

行政院國家科學委員會專題研究計畫 成果報告

小球藻受光調節之 SBPase 啟動子功能研究

計畫類別：個別型計畫

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中 華 民 國 95 年 10 月 27 日

(一) 計畫中文摘要

光照是進行光合作用的要素之一，由光誘導之啟動子(light-responsive promoter)，大部分與葉綠體基因功能之表現有關，受光調節之啟動子上游區域常具有一些重要的光調控 motif 位置，例如 GATA、G-box (CACGTC/G)、I-box (GATAA) 和 GT-1 box (GGTTAA) 等，這些都是在光調節反應中與順勢作用元件(cis-acting elements)結合的重要位置。SBPase (sedoheptulose-1,7-bisphosphatase) 的重要特性為（一）在卡爾文循環(Calvin cycle)中碳釋出(carbon flux)之主要調節酵素。（二）SBPase 的催化活性主要是由光來調控其 cysteines 之還原氧化(thioredoxin)狀態，且其需在還原狀態下才有活性。

小麥和阿拉伯芥兩者之 SBPase 基因序列有 79% 之相同性(identities);而高等植物與單胞藻(*Chlamydomonas reinhardtii*) 有 73% 之相同性，小球藻(*Chlorella pyrenoidosa*)與單胞藻(*C. reinhardtii*) 有 80% 之相同性，若能研究開發增強小球藻(*Chlorella pyrenoidosa*) SBPase 之光誘導啟動子效率，除了能提高其固碳率與產量外，亦可釐清其光誘導啟動子之作用機制。

關鍵詞：光誘導啟動子，順勢作用元件，卡爾文循環，小球藻，SBPase

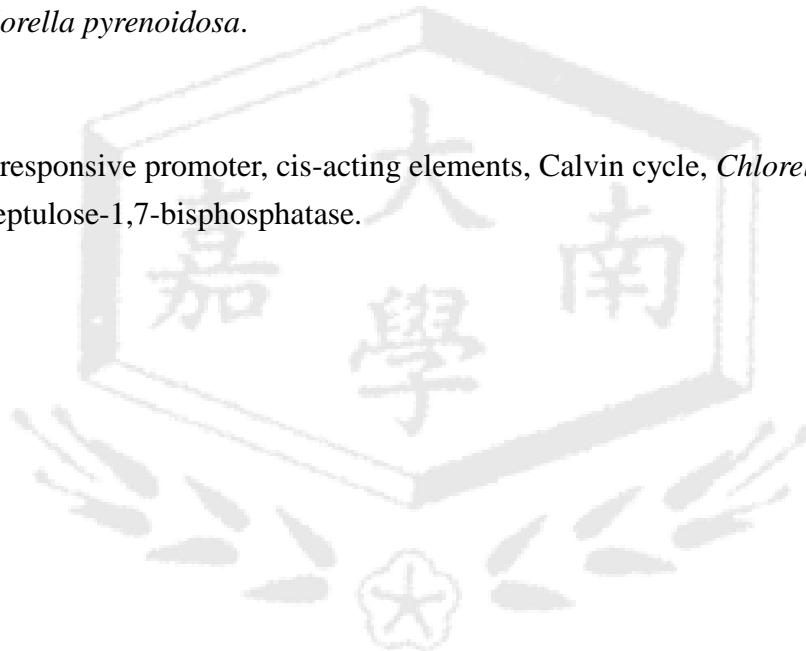


(二) 計畫英文摘要

Light is essential for photosynthesis in plant. In general, the regulation of light-responsive promoter (LRP) is related to plastid genes expression. However, the extent to which illumination controls plastid gene expression varies depending on plant species and the developmental stages of the tissue. The LRP is able to enhance the transcription rate of genes. Some Photo-regulated motif are localized in the upstream of light-regulated promoter. Such as the GATA motif, G-box (CACGTC/G), I-box (GATAA) and GT-1 box (GGTTAA) sites which interact with the cis-acting elements. It has shown that SBPase is a key regulator of carbon flux in Calvin cycle and the redox active cysteines are responsible for the regulation of SBPase catalytic activity by light.

A comparison of the wheat and *Arabidopsis* SBPase gene sequences revealed that they are 79% identity. Then, the algal has around 73% and 80% identity with SBPase from higher plants and *C. reinhardtii*, respectively. Study on the mechanism of LRP to increase the rate of carbon fixation and production in *Chlorella pyrenoidosa*.

Key words: light-responsive promoter, cis-acting elements, Calvin cycle, *Chlorella pyrenoidosa*, sedoheptulose-1,7-bisphosphatase.



(三) 報告內容：

前言及研究目的

光照對藻類或高等植物的生長及發育是必需的因子，因為這是進行光合作用的要素，若無光源則不能進行正常的合成與代謝反應，導致無法適時獲得能量與製造生長所需的成分，故很多酵素或蛋白質皆需靠受光調節之啟動子(light-regulated promoter)經由轉錄(transcription)及轉譯(translation)等過程，才能誘發其酵素或蛋白質合成，此類由光誘導之啟動子(light-responsive promoter)，大部分與葉綠體基因功能之表現有關，例如(一) psbD-psbC 啟動子上游區域有一個會被藍光迅速誘導而大量進行此基因群之轉錄(To *et al.*, 1996)，psbD 及 psbC 分別為水稻第二光合作用系統(PS II)中心 D2 蛋白質及葉綠素 a 結合蛋白 CP 43 之基因，此光誘導之啟動子序列中 -60/-37 含有 AAAG 重複序列可與葉綠體中的 DNA-結合蛋白因數(36 kDa)結合，且此段重複序列在單子葉和雙子葉植物中的相似性皆很高(Christopher *et al.*, 1992)，受光調節之啟動子上游區域常具有一些重要的光調控基因 motif 位置，例如 GATA、G-box (CACGTC/G)、I-box (GATAA) 和 GT-1 box (GGTTAA) 等，這些都是在光反應中與順勢作用元件(cis-acting elements)結合的重要位置。(二) COP 1 (constitutive photomorphogenic 1) 是在阿拉伯芥(*Arabidopsis thaliana*)高等植物中發現的一個光型態發生的抑制者，其能被紅光、遠紅光及藍光調節，是一蛋白質分子量為 76 kDa 之可溶性核蛋白，它可與 HY5 結合，進而導致 HY5 在黑暗中經由 ubiquitin 為媒介而被分解(Ma *et al.*, 2002)。(三) 光敏素 A(phytochrome A) 亦是另一受遠紅光(FR)調控誘發表現的基因，其啟動子上游區域亦發現具有與轉錄因數結合之 G-box (CACGTG) 序列(Hudson and Quail, 2003)。(四) SBPase (sedoheptulose-1,7-bisphosphatase) 是卡爾文循環(Calvin cycle)中受光調節的重要碳脫放(carbon flux)酵素，在小麥與阿拉伯芥中 SBPase 之 mRNA 表現量，在有光照條件下者是黑暗中的至少 20 倍(Willingham *et al.*, 1994; Raines *et al.*, 2000)。

高等植物中的酵素 SBPase 在卡爾文循環內扮演固碳作用之重要路徑，此循環包含 11 種不同酵素催化 13 個反應，且利用光合作用的產物 ATP 和 NADPH 來固定大氣中之 CO₂，用以合成澱粉及蔗糖的碳骨架(見附錄圖-1)(Raines *et al.*, 1999)，此循環可分三時期(1) ribulose-1,5-bisphosphate (RubP) 接收 CO₂ 之 carboxylation 反應。(2) 消耗 ATP 和 NADPH 並產生 triose phosphate (glyceraldehydes-3-phosphate 和 dihydroxyacetone phosphate) 的還原反應。(3) 再生時期(regenerative stage)，此時 SBPase 扮演磷酸水解酶之重要角色，將 sedoheptulose-1,7-bisphosphate 變成 sedoheptulose-7-bisphosphate，再經脫放兩個碳之交換作用，產生 ribose-5-phosphate，最後又回到 RubP 之循環週期；或是直接由 dihydroxyacetone phosphate 轉成蔗糖、由 fructose-6-phosphate 轉成澱粉。另外，SBPase 是經由 ferredoxin/thioredoxin 系統來調節循環週期的還原氧化反應(見附錄圖-2)(Raines *et al.*, 1999)，且需在有光源條件下才能順利執行其磷酸水解酶之功能，可見 SBPase 的啟動子亦是一受光調節的基因(Hahn *et al.*, 1998; Sun *et al.*, 2003)。故綜合以上論點得知 SBPase 的重要特性為(一)在卡爾文循環中碳釋出之主要調節者。(二) SBPase 的催化活性主要是由光來調控其 cysteines 之還原氧化狀態，且其需在還原狀態下才擁有活性功能。

SBPase 是由細胞核解碼(nuclear-encoded)的基因，在細胞質轉譯成前驅物蛋白質，經由胺基端的訊息勝肽鏈(N-terminal signal peptide)帶往葉綠體內，其在小麥和阿拉伯芥高等植物中發現是由 393 個胺基酸所組成的蛋白質前驅物，去除訊息勝肽鏈其蛋白質分子量約為 35~36 kDa，小麥和阿拉伯芥兩者之 SBPase 基因序列有 79% 之相同性(identities)；而高等植物與單胞藻(*Chlamydomonas reinhardtii*)有 73% 之相同性(Hahn *et al.*, 1998)。在阿拉伯芥 SBPase 基因上游含有兩個 GATA motif(Willingham *et al.*, 1994)，此與 I-box 很相似，故其亦

為受光調節之啟動子基因 (Gilmartin *et al.*, 1990)，在小麥發現有 WF-1 的結合位置位於 SBPase 啟動子區域內，且包含兩個順勢作用元件之 ACGT motif 結合區域 (Katagiri and Chua, 1992)，但在單胞藻類尚未有報導其轉錄因數的作用機制為何？

在高等植物如小麥和阿拉伯芥之光誘導啟動子研究上，包括有 psbD-psbC、CPO 1、phytochrome A、rubisco activase、SBPase 等，可見這些光誘導啟動子與光合作用之效率提升是極為相關而重要的因素之一。而小球藻(*Chlorella pyrenoidosa*) 之光誘導啟動子序列研究上，尚未有效開發研究其結構與功能，但小球藻俗稱綠藻是一個非常優良又便利研究 light/dark transition 的遺傳程式 (genetic programs) 模式系統，若能開發增強其 SBPase 光誘導之啟動子效率，除了能提高綠藻的固碳率與產量外，亦可從中篩選一些 suppressor mutants，作為釐清光誘導啟動子作用機制之目的。除此之外，本研究計畫欲利用 SBPase 光誘導啟動子構築一個 chimeric 系統來表現一些抗氧化基因 (anti-oxidative genes) 及光修復基因 (light-repaired genes)，將此融合質體轉殖入綠藻中以提高其抗逆境為目的。

研究方法

(A) Preparation of total RNA (TRIzol total RNA isolation kit, GIBCO BRL)

Chlorella cells were homogenized in 1 mL of TRIzol reagent per 100 mg of sample using a mortar and pestle at liquid nitrogen temperature. The homogenate was centrifuged at 12,000×g for 10 min at 4°C and the supernatant was collected. After incubation for 5 min at room temperature, 0.2 mL (-20 °C) cold chloroform was added. It was followed by shaking vigorously by hand for 15 sec and incubating at 25 °C for 3 min. The sample was centrifuged again at 12,000×g for 15 min at 4°C. The colorless upper aqueous phase was collected and RNA was precipitated by mixing with 0.5 mL of isopropanol. The RNA pellets were washed with 1 mL of 70% ethanol and briefly air dried for 5-10 min, then dissolved in DEPC (diethylpyrocarbonate)-treatment water.

(B) First-strand cDNA synthesis (First-strand cDNA synthesis kit, Amersham, UK)

Place the RNA sample in a microcentrifuge tube and add RNase-free water, if necessary, to bring the RNA to the appropriate volume (8 µl). Heat the RNA solution to 65 °C for 10 min, then chill on ice. Gently pipette the Bulk first-strand cDNA reaction mix to obtain a uniform suspension. (Upon storage, the BSA may precipitate in the mix; this precipitate will dissolve during incubation). The appropriate volume of the Bulk first-strand cDNA reaction mix (5 µl) was added to a sterile 1.5 or 0.5 ml of microcentrifuge tube. Adding 1 µl of DTT solution, 1 µl of your chosen primer at the appropriate concentration, and the heat-denatured RNA to this tube. Pipetting up and down several times to mix the complex and incubated them at 37°C for 1 hour. The completed first-strand cDNA reaction product was ready for immediate second-strand cDNA synthesis or PCR amplification.

(C) Conserved cDNA fragment preparation

The extracted total RNA was used as a template of RT-PCR to synthesize the first strand cDNA. Two degenerated primers, sense primer 5'-CACGAGTTCTGCTGATGGAT/CGACG-3', and antisense primer 5'-TGCCA/GTACAG/TGTACTCCTCA/GAAGCG-3' were used. The annealing temperature was 45 °C and proceeded for 35 cycles. The conserved sequence region of SBPase was 450 base pairs in length. Finally, full length of cDNA was cloned by 5'-RACE and

3'-RACE techniques.

(D) 5'-RACE system (Rapid Amplification of cDNA Ends kit, GIBCO BRL)

First-strand cDNA synthesis. The mixtures, total RNA (1-5 µg), SBPase gene-specific primer (2.5 pmoles, 5'-GCAAGCCCTCACCGATCTCTG-3') and DEPC-treated water, were incubated 70°C for 10 min then chilling on ice for 1 min. To add the following components, 1×PCR reaction buffer, 25 mM MgCl₂, 10 mM dNTP mix and 0.1M DTT, in the order to the previous mixtures, and mixed gently at 42 °C for 1 min, then added 1µl (200U) of SuperScript II reverse transcriptase to react at 42°C for 50 min in the tube. To purify the cDNA by the spin column, adding 120 µl of binding buffer to the First-strand cDNA synthesis reaction, then transferring to spin column and centrifuged at 13,000×g for 20 sec. To add 0.4 mL of cold wash buffer into the spin column and centrifuged at 13,000×g for 20 sec, and this step was repeated 3 times. It was necessary to wash 2 times with 0.4 mL of 70% ethanol then spin briefly. Finally, adding 50 µl of pre-heated to 65°C of sterile ddH₂O to elute the cDNA by centrifugation at 13,000×g for 20 sec. The purified cDNAs (10 µl) were tailed by adding 2 mM of dCTP, 1×tailing buffer, then incubated at 94°C for 3 min and chilling on ice for 1 min and added 1 µl of terminal deoxynucleotidyl transferase (TdT) to react at 37°C for 10 min. These tailing of cDNAs were amplified by PCR and nested PCR methods, individually.

(E) 3'-RACE system

First-strand cDNA synthesis was as described in Section 2. The 5 µl of first-strand cDNAs were mixed with 3 µl of 2.5 mM dNTP, 5 µl of 10×PCR reaction buffer, 2 µl of 10 µM oligo-d(T)₁₈ primer, 2µl of 10 µM SBPase specific primer (5'-CTATGACCAGCGCACTGAGATCTGC-3') and 0.5 µl of Taq DNA polymerase (TaKaRa). The final volume was up to 50 µl by adding sterile ddH₂O. The programs of PCR were proceeded by 35 cycles of denaturing at 94°C for 1 min , 45°C for 1 min, and 72°C for 2 min.

(F) Primer Extension Analysis

The purified oligodeoxynucleotide primers are designed and utilized in this analysis experiments. They are complementary to nucleotide positions of the RNA-like strand of the SBPase gene. Primers (30 ng) were labeled at the 5' end using T4 polynucleotide kinase and γ -³²P-ATP. A total of 10⁵ dpm of ³²P-labeled primer was co-precipitated with either chloroplast RNA (2.05 x 10⁶ plastids/reaction) or with total cellular RNA (12 to 14 µg). The annealing and primer extension reactions were conducted as normal method, except final deoxynucleotide triphosphate concentration was 400 µM.

結果與討論

圖一：經 5'-RACE 與 3'-RACE 方法選殖之小球藻(*Chlorella pyrenoidosa*) SBPase 的全長 cDNA 基因序列: 1507 bps

[ATG]GCCACTGCATTGCCACCGTACTCAAACCGCAGCAGGCTGCGTGGCCCAGCAGGCCTCAGCAAAGGGCGTTGTGCTTCAGCTGGCTGGTAAGGCCACCGTGAGGTCGTGGCCTCAGCCGCCAGAGCACCTTGCAAGGAATGGCGTCTTACAGCACCCACCCAGCAAAGCATGCGTGGCATGCGTGTGCTCACGCAGGAGCGCTGTGACAACCAAGGCAAAGCTGGAAACCTCCGCTCGAGTTCTGGGGAGGCAACCCCTGACACCAAGCTGCCAGCTCTGATGAGCATGTCCAAGGCACTCCGACAATCGCCTCAAGGTCCGACGGCTTCTGCGCTGGTACCGCCTGCGTACGAGCTTGGTGACGAGCAGCTGGCTTGGACATGCTGGCTGACAAGCTGCTGTTGAGGCTCTCGCTTCTCACATGTGTGCAAGTACGCCGCTCAGCGAGGTCCTGAGCCAGTTGATGTTGGTGAGAAGGCTTCTGTGTGCCCTCGACCCACTGGACGGCAGCAGCAGCATTGTTGACACAAACTTCGCTGTGGCACCATCTTGGTGTGTGGCCCGTGACAAGCTCAAGGACATTGATGGCAGGCAGCAGGCTGCTGCTGTTGGACATCTATGGTCCCCGCACCGTGTCTGCCTGGCAGTTGCTGGCTACCCCGCACTCACGAGTTCTGCTGATG GATGACGGCAAGTGGGTGCATGTGAAGGAGACCACAGAGATCGGTGAGGGCAAGCTGTTGCACCCGGAACCTGAGGGCACCTTTGACAACCCAGCCTACGAGGCCCTCATCAGCCACTACCTGGAGAGAAGTAACACACTGAGGTACACTGGTGGTATGGTCCCAGATGTGTTCCAGATCATTGTCAGGAGAAGGGTGTGTCACAAACGTCACCAGCCAAAGCACAAAGGCAAAGCTCCGATCTGCTGCTGCTGCTGAGGCTGAGGAGTACCTGTATGGCAACTCACCTCGCTCAGCGTGCACAGAGCTAGAGGCCAACCTCGCCAG[TAA]GCG TTGTCATGTTGTCAGCACCCCTCCCACAGCAGTGGCTGCGTGCCTGAGAATGCTGGTGGTGCAGCTGTGACGGCAAGTGCTTGGAGGAGTCTTGTATGCTTGTGTTGGACCCCTGAGAAGAACAGTACGTATAGCAAGTAGGCTGGTTGAGTGTGGGTGGCCAAGTGCATTAAGTGCATGGGCCAATGGGAAGCGTGTGTTGGTAAAGTGGTCGGCCACTGAGCCTGCAGTGATTGTAAGTGGTACAGACTGCCAAAAAA

圖二：選殖之小球藻(*Chlorella pyrenoidosa*) SBPase 的全長 amino acids 序列: 398 aas

MATAFATRTQTAAGCVAQQASAKGVVRPFAGKATVKVVVASARQSTFAGNGVFTAPQQSM RGMRAASRRSAVTTKAKLGNSLAEFLGEATPDTKLRLQLLMSMSKALRTIAFKVRTASCAGT ACVSSFGDEQLAVDMLADKLLFEALRFSHVCYACSAEVPEPVDFVGEGFCVAFDPLDGSS IVDTNFAVGTIFGVWPGDKLKIDGRQQAAAGMGIYGPRTVFCLAVAGYPGTHEFLMDD GKWHVHKETTEIGEGKLFAPGNLRATFDNPAYERLISHYLGEKYTLRYTGGMVPDVFQIIV KEKGVFTNVTPSTKAKLRLIFEVAPLALLVENAGGASSCDGKCVSALDIPILNQDQREICF GSIGEVRRFEEYLYGNSPRSVANELEANPRQ

圖三：經 5'-RACE 方法選殖之小球藻(*Chlorella pyrenoidosa*) SBPase 啟動子的 upstream 部份序列結果: 172 bps.

GCTACAGAGTGCTTACGTGCGCGTATGCTCGGGTGCCTGATCAACCATCCGTATTG AGTTGCAGAGTTAACGTCAAGCCTCGCTTGCTTGAGCCCGTATGCTTGCCTTCTTGC AGCTGTTAAACCCAGCAGCAGCGCACTTATTGGCAAATAATAGGCAGGT

圖四：將選殖之小球藻(*Chlorella pyrenoidosa*) SBPase 的 cDNA 序列與 *Chlamydomonas reinhardtii* 之 SBPase cDNA (1610 bps, X74418) 比較有 80% 相同度[Identities = 737/917 (80%), Gaps = 2/917 (0%)]

Query: 代表 *Chlorella pyrenoidosa* SBPase cDNA

Sbjct: 代表 *Chlamydomonas reinhardtii* SBPase cDNA

Query 243 CCTCGCTGAGTTCTGGGGAGGCAACCCCTGACACCAAGCTGCGCCAGCTTCTGATGAG 302

A horizontal bar divided into five segments by vertical tick marks. The first four segments are of equal length, while the fifth segment is half as long.

Sbjct 239 CCTGGCTGAGTTCTGGTCGAGGCCACCCCCGACCCGAAGCTGCGCACGTGATGAG 298

Query 303 CATGTCCAAGGCACTCCGACAATGCCCTCAAGGTCCGACGGCTTCATGCGCTGGTAC 362

||||| | ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

Sbjct 299 CATGGCTGAGGCCACCGCACCATCGCCCACAAGGTCCGCACCGCCTCGTGCGCCGGTAC 358

Query 363 CGCCTGCGTGAGCAGCTTGGTACGAGCAGCTGGCTGGACATGCTGGCTGACAAGCT 422

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Sbjct 359 CGCCTGCGTGAACAGCTCGCGATGAGCAGCTGGCGTCACATGGTGGCTGACAAGCT 418

Query 423 GCTGTTGAGGCTCTCCGTTCTCACATGTGTGCAGGTACGCCCTGCTCAGCGGAGGTCCC 482

Digitized by srujanika@gmail.com

Sbjct 419 GCTGTTCGAGGCCCTGAAGTACTCGCACGTGTGCAAGCTGGCCTGCTCCGAGGAGGTGCC 478

Query 483 TGAGCCAGTTGATGTGGAGAAGGCTCTGTGTGGCCTCGACCCACTGGACGGCAG 542

||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

Shift 479 CGAGCCCCCTGGACATGGGGGGGGAGGGCTTCTGGCTGGCTTCTGACCCCTGGACGGCT- 537

Query 543 CAGCATTGTT-GACACAACTTCGCTGTGGCACCATCTTGGTGTGGCCCGGTGACA 601

— 1 —

Shict 538 CGTCATCGTCCGACACCAACTTCGGCGTGCGACCATCTTGGGGGTGTGGCCCCGGACA 597

Query 602 AGCTCAAGGACATTGATGGCAGGCAGCAGGCTGCTGCTGGTATGGCATCTATGGTCCCC 661

||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| | ||||| |

Shict 598 AGCTGACCAACATCACCGGCCCCAGCAGGTGGCTGCCGGCATGGGCATCTACGGTCCCC 657

Query 662 GCACCGTGTCTGCCCTGGCAGTTGCTGGCTACCCGGCACTCACGAGTTCTGCTGATGG 721

Sbict 658 GCACCGTGTCTGCAATTGCCCTGAAGGACGGCCCCGGCTGCCACGAGTTCTGCTGATGG 717

Query 722 ATGACGGCAAGTGGGTGCATCTGAAGGAGACCACAGAGATCGGTGAGGGCAAGCTGTTTG 781

Sbct_718 ACCACGGCAAGTGGATGCACGTCAAGGAGACCACCCCCATCGGTGAGGGCAAGATGTTCG 777

Query	782	CACCCGGAACCTGAGGCCACCTTGACAACCCAGCCTACGAGCGCTCATCAGCCACT	841
Sbjct	778	CCCCCGCAACCTGCGGCCACCTCGACAACCCCGCTACGAGCGCTGATCAACTTCT	837
Query	842	ACCTGGGAGAGAAGTACACACTGAGGTACACTGGTGGTATGGTCCCAGATGTGTTCCAGA	901
Sbjct	838	ACCTGGCGAGAAGTACACCTGCGCTACACCGCGGCATCGTCCCCACTTGTCCAGA	897
Query	902	TCATTGTCAAGGAGAAGGGTGTGTTACAAACGTACCAGCCAAGCACAAAGGCAAAGC	961
Sbjct	898	TCATTGTCAAGGAGAAGGGTGTGTTACCAACTTGACCTCGCCACCACCAAGGCCAAGC	957
Query	962	TCCGTATCCTGTTCGAGGTTGCCCTCTCGCTCTGCTTGTGAGAATGCTGGTGGTGC	1021
Sbjct	958	TCCGCATCCTGTTCGAGGTTGCTCCCTGGCCCTGCTGATCGAGAAGGCCGGTGCCT	1017
Query	1022	GCAGCTGTGACGGCAAGTGTGTGTCAGCCCTGGACATCCCCATCCTAAACTATGACCAGC	1081
Sbjct	1018	CCAGCTGCGACGGCAAGGCCGTGTCGCTCTGGACATCCCCATCCTGGTGTGCGACCAGC	1077
Query	1082	GCACTGAGATCTGCTTCGGTAGCATCGCGAGGTAGGCGCTTGAGGAGTACCTGTATG	1141
Sbjct	1078	GCACCCAGATCTGCTACGGCTCGATCGCGAGGTCCGCCCTCGAGGAGTACATGTACG	1137
Query	1142	GCAACTCACCTCGCTTC	1158
Sbjct	1138	GCACCTCGCCCCGCTTC	1154

圖五：將選殖之小球藻(*Chlorella pyrenoidosa*) SBPase 的 amino acids 序列與 *Chlamydomonas reinhardtii* 之 SBPase amino acids (X74418) 比較有 77% 相同度 [Identities = 286/367 (77%), Positives = 318/367 (86%), Gaps = 3/367 (0%)]

Query: 代表 *Chlorella pyrenoidosa* SBPase amino acids

Sbjct: 代表 *Chlamydomonas reinhardtii* SBPase amino acids

Query	23	KGVVRPFAGKATVK--VVASARQSTFAGNGVFTAPQQXXXXXXXXXXXXVTTAKLGN	80
		+ V P G+A VVASA S F G V TA + S R R SRR+AV T+AK+G+	
Sbjct	19	RSAVAPKMGRAATAPVVVASANASAFKGAAV-TARVKASTRAARVQSRRTAVLTQAKIGD	77
Query	81	SLAEFLGEATPDTKLRLQIIMSKALRTIAFKVRTASCAGTACVSSFGDEQLAVDMLADK	140
		SLAEFL EATPD KLR ++MSM++A RTIA KVRTASCAGTACV+SFGDEQLAVDM+ADK	
Sbjct	78	SLAEFLVEATPDPKLRHVMMMSMAEATRTIAHKVRTASCAGTACVNSFGDEQLAVDMVADK	137

Query 141 LLFEALRFSHVCYACSAEVPEPVDGEGFCVAFDPLDGSSIVDTNFAVGTIFGVWPGD 200
 LLFEAL++SHVC+ ACS EVPEPVG+GGEGFCVAFDPLDGSS DTNFAVGTIFGVWPGD
 Sbjct 138 LLFEALKYSHVCKLACSEEVPEPVDMGGEGFCVAFDPLDGSSSDTNFAVGTIFGVWPGD 197

Query 201 KLKDIDGRQQAAAGMGIYGPRTVFCЛАVAGYPGTHEFLLMDGKWHVVKETTEIGEGKLF 260
 KL +I GR+Q AAGMGIYGPRTVFC+A+ PG HEFLMDDGKW+HVKEETT IGEGL+F
 Sbjct 198 KLTNITGREQAAGMGIYGPRTVFCIALKDAPGCHEFLLMDGKWMHVKEETTHIGEGKMF 257

Query 261 APGNLRATFDNPAYERLI SHYLGEKYTLRYTGGVPDVFQI IVKEGVFTNVTSPTKAK 320
 APGNLRATFDNPAYERLI+ YLGEKYTLRYTGG+VPD+FQI IVKEGVFTN+TSP+TKAK
 Sbjct 258 APGNLRATFDNPAYERLINFYLGEKYTLRYTGGIVPDLFQI IVKEGVFTNLTSPTTKAK 317

Query 321 LRILFEVAPLALLVENAGGASSCDGKCVSALDIPILNQDQRTIEFGSIGEVRRFEELY 380
 LRILFEVAPLALL+E AGGASSCDGK VSALDIPI DQRT+IC+GSIGEVRRFEELY+Y
 Sbjct 318 LRILFEVAPLALLIEKAGGASSCDGKAVSALDIPILVCDQRTQICYGSIGEVRRFEEMY 377

Query 381 GNSPRFS 387
 G SPRFS
 Sbjct 378 GTSPRFS 384

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(四) 計畫成果自評

已完成定序確認小球藻 SBPase 之 cDNA 全長基因，並分析小球藻 SBPase 之光誘導啟動子部分基因。未來將可達成預期目標，即可將研究成果在 SCI 學術期刊上發表。