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Functions of PUFAs:

GLA Anti-aging Weight loss Anti-cancer Hairdressing Anti-lipid oxidation Anti-bacterial Activity



Intermediate of many other PUFAs



acid's transformation to GLA



Where is it ?

Main Derivations of PUFAs:



 high production costs

 diminishing feedstock

 Image: Second state

 limit PUFAs' supply and usage

**Seek New resourses is no time to delay.** 



### algae



### Fish oil in the deep sea



### **Borage seed Oil**



### **Evening Primose Oil**





# **Conclusions:**

- New resources: reduce costs, increase production
- make up for the lack of animal and plant resources
- improve the standards of people's living

# **Purposes and Significances**





### Purposes

- Clone the genes of  $\triangle$ 6-fatty acid desaturase
- Construct plasmid with strong promoter
- Transform the genes into oleaginous yeast
- Realize the heterogenous expression
- Increase GLA's contents.



### Significances

- Yeast, especially <u>oleaginous yeast</u> has a <u>high oil content</u> and <u>high</u> <u>linoleic acid rate</u> <u>high content GLA</u> with △6-desaturase.
- Oleaginous yeast as a host strain expressing foreign genes has not been published yet, so it is a <u>new and challenging work</u> for us.



transformation to GLA

# **Resources of Strains and Genes**





# **Resources of Strains and Genes**

Strains: Rhizopus stolonifer (49% GLA of total oil content)

**Rich in PUFAs** 

Cunninghamella echinulata

# Genes:

 $\triangle$ 6-Desaturase (D6DRs) from *Rhizopus stolonifer* 

 $\triangle$ 6-Desaturase (D6DM) from *Cunninghamella echinulata* 

GenBank, numberred DQ291156 and DQ177498, respectively.

# **Resources of Strains and Genes**

### **Host strains**

- Rhodotorula glutinis
- Lipomyces starkeyi
- Lipomyces kononenkoae
- **Trichosporon cutaneum**
- **Trishosporon fermentans**
- Trishosporon sp
- Pichia pastoris
- Saccharomyces cerevisiae





### **Functional identification of** $\triangle$ **6-fatty acid desaturase**

- We have constructed several plasmids:
- **pHBM605(pHBM906**<sub>CN</sub>+D6DM) / Pichia pastoris GS115
- **pHBM615(pHBM906**<sub>CN</sub>+**D6DRs)** / *Pichia pastoris* GS115







### **Composition Changes of Fatty acid in** *Pichia pastoris* GS115/pHBM605 before and after transformation

Fatty acid Percentage Number	Oleic acid	Linoleic acid	GLA	ALA
-CK	49.56	16.24	0.19	2.86
1	12.33	3.54	3.02	10.77
2	8.03	0.09	3.03	14.31
3	10.01	1.11	2.95	11.64

**GLA is accumulated After transformation.** 



Our major work is to construct a novel vector which can be used in oleaginous yeast.

- Integration Expression Plasmid of Lipomyces kononenkoae

**Integration Expression Plasmid of Trishosporon** fermentans



YPD without Hygromycin B Hygromycin B, 80ug/ml YPD

Fig. 4.4 Growing Conditions of L. kononenkoae before and after transformation

### Lipomyces kononenkoae



Fig. 4.5 Growing conditions of *L. kononenkoae* before and after transformation in Fluorescence microscopy



Trishosporon sp.

Conserved sequence of phosphoglycerokinase protein sequence in *Trichosporon sp* 

**Chromosome walking: The promoter sequence** 

Integration Expression Plasmid of Trichosporon fermentans.



### Trishosporon sp.

ATACGACTCACTATAGGGCACGCGTGGTTGACGGCCCGGGCTGGTCTGATAACTCTGGCTTC TCCCAATCCAAT TATAATGTATTTTTTGCTTTAGAATTTGAAAGGGTTCAAATAAAGTTGGTG TCAAATATTTAGTGCAGATGTGTCAAATTTATATGGTTTCTAGGAAAGACTAGACTATTACCTC AAAGGTCCTATCCTAGATTGACACCCTAGATCAATACTAGTCTCCTCTGAAAACGGTCTAGC AAAACCAATTCGCGAACAAGATTTTTAATTGCACCATGAGACGATCAAGAGTGAAAAATTG TATGATCTGAAGCACAAGGTACACTTTGATGGTTAAATGAGCTGGAATTGTTAGGTGATTGG GTATGTACAACGTGTCTGAGGCTAGAGACATTCTATCCCAGTCGTAGTCAGCCTGGTGCTCTT AATTCCTTGTACTACACTCTGAGCTGTACTTCTCTGATTGTATGTGAATGCTCGTGGTGAGCC TTTCTATCAGAGATGAGCTAGCATTGACACATAATAAATCATCACTCTCAATTAAAC CAATCCAGATCAATTCCTTCTGTAGTCTTCCATAATCTTCCATAATCCTCTATAATCCACTCTAT TACTCTAATTATCACTCCCAGTTTACCCTTCTTACAAAATTTGCATCCCACCGTTGACTCGTCC GTTGATGTCGTTGACGTTCGTGGTGTGTGGCCCGGCACACACGCTCACACGGTGCAACATC ATCCCCCTGTTGCTTACAACCTTCTTTACTACCACATCAACAATACTTAGAGTATTAAACAAA GTAAGCACAGTGAACACTCAATTGACATCTCAATAGCTAACCCCTTAACCCTAGTTTTACAA TTACCAACCATCCAATACGTTGCTGAACATAAACCAAAAGCTATCATCTTGGCTTCTCATTTAGGTAG ACCAAATGGTGAAAGAAATGAAAAAATATTCTTTAAAACCTGTTGTTGCTGAATTATCTTCATTATTAGG TAAACAAGTTACTTTCTTAAACGATTGTGTTGGTGATGAAGTTGAAAAAGCCGTTAATGGTGCCA+

### Fig. 4.6 Sequence analysis of PGK promoter

Grey frames: TATA box, CAAT box; White frames: AGAGbox, TGTG cis-acting element; "\_": initiation codon



YPD without Hygromycin B Hygromycin B, 80ug/ml YPD Fig. 4.8 Growing Conditions of *L. kononenkoae* before and after transformation

# Next plans





D6DM and D6DRs expressed In Lipomyces kononenkoae and Trichosporon fermentans respectively.

 $\bigcirc$   $\triangle$  5-,  $\triangle$  4-,  $\triangle$  12-,  $\triangle$  15- Fatty acid desaturases and elongases

