Development of Algae-based biofuels at CPC Corporation, Taiwan

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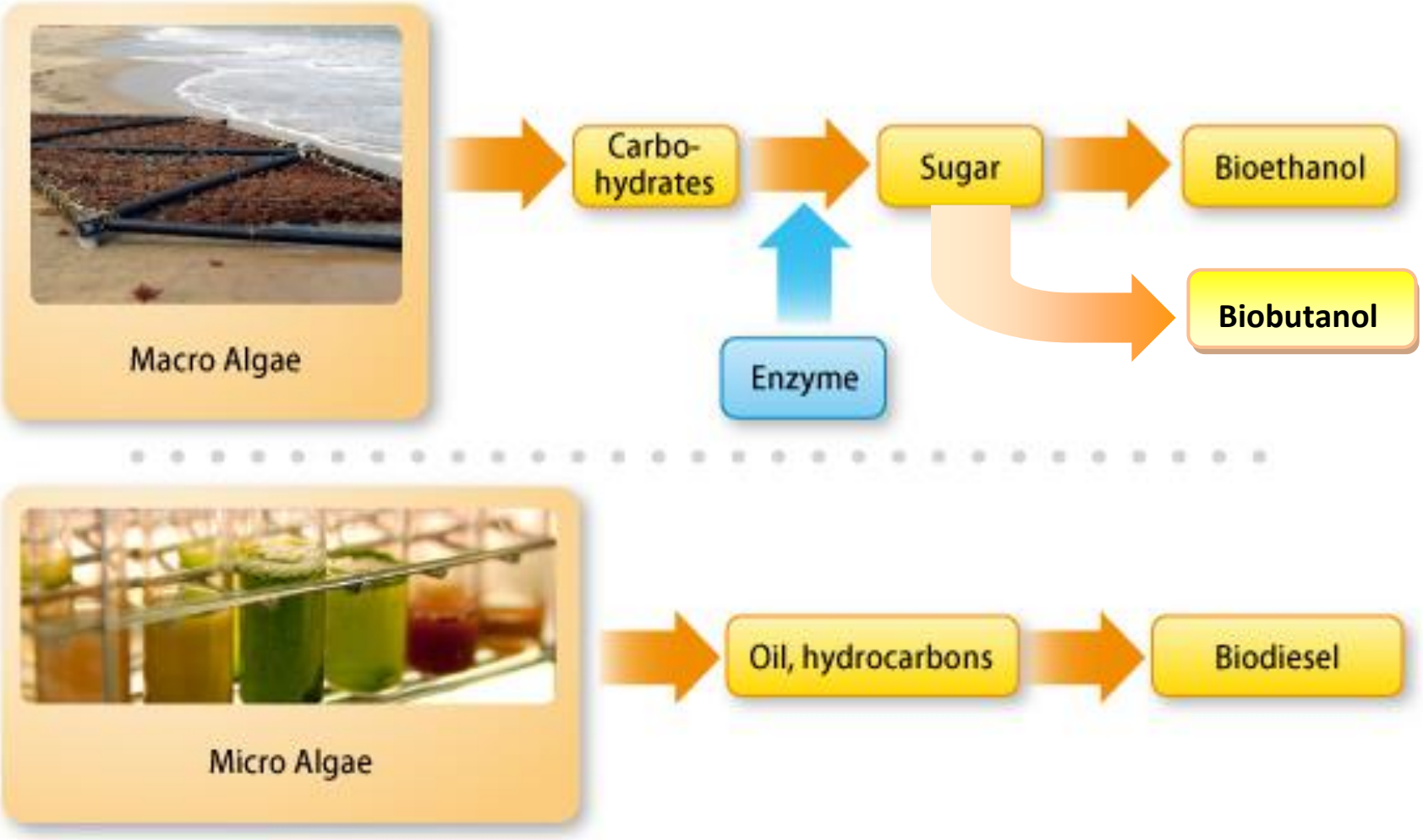
Gtr GREEN TECHNOLOGY RESEARCH INSTITUTE 台灣中油 綠能科技研究所

Algae Biofuel



(Modified from “Sea6 Energy biofuel from the oceans”)

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1. **Introduction of GTRI**
2. **Current R＆D of microalgae at GTRI**
3. ***Chlamydomonas orbicularis* CPC1215**

   -- a candidate in commercial and industrial algal oil producer
4. **Perspectives**

Introduction of CPC Corporation, Taiwan (CPC)

Listed in Fortune Magazine

Annual sales: 40 billion USD

Employees: ~15,000

Production : 8.9 million kL gasoline
7.0 million kL diesel
7.2 million kL fuel oil
842,000 T ethylene
670,000 T propylene

Sites: oversea exploration sites,
3 refineries,
1 petrochemical plant,
2 LNG receiving terminals

Business Field of CPC

台灣中油股份有限公司
CPC Corporation, Taiwan

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Green Technology Research Institute (GTRI)

- The GTRI was established on March 1st in 2012.

Dr. Tung-Li Huang

Director

Deputy Director

Mr. Yu-Feng Zeng

Biotechnology Department

Bioethanol, Biobutanol and Biomass cultivation

Renewable Energy Department

Solar energy, hydrogen energy and biofuels

Material Technology Department

LED , Coating, energy-storage materials

Environmental Technology Department

Algae and environmental technology

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Biotechnology Department

GTRI Headquarter

Green Technology Demonstration Area

Biotechnology Department

Alishan national scenic area

Green Technology Demonstration Area

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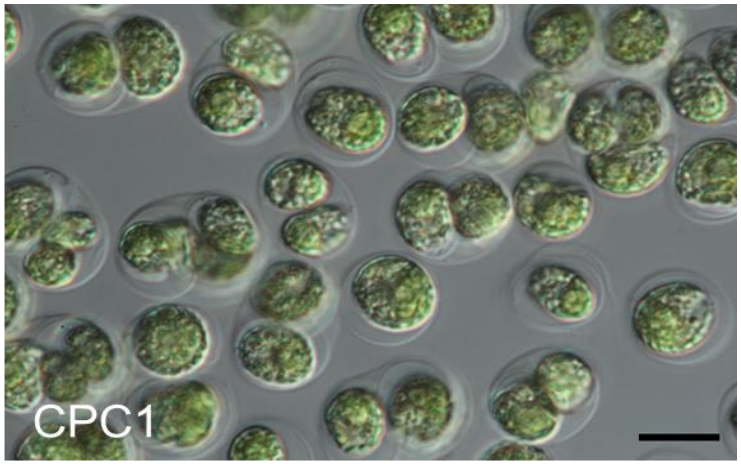
Algae-based biofuels at CPC

- In 2010 to response with governmental policy to develop renewable energy, CPC began to search suitable feedstock for biodiesel production.

  -- Microalgae is the third generation biofuel producer.

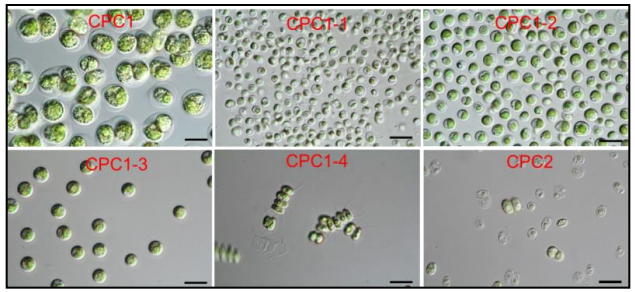
- Microalgae selection for biodiesel production in CPC was commissioned to Prof. Jo-Shu Chang in National Cheng Kung University.

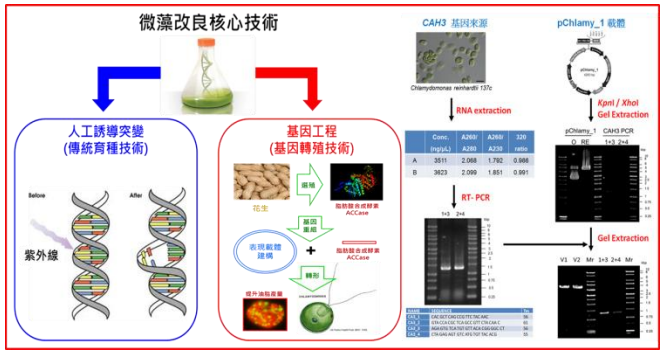


The GTRI Research Groups of Microalgae

Biotechnology

開發低成本纖維酒精發酵技術
生質醇發酵技術改良
生質能料源培育技術開發
(微藻品種改良技術開發)
生質化學品合成技術開發





再生能源
高值低碳
環保節能
之
綠能產業

Environmental Technology

環保觸媒及綠色製程開發
節能燃燒與減排技術開發
微藻培育減碳及產油技術開發
環境節能與整治技術開發

翁堉翔博士提供

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Contents

3. Chlamydomonas orbicularis CPC1215

-- a candidate in commercial and industrial algal oil producer

Topic : The establishment and application of *Chlamydomonas orbicular* CPC1 ultraviolet-induced mutants

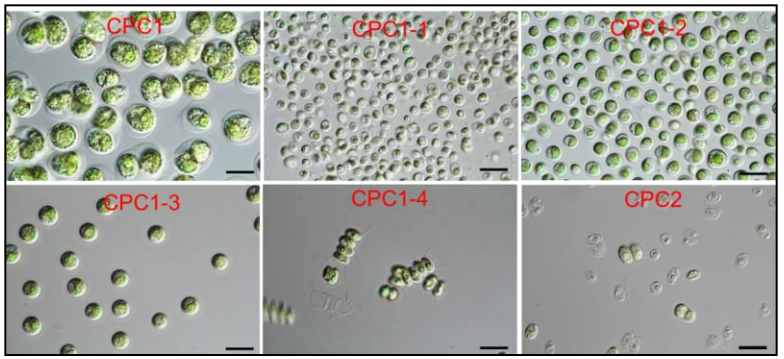
Author : Ai-Ling Kao, Zheng-Chia Tsai, Chun-Yen Chen, Jo-Shu Chang, Mao-Yuan Tu, Tung-Li Huang

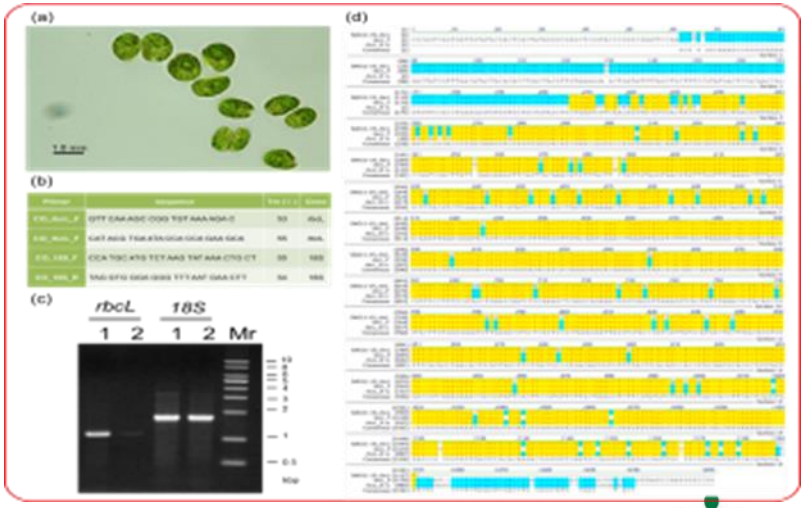
Paper was accepted by Journal of Petroleum in July 2015.

4. Perspectives

Chlamydomonas orbicularis CPC1

- CPC1 is olated from seawater in southern Taiwan
- Lipid content:
  - > 40% (laboratory)
  - 20-30% (outdoor)
- Outdoor cultivation:
  - variation in light intensity
  - high temperature
  - contamination





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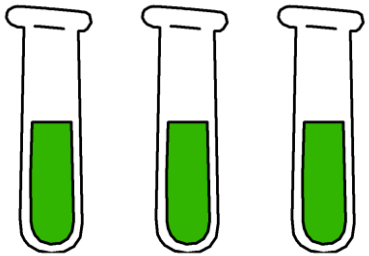
UV mutagenesis of microalgae to enhance lipid production

6W UV light

12.5 cm
2 min

Chlamydomonas orbicularis CPC1
Exponential phase

24h in the dark
to prevent photoreactivation



mutant selection

High-throughput Nile red method

- ✓ Rapid analysis
  - -- about 10~20 mins
- ✓ Low cost
  - -- one dye and spectrophotometer
- ✓ Small sample volume
  - -- 1 mL sample at OD680 = 0.06

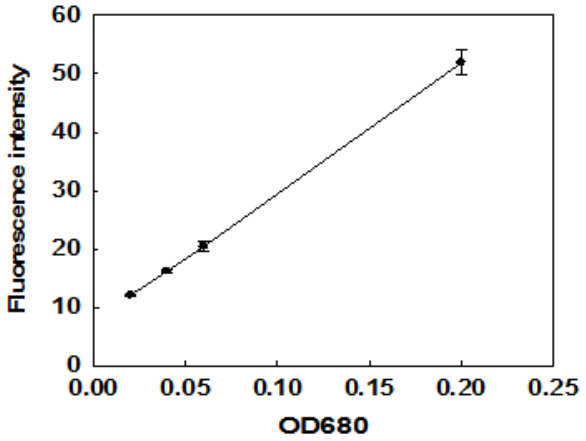
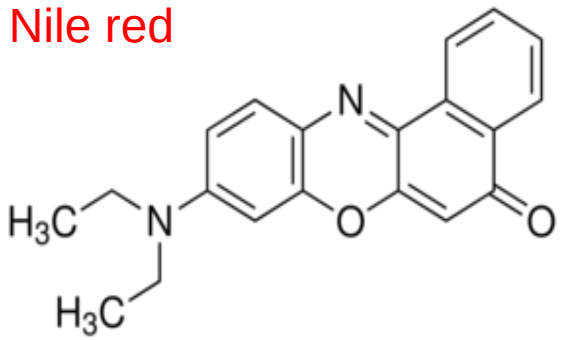
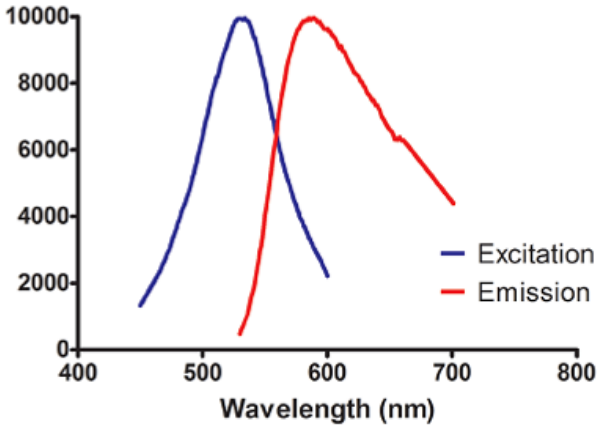


Fig 2、藻體濃度 (OD680) 與 Nile red 螢光強度的線性關係。

Nile red



Fluorescent probe of intracellular lipids



(From BioTek's website)

Mutant selection procedures

5 mL in test tube

320 mutants

250 mL photobioreactor

16 mutants

1 L photobioreactor

3 mutants

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First-stage screening at 5 mL scale

Table 2、、第一階段篩選 Nile red 螢光強度高於 CPC1 野生型之突變藻種

Mutant no.	% increase in Lipid content vs. wild type	Mutant no.	% increase in Lipid content vs. wild type
23	[illegible]	[illegible]	32.2
[illegible]	[illegible]	[illegible]	[illegible]
54	[illegible]	[illegible]	[illegible]
81	[illegible]	[illegible]	26.5
91	22.7	[illegible]	11.2
183	14.4	291	9.6
190	14.7	300	17.3
210	3.5	311	25.3

16 mutants

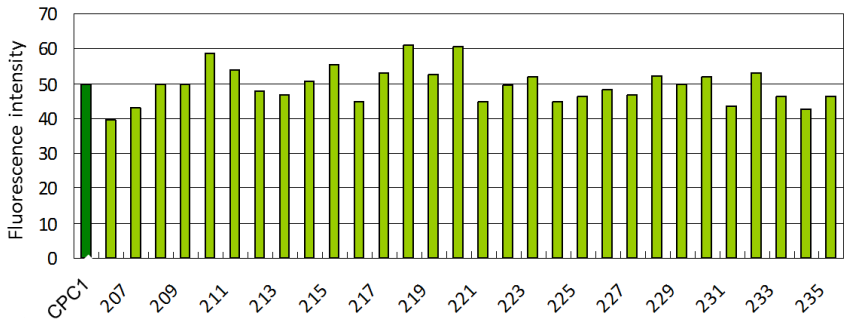
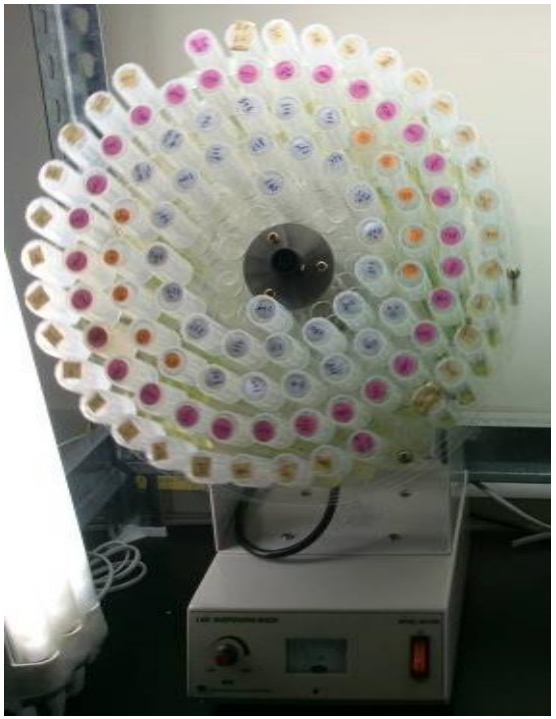


圖 3、 CPC1野生型與突變藻種之Nile red螢光染色強度比較

5 mL in test tube



320 mutants

Second-stage screening at 250 mL scale

Table 3、Lipid accumulation of CPC1 mutants 250 mL photobioreactor

Strain	Ni…ee…	Lipid content (%)
WT		…9
81		42.2
91		…6
215		44.8
218	8…5	43.6
266	6…1.5	42.9

3 mutants
CPC1215
CPC1218
CPC1266

250 mL in PBR

16 mutants

Third-stage screening at 1L scale

表 4、 CPC1野生型與突變株CPC1215、CPC1218、CPC1226之油脂生產速率比較

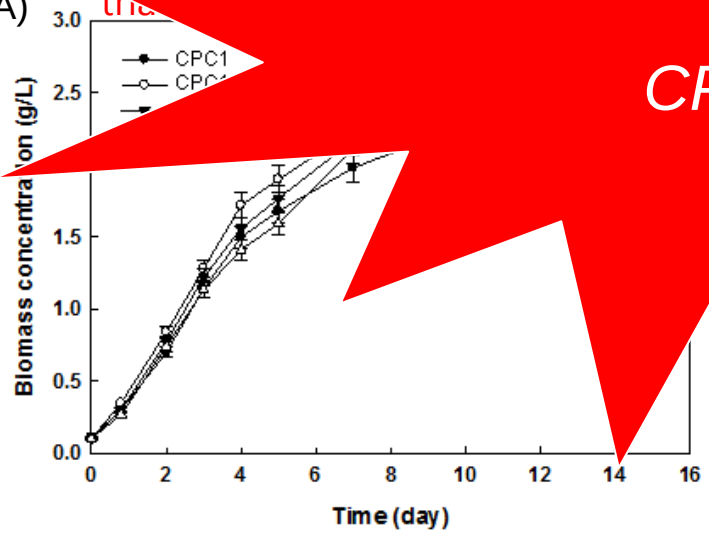
Lipid productivity (mg/L/day)				
	Day 7	Day 9	Day 11	Day 14
CPC1	87.8±2.6	93.2±2.7		73.9±2.2
CPC1215	118	121		90.3±2.7 (51.8%)
CPC1	11			83.0±2.4
CPC1266				87.6±2.6

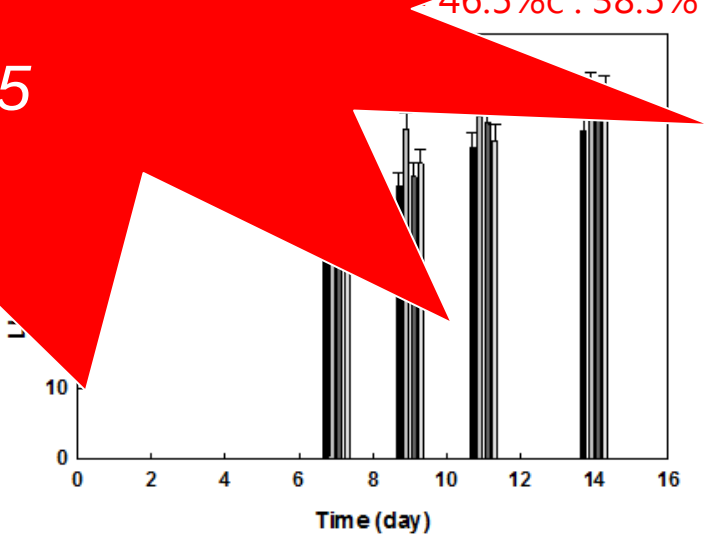
increase 30.8%

1 high lipid-producing mutant

CPC1215

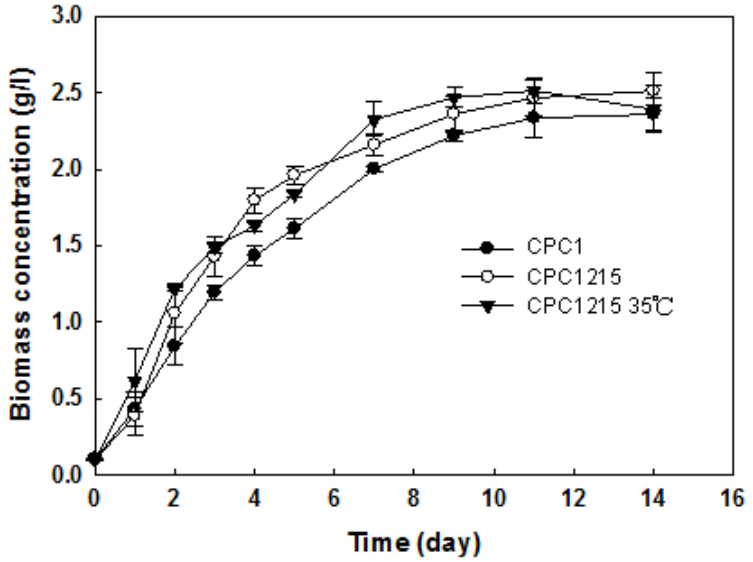
(A)





Thermo-tolerance of CPC1215

(A)



(B)

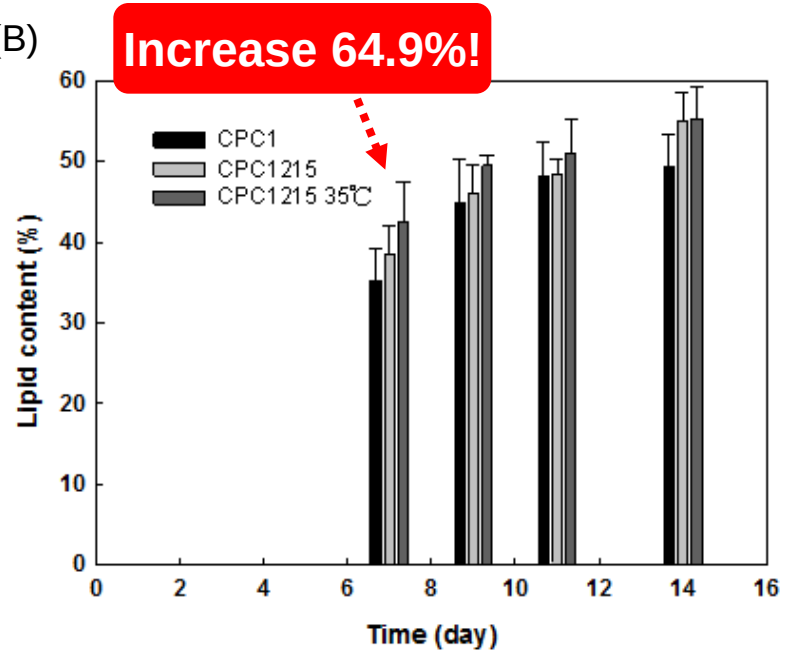
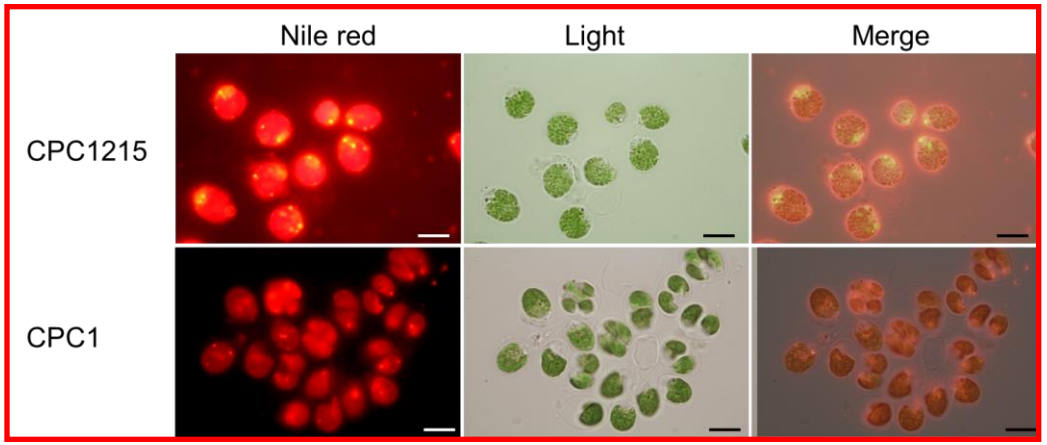


表 5、 CPC1215 高溫培養之油脂生產速率

Lipid productivity (mg/L/day)				
	Day 7	Day 9	Day 11	Day 14
CPC1	100.7±3.0	110.6±3.3	102.6±3.1	83.2±2.5
CPC1215	118.4±3.6	121.0±3.6	108.6±3.2	98.7±2.9
CPC1215 35℃	140.8±4.2	124.8±3.7	109.5±3.3	95.5±2.8

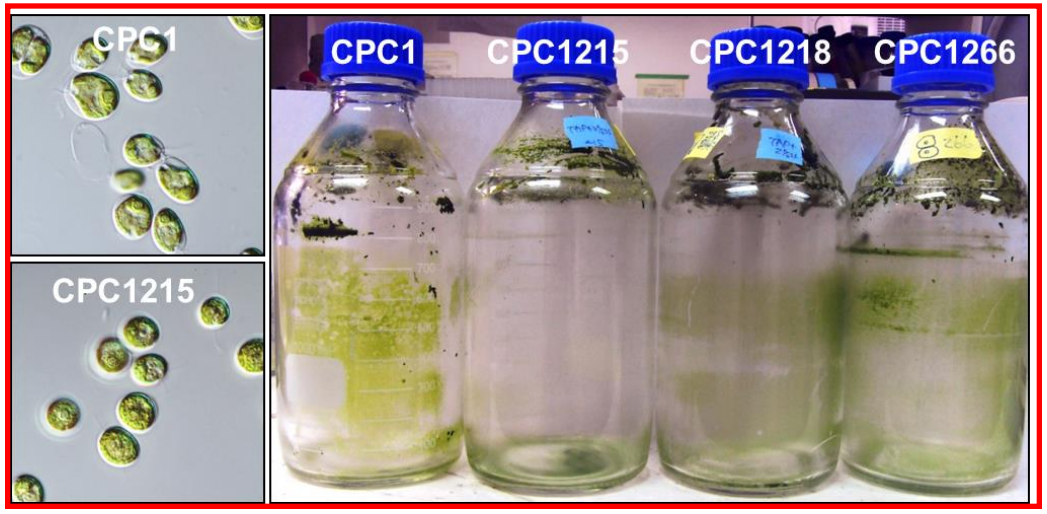
CPC1215 showed the low adhesion and rapid gravitational sedimentation properties

(A) High lipid accumulation



(B) Rapid sedimentation by gravity

(C) Low adhesion to photoreactor



Outdoor cultivation in 50 L photobioreactor

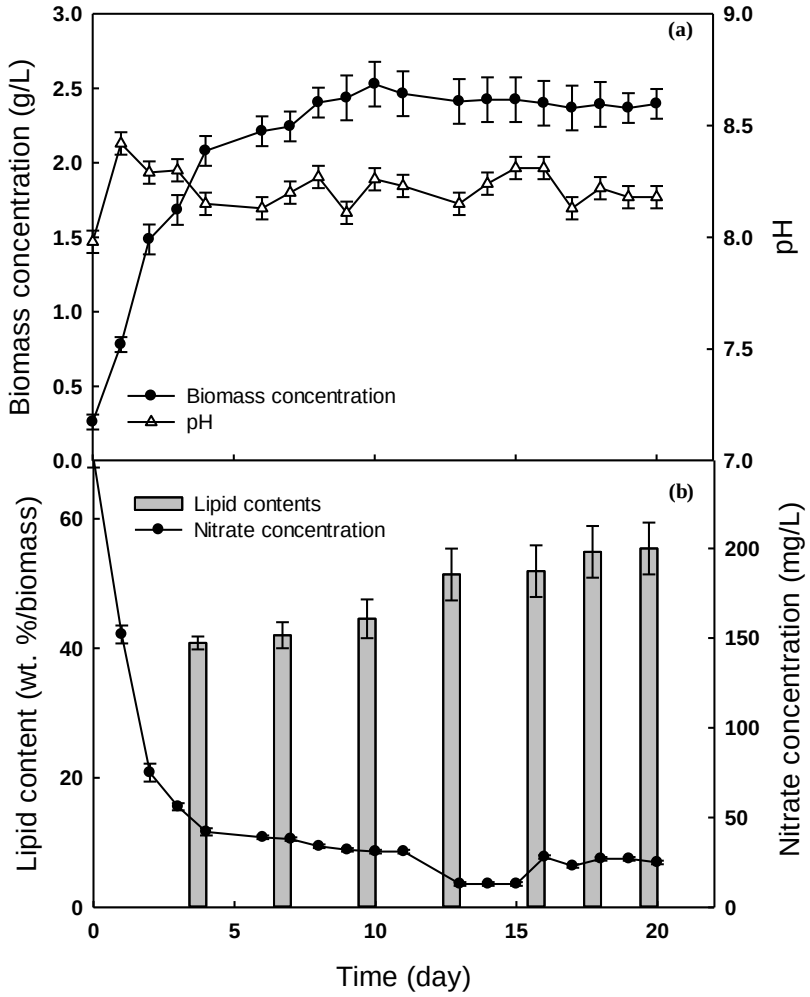


圖 8、 CPC1215戶外50L光生物反應器培養之生長速率與產油能力分析結果

表 6、CPC1215 與 CPC1 戶外 50L 養殖生長速率與產油能力比較表

	Days	Biomass concentration (g/L)	Lipid content (FAME:%)	Lipid productivity (mg/L/day)
CPC1	28	2.3	54.1%	45.4
CPC1215	20	2.5	55.4%	95.4

***C. orbicularis* CPC1215 would be a great candidate in commercial and industrial algal oil producer according to its high lipid productivity under 1% salinity, low adhesion, and easily collection by gravity sedimentation.**

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Perspectives of Algae Research in GTRI

Use Green technology to reach the goal in creation of the company's revenue

Acknowledgment

- All members in the department of biotechnology, GTRI
- Prof. Jo-Shu Chang,

  National Cheng Kung University, Taiwan
- Dr. Chun-Yen Chen,

  National Cheng Kung University, Taiwan

Thank you for your attention !

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