江蘇科技大學 篤學明德 經世致用

Jiangsu University of Science and Techenology

Inositol enhances lipid production by *Schizochytrium limacinum* SR21 using defatted silkworm pupae hydrolysate

Zhao-Xin Liu, Bin-Peng Tang, Bo Wang, Sheng Sheng, Jun Wang*, Fu-An Wu*

School of Biotechnology, Jiangsu University of Science and Technology

Sericultural Research Institute, Chinese Academy of Agricultural Sciences

Zhenjiang 212018, PR China

E-mail: wangjun@just.edu.cn

6th International Conference on Sustainable Solid Waste Management,

Naxos Island, Greece, 13–16 June 2018

CONTENT

- Background
- Previous study
- Present study
- Conclusions
- Acknowledgments

Background

Energy shortage

Oil excavation

New oil source should be found…

Silkworm pupae

Embroidery

Silk Road

Silk

Silkworm pupae

Silkworm

0.5 Million tons/year

Previous study

Biorefinery of silkworm pupae

Silkworm pupae

Silkworm pupa oil

Unsaturated fatty acid

Biodiesel

Structured lipids

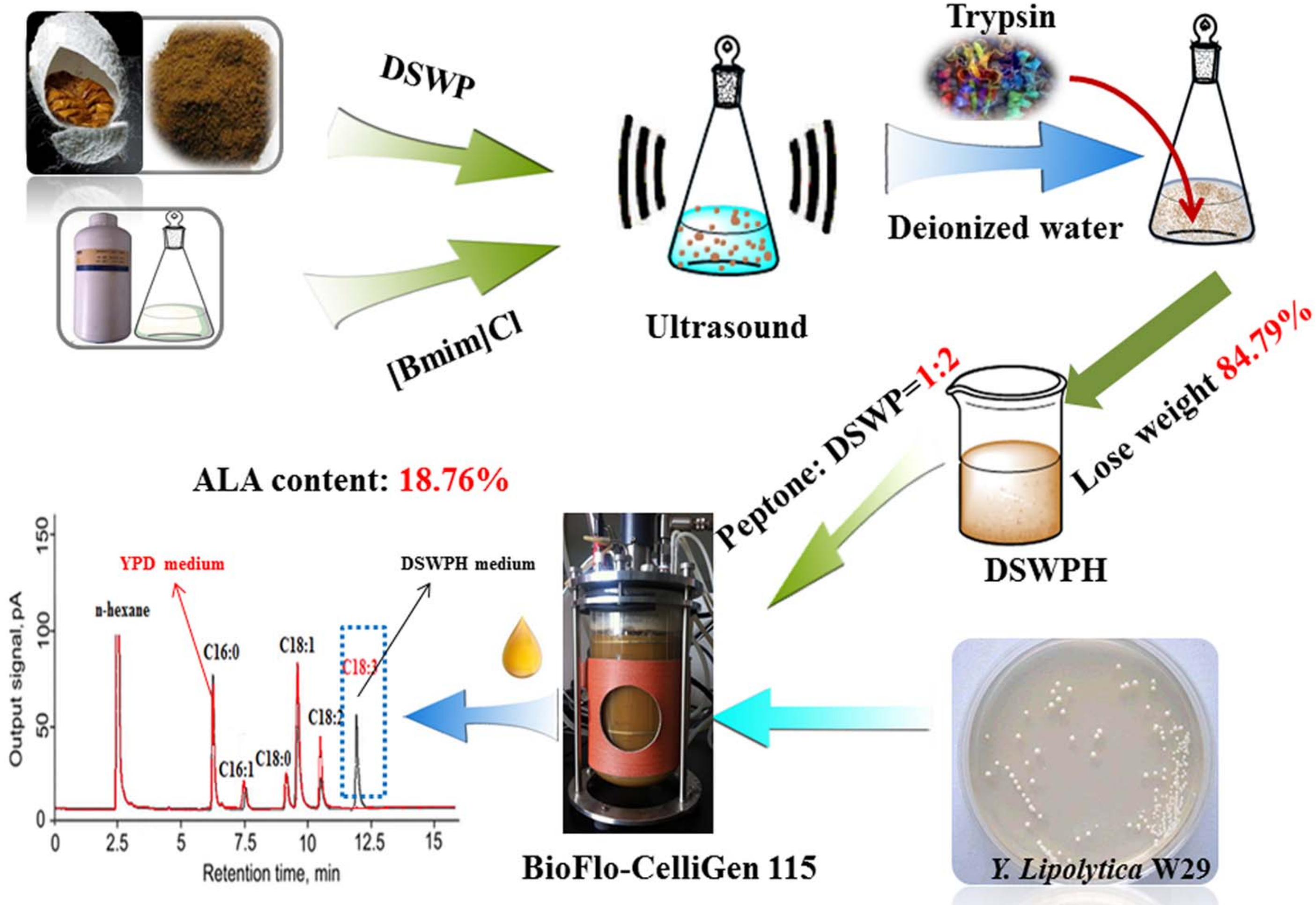
Silkworm pupa residue

Yeast lipid

Yang L I F, Siriamornpun S, Li D. *Journal of Food Lipids*, 2006, 13(3): 277-285

Manzano-Agugliaro F, Sanchez-Muros M J, Barroso F G, et al. *Renewable and Sustainable Energy Reviews*, 2012, 16(6): 3744-3753.

Converting defatted silkworm pupae by *Yarrowia lipolytica* for enhanced lipid production



Shi XY, Wang J*, et al. *European Journal of Lipid Science and Technology*. 2017, 119, 1600120.

Present study

High unicellular growth rate ?

Rapid lipid accumulation ability ?

Microbial oils

High-value oil and fat products

Problem: The cost of nitrogen and carbon sources

The cost of nitrogen source is about five times of carbon source

Feasibility of *Schizochytrium limacinum* SR21 using DSWP as a new nitrogen source

High yield

High protein content (85.2%)

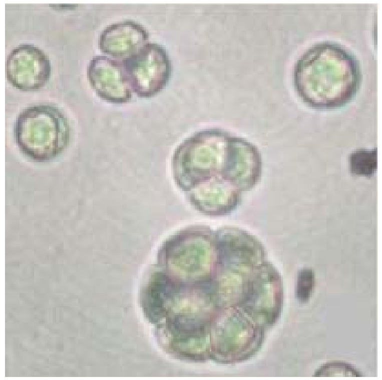
Defatted silkworm pupae (DSWP)

↓ hydrolysis

Soluble polypeptides → Cultivating

After 5d

Biomass	**39.27 g/L**
lipid yield	**22.44 g/L**
DHA productivity	**62.63 mg/(L·h)**



Schizochytrium limacinum SR21

Methods for improving lipid accumulation in microalgae

Novel approaches	Advantages	Challenges
Cultivation	High biomass production at first stage High lipid accumulation in second stage	Large scale trails are required
Combined nutrient and abiotic	High biomass and lipid productivity Suitable fattyacidprofile Easily scalability	Large scale trials are required Need to find cheap nutrient sources
Additives	High growth rate High biomass High lipid productivity	Need further research and optimization
Co-cultivation	High lipid productivity High growth	Bacterial population may affect the fatty acid composition Need further research to understand mechanism

Renewable and Sustainable Energy Reviews. 2016, 55: 1–16
Renewable Energy. 2016, 98: 72-77
Journal of the Energy Institute. 2016, 89: 330-334

Effects of inositol feeding on the fermentation process of *S. limacinum* SR21

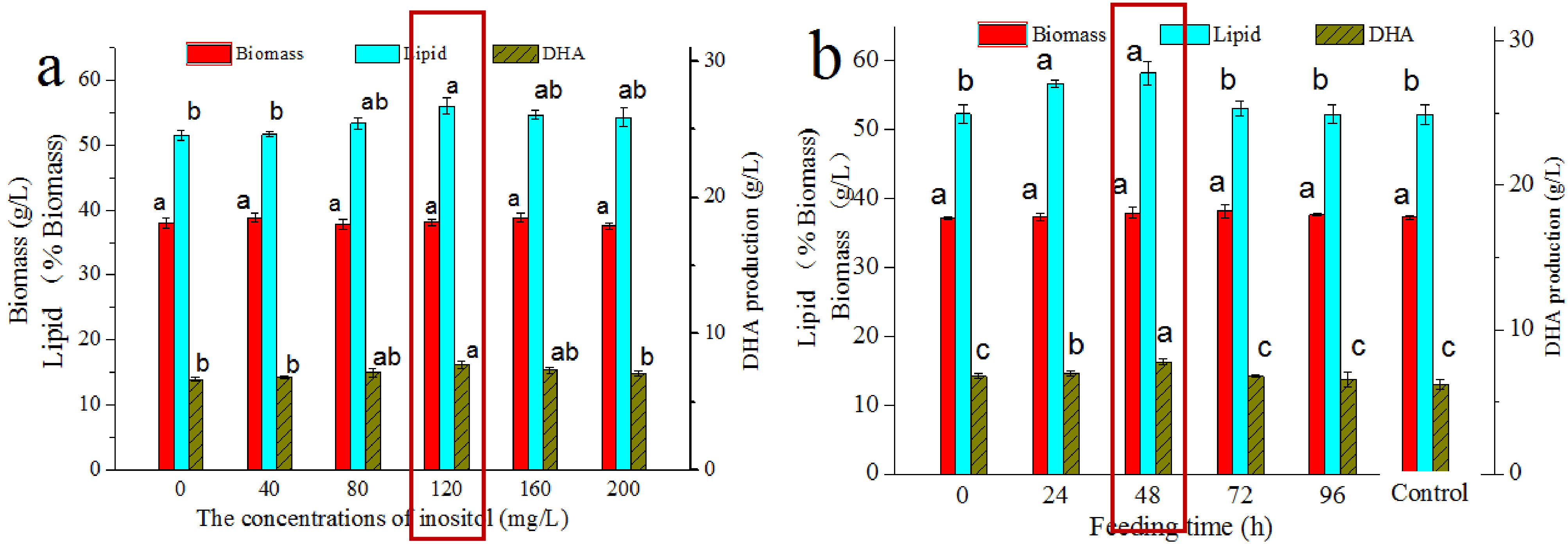


Fig. 1. Effects of different concentrations and feeding of inositol time on biomass, lipid content and DHA yield. (a) Feeding concentrations of inositol; (b) Feeding tine of inositol.

Changes of biomass, lipid content and DHA yield with and without inositol

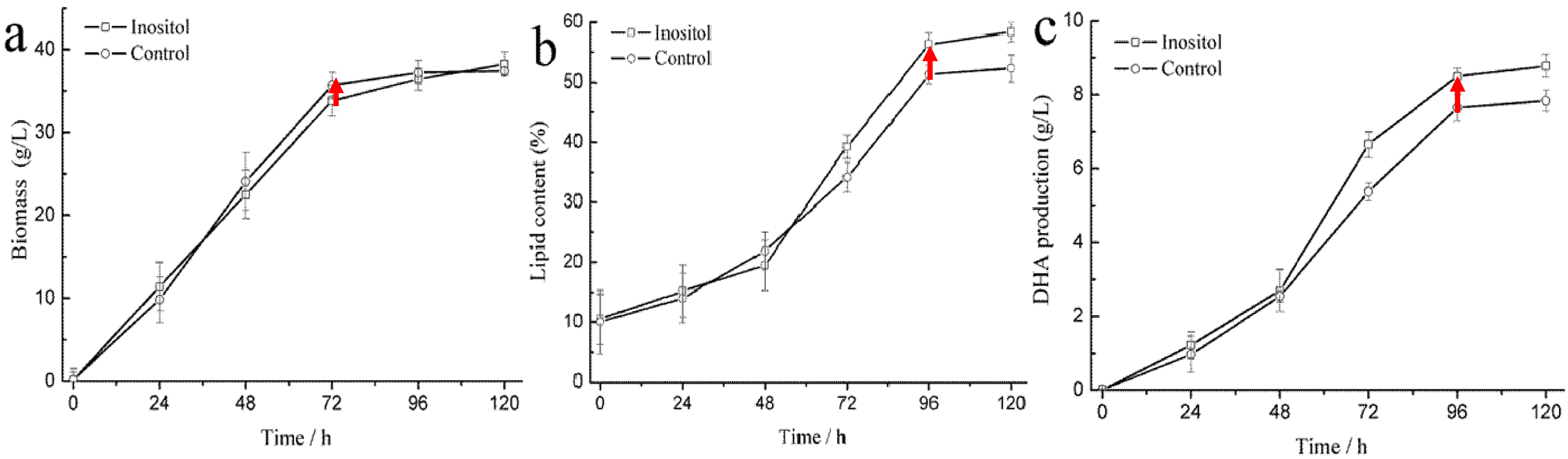


Fig. 2. Change of biomass, lipid content and DHA yield with and without inositol.
(a) Biomass; (b) Lipid yield; (b) DHA yield.

Micrograph of cells stained with nile red with and without inositol

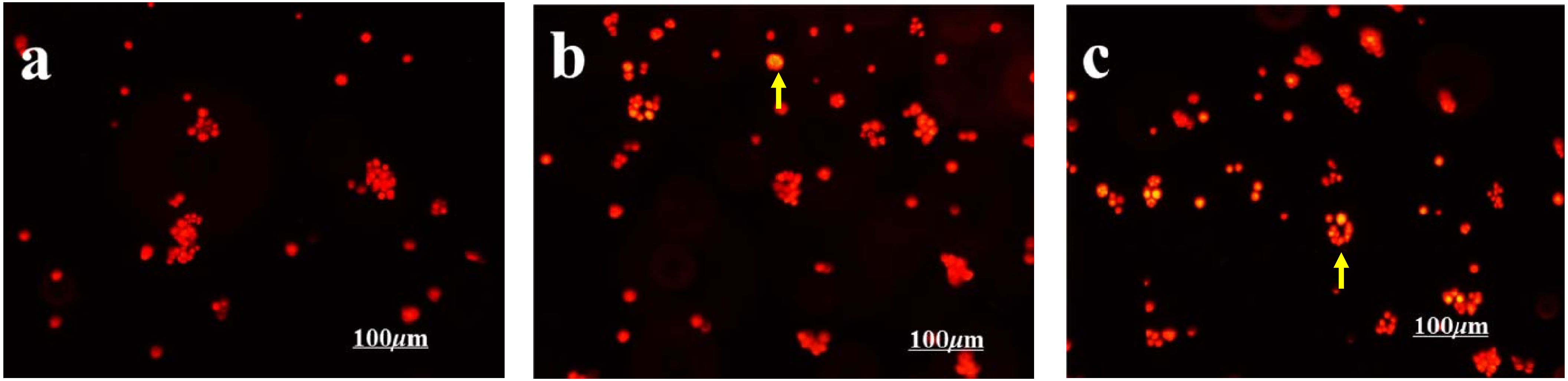


Fig. 3. Micrograph of cells stained with nile red for detection of total cellular lipids after 96 h of cultivation.
(a) Medium without inositol;
(b) Medium with inositol being added before the culture;
(c) Medium with inositol being supplemented at 48 h.

Table 1 Effect of inositol on fatty acid profiles and contents of produced lipids, and UFAs/SFAs.

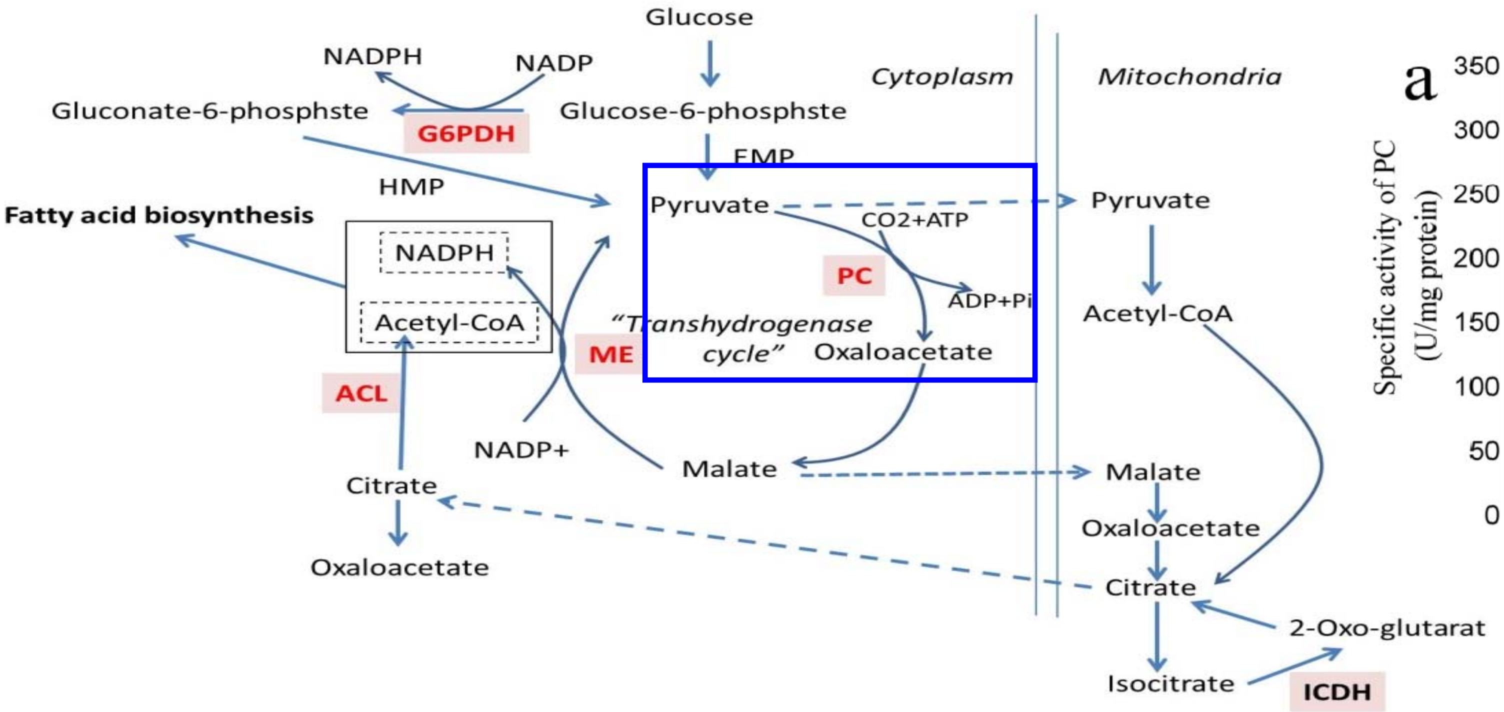
FAs（%）	Treatment		
	Control	0 h	48 h
C12:0	0.27 ± 0.16^{a}	0.21 ± 0.14^{a}	0.25 ± 0.13^{a}
C14:0	7.66 ± 0.57^{a}	6.97 ± 0.63^{ab}	6.63 ± 0.12^{b}
C15:0	3.35 ± 0.08^{a}	3.29 ± 0.31^{a}	3.01 ± 0.15^{a}
C16:0	44.48 ± 3.10^{a}	42.85 ± 0.40^{a}	40.54 ± 1.12^{a}
C17:0	0.58 ± 0.04^{a}	0.58 ± 0.01^{a}	0.56 ± 0.03^{a}
C18:0	0.26 ± 0.16^{a}	0.28 ± 0.10^{a}	0.37 ± 0.00^{a}
C18:1	0.79 ± 0.09^{a}	0.84 ± 0.03^{a}	0.79 ± 0.03^{a}
C18:3	0.21 ± 0.07^{a}	0.19 ± 0.10^{a}	0.08 ± 0.02^{a}
C20:5 (EPA)	1.23 ± 1.82^{a}	1.32 ± 2.06^{a}	3.62 ± 0.77^{a}
C22:5 (DPA)	6.45 ± 0.24^{a}	6.78 ± 0.55^{a}	6.75 ± 0.22^{a}
C22:6 (DHA)	35.20 ± 0.68^{b}	37.02 ± 2.87^{ab}	37.32 ± 1.27^{a}
UFAs	43.67 ± 2.13^{b}	46.00 ± 1.17^{ab}	48.50 ± 1.35^{a}
SFAs	56.33 ± 2.13^{a}	54.00 ± 1.17^{ab}	51.50 ± 1.35^{b}
UFAs/ SFAs	0.78	0.85	0.94

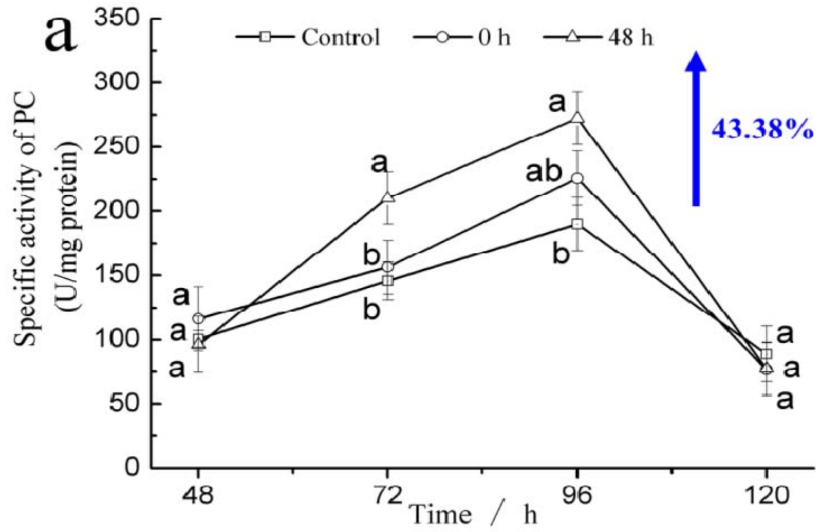
↑ 20.51%

UFAs/SFAs

[a, b, c] The mean values in the same row for *S. limacinum* SR21 lipid TFAs culturing on different media are significantly different ($p < 0.05$). UFAs: unsaturated fatty acids; SFAs: saturated fatty acids; TFAs: total fatty acids. For the *S. limacinum* SR21 , main UFAs are C18:1, C18:3, C20:5, C22:6 and C22:6, main UFAs are C12:0, C14:0, C15:0, C16:0, C17:0 and C18:0.

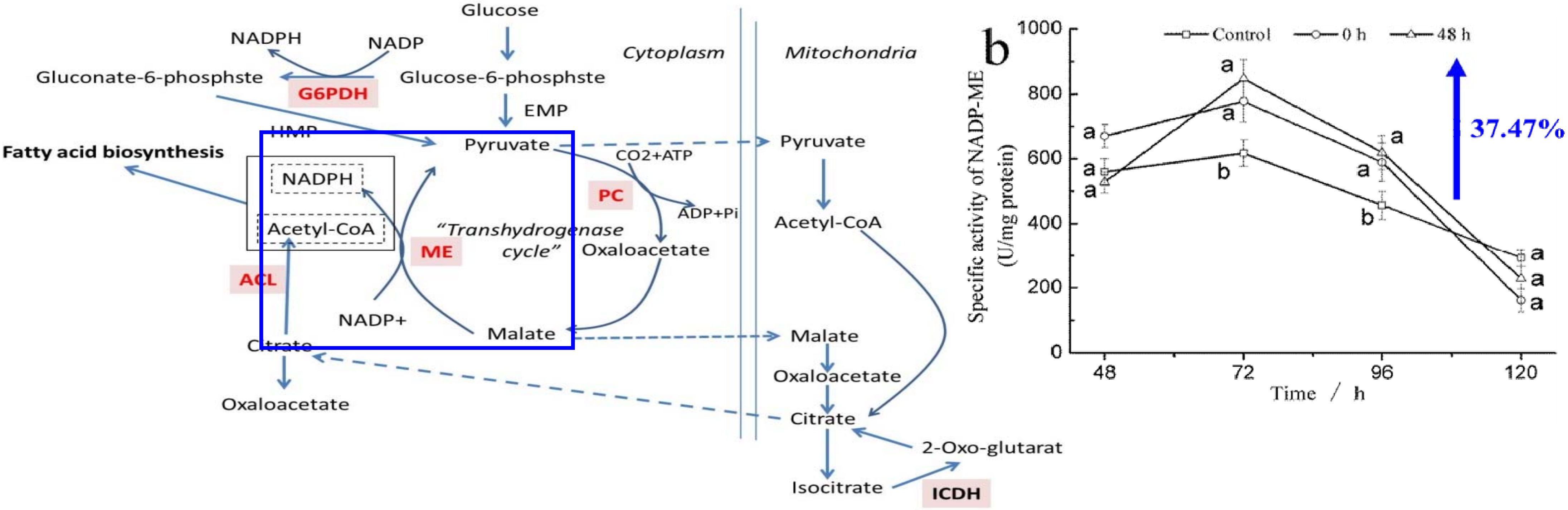
PC activity in *S. limacinum* SR21





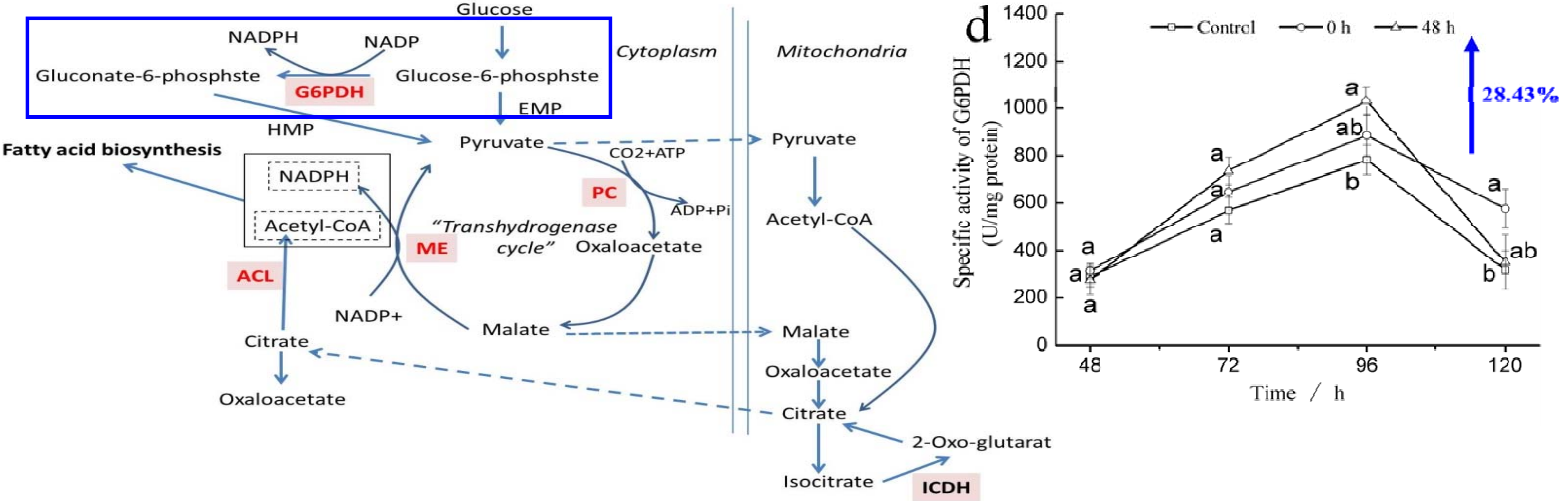
In the lipid producing microorganisms, pyruvate carboxylase (PC) is considered as an acetyl CoA and NADPH played a role in the process of synthesis of intermediate cycle.

ME activity in *S. limacinum* SR21



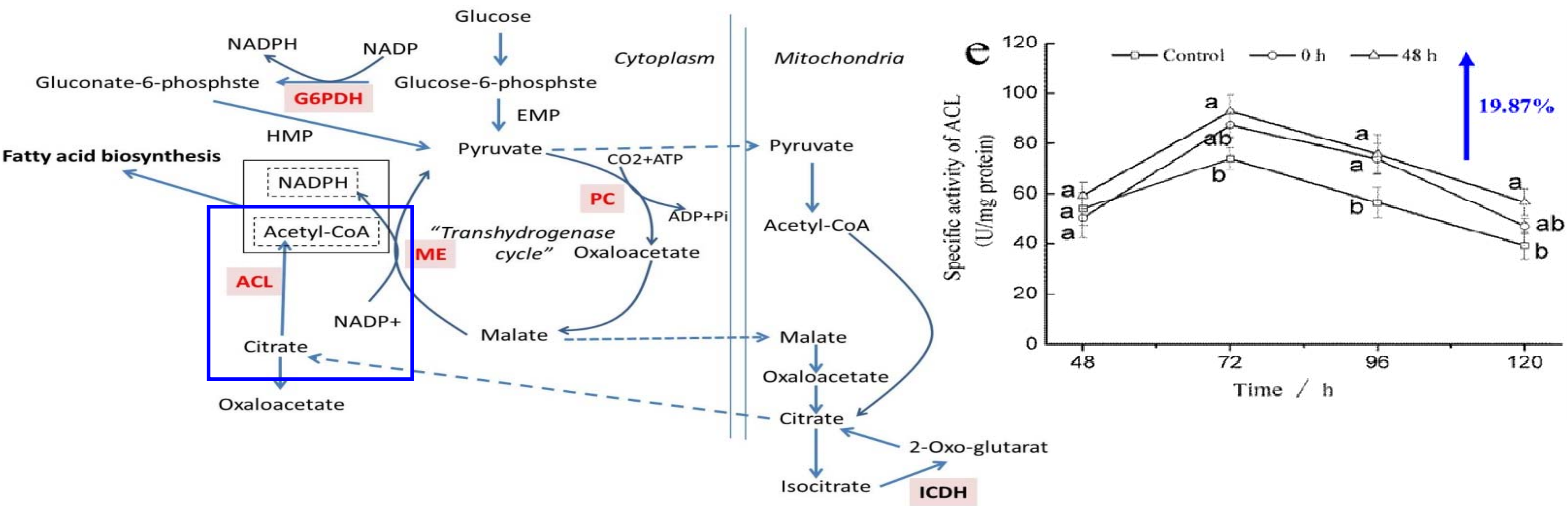
In the lipid synthesis process of eukaryotic microorganism, for NADPH supply, the main enzymes involved are NADP-ME and the enzymes of the HMP pathway, such as glucose 6-phosphate dehydrogenase (G6PDH).

G6PDH activity in *S. limacinum* SR21



In the process of cultivating 48 to 120 h, hexose monophosphate pathway (HMP) is a major source of NADPH for lipid synthesis. A higher G6PDH activity would strengthen the HMP activity and thus produce more NADPH.

ACL activity in *S. limacinum* SR21



ATP-citrate lyase (ACL) is considered to be a key limiting enzyme for lipid synthesis in oleaginous microorganisms. A higher ACL activity would produce more acetyl-CoA.

Conclusions

1. The yield of **lipid** and **DHA** was **13.90%** and **20.82%** higher by adding inositol.

2. The content of unsaturated fatty acids in lipid increased significantly, and UFAs/SFAs increased by **20.51%.**

3. **Inositol** can enhance the lipid accumulation of *S. limacinum* SR21 and change in fatty acid composition, and it can be used as an enhancer for fermentation of *S. limacinum* SR21 .

Acknowledgments

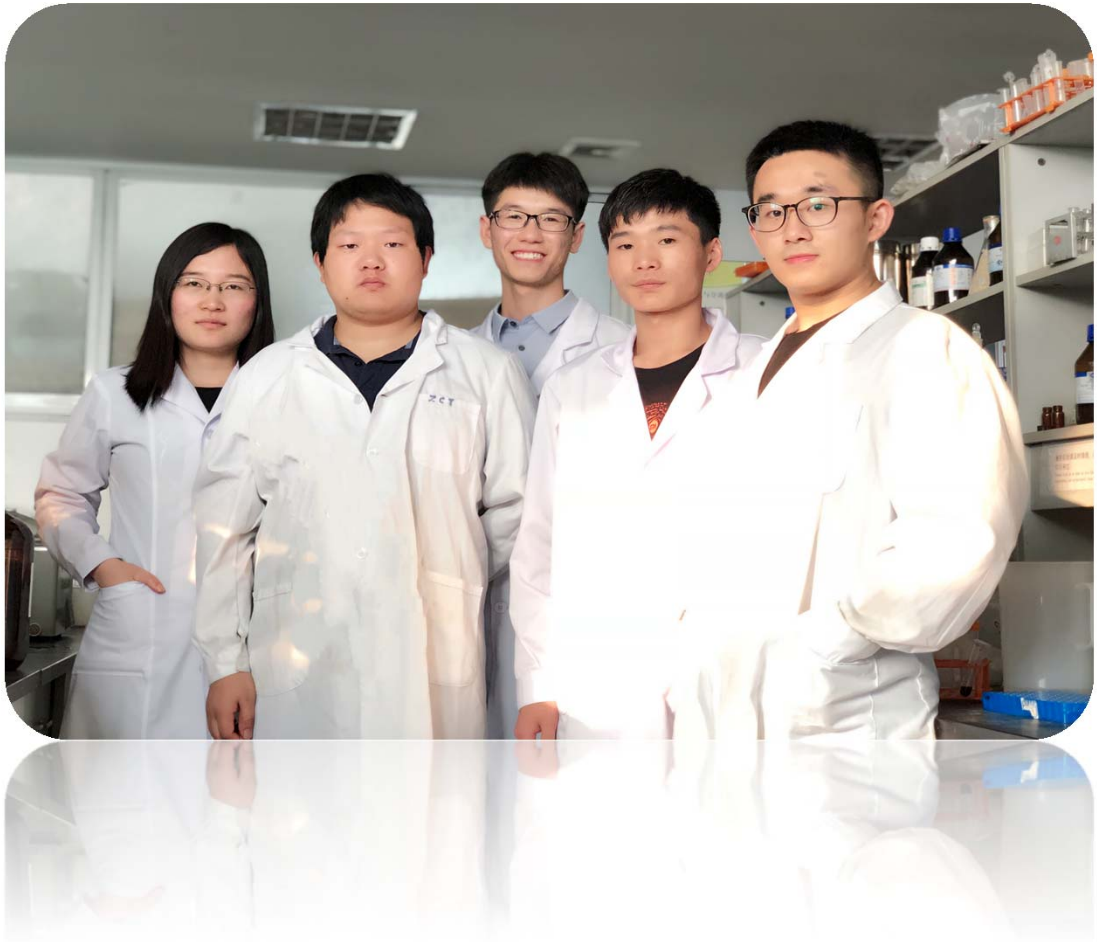
The Key Research and Development Program (Modern Agriculture) of Jiangsu Province (BE2017322)

The Six Talent Peaks Project of Jiangsu Province (2015-NY-018)

The Qing Lan Project of Jiangsu Province (2014)

The Shen Lan Young scholars program of Jiangsu University of Science and Technology (2015),

The China Agriculture Research System (CARS-18- ZJ0305).



Thank you for your kind attention!

Jinshan Temple
(1600 years old)
Zhenjiang City