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研究獎學金

Research Scholarship

建立本土微藻*Chlorella sorokiniana* MB-1-M12之微藻培養技術、二氧化碳捕捉與葉黃素生產程序

Cultivation microalgae-based CO_2 fixation and lutein production processes using an indigenous microalga *Chlorella sorokiniana* MB-1-M12

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研究重點

The low lutein content and high production cost are always the main issues which limits the commercialization of microalgae lutein production. These issues could be resolved through effective engineering operation strategies to comply with higher lutein content and productivity in a large-level scale, hence, both the production cost of microalgae and lutein product cost could be decreased. We had conducted various microalgal lutein production strategies in this study under mixotrophic growth and cultivation modes integration. Developing microalgal strains with the ability to accumulate lutein at high content with an enhanced production rate appears to be the key to the success of commercializing microalgae-based lutein. In this study, we used random mutagenesis as a strategy for strain improvement to enhance the lutein production of *C. sorokiniana* MB-1, while the MB-1-M12 was obtained the best performance on mixotrophic mode under optimal cultivation conditions. The outdoor cultivation of the mutant (MB-1-M12) gave similar lutein content to that obtained in indoor cultivation, whereas a decrease in lutein productivity was observed in the outdoor culture. Therefore, the effective operation strategies were applied to enhance the lutein production performance. On the other hand, microalgal cultivation is highly water dependent, however, they could be cultivated using wastewaters to solve this problem. We utilized aquaculture wastewater acquired from a shrimp cultivation farm to cultivate MB-1-M12. The aim of this study was to achieve high biomass and lutein production using aquaculture wastewater. The MB-1-M12 can produce carotenoids under autotrophic, mixotrophic and heterotrophic conditions. However, the phototrophic growth is self-limiting because of shading effect of light that occurs as cell density increases, while the carotenoids belong photosynthetic pigment led to the heterotrophic growth obtains low lutein content. Therefore, two-stage cultivation of microalgae is not avoided for commercially viable production of microalgal lutein. We conducted the microalgal strains (MB-1 and MB-1-M12) are performed to grow under three different cultivation methods and carried out carotenoids analysis, further metabolic analysis targeted to these cultivation conditions. According to above results, we develop the novel operation strategies for lutein production as combing two cultivation methods (Autotrophic/Heterotrophic and Mixotrophic/Heterotrophic), which was few applied to produce lutein in the previous studies. These strategies have important implications for commercial lutein production.

研究成果

1. Mutant microalgal strains selection and cultivation methods comparison

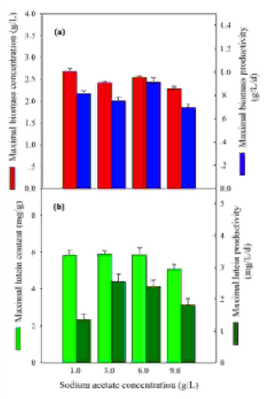

Figure 1.1 Lutein production from mixotrophic cultivation of *C. sorokiniana* MB-1. (a) Effect of sodium acetate concentration on biomass concentration and productivity, (b) effect of sodium acetate concentration on lutein content and productivity.

Table 1.1 Cell growth and lutein production performance of *C. sorokiniana* MB-1 and its mutant strains

Strain	Max. biomass concentration (g/L)	Overall biomass productivity (g/L/d)	Max. lutein content (mg/g)	Max. lutein productivity (mg/L/d)
MB-1	2.26±0.06	0.928±0.04	5.86±0.11	2.39±0.09
MB-1-M3	1.69±0.05	0.643±0.11	6.60±0.15	2.10±0.17
MB-1-M5	1.63±0.04	0.428±0.08	6.32±0.22	2.15±0.21
MB-1-M9	1.99±0.07	0.435±0.06	6.25±0.17	3.60±0.16
MB-1-M12	2.29±0.03	0.932±0.09	7.52±0.12	3.63±0.15

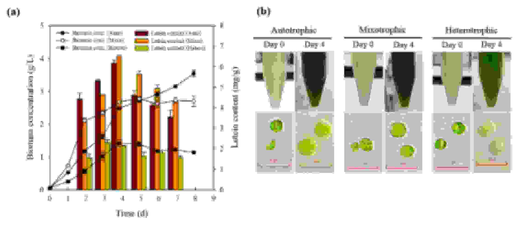

Figure 1.2 The performance of *C. sorokiniana* MB-1-M12 under different cultivation methods. (a) time course profile of biomass concentration and lutein content, (b) the morphology of microalgal cultivation for different cultivation methods.

2. The optimal condition base on outdoor cultivation under mixotrophic growth

Table 2.1 Effect of medium composition on biomass and lutein production using *C. sorokiniana* MB-1-M12 (Means ± SD, n = 3)

Medium	Max. biomass concentration (g/L)	Overall biomass productivity (g/L/d)	Max. lutein content (mg/g)	Max. lutein productivity (mg/L/d)	Medium cost (USD/L)
BG-11	3.28±0.20	1.011±0.018	5.93±0.07	3.57±0.06	0.193
BM	4.72±0.03	0.894±0.016	2.30±0.09	2.05±0.04	0.368
BBM	1.92±0.03	0.826±0.032	0.71±0.10	0.59±0.02	0.139
CZ-M1	3.15±0.04	0.626±0.009	1.44±0.06	0.81±0.01	0.177

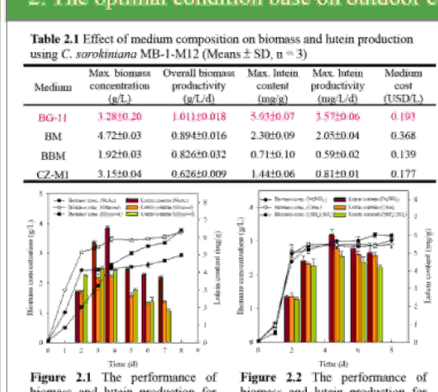

Figure 2.1 The performance of biomass and lutein production for *Chlorella sorokiniana* MB-1-M12 under different carbon sources.

Figure 2.2 The performance of biomass and lutein production for *Chlorella sorokiniana* MB-1-M12 under different nitrogen sources.

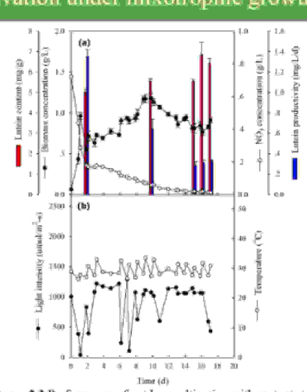

Figure 2.3 Performance of outdoor cultivation with mutant strain *Chlorella sorokiniana* MB-1-M12 under mixotrophic cultivation. (a) Time course profile of lutein content and productivity for strain MB1-M12, (b) time course profile of light intensity of sunlight and outdoor temperature.

Table 2.2 Effect of light-dark cycle on biomass and lutein production using Chlorella sorokiniana MB-1-M12 (Means ± SD, n = 3)

Light/Durk	Max. biomass concentration (g/L)	Overall biomass productivity (g/L/d)	Max. lutein content (mg/g)	Max. lutein productivity (mg/L/d)	Max. lutein production (mg/L)
24hr/00hr	3.21±0.06	1.281±0.014	6.55±0.22	4.90±0.10	18.23±0.05
16hr/08hr	3.01±0.14	1.182±0.027	5.59±0.10	3.66±0.07	14.89±0.34
12hr/12hr	2.82±0.06	0.976±0.030	6.14±0.05	3.16±0.12	16.60±0.57
08hr/16hr	2.07±0.09	0.600±0.028	6.09±0.06	1.93±0.13	10.79±0.44
00hr/24hr	1.24±0.08	0.306±0.021	6.71±0.23	1.46±0.21	7.48±0.35

3. Bioprocess engineering strategies for the enhanced lutein production

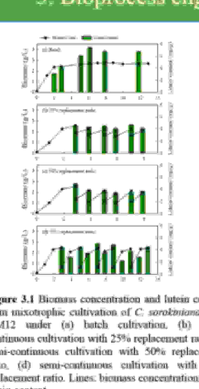

Figure 3.1 Biomass concentration and lutein content from mixotrophic cultivation of *C. sorokiniana* MB-1-M12 under (a) batch cultivation, (b) semi-continuous cultivation with 25% replacement ratio, (c) semi-continuous cultivation with 50% replacement ratio, (d) semi-continuous cultivation with 75% replacement ratio. Lines: biomass concentration; bars: lutein content.

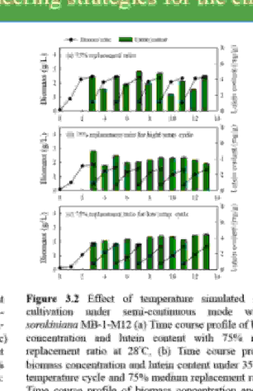

Figure 3.2 Effect of temperature simulated outdoor cultivation under semi-continuous mode with *C. sorokiniana* MB-1-M12 (a) Time course profile of biomass concentration and lutein content with 75% medium replacement ratio at 28˚C, (b) Time course profile of biomass concentration and lutein content under 35˚C/25˚C temperature cycle and 75% medium replacement ratio, (c) Time course profile of biomass concentration and lutein content under 25˚C/15˚C temperature cycle and 75% medium replacement ratio. Lines: biomass concentration; bars: lutein content.

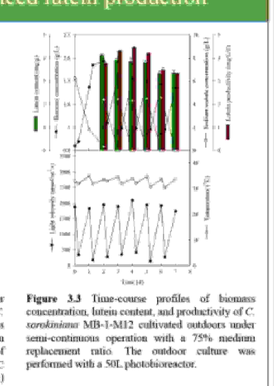

Figure 3.3 Time-course profiles of biomass concentration, lutein content, and productivity of *C. sorokiniana* MB-1-M12 cultivated outdoors under semi-continuous operation with a 75% medium replacement ratio. The outdoor culture was performed with a 50L photobioreactor.

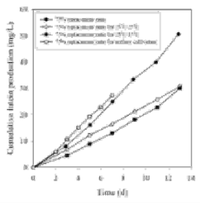

Figure 3.4 Cumulative lutein production profiles of C. sorokiniana MB-1-M12 cultured using semi-continuous strategy. The semi-continuous mode was operated for a period of 13 days in indoor cultivation with 1L photobioreactor and was cultured for a period of 7 days in outdoor cultivation with 50-L photobioreactor, as described in Figs. 3.2 and 3.3.

4. Microalgae cultivation using aquaculture water reuse

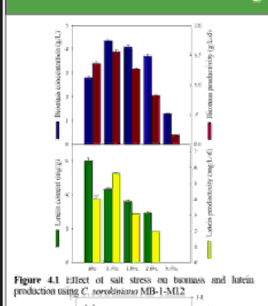

Figure 4.1 Effect of salt stress on biomass and lutein production using *C. sorokiniana* MB-1-M12

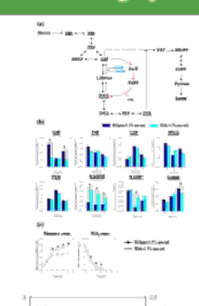

Figure 4.2 Time course analysis of metabolite content of *C. sorokiniana* MB-1-M12 cultivated BG-11 medium with or without 0.5% sea salt. (a) Synthetic pathway of lutein by CO_2 fixation. Abbreviations: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde-3-phosphate; 1,3BPGA, 1,3-bisphosphoglycerate; 3PGA, 3-phosphoglycerate; 2PGA, 2-phosphoglycerate; Ru5P, ribulose-5-phosphate; RuBP, ribulose-1,5-bisphosphate; PEP, phosphoenolpyruvate; PYR, pyruvate; DXP, 1-Deoxy-D-Xylulose 5-Phosphate; MEcPP, methylerythritol cyclodiphosphate, GGPP, geranylgeranyl diphosphate; NADPH, nicotinamide adenine dinucleotide phosphate. (b) Pool size of the metabolites under with (light-blue bar) or without (deep-blue bar) 0.5% sea-salt conditions. Error bars indicate the SD of three replicate experiments (*p < 0.05 by two-way ANOVA). (c) the situation for biomass concentration and nitrogen consumption.

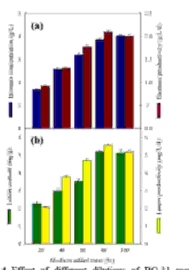

Figure 4.3 Effect of different ratios of shrimp culture wastewater on biomass and lutein production of *C. sorokiniana* MB-1-M12.

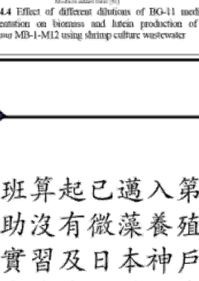

Figure 4.4 Effect of different dilutions of BG-11 medium supplementation on biomass and lutein production of *C. sorokiniana* MB-1-M12 using shrimp culture wastewater

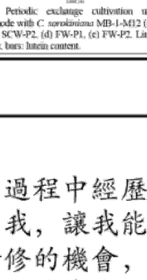

Figure 4.5 Periodic exchange cultivation under semi-continuous mode with *C. sorokiniana* MB-1-M12 (a) SCW, (b) SCW-P1, (c) SCW-P2, (d) FW-P1, (e) FW-P2. Lines: biomass concentration; bars: lutein content.

5. Metabolic profiling under different cultivation methods

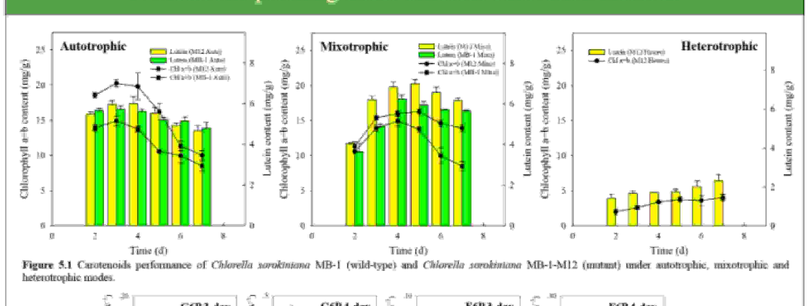

Figure 5.1 Carotenoids performance of *Chlorella sorokiniana* MB-1 (wild-type) and *Chlorella sorokiniana* MB-1-M12 (mutant) under autotrophic, mixotrophic and heterotrophic modes.

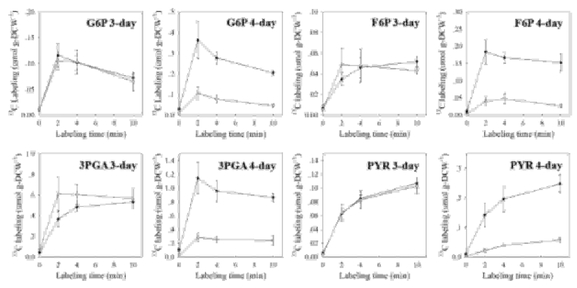

Figure 5.2 Metabolic profiling analysis of *Chlorella sorokiniana* MB-1 and MB-1-M12 under autotrophic condition.

6. Operation strategies for combing cultivation methods

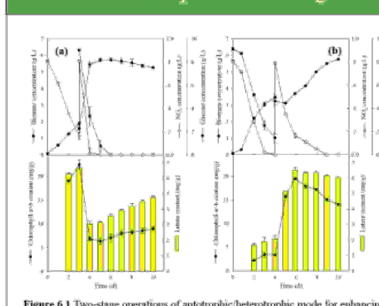

Figure 6.1 Two-stage operations of autotrophic/heterotrophic mode for enhancing lutein production using *Chlorella sorokiniana* MB-1-M12. (a) Autotrophic followed by heterotrophic, (b) Heterotrophic followed by autotrophic.

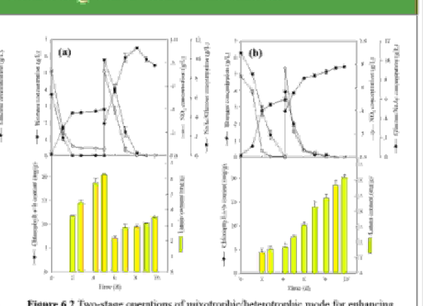

Figure 6.2 Two-stage operations of mixotrophic/heterotrophic mode for enhancing lutein production using *Chlorella sorokiniana* MB-1-M12. (a) Mixotrophic followed by heterotrophic, (b) Heterotrophic followed by mixotrophic.

Table 6.1 Comparison of operation strategies for combining various cultivation modes using *Chlorella sorokiniana* MB-1-M12 (Means ± SD, n = 3)

Type	Max. biomass concentration (g/L)	Overall biomass productivity (g/L/d)	Max. lutein content (mg/g)	Max. lutein productivity (mg/L/d)	Max. lutein production (mg/L)
Auto	3.97±0.08	0.625±0.009	6.01±0.33	3.56.±0.27	16.40±1.05
Mixo	2.75±0.09	1.228±0.008	7.00±0.21	5.15±0.06	18.04±0.69
Hetero	4.54±0.12	1.125±0.073	2.31±0.33	1.88±0.06	7.71±0.22
Auto followed by Hetero	5.74±0.09	3.868±0.315	4.75±0.10	11.75±1.46	24.97±0.55
Hetero followed by Auto	5.77±0.03	0.454±0.068	6.52±0.16	2.86±0.35	34.62±0.27
Mixo followed by Hetero	6.50±0.04	1.984±0.144	3.50±0.10	5.62±0.50	19.07±0.58
Hetero followed by Mixo	5.45±0.07	1.351±0.095	6.17±0.20	3.42±0.19	33.64±1.51

研究生活與心得

我的研究生生涯從碩士班算起已邁入第七年，過程中經歷了許多波折與挑戰，當然也有相當多的新鮮體驗，讓我能夠應付未來的各種挑戰。回想剛進張嘉修老師實驗室時，張老師與實驗室成員們都相當熱心的幫助沒有微藻養殖經驗的我，讓我能夠快速進入微藻研究領域；因緣際會下，我與實驗室學長姐們前往矽谷進行三個月的創業培訓，隨後參加許多創業競賽、國內外研討會、金屬中心研究生實習及日本神戶大學研修的機會，因而使我提升各項能力及豐富我的博士班生活。如今畢業在即，沒有大家的幫忙與鼓勵，我著實無法獨自走完這條崎嶇的路，由衷感謝給予我細心指導及充沛資源的張嘉修老師，讓我能夠擁有這些豐碩的經驗成果，以及在我低潮時不停給我加油打氣的家人，讓我能夠不畏困難的接受挑戰，還有不可或缺的學長姐及學弟妹們，使我能夠順利完成各項任務，最後感謝中技社提供的研究獎學金，這份莫大的鼓勵使我更有動力朝向未來邁進。

財團法人中技社 CTCI FOUNDATION