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耐熱藍綠菌光反應的特殊保護機制─狀態變遷

得獎獎項

一等獎

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作者簡介

「追求卓越，成功自然就會找上門」是商廣明一我奉行的座右銘，喜好出外旅遊增廣自己的世界觀。自從進入建國中學數理資優班後，便對自己動手做實驗產生了濃厚的興趣，從檢索文獻到實驗設計，付諸實行再改善實驗，追求書本上沒有的新知識，心中滿足感便油然而生，盡管這其中難免會碰到挫折、失敗，但這不正是在追求卓越時會面臨的困難，我相信只要不斷地努力、不斷的克服，最後終將邁向成功。

摘要

隨著全球溫度逐年升高，生物如何在高溫下生存成了一個急需解決的問題，本研究探討耐熱藍綠菌(Thermosynechoccus sp.CL1)與常溫藍綠菌(Synechocystis sp. PCC6803)在不同溫度下光合作用光反應的異同，並了解耐熱藍綠菌光反應耐熱的原因。

本研究中發現在高溫下耐熱藍綠菌存在有顯著的狀態變遷(state transition)機制，其藻藍素(phycobilisome)在暗處理的狀況下不會與光系統二結合，而這個機制可以減少光系統二在高溫下高電子傳遞速率對光系統二本身造成的破壞，支持了狀態變遷現象是耐熱藍綠菌在高溫下進行光合作用光反應的一種重要保護機制。

Abstract

As the earth is getting warmer, how the creatures live in a high temperature surrounding becomes an interesting yet need-to-be-solve issue. In the research, the relationship between temperature and photosynthesis light reaction will be well discussed while *Thermosynechoccus sp.CL-1* , one kind of thermophilic cyanobacteria, and another mesophilic one, *Synechocystis sp. PCC6803*. Our result reveals that apparently , the state transition in TCL-1 increases at high temperature (40℃ to 60℃),which means phycobilisomes will be uncoupled when cells are dark-adapted. With this mechanism, state transition can reduce the damage to the photosystem II from high electron transport rate at high temperature. Therefore, our research supports that state transition is a unique protective mechanism of TCL-1 at the high temperature.

目錄

壹、前言

一、研究動機

高溫對於一般的生物來說是一種逆境，但我們已知有些生活在極熱地帶的光合微生物可以有效地進行光合作用以產生能量，為了探討其運作的特殊性，我們想探討兩株對溫度有不同耐受性的藍綠菌之間光合作用光反應的異同，藉此了解耐熱生物其光合作用光反應的耐熱的原因。

二、研究材料

為了研究不同藍綠菌中的光合作用對溫度的耐受程度，我們選取了一株由成功大學朱信教授的實驗室從台灣台東金崙溫泉(pH=9.3,62℃)中所分離的耐熱藍綠菌，這株藍綠菌被命名為(*Thermosynechococcus sp. CL-1* ,簡稱TCL-1)，另外，一般室溫生存的藍綠菌我們則選用(*Synechocystis* sp. *PCC6803* ,簡稱PCC6803)。

三、研究目的

探討耐熱藍綠菌(TCL-1)與常溫藍綠菌(PCC6803)在光合作用光反應上的異同以了解TCL-1耐熱的原因。

貳、 研究方法或過程

一、研究策略

有鑑於光合作用光反應在分階段的進行，因此我們將分段測量其活性，以縮小其耐熱主因發生部位的範圍，進而進行更深入的研究。

二、研究儀器與試藥

培養箱

無菌操作台

震盪器

離心機

分光光度計

氧電極儀

Dual-PAM-100

螢光光譜儀

BG-11(配製如附件)

methanol (CH_3OH)

phosphate(Na_2HPO_4)

alcohol(C_2H_5OH)

carbon dioxide($CO_{2(s)}$)

nitrogen($N_{2(l)}$)

三、研究流程

流程圖


培養藍綠菌
• Thermosynechococcus sp.CL-1
• Synechocystis sp. PCC6803
觀察藍綠菌的型態
• 電子顯微鏡(TEM)
測量光合作用活性
• 氧電極
測量電子傳遞速率
• ETR
測量光系統二活性
• 光系統二螢光活性(Y(II))
發現TCL-1狀態變遷
• 光系統二螢光活性(Y(II))
• 77K螢光發散光譜
證明TCL-1狀態變遷
• 光系統二螢光活性(Y(II))+磷酸鹽
證明TCL-1狀態變遷是一種特殊的保護機制
• 光系統二螢光譜線(Fluorescence Induction)

四、研究方法

1. 培養藍綠菌 TCL-1 與 PCC6803

   i. 以 BG-11 為緩衝溶液 100mL，原菌液 5mL 為菌溶液。

   ii. 菌溶液全天候照光與搖晃，光照條件為 25~30μE/m^2/s。

   iii. 培養至 OD_{730} 約為 0.8~1.2 即可進行實驗。

2. 觀察藍綠菌的型態

   i. 將 TCL-1 與 PCC6803 分別培養在 30℃與 45℃。

   ii. 將樣品送電顯室，拍照。

3. 測量光活作用活性(氧電極)

   i. 測量葉綠素濃度

      a. 取培養好的菌溶液兩管各 45mL。

      b. 以ω = 3400 r.p.m. 離心 7 分鐘。

      c. 將上清液倒出，震盪，將兩管合為一管，震盪，備用。

      d. 取三管 1.5mL 微量離心管各裝入 1000μL 甲醇。

      e. 取 20μL 菌溶液裝入兩管裝有 1000μL甲醇的 1.5mL 微量離心管。

      f. 以ω = 13000 r.p.m. 離心 2 分鐘。

      g. 以甲醇做歸零測兩管的 OD_{665}。

      h. 帶入公式計算葉綠素濃度 $\frac{\overline{OD_{665}} \times \frac{1}{2}}{0.7924}$ 。

   ii. 將恆溫水浴槽溫度調製到所需要測量的溫度。

   iii. 校正該溫度下的 100%氧氣濃度。

iv. 計算含有 16μg 葉綠素的菌溶液體積。

v. 加入人工電子接收劑(artificial electron acceptor)

本實驗使用 0.5mM 的 $NaHCO_3$。

vi. 加入緩衝溶液(BG-11)與樣品使總體積達到 1.6mL。

vii. 靜候約兩分鐘，開始測量氧氣濃度增加斜率。

viii. 帶入公式計算光合作用活性。

4. 測量電子傳遞速率

i. 測量光系統二螢光譜線帶入公式計算(ETR)。

5. 測量光系統二螢光活性(Y(Ⅱ))

i. 測量葉綠素濃度

a. 取培養好的菌溶液兩管各 45mL。

b. 以ω = 3400 r.p.m. 離心 7 分鐘。

c. 將上清液倒出，震盪，將兩管合為一管，震盪，備用。

d. 取三管 1.5mL 微量離心管各裝入 1000μL 甲醇。

e. 取 20μL 菌溶液裝入兩管裝有 1000μL甲醇的 1.5mL 微量離心管。

f. 以ω = 13000 r.p.m. 離心 2 分鐘。

g. 以甲醇做歸零測兩管的 OD_{665}。

h. 帶入公式計算葉綠素濃度 $\frac{\overline{OD_{665}} \times \frac{1}{2}}{0.7924}$ 。

ii. 將恆溫水浴槽溫度調製到所需要測量的溫度。

iii. 計算含有 20μg 葉綠素的菌溶液體積。

iv. 加入緩衝溶液(BG-11)與樣品使總體積達到 2mL。

v. 改變光照條件。

a. 暗處理 5 分鐘。

b. 暗處理 5 分鐘，遠紅光處理 1 分鐘。

vi. 測量光系統二活性(Y(Ⅱ))。

6. 77K 的螢光發散光譜(The 77K fluorescence emission spectra)

i. 計算含有 20μg的葉綠素菌溶液體積。

ii. 將 BG-11 加至總體積 1mL。

iii. 光照條件，如下。

a. 暗處理 10 分鐘。

b. 遠紅光處理 10 分鐘。

iv. 將 200mL 的樣品放入石英管中。

v. 將石英管放入乾冰的純酒精溶液。

vi. 將石英管外酒精擦乾後放入液態氮中。

vii. 測量激發波長為 435nm 的發散光譜。

7. 光系統二螢光活性(Y(II))+磷酸鹽

i. 準備步驟同實驗 4.

ii. 改變溫度條件，如下：

30℃	30℃添加磷酸鹽(0.5M Na_2HPO_4)
50℃	50℃添加磷酸鹽(0.5M Na_2HPO_4)

iii. 第一次測量後打開遠紅光。

iv. 每一分鐘測量一次光系統二螢光活性。

8. 證明 TCL-1 狀態變遷(state transition)是特殊的保護機制

i. 準備步驟同實驗 4.。

ii. 改變條件，如下。

a. 50℃暗處理 5 分鐘

b. 50℃暗處理 5 分鐘，遠紅光 5 分鐘。

c. 50℃暗處理 5 分鐘，加入磷酸鹽(0.5M Na_2HPO_4)後再靜候 5 分鐘。

d. 50℃暗處理 5 分鐘，遠紅光 5 分鐘後，加入磷酸鹽(0.5M Na_2HPO_4) 後再靜候 5 分鐘。

iii. 測量光系統二螢光譜線。

參、 研究結果與討論

一、 觀察藍綠菌的型態

就文獻上來看，TCL-1 的最適生長溫度約在 45℃~55℃之間，而 PCC6803 約在 30℃，因此我們便想要觀察這兩株藍綠菌在其最適生長溫度下，與逆境溫度下，在形態上的異同，結果如 Fig.1

就外型來看，TCL-1 是屬於桿狀而 PCC6803 是屬於球狀；就類囊膜的型態來看，我們可以發現，PCC6803 在 30℃的膜系是很平滑的排列在細胞的外側，但當溫度升高到 50℃時已無法生存；TCL-1 在 50℃的膜系呈現出較鬆散的樣子，但當培養溫度降到 30℃時其膜系又呈現平滑排列，而其膜的型態，與光合作用活性是否有直接的相關性，還待進一步實驗確認。

二、 測量光合作用活性

再者，我們先以氧電極的方法初步測定在不同溫度下耐熱藍綠菌(TCL-1)與常溫藍綠菌(PCC6803)的活性，結果如 Fig.2。我們發現，PCC6803 在 50^{o}C 活性下降，TCL-1 在 50^{o}C 仍保有活性，而兩株藍綠菌在其適應溫度範圍下，光合作用活性隨著溫度升高而升高。

三、 測量電子傳遞速率

結果如 Fig.3，兩株藍綠菌的電子傳遞速率皆隨著溫度升高而升高，也造成了如 Pic.1 的光合作用活性隨著溫度升高而升高。

四、 測量光系統二螢光活性(Y(Ⅱ))

為了近一步研究光合作用耐熱的原因，且光反應的第一步就是在光系統二進行的，因此我們便使用螢光的方式來測量光系統二活性(Y(Ⅱ))，結果如 Fig.4。

經觀察後我們可以發現，在暗處理 5 分鐘的條件下時，PCC6803 的活性有隨著溫度上升而下降的趨勢，而 TCL-1 的活性並沒有明顯的改變，因此，這說明了在溫度逐漸升高的環境下，TCL-1 的光合作用活性的上升不是因為光系統二對高溫有較好的活性所造成。

為了近一步的研究 TCL-1 光反應的耐熱性，我們在暗處理過後給予一分鐘的遠紅光(FR)處理，再測量光系統二活性(Y(Ⅱ))，一般認為遠紅光(FR)可激發光系統一使色素體醌(plastoquinone , PQ)被氧化，而電子就可以順利的從光系統二傳遞給色素體醌使光反應順利進行，而我們的實驗結果發現，耐熱藍綠菌(TCL-1)與常溫藍綠菌(PCC6803)的活性雖然都有升高，但我們訝異的是，TCL-1 在 40℃~60℃時活性卻有高達 40%以上的成長。

五、 77K 的螢光發散光譜

同樣的，我們在進行常溫藍綠菌(PCC 6803)與耐熱藍綠菌(TCL-1)的 77K 螢光光譜實驗時，實驗結果 Fig.5 顯示，兩株藍綠菌在照遠紅光處理後，且以光系統一標準化，耐熱藍綠菌(TCL-1)光系統二的螢光值會有別常溫藍綠菌(PCC6803)而大量上升。

綜合以上所述，我們認為這種在耐熱藍綠菌(TCL-1)裡大量表現的現象，可能是因為大量的狀態變遷所造成，而在耐熱藍綠菌(TCL-1)中，又以高溫下才會有這個現象，這不禁讓我們覺得，狀態變遷(state transition)應該是一種在對耐熱藍綠菌而言，在高溫環境下的特殊保護機制。

狀態變遷(state transition)是一種可以調節並且分配以平衡兩個光系統激發能量的一種生理機制，而在藍綠菌中的這個機制，則是由藻藍素所完成，藻藍素(phycobilisome)，是一種在藍綠菌中特殊的光捕捉蛋白，藉由移動與結合，增加光系統的激發能量。當藻藍素移動而結合在光系統二上，這種情況稱之為<State 1>，一般發生在藍綠菌被遠紅光(700nm)照射時，這時藻藍素可以接收光子以利光系統二進行激發，反之，<State 2>則是藻藍素結合在光系統一，通常這種情況發生在照射紅光(650nm)，而目前的研究指出，當常溫藍綠菌在暗處理時，大部分的藻藍素會與光系統二結合，但我們發現在耐熱藍綠菌(TCL-1)中，特別是在高溫的環境下，光系統二的螢光活性在照遠紅光後才會明顯的升高，這是否因為在高溫下，耐熱藍綠菌(TCL-1)中的藻藍素需要移開光系統二，而造成的大量的狀態變遷，而這是否又與耐熱藍綠菌的耐熱性有關，還待接下來的實驗證明。

六、 證明 TCL-1 狀態變遷

為了確定我們看到的現象是狀態變遷,我們便使用磷酸鹽(0.5M Na_2HPO_4)這種高濃度的緩衝溶液來抑制狀態變遷的現象，分別在 30℃與 50℃下，測量其光系統二螢光活性(Y(Ⅱ))在持續照遠紅光(FR)的情況下隨著時間的變化。結果如 Fig.6。

結果顯示，在無添加磷酸鹽的兩條曲線都有光系統二營光活性(Y(Ⅱ))逐漸上升的趨勢，但暗處理過且在添加了磷酸鹽以後，兩組的光系統二營光活性(Y(Ⅱ))隨著時間都無明顯上升，這解釋了在耐熱藍綠菌 TCL-1 裡有確實有狀態變遷的現象。

另外有趣的是,我們發現在 30℃與 50℃兩種溫度下,耐熱藍綠菌(TCL-1)50℃時狀態變遷有進行較快與比率較高的現象，推測是因為耐熱藍綠菌的膜系在高溫時排列得比較鬆散，使得藻藍素可以很快速的移動到光系統二上所造成；而關於比率較高的原因就是因為在越高溫的情況下，越高比率的狀態變遷才可保護 TCL-1 的光系統二不受高溫下過多的電子造成的傷害。

七、 證明 TCL-1 狀態變遷是一種特殊的保護機制

在證明是狀態變遷後，我們必須進一步證明狀態變遷是一種在高溫下 TCL-1 光合作用的保護機制,因此我們測量 TCL-1 暗處理(Dark)與照遠紅光(FR)後的螢光光譜，並與加入磷酸鹽(0.5M NaH_2PO_4)的對照比較，結果如 Fig.7。

很明顯的，我們可以發現在沒有加磷酸鹽的螢光光譜中，暗處理的光譜的螢光值會在開燈以後升高，即藻藍素移動到光系統二上，但漸漸地又因為高溫會產生過多的電子，故藻藍素又移開光系統二；而遠紅光處理的光譜中，可以發現一開始的螢光值即升高，說明藻藍素在遠紅光處理的過程中移動到光系統二上，而開燈後，藻藍素又必須漸漸的移開光系統二，造成螢光值的下降。

而觀察暗處理後隨即加入磷酸鹽的螢光光譜，不難發現不管在關燈或開燈時都無明顯的螢光值波動，說明狀態變遷明顯的被抑制，但反觀在遠紅光處理過後加入磷酸鹽的螢光光譜，可以發現原本應該保持不變的螢光值，卻呈現一直下降的趨勢，這說明了當藻藍素被固定在光系統二後，在給予光照後，因為高溫造成的激發能量而產生過多的電子對光系統二的蛋白造成破壞的速率比其生成速率要

慢，所以光系統二的螢光值就會下降，這也證明了耐熱藍綠菌(TCL-1)中，尤其是在高溫環境下，藻藍素必須移開光系統二進行狀態變遷，否則光系統二蛋白就會受到破壞，也就是說，狀態變遷在耐熱藍綠菌中是一種保護機制。

綜合以上所述，我們對不同耐受溫度的藍綠菌提出了模型，如下。

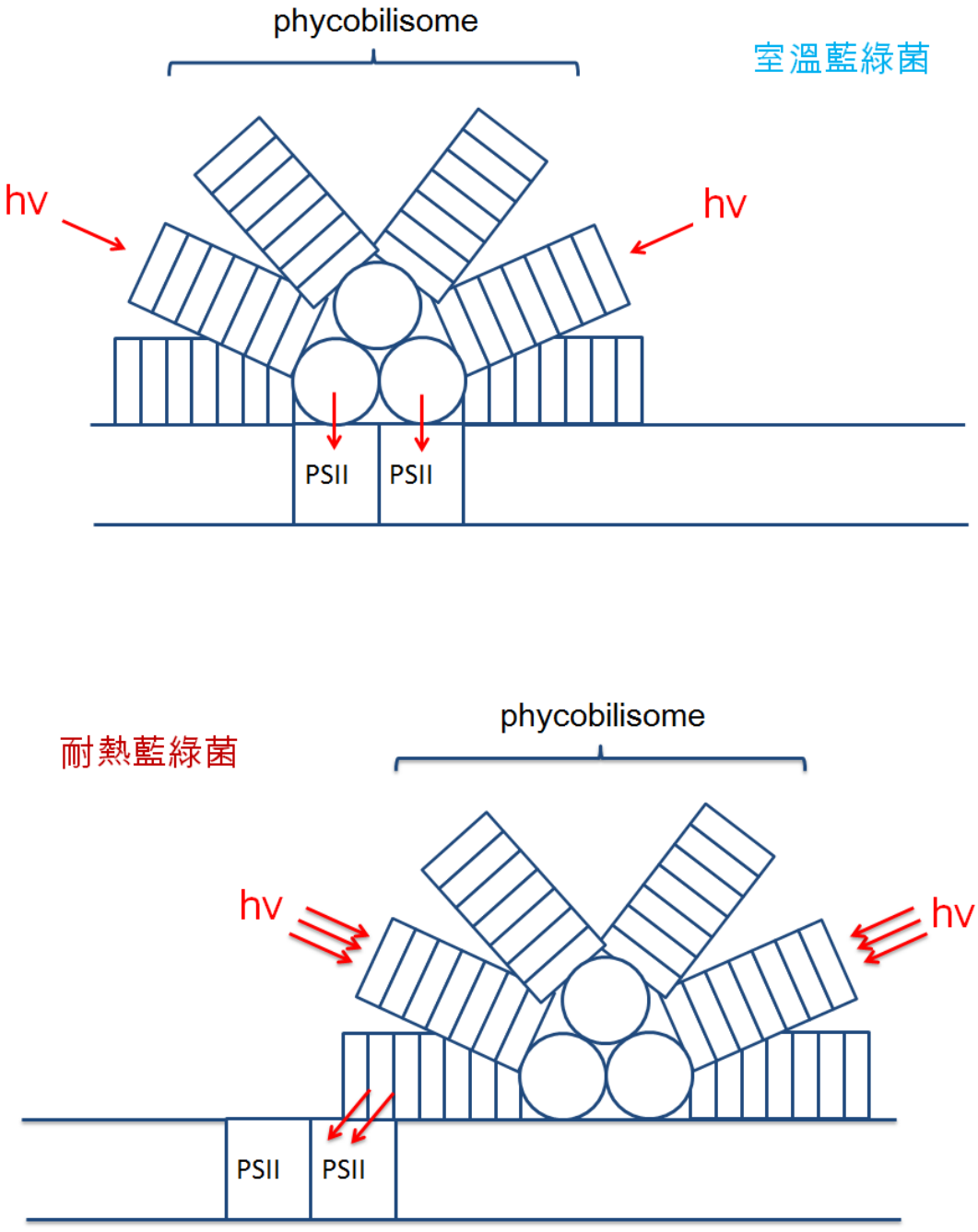

Fig.8 The light reaction model of mesophilic and thermophilic cyanobacteria.

肆、 結論與應用

一、結論

1. TCL-1 的膜系隨著培養溫度而改變。
2. TCL-1 在高溫下有大量的狀態變遷現象。
3. 狀態變遷是在 TCL-1 中的特殊保護機制，可以減少高溫對光系統二造成的破壞。

二、應用

對於 TCL-1 中大量的狀態變遷現象，在本研究實屬一新發現，不但在 TCL-1 中，同樣的我們也在另一株耐熱藍綠菌 TAO1(*Thermosynechococcus sp. AO-1*)中發現類似的現象，因此，這有可能是一種存在於耐熱藍綠菌中普遍的現象。

而目前的研究，對於藍綠菌進行狀態變遷的機轉並不十分清楚，一部份原因也就是在大部分室溫的藍綠菌中狀態變遷的現象不明顯，而對於耐熱藍綠菌中大量的狀態變遷現象卻提供了未來進行藍綠菌狀態變遷研究的一個更好的材料。

伍、 未來展望

另外從我們實驗的結果整體來看，提出一個假設：耐熱藍綠菌在高溫下為了要進行狀態變遷進行保護作用，必須給藻藍素足夠的移動空間，所以細胞會產生適量的自由基，而自由基會氧化磷脂層進而使類囊膜的排列傾向鬆散，以利狀態變遷這種保護機制的進行。

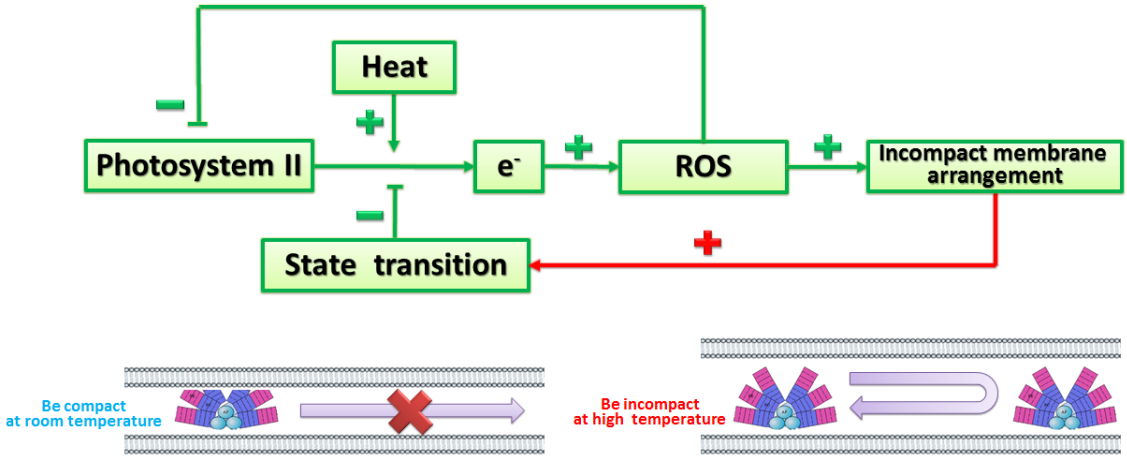

Fig.9 The hypothesis of the thylakoid membrane arrangement induces state transition.

未來的實驗將進行觀察在 PCC68003 會進行狀態變遷的轉殖株中，膜的排列是否也像 TCL-1 在高溫時一樣鬆散，並且利用自由基會氧化磷脂層的特性，測量這兩株藍綠菌類囊膜中被氧化的磷脂層所佔的比例，以說明我們的假設。

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柒、 附圖與附表

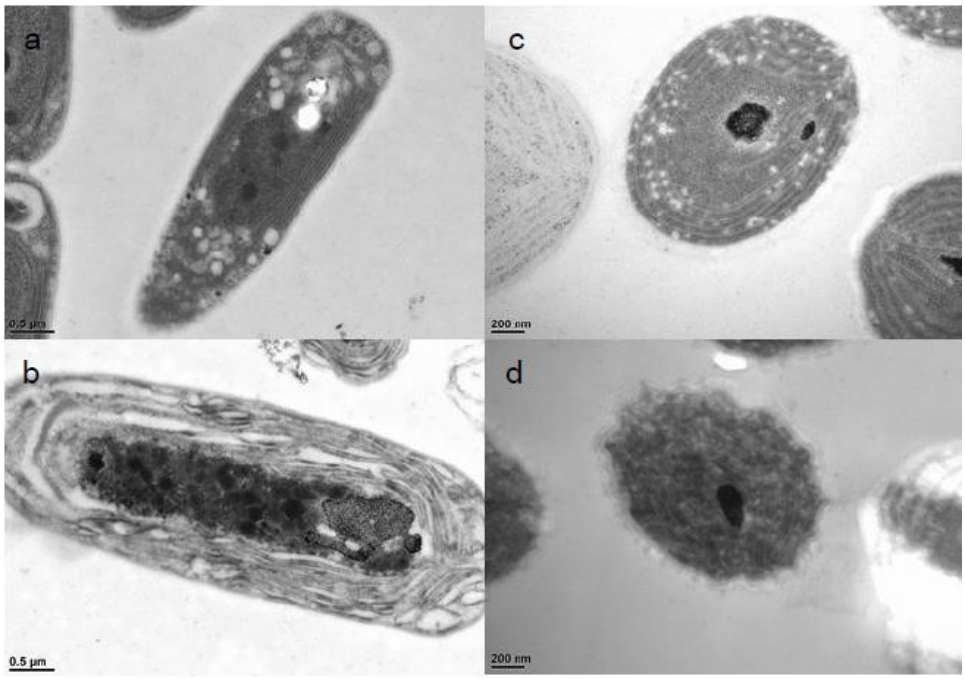


Fig.1 TEMs of cells. *Thermosynechococcus sp. CL-1* grew at 30℃ (a) and at 45℃ (b). *Synechocystis sp. PCC6803* grew at 30℃ (c) and at 45℃ (d).

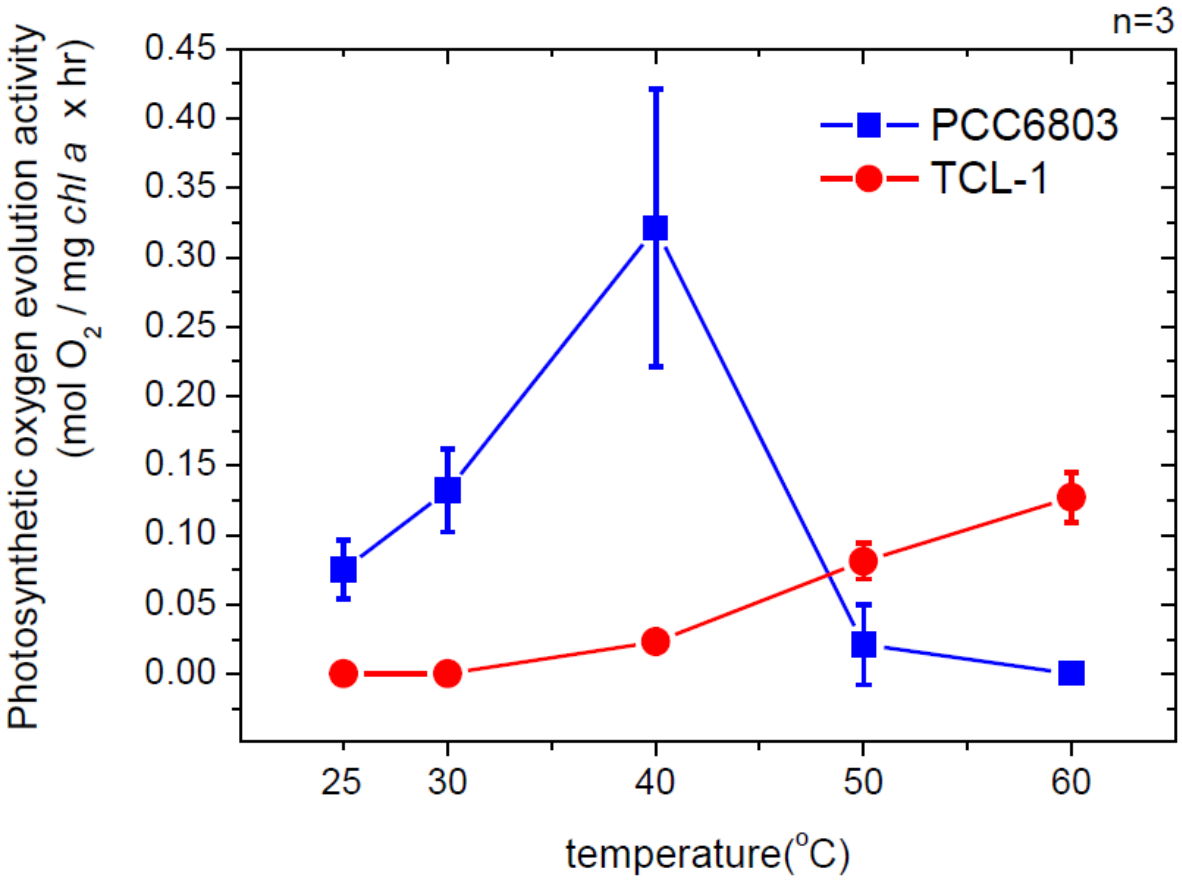


Fig.2 The photosynthetic oxygen evolution activity at five different temperatures. Cells are *Thermosynechococcus sp. CL-1*( )and *Synechocystis sp. PCC6803*( ).

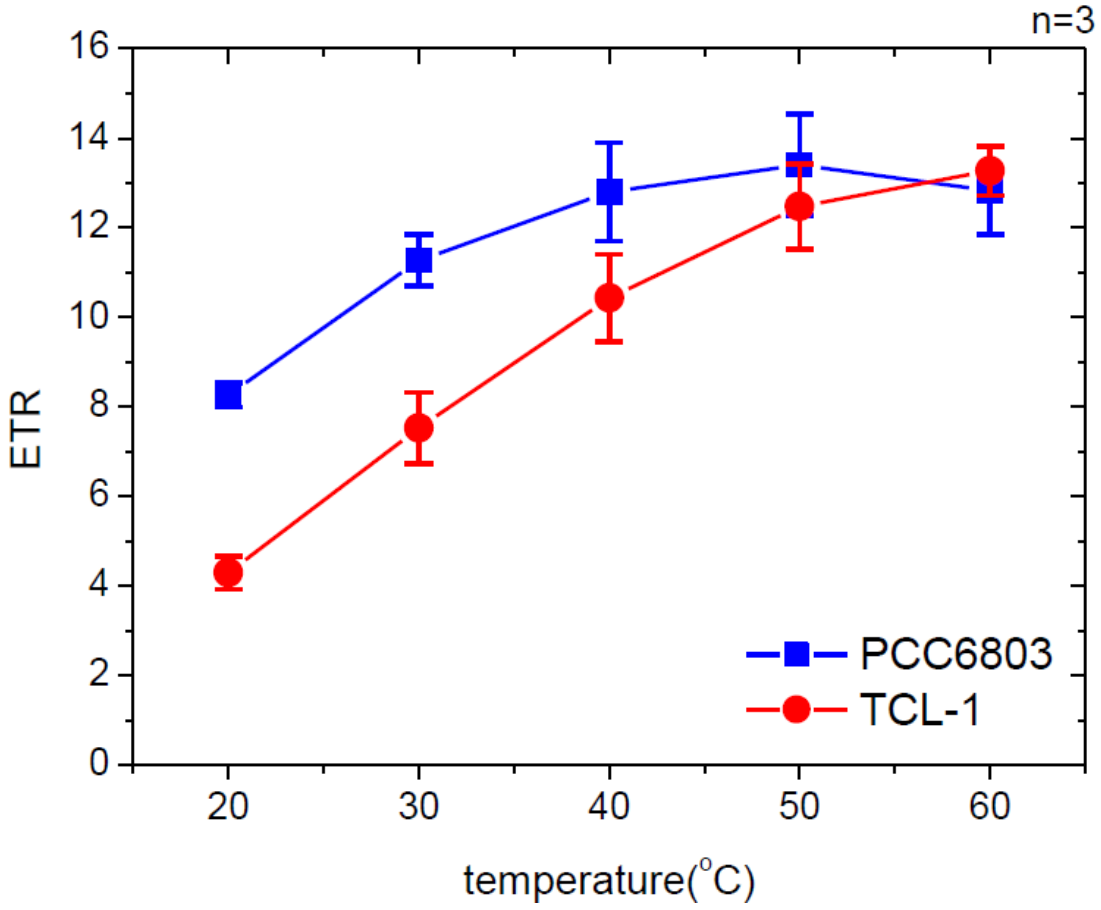


Fig.3 The electron transport rate at five different temperatures. Cells are *Thermosynechococcus sp. CL-1*( )and *Synechocystis sp. PCC6803*( ).

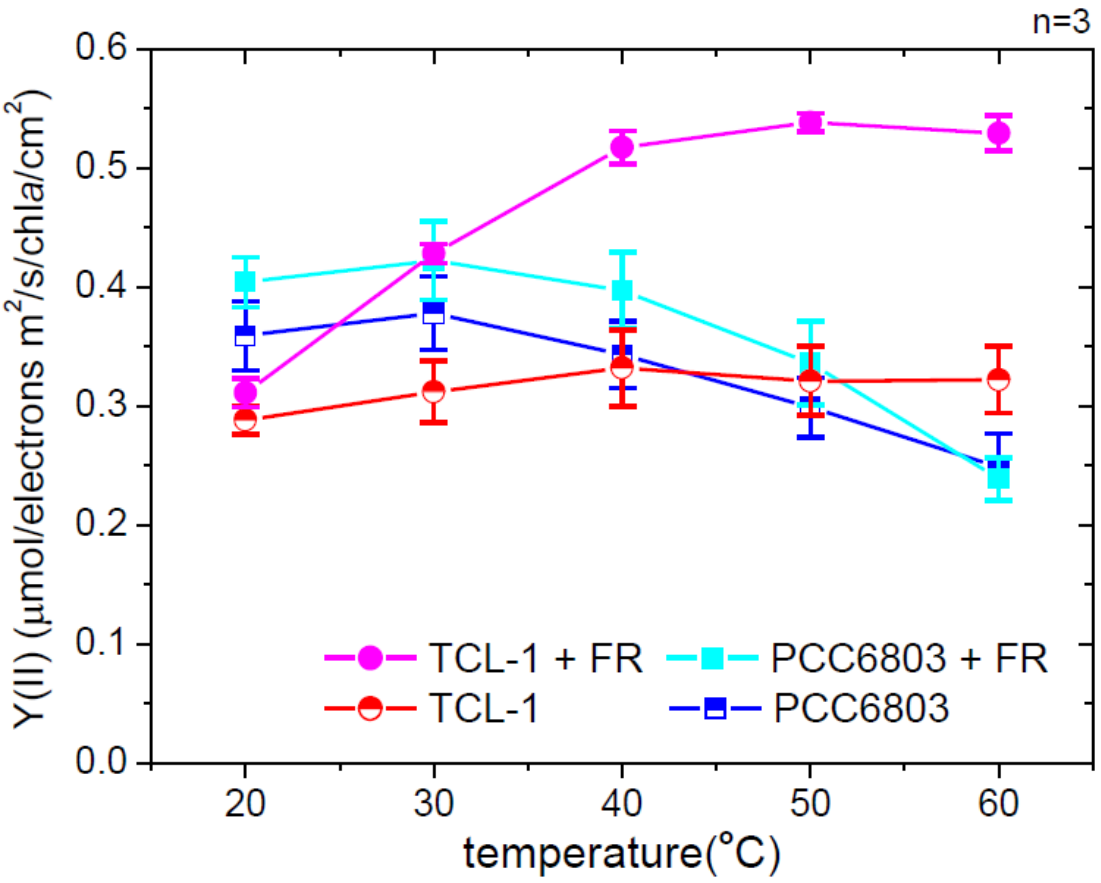


Fig.4 Comparison of the effects of five different temperatures on Y(II) from dark-adapted samples ,*Thermosynechococcus sp. CL-1* ( ) and *Synechocystis sp. PCC6803* ( ) ,and Far-red-light-adapted samples, *Thermosynechococcus sp. CL-1* ( ) and *Synechocystis sp. PCC6803* ( ), using fluorescence Induction.

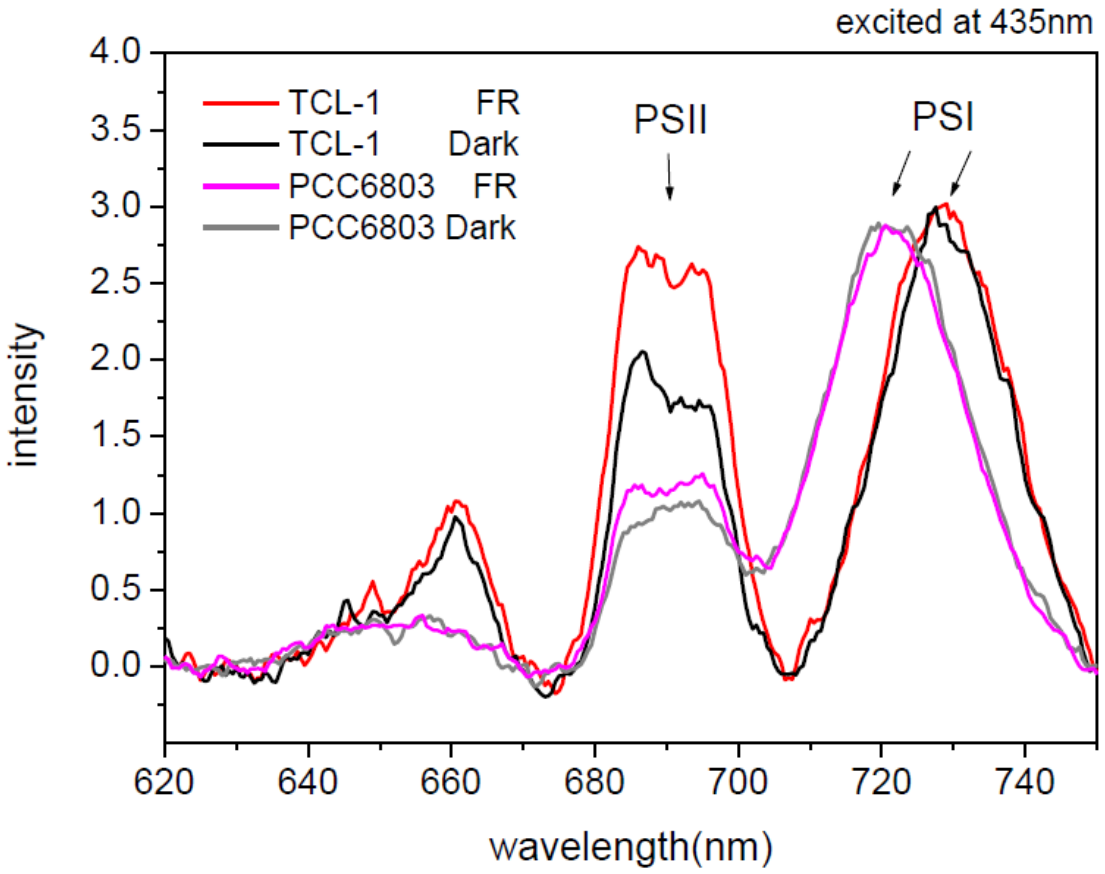


Fig.5 The 77K fluorescence emission spectra for cells of *Thermosynechococcus sp. CL-1* and *Synechocystis sp. PCC6803* adapted to State 1 or to State 2. TCL-1 were adapted to State 1 (——) by incubation in Far-red-light or to State 2 (——)by incubation in the dark ,and PCC6803 were adapted to State 1 (——) by incubation in Far-red-light or to State 2 (——)by incubation in the dark , both of which are freezing in liquid nitrogen. Fluorescence were recorded with excitation at 435nm and normalized to photosystem I peak.

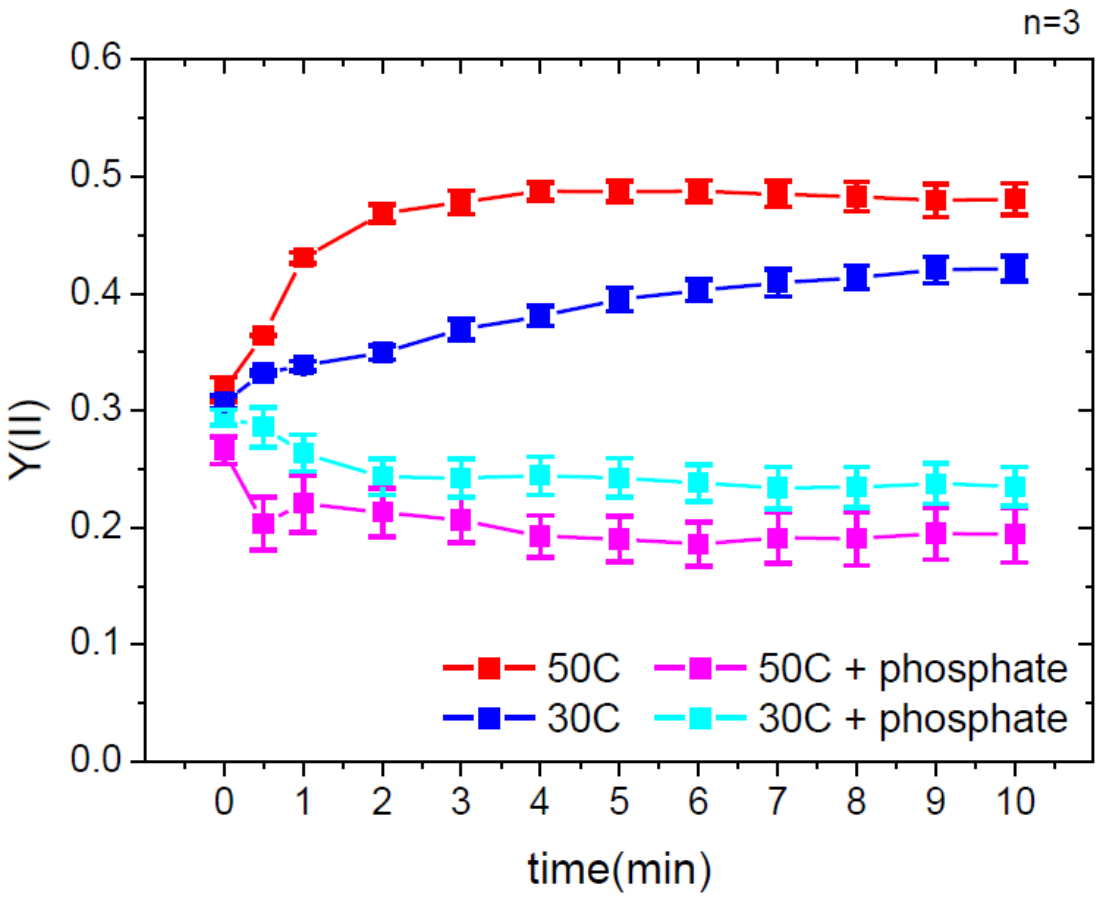


Fig.6 Time course of Far-red-light induces changes in *Thermosynechococcus sp. CL-1*. 30℃ with no phosphate (—■—). 30℃ with phosphate (—■—). 50℃ with no phosphate (—■—). 50℃ with phosphate (—■—).We uses 0.5M Na_2HPO_4 as phosphate.

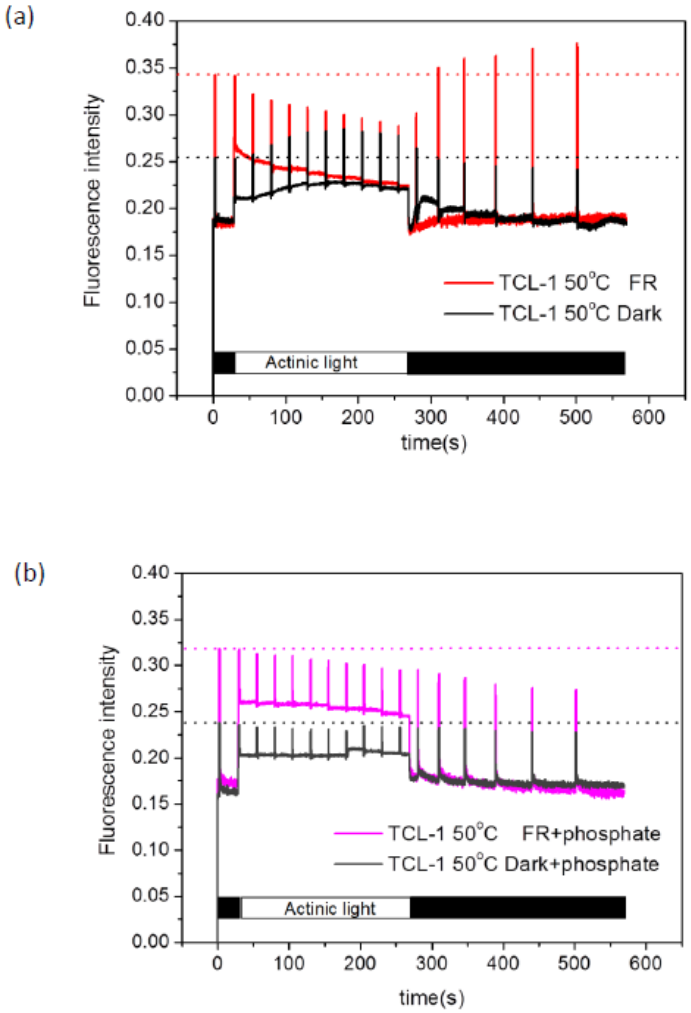


Fig.7 Transients of PSII fluorescence in *Thermosynechococcus sp. CL-1* in the presence and absence of actinic light.(a) is adapted by Far-red-light and Dark without phosphate.(b) is adapted by Far-red-light and Dark with phosphate(0.5 M Na_2HPO_4).

BG-11 Medium for cyanobacteria

STOCK SOLUTIONS：

Trace Metal Mix (1 Liter)：

H_3BO_3	2.86g
$MnC1 \cdot 4H_2O$	1.81g
$ZnSO_4 \cdot 7H_2O$	0.222g
$Na_2MoO_4 \cdot 2H_2O$	0.39g
$CuSO_4 \cdot 5H_2O$	0.079g
$Co(NO_3)_2 \cdot 6H_2O$	0.0494g

Bring to 1 Liter with ddH_2O.

100X BG-11 Stock (1 Liter)：

$NaNO_3$	149.58g
$MgSO_4 \cdot 7H_2O$	7.49g
$CaCl_2 \cdot 2H_2O$	3.6g
Citric acid	0.6g
diNaEDTA (Mg salt)	0.1g

100 mLs Trace Metals mix

Bring to 1 Liter with ddH_2O. Autoclave

175 mM K_2HPO_4： 3.05g/100 mLs─autoclave and store at 4℃

189 mM Na_2CO_3： 2g/100mLs─Filter sterilize and store at 4℃

Ferric Ammonium Citrate：0.6g/100mLs-autoclave and store at 4℃

1M TES, pH8.0：23g/100mL─Autoclve and store at 4℃.

2M Glucose：Dissolve in ddH_2O, filter sterilize , store as 1mL aliquots at -20℃.

10 mM DCMU： Dissolve in Absolute EtOH, store at -20℃.

Antibiotics：

20mg/mL Kanamycin (Km)：

Dissolve 1g Kanamycin monosulfate in 50 mLs ddH_2O, Filter Sterilize, Store as 5 mL liquots at -20℃.

20 mg/mL Specionmycin (Sm)：

Dissoorlve 1 g Spectionmycin dihydrochlride in 50 mLs ddH_2O, Filter Sterilize,Store as mL aliquots at -20℃.

10 mg/mL Chloramhenicol (Cm)：

Dissolve 500 mg Chloramphenicol in 50 mLs absolute EtOH, store as 10 mL aliquots at -20℃.

20 mg/mL Streptomycin (St)：

Dissolve 1 g Streptomycin sulfate in 50 mLs ddH_2O, Filter Sterilize, Store as 5 mL aliquots at -20℃.

0.4 mg/mL Erythromycin (Em)：

Dissolve 20 mg Erthromycin in 50 mLs ddH_2O, Filter Sterilize, Store as 5 mL aliquots at -20℃

BG-11 Liquid medium (1 Liter)：

1 Liter dd H_2O

1 mL 175 mM K_2HPO_4

1 mL 189 mM Na_2CO_3

1 mL 6 mg/mL Ferric Ammonium Citrate

10 mLs 100x BG-11

5 mLs 1M TES , pH 8.0

Autoclave and store at Room Temperature.

Optional supplements【per 100 mLs of BG-11 with TES (add just before use)】：

For 5 mM glucose ： add 250 μL 2 M glucose

For 5μg/mL Km： add 25 μL 20 mg/mL Kanamycin monosulfate

For 2.5 μg/mL Cm： add 25 μL 10 mg/mL Chloramphenicol

For 20 μg/mL Sm： add 100 μL 20 mg/mL Spectionmycin dihydrochloride

For 0.1 μg/mL Em： add 25 μL 0.4 mg/mL Erythromycin

For 10 μg/mL St： add 50 μL 20 mg/mL Streptomycin sulfate

For 10 μM DCMU ： add 100 μL 10 mM DCMU

SOLUBILITY OF SELECTED GASES IN WATER

The values in this table are taken almost exclusively from the International Union of Pure and Applied Chemistry "Solubility Data Series." Unless noted, they comprise evaluated day fitted to a smoothing equation. The data at each temperature are then derived from the smoothing equation which expresses the mole fraction solubility X_1 of the gas in solution as:

$$\ln X_1 = A + B/T^* + C \ln T^*$$

Where

$$T^* = T/100\ K$$

All values refer to a partial pressure of the gas of 101.325 kPa (one atmosphere)

T/K	Solubility(X_1)	Equation constants
288.15	2.756×10^{-5}	A=-66.7354
293.15	2.501×10^{-5}	B=87.4755
298.15	2.293×10^{-5}	C=24.4526
303.15	2.122×10^{-5}	Std.dev.=±0.36%
308.15	1.982×10^{-5}	Temp.range=273.15~348.15

評語

1. 能結合光化學分析探討耐熱藍綠菌之生理變化。

2. 能依據實驗數據建立新穎模型以解釋耐熱機制。

3. 建議加強 Phycobilisome 之定位分析以釐清其角色。

2011 年臺灣國際科學展覽會

優勝作品專輯

編號：070001

作品名稱

State transition mechanism protects photosynthetic light reaction in thermophilic cyanobacteria

得獎獎項

一等獎

美國正選代表:美國第 62 屆國際科技展覽會

作者姓名

Kuang-Ming Shang

Abstract

With so much attention being placed on global warming, how thermophilic hot spring cyanobacteria photosynthesize at higher temperature becomes an interesting and pertinent issue. In this study, the photosynthetic characteristics of the thermophilic cyanobacterium, *Thermosynechoccus sp.CL-1*, and the mesophilic cyanobacterium, *Synechocystis sp.PCC6803*, were compared. Chlorophyll *a* fluorescence induction analysis and 77K fluorescence emission spectra revealed that phycobilisomes, the light harvesting antenna of photosystem II in cyanobacteria, were highly mobile and could readily couple and uncouple with photosystem II. This so-called state transition frequently occurs in *Thermosynechoccus sp.CL-1*, but not in *Synechocystis sp.PCC6803*. Moreover, increasing temperature induced greater effects on state transition in *Thermosynechoccus sp.CL-1*. To study the physiological impact on state transition of *Thermosynechoccus sp.CL-1* at higher temperatures, state transition was suspended using a high concentration of betaine. When state transition was impaired by betaine, photoinhibition occurred in *Thermosynechoccus sp.CL-1*, suggesting that state transition protects the photosynthetic apparatus at higher temperatures by reducing the energy delivered from the phycobilisomes to photosystem II. Taken together, these results suggest that state transition in thermophilic cyanobacteria plays an important role in protecting thermophilic cyanobacteria from photoinhibition at higher temperatures.

Table of Contents

1. Introduction

1.1 Literature Review of State Transition

In cyanobacteria, "state transition" is a rapid response to light. Different states are induced by mobile phycobilisomes (PBSs), which are the light-harvesting antenna of cyanobacteria [10].

During state transition, photosynthetic light reactions regulate and distribute excitation energy between two photosystems. The state transition mechanism involves the movements of PBSs between photosystem II (PSII) and photosystem I (PSI). It is known that different light conditions can induce different states [12]. For example, Far-Red light (FR) induced state 1 (**Fig. 1A**) when PBSs moved to PSII and Orange light induced state 2 when PBSs move to PSI (**Fig. 1B**). However, the exact molecular mechanisms of state transition in cyanobacteria are still unclear [4].

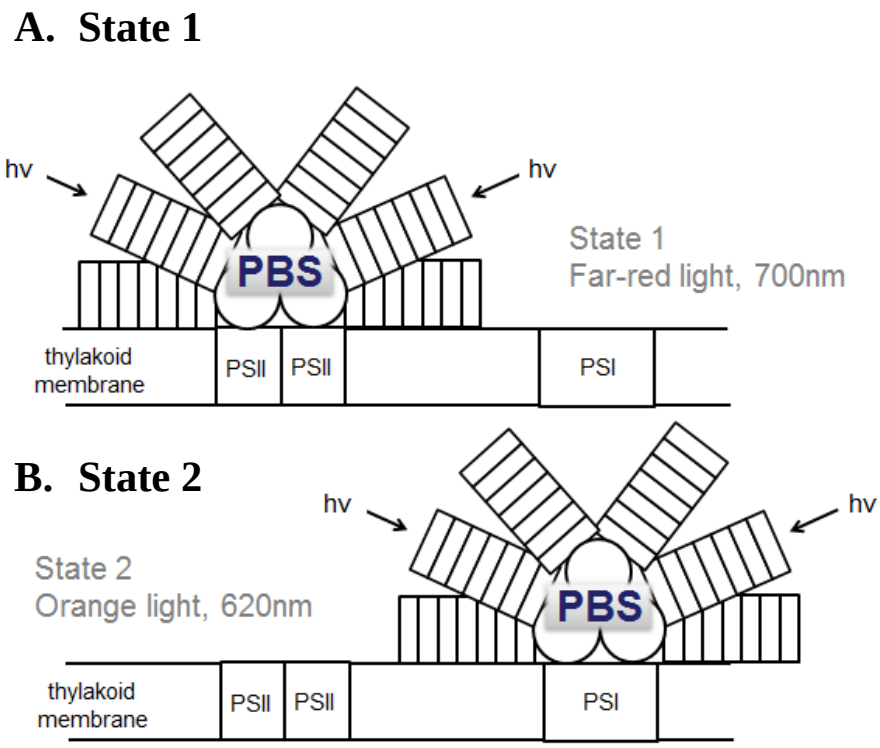


Fig. 1 State transition models in cyanobacteria: (A) When state 1 was induced by Far-Red light, PBSs are coupled with PSII; (B) When state 2 was induced by Orange light, PBSs are uncoupled with PSII.

1.2 Motivation and Goal

To understand how photosynthetic light reactions operate in thermophilic cyanobacteria at higher temperature.

2. Materials and Methods

2.1 Cyanobacteria Strain and Culture Condition

Thermophilic cyanobacterium, *Thermosynechoccus sp.CL-1*, was isolated from Chin-Lun (CL) hot spring (pH 9.3, 62^{o}C) in eastern Taiwan [7]. *Synechocystis sp.PCC6803* was used as the model system of mesophilic cyanobacterium. Both TCL-1 and PCC6803 were cultivated in BG-11 medium at 45^{o}C and 30^{o}C respectively. Cells were grown under 25-30 μE m^{-2} s^{-1} until OD_{730} reached 0.7-1.2.

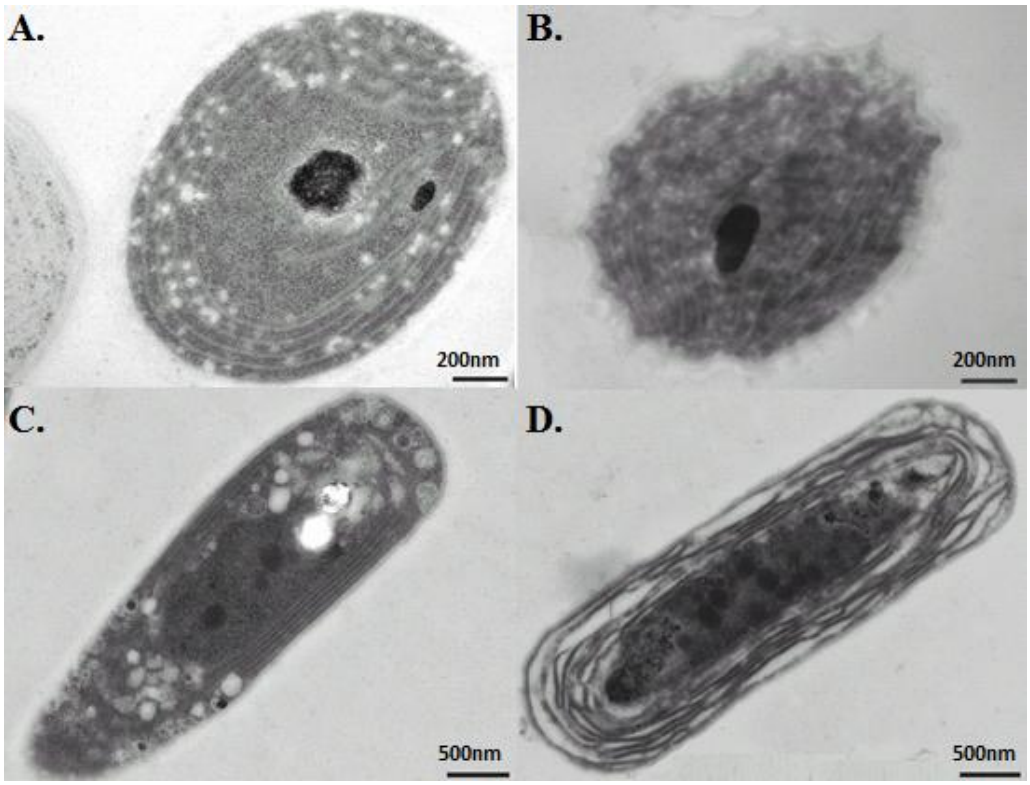


Fig. 2 Transmission electron micrographs of cyanobacteria: PCC6803 grew at 30^{o}C (A) and at 45^{o}C (B).TCL-1 grew at 30^{o}C (C) and at 45^{o}C (D).

2.2 77K Fluorescence Emission Spectra

Fluorescence emission spectra were recorded with a fluorescence spectrometer (Jasco model FP-6500). All the measurements were carried out at 77K, using cell suspensions at a chlorophyll concentration of 20 μg/mL. The excitation light wavelength used for exciting chlorophyll was 435 nm (excitation band width 5 nm, emission band width 1 nm) [3].

2.3 Phycobilisomes Isolation

All steps were carried out at room temperature. 500mL cultures were harvested and washed twice in 0.8 M potassium phosphate buffer, pH 7.0 (KP). The cells were re-suspended in 10 ml KP, and then passed twice through a French pressure cell at 20000 psi. Triton X-100 was added to the broken cells to a final concentration of 2% (v/v). After incubation for 30 min, in the dark, with occasional shaking, unbroken cells and debris were removed by centrifugation at 17000 × g for 20 min at 20^{o}C. To reduce chlorophyll contamination, the supernatant was carefully removed from beneath the floating chlorophyll layer, leaving approx. 1-2 ml of supernatant on top of the greenish-grey pellet. 1 ml of supernatant was layer on top of the following sucrose step-gradients prepared in 12 ml ultracentrifuge tubes: I ml of 2.0 M, 3 ml of 1.0 M, 2.5 ml of 0.75 M, 2.5 ml of 0.5 M, and 1 ml of 0.25 M sucrose solutions in 0.8 M KP. The gradients were spun 20 h in a Beckman SW41 rotor at 35000 rpm at 20℃.The blue-colored bands were isolated from the gradients and stored at 4℃ as sucrose solutions. These samples were examined by SDS polyacrylamide gel electrophoresis and by spectroscopy within 48 hours of isolation. [6, 8]

2.4 PAGE Analysis

PBSs proteins were analyzed by SDS-PAGE on 4–12% Bis-Tris gels (NuPAGE, Novex) in MOPS-SDS buffer. OD at 620 nm was used to ensure approximately equal loading of different PBSs samples (100 μL from a sample at OD_{620}=3). PBS-containing samples were concentrated by precipitation with 10% (w/v) trichloroacetic acid. Proteins were visualized by using Coomassie Brilliant Blue stain.[2]

2.5 Chlorophyll Fluorescence Analysis.

When the solar-emitted photons excite the chlorophyll in the photosynthetic organisms, the excited chlorophyll can lose its energy through one of three competing process (**Fig. 3**). Under normal conditions, >95% energy can be either used to drive photosynthetic light reactions, be dissipated as heat, or be remitted as fluorescence [5]. Since the total rate remains stable, any increase in the efficiency of one process will result in a decrease in the yield of the other two. Therefore, measuring the yield of chlorophyll fluorescence will give information about changes in photosynthetic efficiency.

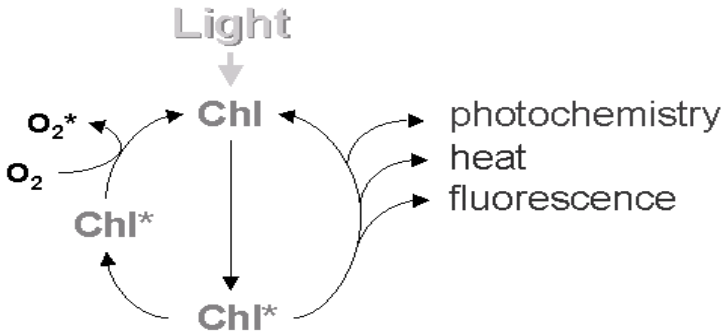


Fig. 3 Possible outcomes of excited chlorophyll. (J. Leipner, 2007)

Fig. 4A shows a typical measurement of chlorophyll fluorescence of cyanobacterial culture.

Before measurement, cells are dark-adapted to the temperatures for five minutes. Ground state values of fluorescence (F_0, dark fluorescence yield) are determined by the measuring light (about 1 μE m^{-2} s^{-1}). While the 800-ms saturating pulse (8000 μE m^{-2} s^{-1}) is applied, fluorescence is raised to F_{Mdark} (maximum fluorescence yield). At this moment, PSII is fully reduced and unable to drive photosynthesis, so the variable fluorescence over the maximum fluorescence generally represents the maximum quantum PSII yield (F_V/F_{Mdark}) (**Fig. 4A**).

Actinic light (about 100 μE m^{-2} s^{-1}) is then turned on to measure the $\Delta F/F_M'$, which represents the effective PSII quantum yield in the illumination sample. Different levels of maximum fluorescence yield during illumination (F_M') are compared to the first peak (F_{Mdark}). Due to the actinic light, different reactions of PSII can be observed.

Under normal conditions, F_M' will be close to F_{Mdark} (**Fig. 4A**). However, under actinic light illumination, F_M' may decrease under actinic light illumination due to the occurrence of non-photochemical quenching (NPQ). There are generally two reasons for the presence of NPQ in cyanobacteria [11] : One is through the state transition mechanism. When this occurs, F_M' return to F_{Mdark} once the actinic light is turned off (**Fig. 4B**). The other is photoinhibition. This occurs when F_M' cannot return to F_{Mdark} once the actinic light is turned off (**Fig. 4C**).

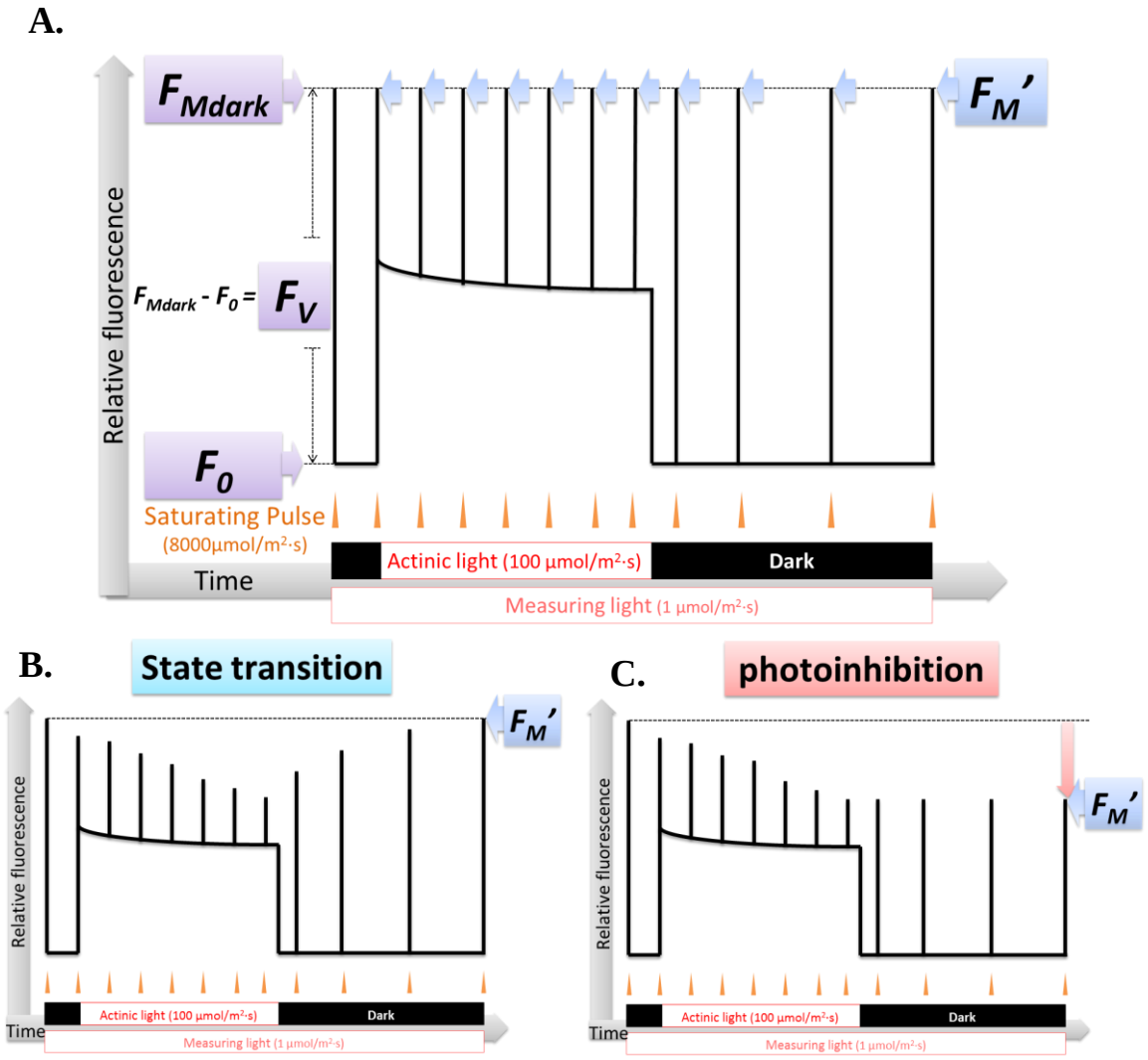


Fig. 4 Schematic diagram of chlorophyll fluorescence analysis of cyanobacterial cultures: (A) under normal conditions, (B) under state transition, and (C) under photoinhibition. F_V/F_{Mdark} represents the maximum PSII quantum yield. $\Delta F/F_M'$ represents the effective quantum yield of PSII.

3. Results

3.1 FR enhanced PSII Activity in TCL-1

Based on the dark-adapted data, maximum PSII quantum yield (F_V/F_{Mdark}) of PCC6803 declined as temperature increased (**Fig. 5A**), but F_V/F_{Mdark} of TCL-1 remained steady with the temperature (**Fig. 5B**). Far-red light (FR) was used to induce state 1. Under FR conditions, PBSs coupled with PSII. With FR (5 μE m^{-2} s^{-1}) treatment, PCC6803 showed a slight increase in F_V/F_{Mdark} (**Fig. 5A**). Surprisingly, TCL-1 showed vigorous state transition (up to five times greater than for PCC6803) as temperature increased (**Fig. 5B**).

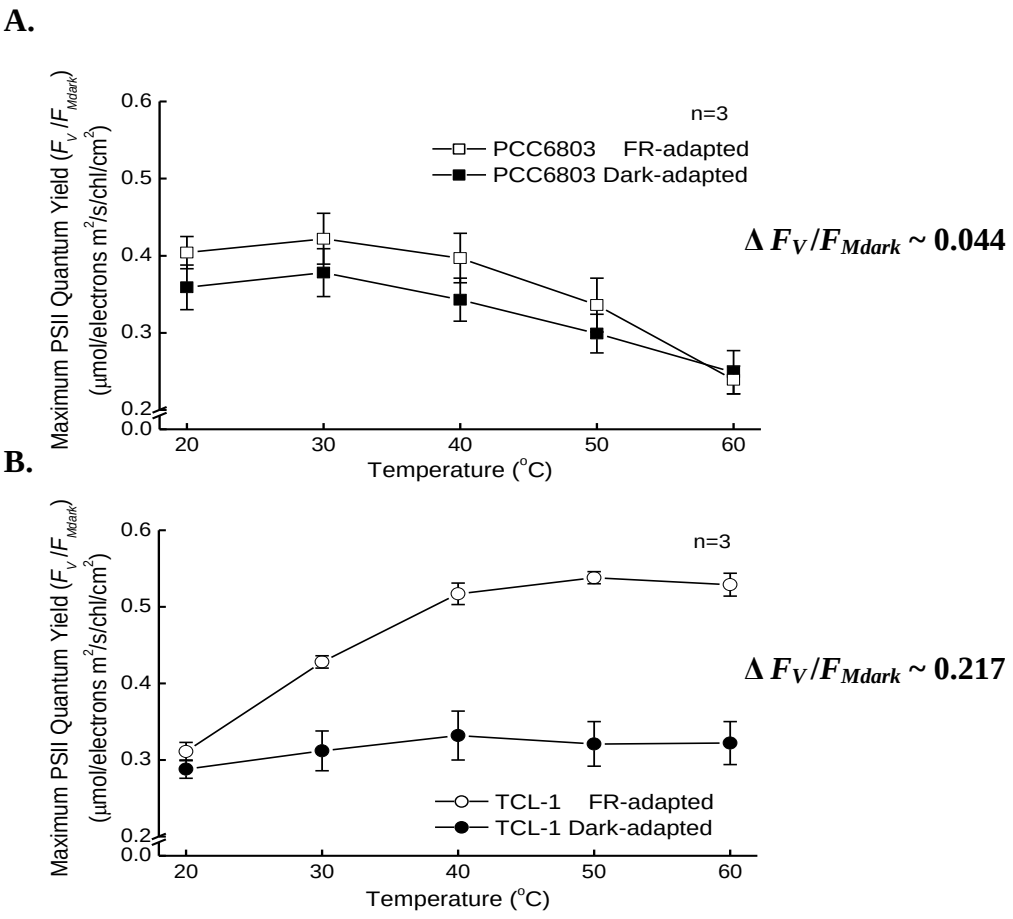


Fig. 5 Maximum PSII quantum yield (F_V / F_{Mdark}) of PCC6803 (A) and TCL-1 (B) measured from 20-60°C. Samples are measured after five-minute adaptation to temperatures and different light conditions, FR or Dark.

Another useful technique to study state transition is 77K fluorescence measurement. **Fig. 6** shows the fluorescence emission spectra of PCC6803 (A) and TCL-1 (B). The emission bands at 680-700 nm arose from PSII, those at 700-740 nm arose from PSI. PSI maximum emission at 723nm for PCC6803 and at 729nm for TCL-1 were used to normalize fluorescence spectra.

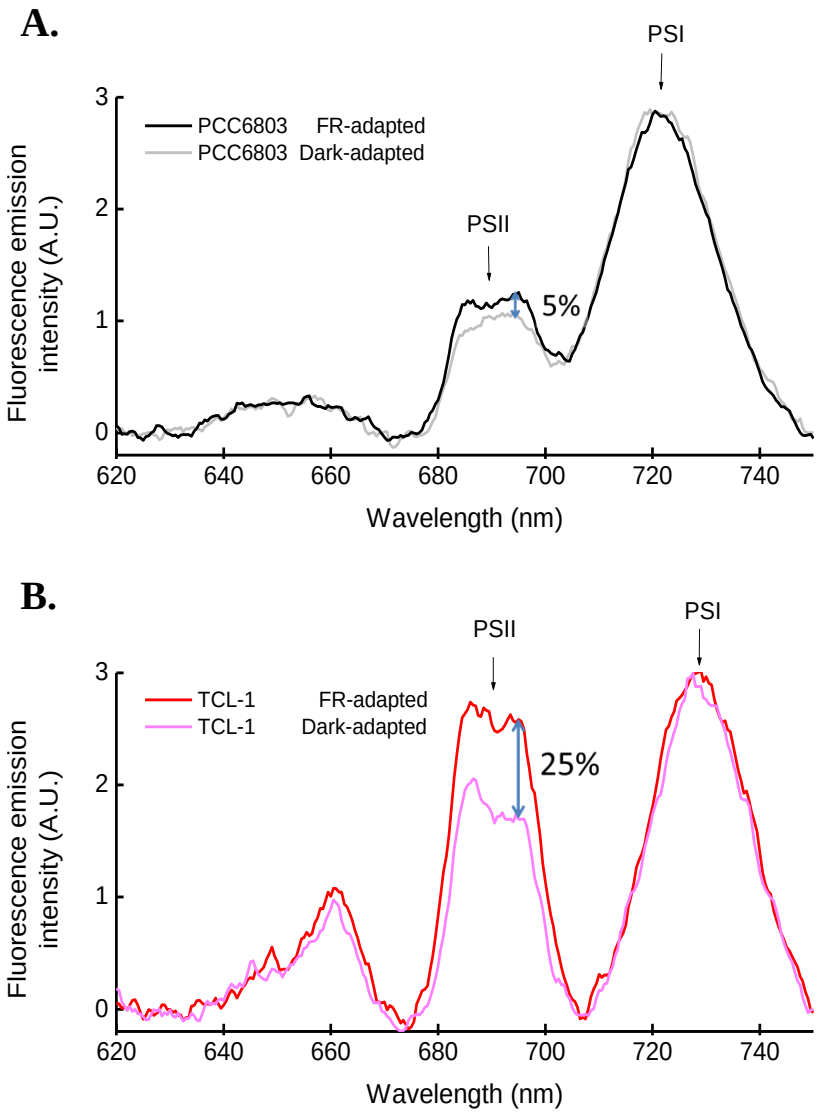


Fig. 6 77K fluorescence emission spectra of PCC6803 (A) and TCL-1 (B): PCC6803 was dark-adapted or FR-adapted at 30°C, and TCL-1 was dark-adapted or FR-adapted at 50°C. After the light treatment, samples were frozen into liquid nitrogen (77K) then excited at 435nm. Maximum PSI emission is used to normalize (723nm at PCC6803; 729nm at TCL-1)

The PSII emission peak under FR was about 5% higher than that in dark-adapted PCC6803 PSII samples. In contrast, the PSII emission peak under FR was about 25% higher than that in TCL-1 PSII samples (**Fig. 6**). The increase in PSII emission bands after FR-adaptation can be attributed to state transition [13]. Therefore, 77K fluorescence results under experimental conditions also demonstrated a high occurrence of state transition in TCL-1, but only a slight one in PCC6803.

Because the FR-induced changes were measured within five minutes, the possibility of this phenomenon could not be caused by long-term biosynthesis. It must be due to a short-term "state transition" induced by PBSs movements [4]. That is to say, PBSs in TCL-1 were not coupled with PSII in the dark (state 2) and became coupled with PSII under FR illumination (state 1) (**Fig. 7**).

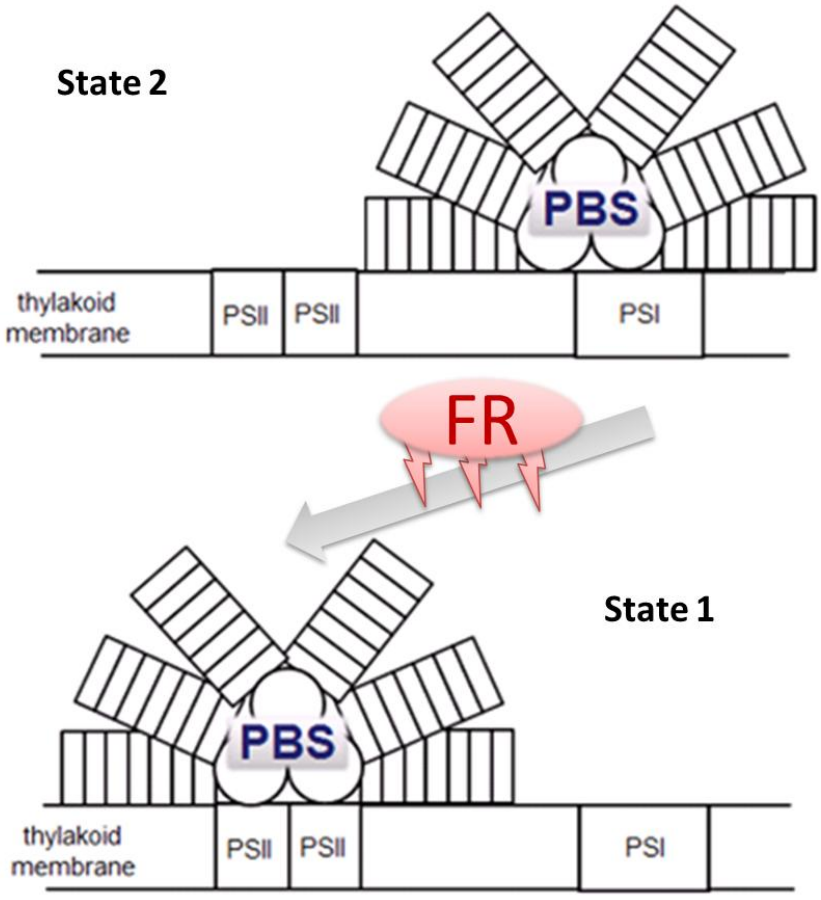


Fig. 7 Schematic diagram of FR-induced state transition

3.2 State Transition is Responsible for FR-Enhanced PSII Activity

In order to understand the physiological function of state transition in TCL-1, a high concentration (0.8M) of betaine ($C_5H_{11}NO_2$) was used to suspend "state transition" [13].

Fig. 8 shows time required for FR-induced changes in TCL-1 at 30°C and 50°C, with and without of betaine. FR-induced the increase in maximum PSII quantum yield (F_V / F_{Mdark}) of TCL-1 at 30°C and 50°C, and the rate is faster at 50°C than at 30°C. This increase in F_V / F_{Mdark} originated from the state transition, indicated by the faster movement of PBSs at 50°C.

In addition, the increasing F_V / F_{Mdark} suspended by betaine also showed that the FR-induced change in TCL-1 was due to "state transition".

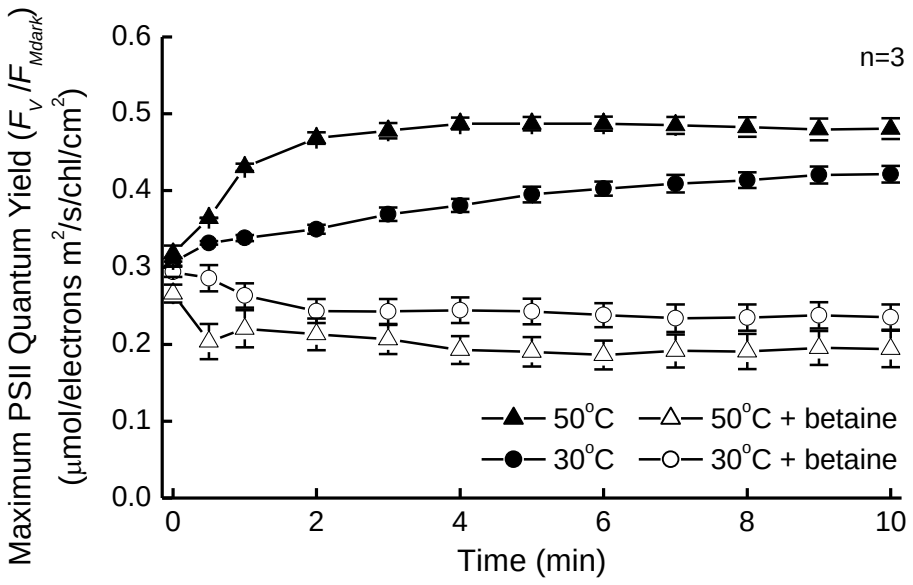


Fig. 8 Time required for of FR-induces changes of F_V / F_{Mdark} in TCL-1 at 30°C and 50°C, with or without betaine.

3.3 Different Sizes of Linker Core Membrane

In order to understand what trigger the vigorous state transition in TCL-1. **Fig. 9A** shows the sucrose density gradient of PCC6803 and TCL-1. The blue-colored PBSs' band in TCL-1 was presence in the lower fraction of the sucrose gradient than in PCC6803. It indicated that the molecular weight of PBSs in TCL-1 is larger than that in PCC6803.

Then, we isolated the blue-colored band of PBSs-contained protein to do the SDS-PAGE analysis. **Fig 9B** shows the major difference between PCC6803 and TCL-1 is the molecular size of Linker Core Membrane (LCM). LCM is the linker protein that connects PBSs to PSII, so the different size of LCM may play a specific role in modifying distinct state transition in TCL-1.

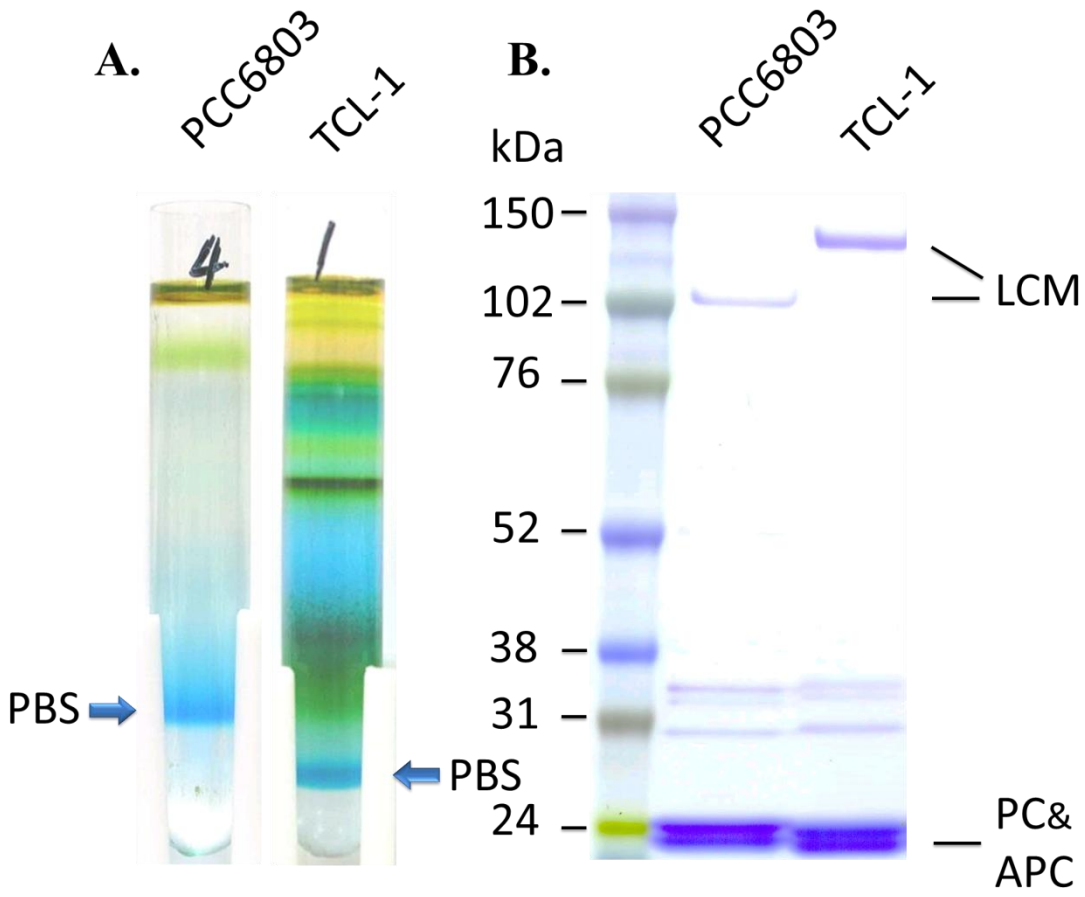


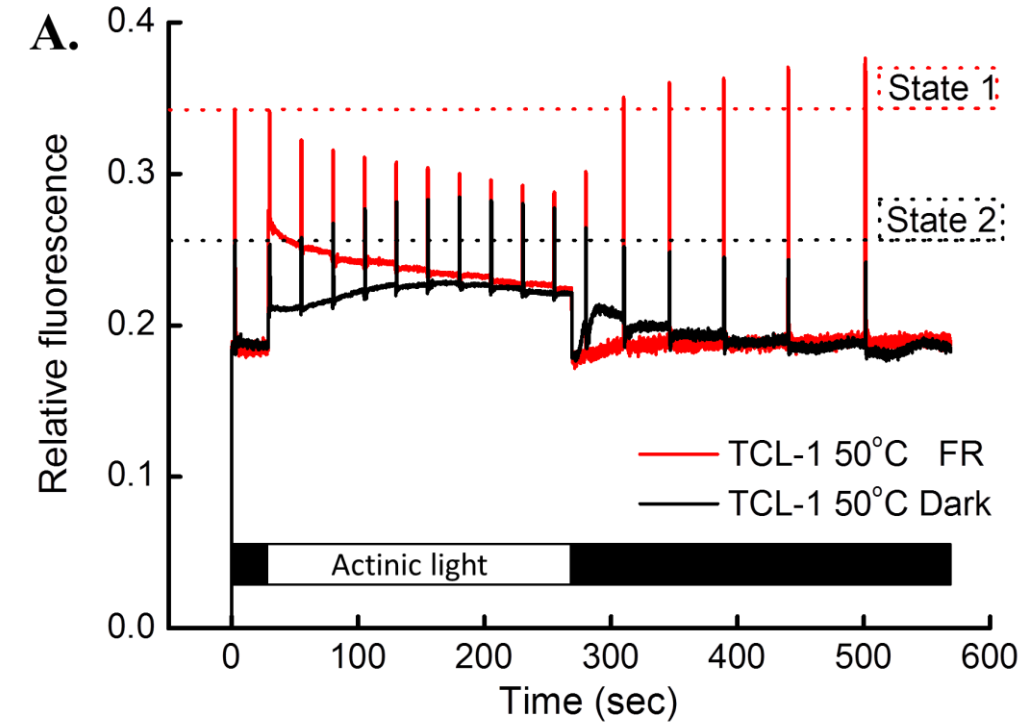
Fig. 9 (A) Isolation of PBSs from sucrose gradient and (B) SDS-PAGE analysis of PBS subunits from PCC6803 and TCL-1.

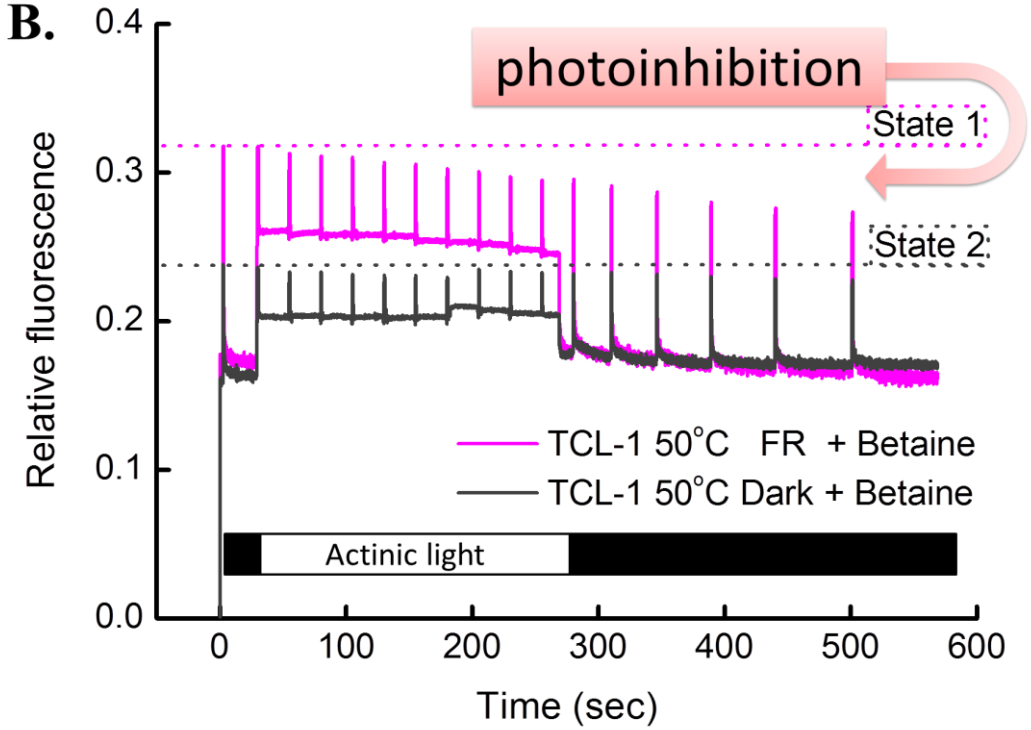
3.4 State Transition as a Thermal Protection

To test whether or not state transition is a thermal protective mechanism in TCL-1, we performed an analysis of chlorophyll fluorescence induction and recovery with or without betiane (**Fig. 10**). **Fig. 10A** showed the effect of state transition in TCL-1 at 50^{o}C in the "absence" of betaine. The maximum fluorescence yield (F_M') of dark-adapted TCL-1 (state 2) increased slightly under actinic light illumination, and returned to the F_{Mdark} once the actinic light was turned off. The F_{Mdark} of FR-adapted TCL-1 (state 1) reached a higher level than that of the dark-adapted sample (state 2). F_M' of FR-adapted TCL-1 decreased significantly under actinic light illumination, and returned to the F_{Mdark} once the actinic light was turned off. The change of F_M' during actinic light illumination is due to state transition.

Fig. 10B shows the effect of photoinhibition in TCL-1 at 50^{o}C in the "presence" of betaine. Betaine was used to trap FR-adapted samples at state 1 or dark-adapted samples at state 2. The F_M' of dark-adapted TCL-1 (state 2) remained steady as F_{Mdark} under and after actinic light illumination. However, the F_{Mdark} of FR-adapted TCL-1 (state 1) decreased under and after actinic light illumination. Results showed that photoinhibition occurred in the FR-adapted sample (state 1) when state transition was impaired.

In contrast, a weak state transition was observed in PCC6803 without betaine (**Fig. 10C**) and a weak photoinhibition effect was observed with betaine (**Fig. 10D**). Taken together, state transition may play an important role in protecting TCL-1 from photoinhibition at hot-spring temperatures (40-60^{o}C).


A.
Relative fluorescence
0.4
0.3
0.2
0.1
0.0
State 1
State 2
TCL-1 50°C FR
TCL-1 50°C Dark
Actinic light
0
100
200
300
400
500
600
Time (sec)


B.
Relative fluorescence
0.4
0.3
0.2
0.1
0.0
photoinhibition
State 1
State 2
TCL-1 50°C FR + Betaine
TCL-1 50°C Dark + Betaine
Actinic light
0
100
200
300
400
500
600
Time (sec)

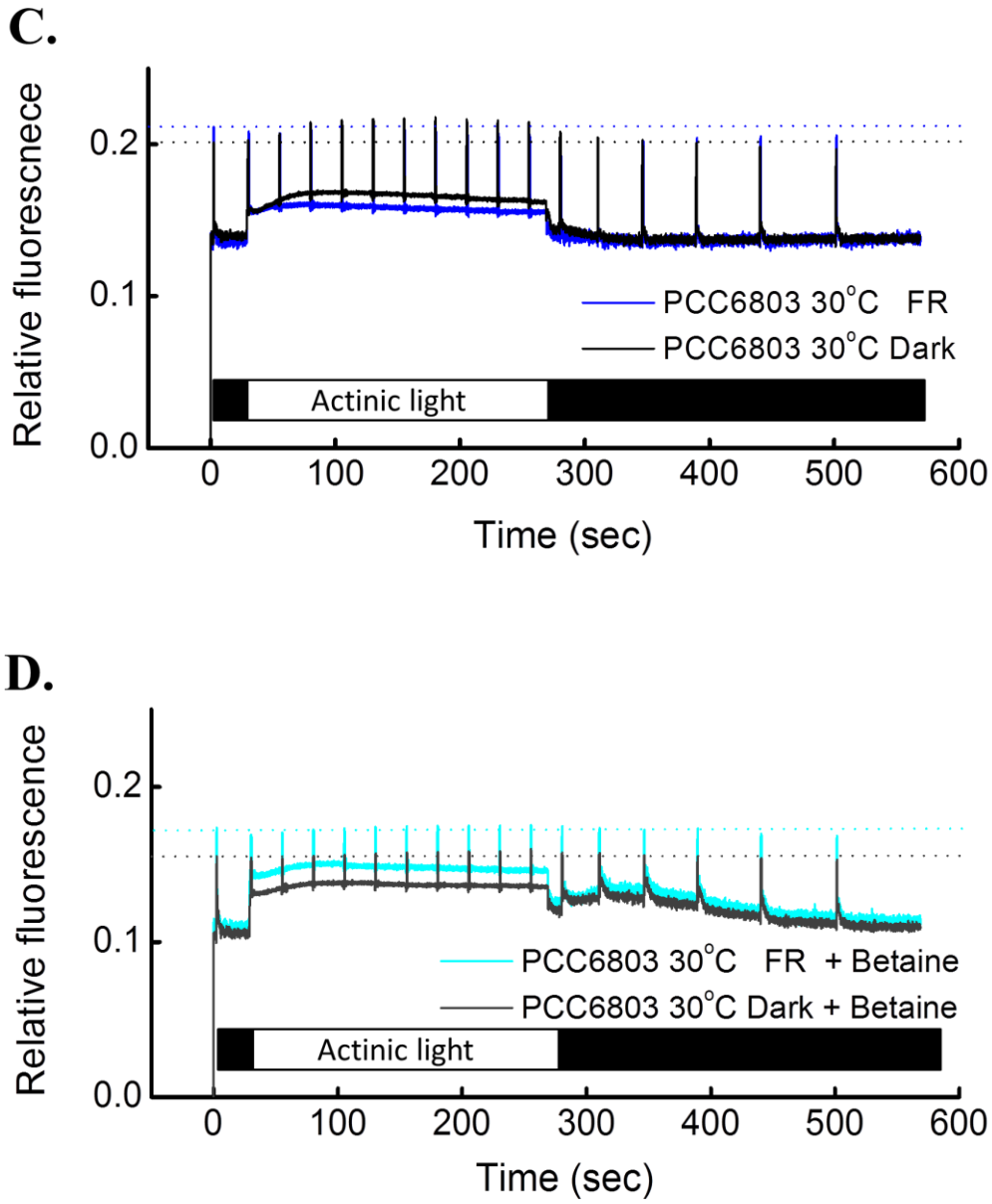


Fig. 10 Chlorophyll fluorescence induction and recovery analysis of PSII in the presence and absence of actinic light: Both TCL-1 and PCC6803 had prior FR or dark treatment. TCL-1 was measured at 50°C in the absence of betaine (A) or in the presence of betaine (B), and PCC6803 was measured at 30°C in the absence of betaine (C) or in the presence betaine (D).

4. Discussion

In this study, we discovered that a distinct state transition phenomenon occurs in TCL-1 under light at higher temperatures (40-60^{o}C).

Moreover, due to the different sizes of LCM and distinct state transition in TCL-1, it is proposed that LCM might play a specific role.

To understand the physiological significance of state transition in TCL-1, two possibilities are proposed. The first one involves the reduction of the light energy transfer to PSII. Because photosynthesis is a photochemical reaction, the reaction rate is accelerated as the temperature increases. At higher temperature, more electrons are produced in photosynthetic light reactions, and so are the by product, reactive oxygen species (ROS). ROS has a high oxidation power, which is able to oxidize proteins, membrane lipids, and pigments in the photosynthetic apparatus. Therefore, reduction of the light energy transfer to PSII can protect the photosynthetic apparatus from photoinhibition. Also, under this condition the excitation energy in PBSs transfers to PSI. PSI reduces the electrons accumulated between PSII and PSI. Therefore, fewer ROS will be produced. Taken together, state transition plays an important role in protecting photosynthetic light reaction at hot-spring temperatures (40-60^{o}C).

A similar phenomenon was observed for the thermophilic cyanobacterium *Thermosynechococcus sp. AO-1* (data not shown). The state transition mechanism must thus be a general protective mechanism for thermophilic cyanobacteria.

All previous studies on state transition of cyanobacteria used mesophilic cyanobacteria, such as PCC6803. Present studies, thermophilic cyanobacteria, TCL-1, show a distinct state transition phenomenon in thermophilic cyanobacteria, TCL-1 compared to mesophilic cyanobacteria. Therefore, thermophilic cyanobacteria, such as TCL-1, appear to be an ideal system to study the mechanism of “state transition”.

5. Conclusions

*5.1 Thermophilic cyanobacterial phycobilisomes move dynamically between state 1 and state 2 during photosynthesis (**Fig. 11A**) when compared to those of mesophilic ones (**Fig. 11B**).*

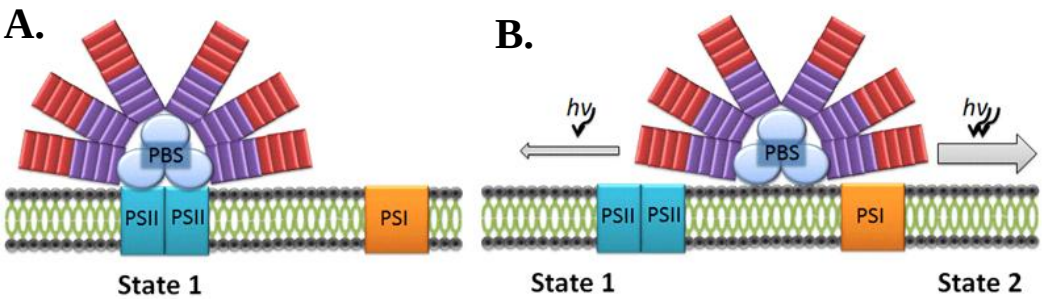


Fig. 112 Photosynthetic light reaction models of mesophilic cyanobacteria (A) and thermophilic cyanobacteria (B).

5.2 "State transition" mechanism of thermophilic cyanobacteria plays an important role in protecting photosynthetic light reaction from photoinhibition at hot-spring temperatures (40-60^{o}C).

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