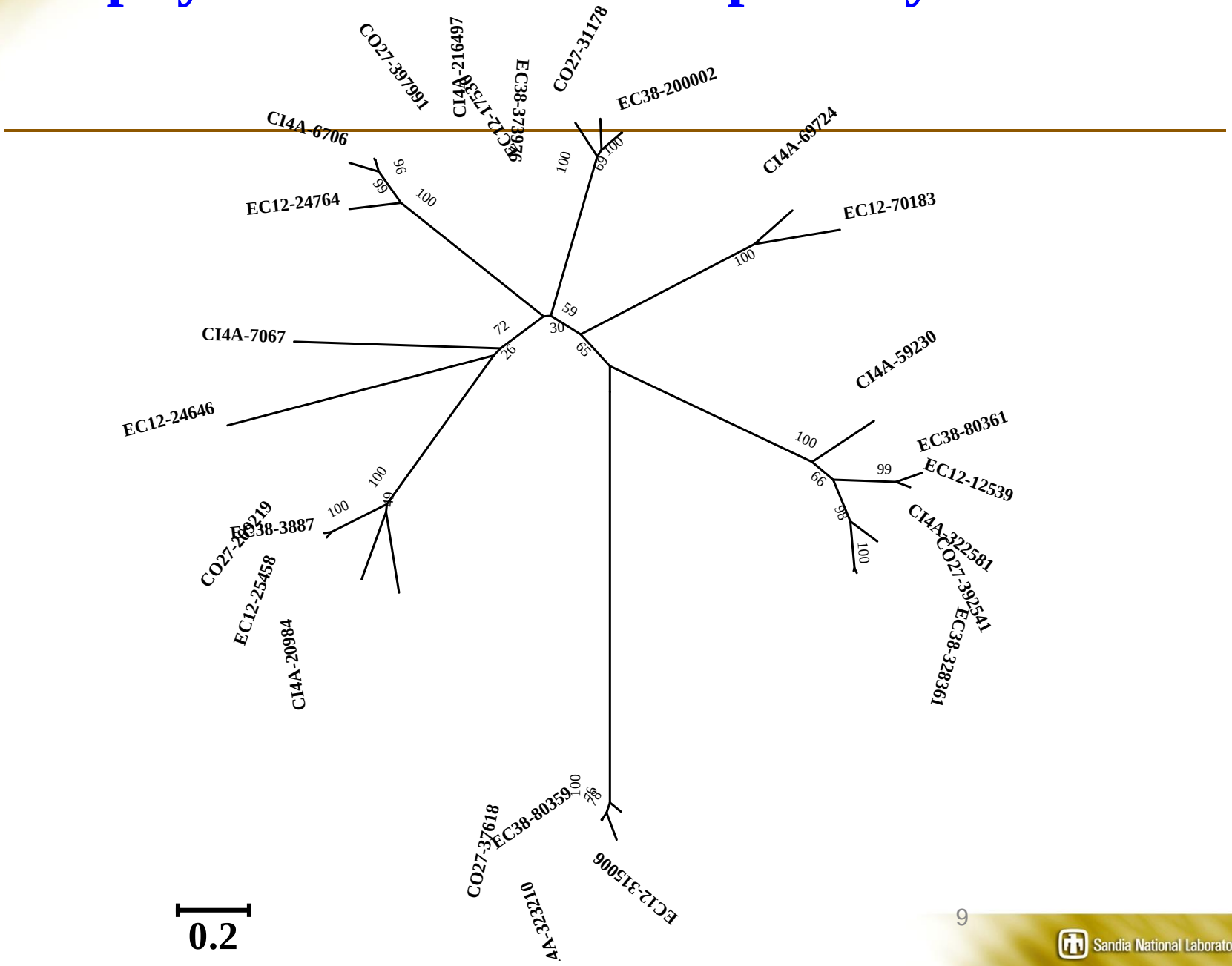
•Enzymes from Mevalonate (MEV)pathway are present!



# Endophytes are Rich in Terpene Synthases

| Protein ID | Aspartate Rich Motif | NSE/DET Triad |
|---|---|---|
| **Cluster 1** | DDXXE | NDXXSXXKE |
| CI4A-20984 |  |  |
| CO27-269219 |  |  |
| EC38-3887 |  |  |
| EC12-25458 |  |  |
| **Cluster 2** | DDXXD | NDXXSXXKE |
| CI4A-216497 |  |  |
| CO27-31178 |  |  |
| EC12-17536 |  |  |
| EC38-200002 |  |  |
| **Cluster 3** | DDXXD | NDXXSXXKE |
| CI4A-6706 |  |  |
| CO27-397991 |  |  |
| EC12-24764 |  |  |
| EC38-373976 |  |  |
| **Cluster 4** | DDXXE | NDXXSXXKE |
| CI4A-322581 |  |  |
| CO27-392541 |  |  |
| EC12-12539 |  |  |
| EC38-328361 |  |  |
| EC38-80361 |  |  |
| CI4A-59230 |  |  |
| ***Cluster 5*** | DDXXE | XDXXXSXXRE |
| CI4A-323210 |  |  |
| CO27-37618 |  |  |
| EC38-80359 |  |  |
| EC12-315006 |  |  |
| Non-Clustered | DDXXD | NDXXSXXK(E/D) |
| CI4A-7067 |  |  |
| EC12-24646 |  |  |
| CI4A-69724 |  |  |
| EC12-70183 |  |  |

# Endophytes are Rich in Terpene Synthases



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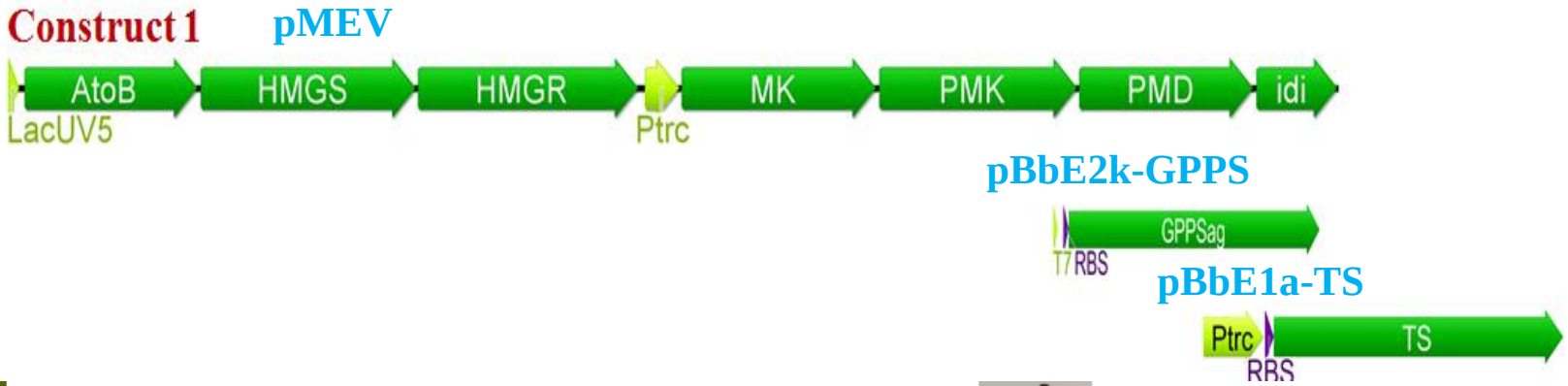
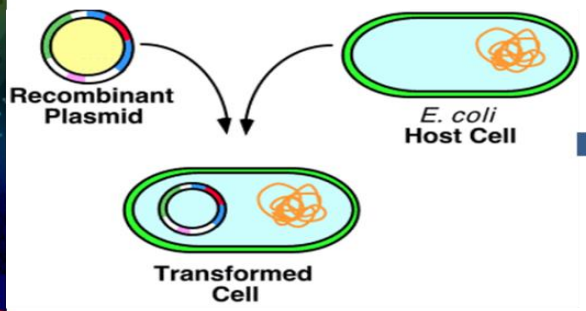
# Reconstruction of Terpene Pathway-TSs Screening

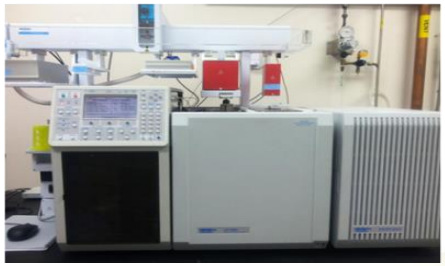


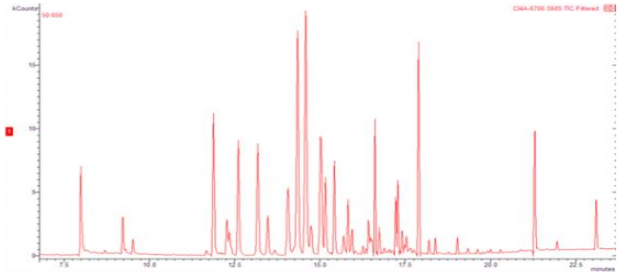

TS Expression

SPME

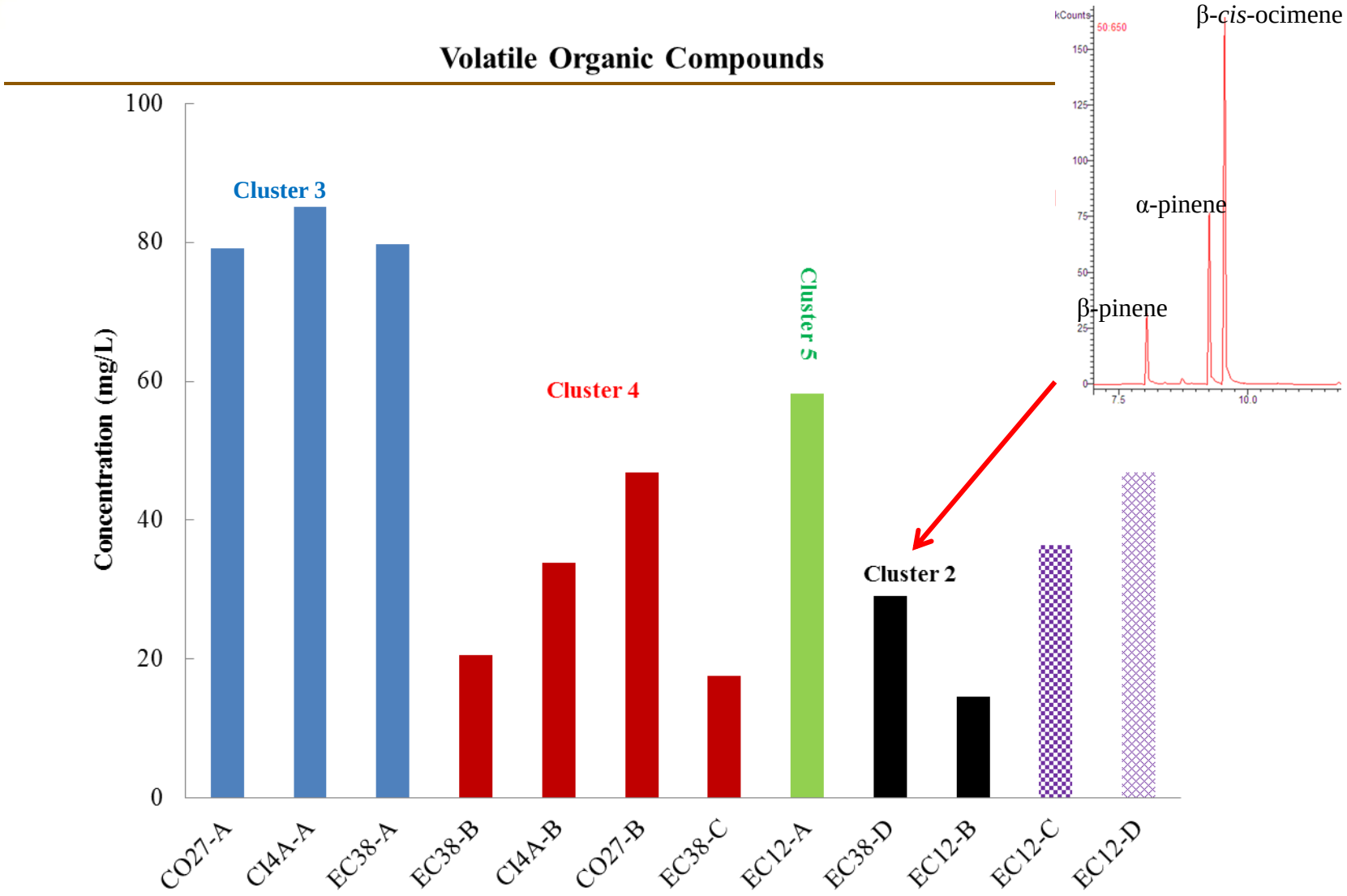
Terpene Extraction



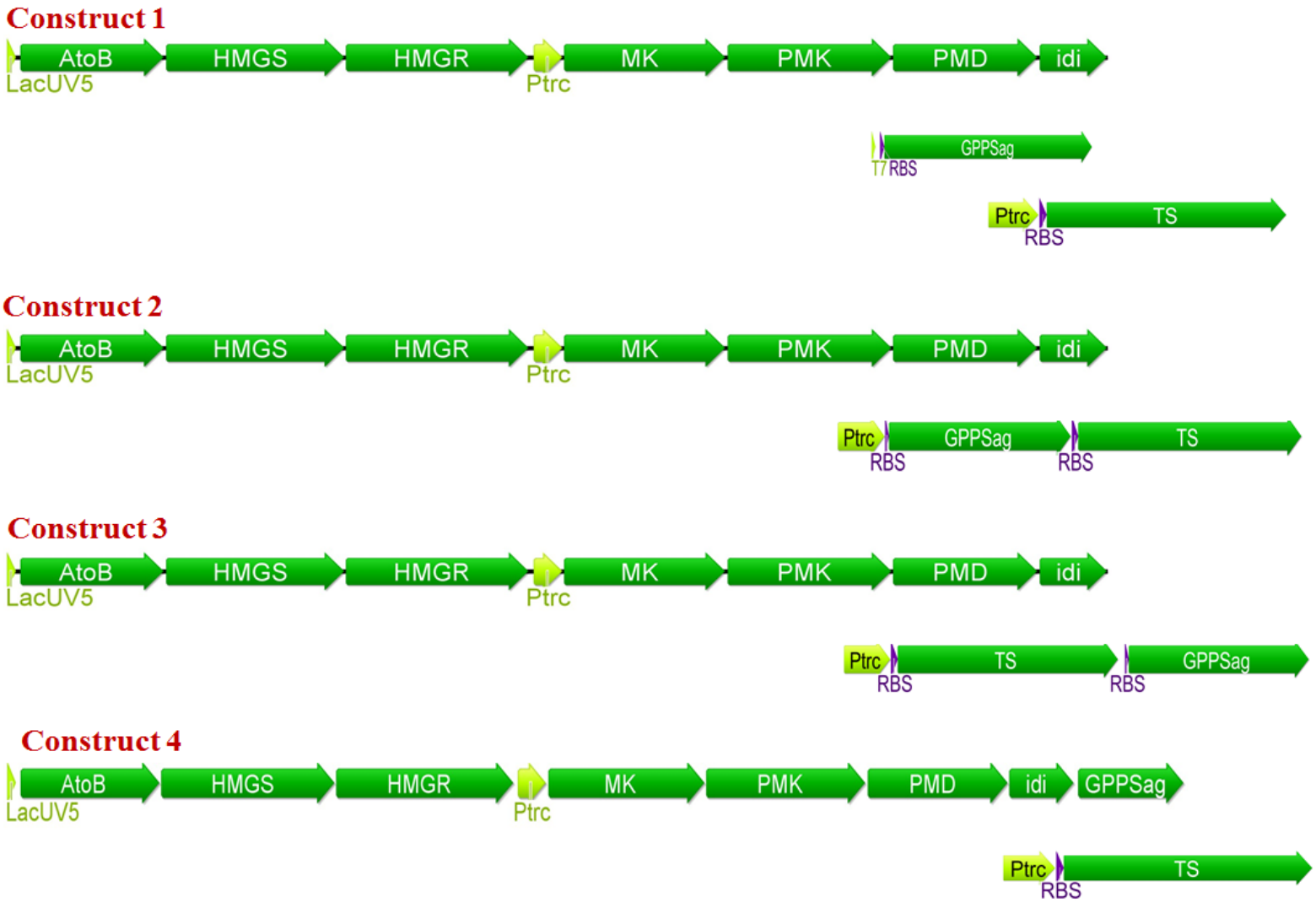
Data Analysis



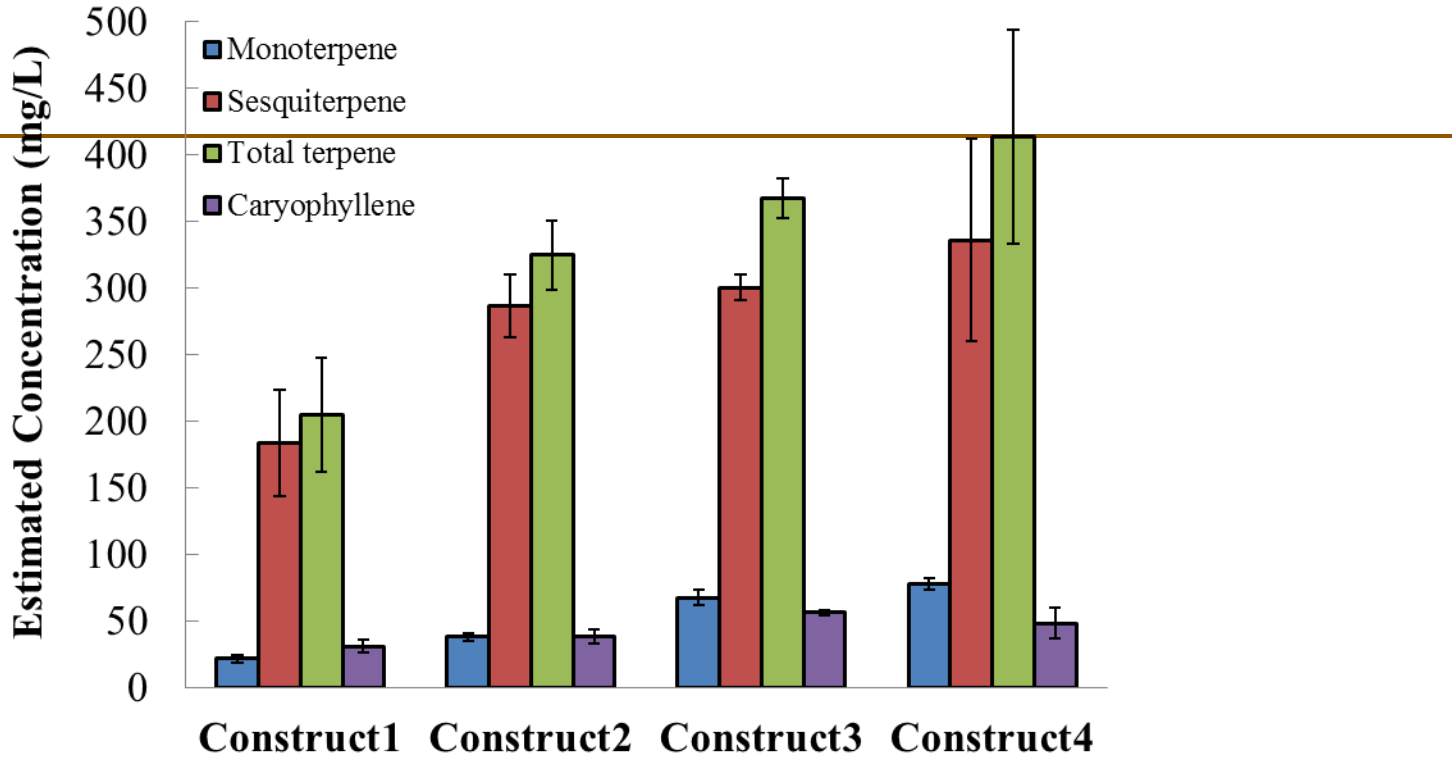
# Twelve Novel TS Genes were Discovered



# Driven the Metabolic Flux to Terpene Formation



# Construct 4 Produced Highest Terpene Yield




| β-Caryophyllene | Isolongifolene | β-Pinene | α-Guaiene |
|---|---|---|---|
| ~15.1% | ~12.2% | ~7.2% | ~5.5% |

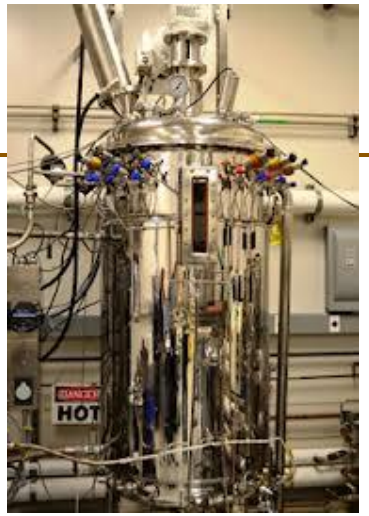
# Algal Biofuel R&D Challenge- Reduction of Process Cost

**Algae Cultivation**

**Strain Selection**:
- Productive
- Stable and resilience

**Cultivation Strategies and productivity and economics**
- Open, close, or offshore
- CO2, H2O sources, energy
- Nitrogen and phosphorous
- Land use

- Energy efficient **Harvesting** and **dewatering systems**
- Biomass **extraction** and **fractionation**
- Product **purification**




**Biomass Harvesting and Recovery**

Process Optimization
- Thermochemical
- Biochemical

Fuel Characteristics

Co-Products

**Conversion and End-use**

Cheaper… Cheaper… Cheaper…

http://www.dailymail.co.uk/news/article-1031444/Chinas-blooming-algae-problem-thats-swamping-Olympics.html
https://biosarch.wordpress.com/page/6/



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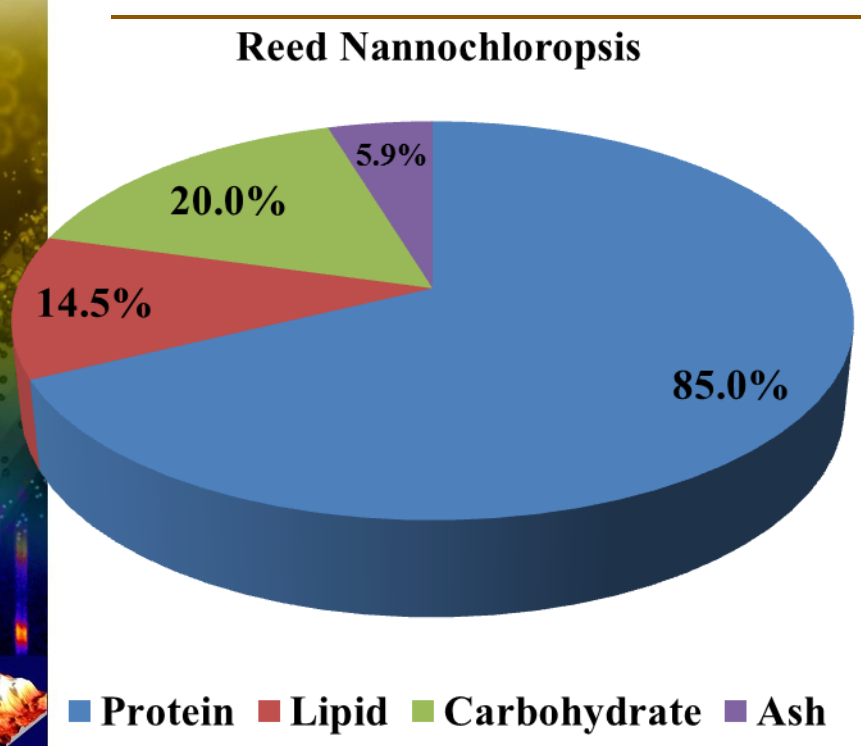
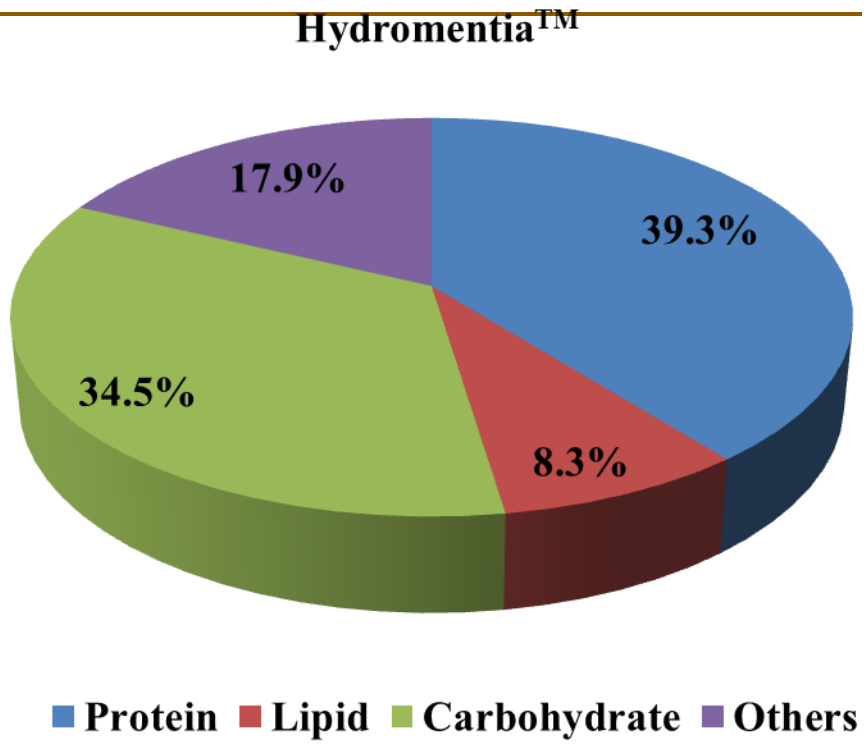
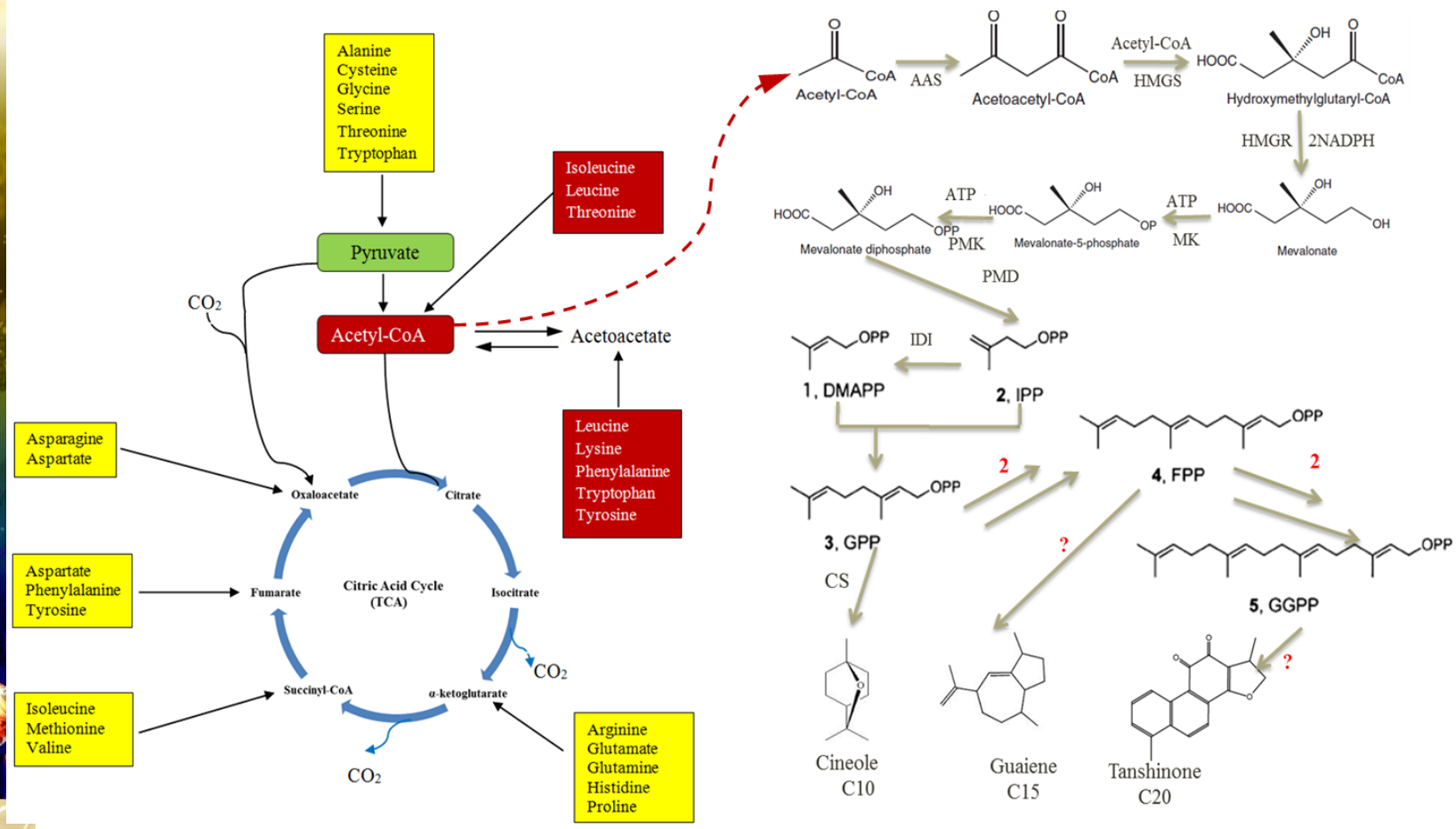
# Carbohydrate and Protein account for ~65% of Algal Biomass



# Amino Acids Assimilation Connected with Terpene Biosynthesis: Acetyl-CoA Niche

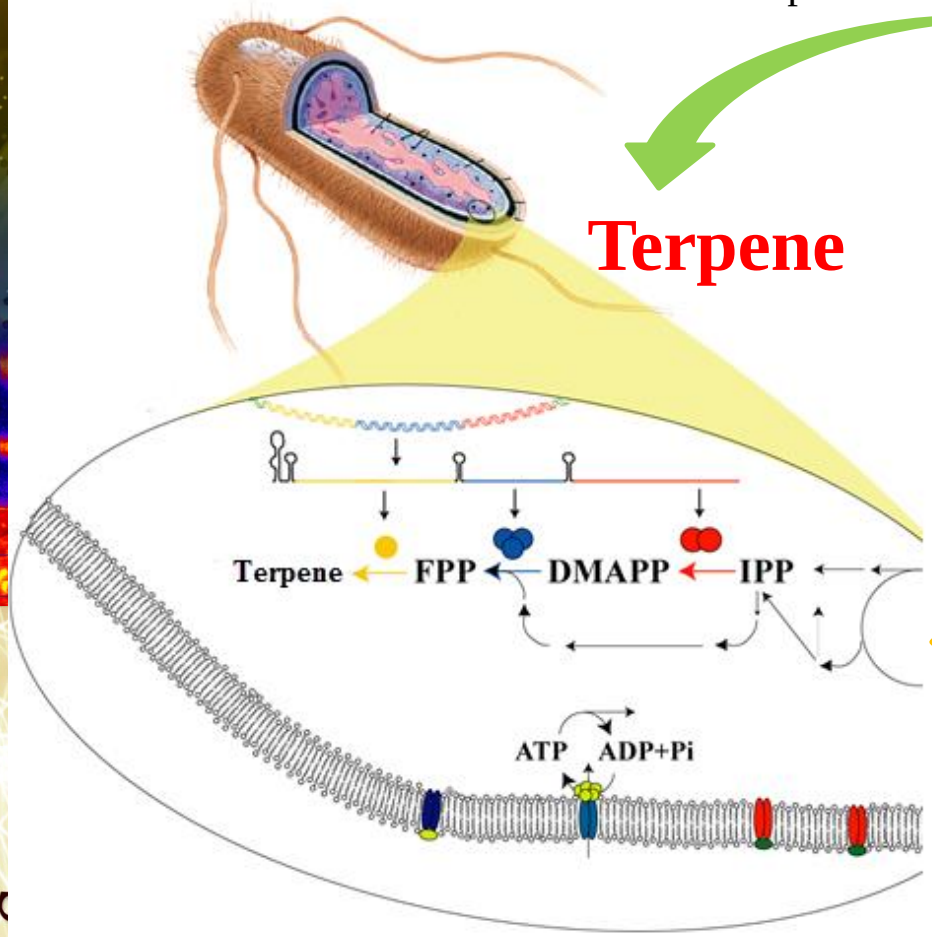



**Amino Acids Assimilation** → **Terpene Biosynthesis**

# Bioconversion of Proteins to Terpene in Engineered *E.coli* Strain YH40

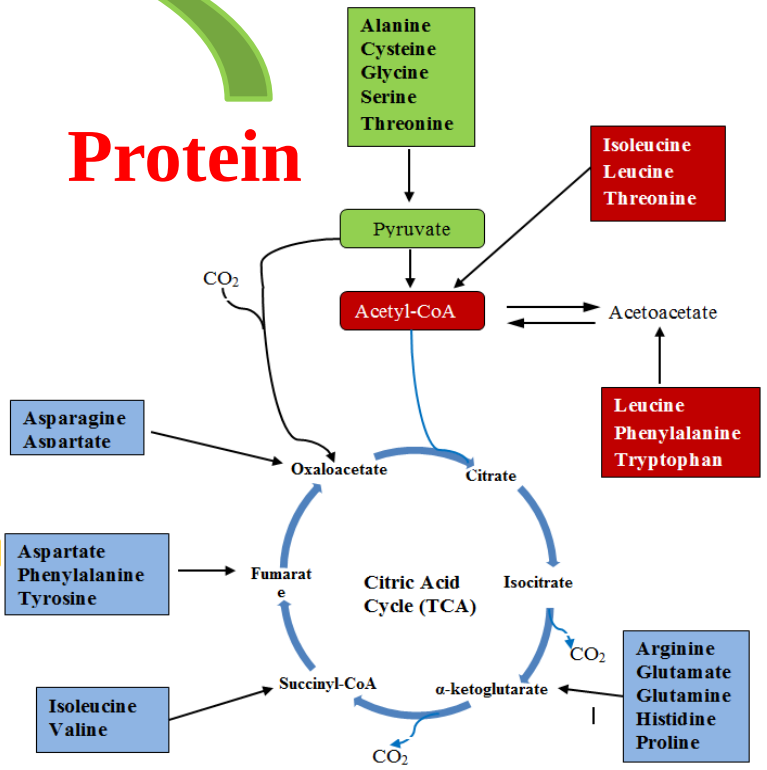
Chemical Mutated *E.coli* strain **YH40** with *ΔglnA*, *ΔgdhA*, *ΔluxS*, *ΔlsrA*-Courtesy of Professor James C. Liao from UCLA


Terpene Biosynthesis Pathway

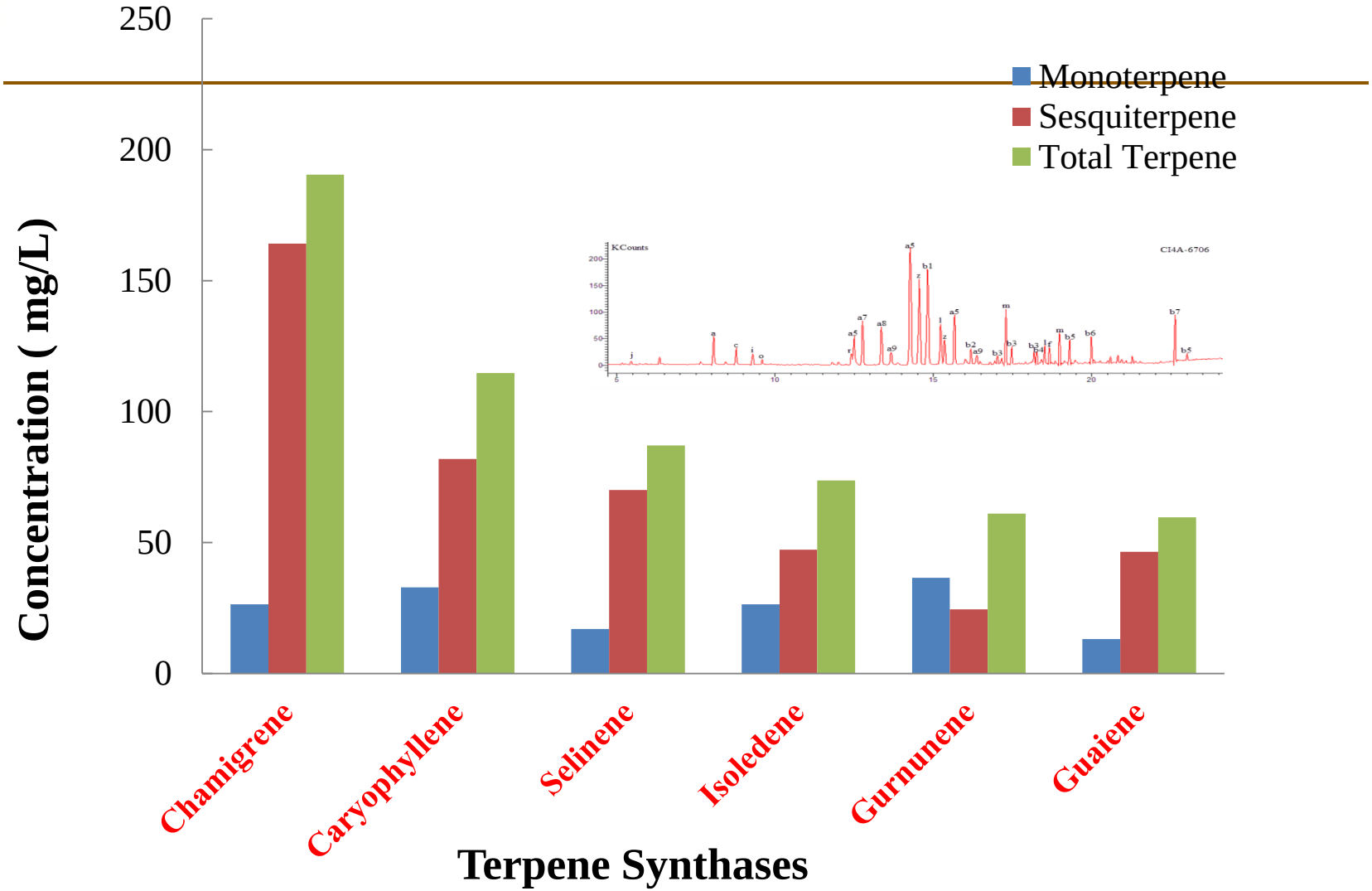



Terpene

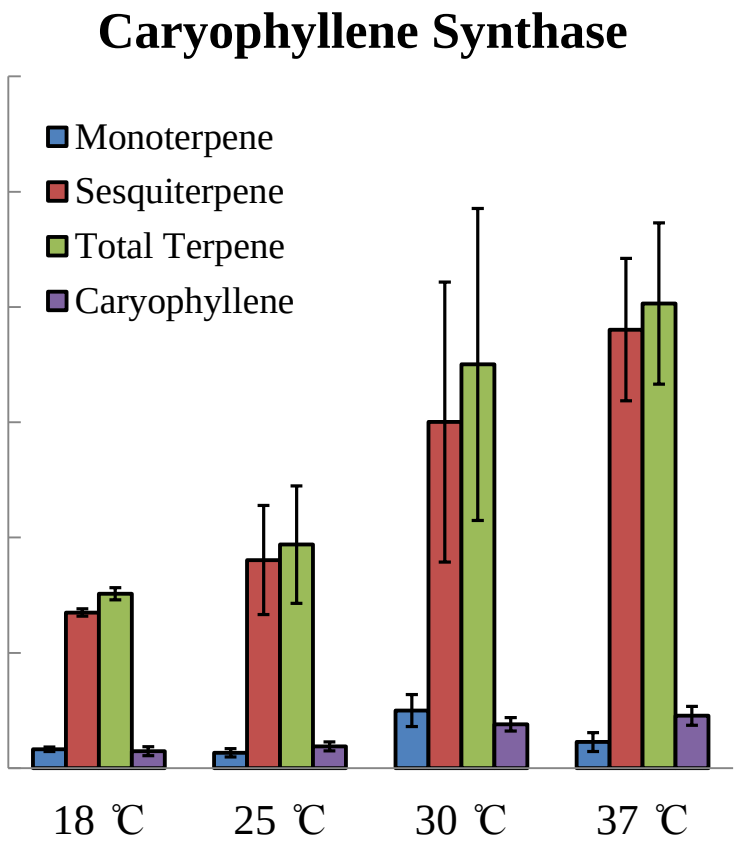
Protein

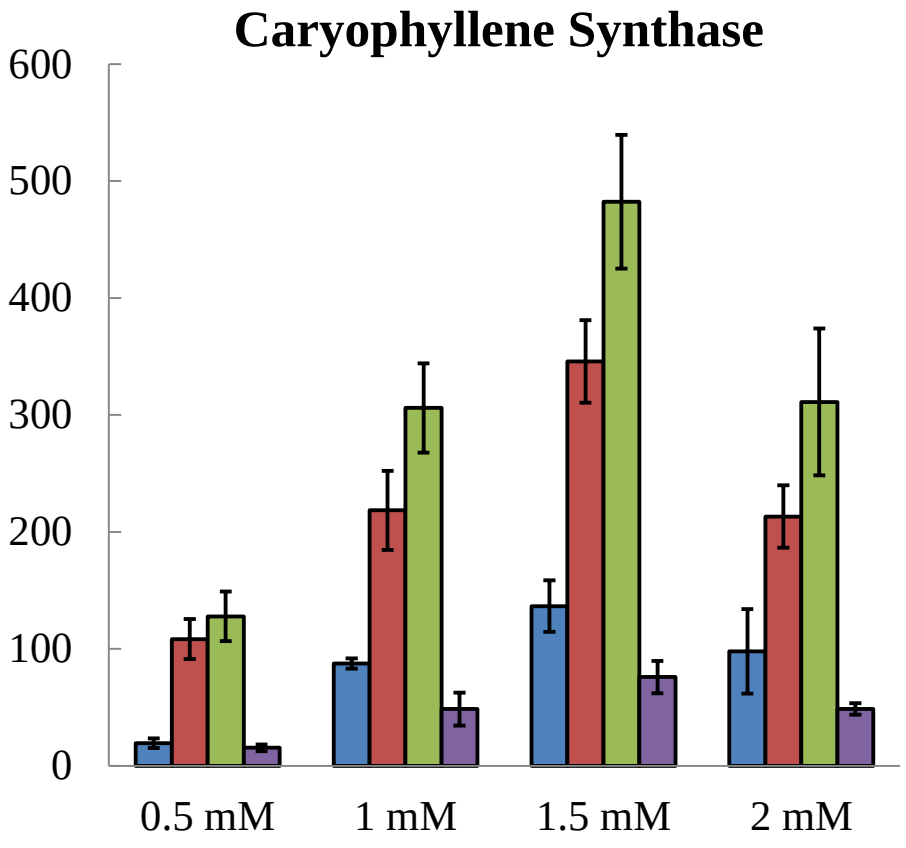


# Various Terpene Compounds Produced from Amino Acids Mixture



# Terpene Yield Improved with Optimized Fermentation Conditions

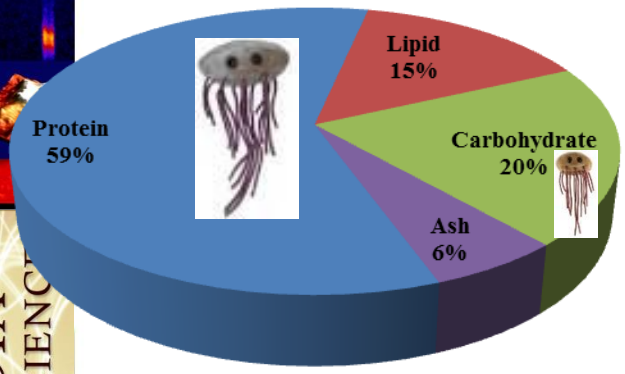


# "One-Pot" Bioconversion of Algal Carbohydrate and Protein with an Engineered Microbial Consortium


*E.coli* Mutant YH40

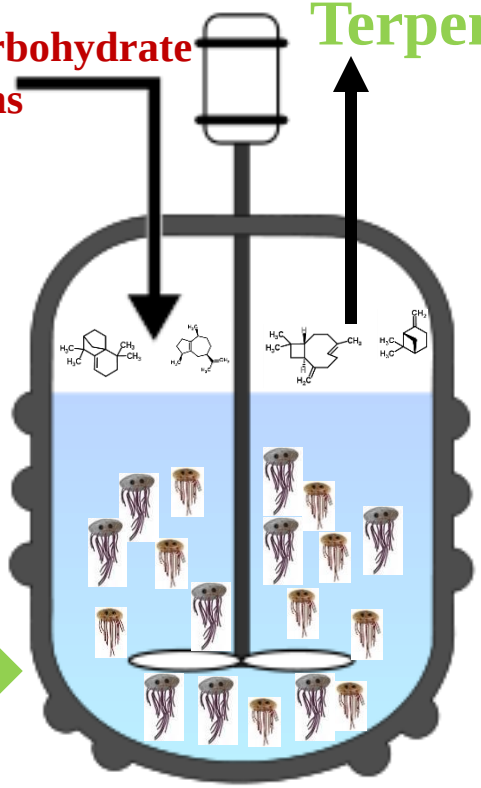
*E.coli* Strain DH1




*Reed nannochloropsis*

Algal Carbohydrate & Proteins

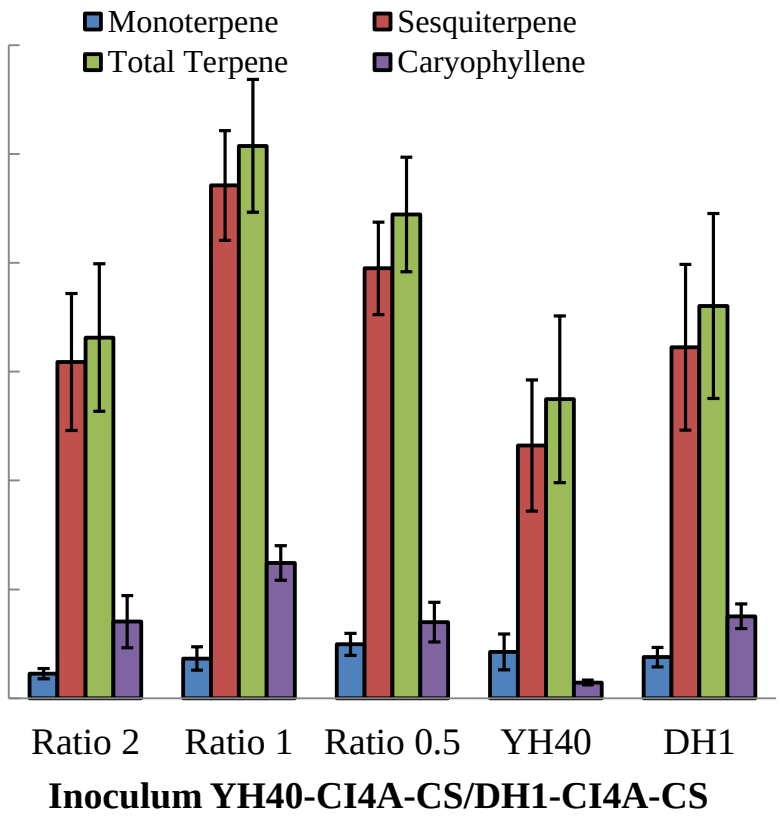
Terpenes

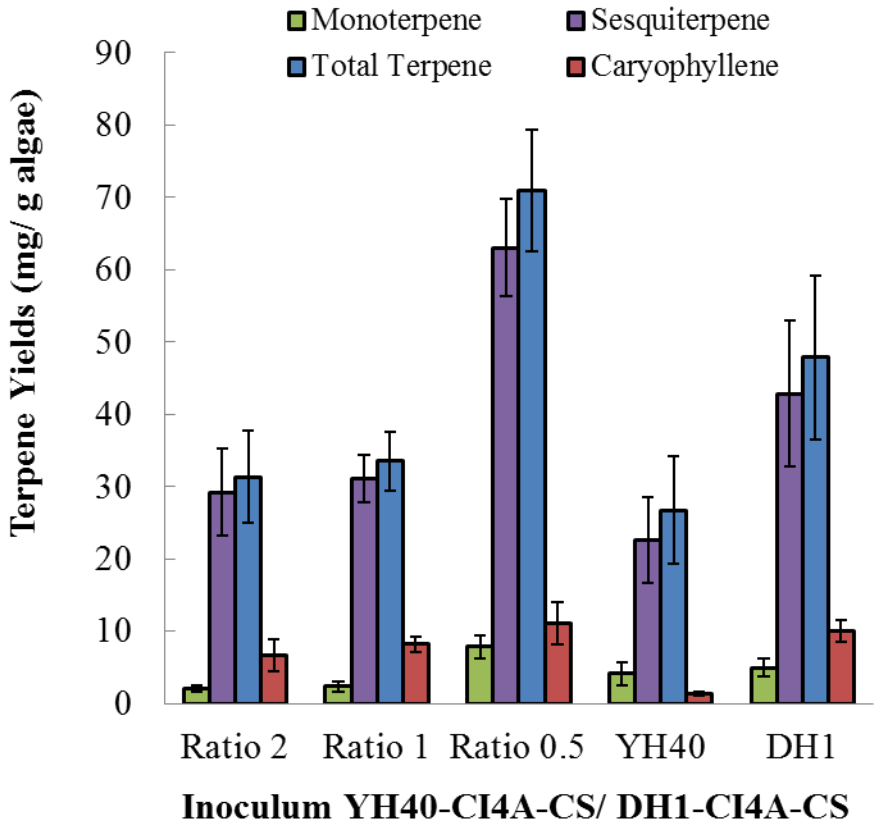


YH40-TS+DH1-TS

http://www.giantmicrobes.com/uk/products/ecoli.html

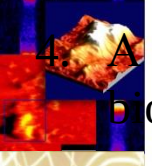
# Inoculum Ratio 1 of Two Strains Yielded the Highest Terpene Titer



# Take Home Message

1. Terpene biosynthetic pathway was rewired in *E.coli*: **12 novel TS genes discovered**
2. **Homologous expression of intermediate pathway genes** achieved the highest terpene titer
3. Successfully demonstrated the feasibility of bioconversion of **protein to terpenes**
4. A synthetic microbial consortium achieved “one-pot” production of terpene from algae biomass



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# Questions?