

Study on Production of Phycobiliprotein by Various Microorganisms

許銘展、吳建一

E-mail: 386684@mail.dyu.edu.tw

ABSTRACT

0

Keywords : Phycocyanin、Purification、Light intensity、Salinity

Table of Contents

封面內頁

簽名頁

中文摘要iii

英文摘要iv

誌謝v

目錄vii

圖目錄x

表目錄x

1.前言1

2.文獻回顧4

2.1藍菌(*Cyanobacteria*)概述4

2.2藻藍蛋白結構9

2.3藻藍蛋白合成調控12

2.4藻藍蛋白的應用14

2.4.1螢光探針14

2.4.2食品添加劑與健康食品16

2.4.3營養醫學補充品與醫藥品17

3.材料與方法20

3.1實驗器材20

3.1.1實驗藥品20

3.1.2儀器設備21

3.1.3藍菌來源與篩選23

3.1.4菌株之鑑定28

3.1.5掃描式電子顯微鏡28

3.1.6傅立葉轉換紅外線光譜儀 29

3.2藍菌之培養30

3.2.1 *Oscillatoria* sp. Wu1、*Thermosynechococcus* sp. 及
Spirulina sp. 之培養30

3.3藍菌代謝產物分析31

3.3.1總糖分析31

3.3.2氨氮分析32

3.3.3硝酸根與亞硝酸根離子分析34

3.3.4尿素含量分析 35

3.4藍菌中色素蛋白萃取及分析方法37

3.4.1藻膽蛋白萃取及含量測定 37

3.4.2葉綠素a萃取及含量測定38

3.5藻膽蛋白之純化分析38

3.5.1SDS-Polyacrylamide gel eletrophoresis38

3.5.2膠體大小排阻層析	39
3.5.3質譜儀樣品製備及檢測	39
4.結果與討論	41
4.1藻藍蛋白生產藍菌之篩選	41
4.2藍菌最適基本培養基選擇	47
4.3培養基成份之探討	59
4.3.1氮源種類對藍菌生長及藻藍蛋白含量之影響	59
4.3.2氮源濃度對藍菌生長及藻藍蛋白含量之影響	77
4.3.3碳源種類對Thermosynechococcus sp.生長及藻藍蛋白含量之影響	106
4.3.4碳源濃度對 Thermosynechococcus sp.生長及藻藍蛋白含量之影響	117
4.4環境因子之探討	125
4.4.1NaCl添加濃度對藍菌生長及藻藍蛋白含量之影響	125
4.4.2光照強度對藍菌生長及藻藍蛋白含量之影響	142
4.5藻藍蛋白之純化與分析	160
4.5.1藻藍蛋白之純化與光譜分析	160
4.5.2液相層析串聯式質譜儀(LC / MS / MS)蛋白質身分鑑定	176
5.結論	183
參考文獻	187

圖目錄

Fig.1-1Schematic description of this study	3
Fig.2-1The structure of phycobilisome (MacColl, 1998)	12
Fig.3-1Sift flow chart of cyanobacteria	27
Fig.3-2Pre-treatment flow chart of SEM	29
Fig.3-3The calibration curve of total sugar using glucose as a standard agents	32
Fig.3-4The calibration curve of NH ₄ ⁺ -N.	33
Fig.3-5The ion chromatography spectrum of various anion	34
Fig.3-6The calibration curve of NO ₃ ⁻ and NO ₂	35
Fig.3-7The calibration curve of urea	36
Fig.4-1Morphology of Thermosynechococcus sp. : (A) Optical microscopy of Thermosynechococcus sp., (B) Scanning electron micrograph of Thermosynechococcus sp.(× 15000), (C) Scanning electron micrograph of Thermosynechococcus sp. (× 30000), (D) (a) the photo of phycocyanin ; (b) the fluorescent photo of phycocyanin.	43
Fig.4-2Morphology of helicoidal trichomes of Spirulina sp : (A)Optical microscopy of Spirulina sp., (B) Scanning electron micrograph of Spirulina sp (× 1000), (C) Scanning electron micrograph of Spirulina sp (× 10000),(D)(a) the photo of phycocyanin ; (b) the fluorescent photo of phycocyanin.	44
Fig.4-3Morphology of linearly trichomes of Spirulina sp : (A) Optical microscopy of Spirulina sp., (B) Scanning electron micrograph of Spirulina sp (× 1000), (C) Scanning electron micrograph of Spirulina sp (× 10000),(D)(a) the photo of phycobiliprotein ; (b) the fluorescent photo of phycobiliprotein.	45
Fig.4-4The phycobiliprotein content and growth of Spirulina sp. at different form after 7day culture	46
Fig.4-5Time behavior of the cell concentration and phycocyanin concent during Spirulina sp. cultivations performed in various medium with inoculum level of 10% at aerobic 0.6 vvm and anaerobic conditions	50
Fig.4-6Time behavior of the nitrate, nitrite, ammonia and phycocyanin concent during Spirulina sp. cultivations performed in various medium with inoculum level of 10% at aerobic 0.6 vvm and anaerobic conditions	51
Fig.4-7The specific growth rate (μ max) and doubling time (d) for the culture of Spirulina sp. in Schl?sser medium (SLM), BG11medium (BGM) and Zarrouk's medium (ZRM) with inoculum level of 10%	52
Fig.4-8Time behavior of optical microscopy during Spirulina sp. cultivations performed in BG11medium with inoculum level of 10% at aerobic (0.6 vvm) conditions	53
Fig.4-9Time behavior of optical microscopy during Spirulina sp. cultivations performed in BG11medium with inoculum level of	

- 10% at anaerobic conditions.54
- Fig.4-10 Time behavior of optical microscopy during *Spirulina* sp. cultivations performed in Schl?sser medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.55
- Fig.4-11 Time behavior of optical microscopy during *Spirulina* sp. cultivations performed in Schl?sser medium with inoculum level of 10% at anaerobic conditions.56
- Fig.4-12 Time behavior of optical microscopy during *Spirulina* sp. cultivations performed in Zarrouk ' s medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.57
- Fig.4-13 Time behavior of optical microscopy during *Spirulina* sp. cultivations performed in Zarrouk ' s medium with inoculum level of 10% at anaerobic conditions.58
- Fig.4-14 Time behavior of pH and biomass during *Termosynechococcus* sp. cultivation under different inorganic nitrogen sources (1.0 g / L) performed in BG11 medium with inoculum level of 10% at aerobic (0.6 vvm) conditions. 67
- Fig.4-15 Time behavior of pH and biomass during *Thermosynechococcus* sp. cultivation under different organic nitrogen sources (1.0g / L) performed in BG11 medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.68
- Fig.4-16 Phycobiliprotein contents and biomass of *Thermosynechococcus* sp. cultivation under different inorganic nitrogen sources (1.0 g / L) performed in BG II medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.69
- Fig.4-17 Phycobiliprotein contents and biomass of *Thermosynechococcus* sp. cultivation under different organic nitrogen sources (1.0 g / L) performed in BG11 medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.70
- Fig.4-18 The phycobiliprotein content and growth of *Thermosynechococcus* sp. at various nitrogen sources after 5day culture.71
- Fig.4-19 Time behavior of pH and biomass during *Spirulina* sp. cultivation under different inorganic nitrogen sources performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.72
- Fig.4-20 Time behavior of pH and biomass during *Spirulina* sp. cultivation under different organic nitrogen sources. (1.0g / L) performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions. 73
- Fig.4-21 Phycobiliprotein contents and biomass of *Spirulina* sp cultivation under different inorganic nitrogen sources performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.74
- Fig.4-22 Phycobiliprotein contents and biomass of *Spirulina* sp. cultivation under different organic nitrogen sources performed in Zarrouk's medium with inoculum level of 10% at aerobic 0.6 vvm conditions.75
- Fig.4-23 The phycobiliprotein content and growth of *Spirulina* sp. at various nitrogen sources after 7day culture.76
- Fig.4-24 Time behavior of phycocyanin concen, pH, ammonia nitrogen (mg / L) and biomass (g / L) during *Thermosynechococcus* sp. cultivation under different NH4H2PO4 concentrations performed in modified BG11 medium with inoculum level of 10% at aerobic 0.6 vvm conditions.85
- Fig.4-25 Phycobiliprotein contents and biomass of *Thermosynechococcus* sp. cultivation under NH4H2PO4 concentrations performed in BG11 medium with inoculum level of 10% at aerobic 0.6 vvm conditions.86
- Fig.4-26 The phycobiliprotein content and growth of *Thermosynechococcus* sp. at various NH4H2PO4 concentration after 5day culture.87
- Fig.4-28 Time behavior of the nitrate, nitrite, ammonia and phycocyanin concen during *Spirulina* sp. cultivation under different NaNO2 concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions89
- Fig.4-29 Time behavior of the urea, ammonia and phycocyanin concen during *Spirulina* sp. cultivation under different urea concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.90
- Fig.4-30 Time behavior of the nitrate, nitrite, ammonia and phycocyanin concen during *Spirulina* sp. cultivation under different NH4Cl concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.91
- Fig.4-31 Time behavior of the nitrate, nitrite, ammonia and phycocyanin concen during *Spirulina* sp. cultivation under different NH4NO3 concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.92
- Fig.4-32 Time behavior of the nitrate, nitrite, ammonia andphycocyanin concen during *Spirulina* sp. cultivation under different NH4H2PO4 concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.93
- Fig.4-33 Phycobiliprotein contents and biomass of *Spirulina* sp cultivation under NaNO3 concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions..94
- Fig.4-34 Phycobiliprotein contents and biomass of *Spirulina* sp. cultivation under NaNO2 concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.95
- Fig.4-35 Phycobiliprotein contents and biomass of *Spirulina* sp. cultivation under Urea concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.96
- Fig.4-36 Phycobiliprotein contents and biomass of *Spirulina* sp. cultivation under NH4Cl concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions97
- Fig.4-37 Phycobiliprotein contents and biomass of *Spirulina* sp. cultivation under NH4NO3 concentrations performed in Zarrouk's

- medium with inoculum level of 10% at aerobic (0.6 vvm) conditions98
- Fig.4-39The phycobiliprotein content and growth of Spirulina sp. at various NaNO₃ concentrations after 7 day culture.100
- Fig.4-40The phycobiliprotein content and growth of Spirulina sp. at various NaNO₂ concentrations after 7 day culture.101
- Fig.4-41The phycobiliprotein content and growth of Spirulina sp. at various urea concentrations after 7 day culture.102
- Fig.4-42The phycobiliprotein content and growth of Spirulina sp. at various NH₄Cl concentrations after 7 day culture.103
- Fig.4-43The phycobiliprotein content and growth of Spirulina sp. at various NH₄NO₃ concentrations after 7 day culture.104
- Fig.4-44The phycobiliprotein content and growth of Spirulina sp. at various NH₄H₂PO₄ concentrations after 7 day culture.105
- Fig.4-45Time behavior of pH, ammonia nitrogen (mg/L) and biomass (g/L) cultivation under different inorganic carbon sources with inoculum level of 10%.112
- Fig.4-46Time behavior of pH, ammonia nitrogen (mg/L) and biomass (g/L) cultivation with different organic carbon sources with inoculum level of 10%.113
- Fig.4-47Phycobiliprotein contents and biomass of Thermosynechococcus sp. cultivation under different inorganic carbon sources (0.42 g/L) performed in BG11 medium with inoculum level of 10% at aerobic(0.6 vvm) conditions.114
- Fig.4-48Phycobiliprotein contents and biomass of Thermosynechococcus sp. cultivation under different Organic carbon sources (0.42 g/L) performed in BG11 medium withinoculum level of 10% at aerobic (0.6 vvm) conditions.115
- Fig.4-49The phycobiliprotein content and growth of Thermosynechococcus sp. at various carbon sources after 5 day culture.116
- Fig.4-50Time behavior of pH, ammonia nitrogen (mg/L) and biomass (g/L) of Thermosynechococcus sp. cultivation under different Proportion of carbonate temperture performed in modified BG11 medium with inoculum level of 10% at aerobic 0.6 vvm conditions.122
- Fig.4-51Phycobiliprotein contents and biomass of Thermosynechococcus sp. cultivation under under different proportion of carbonate performed in modified BG11 medium with inoculum level of 10% at aerobic(0.6 vvm) conditions123
- Fig.4-52The phycobiliprotein content and growth of Thermosynechococcus sp. at various proportion of carbonate after 5day culture.124
- Fig.4-53Time behavior of phycocyanin concnet, pH, ammonia nitrogen (mg/L) and biomass (g/L) during Oscillatoria sp. Wu1 cultivation under with inoculum level of 10%.133
- Fig.4-54Phycobiliprotein contents and biomass of Oscillatoria sp.Wu1 cultivation under different sodium chloride concentrations with inoculum level of 10%.134
- Fig.4-55The phycobiliprotein content and growth of Oscillatoriasp.Wu1. at various sodium chloride concentrations after 7day culture.135
- Fig.4-56Time behavior of phycocyanin concnet, pH, ammonia nitrogen (mg/L) and biomass (g/L) during Thermosynechococcus sp. cultivation under different sodium chloride concentrations performed in modified BG11 medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.136
- Fig.4-57The phycobiliprotein content and growth of Thermosynechococcus sp. at various sodium chloride concentrations after 7day culture.137
- Fig.4-58The phycobiliprotein content and growth of Thermosynechococcus sp. at various sodium chloride concentrations after 7day culture138
- Fig.4-59Time behavior of the nitrate, nitrite, ammonia and phycocyanin concnet during Spirulina sp. cultivation under different sodium chloride concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.139
- Fig.4-60Phycobiliprotein contents and biomass of Spirulina sp. cultivation under different sodium chloride concentrations performed in Zarrouk's medium with inoculum level of 10% at aerobic 0.6 vvm conditions.140
- Fig.4-61The phycobiliprotein content and growth of Spirulina sp. at various sodium chloride concentrations after 7day culture141
- Fig.4-62Time behavior of phycocyanin concnet, pH, ammonia nitrogen (mg/L) and biomass (g/L) during Oscillatoria sp. Wu1 cultivation under different light intensity with inoculum level of 10%.151
- Fig.4-63Phycobiliprotein contents and biomass of Oscillatoria sp. Wu1 cultivation under different light intensity with inoculum level of 10 %.152
- Fig.4-64The phycobiliprotein content and growth ofOscillatoria sp. Wu1. at various light intensity after 7day culture.153
- Fig.4-65Time behavior of phycocyanin concnet, pH, ammonia nitrogen (mg/L) and biomass (g/L) during Thermosynechococcus sp. cultivation under different light intensity with inoculum level of 10%.154
- Fig.4-66Phycobiliprotein contents and biomass of Thermosynechococcus sp. cultivation under different light intensity with inoculum level of 10%155
- Fig.4-67The phycobiliprotein content and growth of Thermosynechococcus sp at various light intensity after 7day culture156
- Fig.4-68Time behavior of the nitrate, nitrite and phycocyanin cultivation under different light intensity performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.157

- Fig.4-69 Phycobiliprotein contents and biomass of Spirulina sp. cultivation under light intensity performed in Zarrouk's medium with inoculum level of 10% at aerobic (0.6 vvm) conditions.158
- Fig.4-70 The phycobiliprotein content and growth of Spirulina sp. at various light intensity after 7day culture. 159
- Fig.4-71 Column chromatography of Thermosynechococcus sp. phycobiliprotein.166
- Fig.4-72 Column chromatography of Spirulina sp. phycobiliprotein.168
- Fig.4-73 Absorption spectrum of phycocyanin from Thermosynechococcus sp. at each step of purification.170
- Fig.4-74 Absorption spectrum of phycocyanin from Spirulina sp. at each step of purification171
- Fig.4-75 12 % SDS gel electrophoresis at each stage of purification of phycocyanin from Spirulina sp. Pure phycocyanin showed two bands of molecular mass range between 10 to 20 kDa correspond to the characteristic and subunits, respectively172
- Fig.4-76 12 % SDS gel electrophoresis at each stage of purification of phycocyanin from Thermosynechococcus sp. Pure phycocyanin showed two bands of molecular mass range between 10 to 20 kDa correspond to the characteristic and subunits, respectively.173
- Fig.4-77 FTIR spectra of phycocyanin of Thermosynechococcus sp. at each stage of purification.174
- Fig.4-78 FTIR spectra of phycocyanin of Spirulina sp. at each stage of purification.175
- Fig.4-79 LC/MS/MS spectra of peptide TPLTEAVAAADSQGR(phycocyanin Subunit). (Q-TOF, Applied Biosystems / QSTAR?Elite).178
- Fig.4-80 LC/MS/MS spectra of peptide YVTYAVFSGDASVLDDR (phycocyanin Subunit).(Q-TOF, Applied Biosystems / STAR?Elite)179

表目錄

- Table2-1 The companies of Spirulina health food in Taiwan6
- Table2-2 The nutritional composition of cyanobacteria7
- Table2-3 Pharmaceutical potentials of phycocyanin, suggested physiological mechanisms responsible for the effects, and experimental system19
- Table3-1 BG11 medium24
- Table3-2 Zarrouk medium25
- Table3-3 Schlegel?Sser medium26
- Table3-4 Oscillatoria sp. Wu1culture medium31
- Table4-1 Determination of spectrophotometric purity of C-PC as compared tototal protein, C-PE and C-APC of Thermosynechococcus sp. at eachstage of purification167
- Table4-2 Determination of spectrophotometric purity of C-PC as compared tototalprotein, C-PE and C-APC of Spirulina sp. at each stage of purification169
- Table4-3 Phycocyanin peptide sequence analysis performed by LC/MS/MS (Q-TOF, Applied Biosystems / QSTAR? XL)180
- Table4-4 Phycocyanin peptide sequence analysis performed by LC/MS/MS (Q-TOF, Applied Biosystems/ QSTAR? XL)181
- Table4-5 Identification of and subunits of purified phycocyanin using ultrafiltrationn by LC/MS/MS (Q-TOF, Applied Biosystems / QSTAR? XL)182

REFERENCES

- 王雪青、苗惠、翟燕。2007。微藻細胞破碎方法的研究。天津科技大學學報 22 (1) : 21-25。
- 史成?、唐欣昀、甘旭華、趙良?。2005。有機碳氮源對螺旋藻生長及葉綠素a含量的影響。安徽大學學報 29 (5) : 92-96。
- 朱永寧、泉本勝利。1999。血?蛋白(Hb)的低?保護劑。吉林大學自然科學學報 1999 (3) : 88-92。
- 朱麗萍、顏世敢、李雁冰、周百成。2010。硫酸銨鹽析條件對多管藻R-藻紅蛋白和R-藻藍蛋白得率和純度的影響。科技導報28 (4): 37-41。
- 李博、康瑞娟、張栩、叢威、譚天偉、蔡昭鈴。2005。銨鹽對螺旋藻生長的影響。過程工程學報5 (6) : 684-687。
- 李華佳。2005。藻膽蛋白的提取技術與生物活性研究。食品科學 26 (7) : 243-246。
- 杜旭東、王明華、鄒玲。2003。光合生物反應器。煙台教育?院學報9 (1):69-71。
- 周鳳娟、許時嬰、楊瑞金、王璋。2007。可溶性絲素蛋白的功能性質。食品科學28 (11) : 71-74。
- 林書吟。2010。頭髮菜絲狀體R型藻藍蛋白之提純與性質分析及光質對其生長及色素含量的影響。國立台灣大學生命科學院漁業科學碩士論文。台北。
- 邱偉倫。2007。1.以質譜法分析DNA加成產物、3-硝基酪胺酸與3-溴基酪胺酸 2. oxanine對溶菌?之後轉譯修飾研究。國立中正大學化學暨生物化學系博士論文。嘉義。
- 施春雷、史賢明。2007。原殼小球藻中不依賴於光的原葉綠素酸酯還原?基因的克隆與分析。食品與生物技術學報26 (1) : 111-115。
- 胡曉宇、張克榮、孫俊?。2003。中國環境中磷苯二甲酸酯類化合物污染的研究。中國衛生檢驗雜志13(1):9-14。
- 孫百暉、王修林、李雁賓。2008。光照在東海近海原甲藻赤潮發生中的作用。環境科學29 (2) : 362-367。
- 孫建明、無垠、桂遠明。2003。海洋微藻全封閉、??式培?初步??。水產科?22 (3):22-24。
- 孫凌、閻元卿、張冬梅、莊源益。2007。無機碳對浮游藻類生長和群落結構的影響。環境污染與防治29 (5) : 352-356。
- 徐寧、孫樹剛、段舜山、李

愛芬、張成武。2010。海洋微藻??活性?定方法的實驗研究。中國環境科學30 (5) : 689-693。17.秦華明、宗敏華、梁世中。2001。糖在蛋白質藥物冷凍乾燥過程中保護作用的分子機制。廣東藥學院學報17(4) : 305-306。18.翁婉琳。2008。探討碳氮源於螺旋藻生長及葉綠素、藻藍蛋白產量之影響。國立中興大學化學工程學系碩士論文。台中。19.馬志珍、季梅芳、陳匯遠。1985。一種可做鮑和海參餌料的底棲舟形藻的培養條件的研究。海洋通報4 (4) : 36-39。20.高培均。2012。以螺旋藻進行二氧化碳固定及藻藍素生產之技術開發與製程最適化。國立成功大學化學工程學系碩士論文。台南21.張玉華、凌沛學、籍保平、張天民。2006糖類在生物活性物質冷凍乾燥中的保護作用及其作用機制。中國生化藥物雜誌 27(4) : 247-249。22.張其德、張世平、張?豐。1992。在植物光合作用中鎂離子的作用。黑龍江大學自然科學學報9 (1) : 82-88。23.張慧苗、顧天青。1991。鈍頂螺旋藻品系6的生物學特性。植物學通報8 (4) : 36-39。24.張靜、韋玉春、王國祥、楊飛、程春梅、夏曉瑞。2013。太湖藍藻水樣中藻藍蛋白提取方法比較。湖泊科學25 (2) : 283-288。25.曹鳳儒。2007。點帶石斑幾丁質?純化、功能分析及基因選殖。國立成功大學生物科技研究所碩士論文。台南。26.梁妍。2009。不同碳源對螺旋藻生長影響及不同乾燥方式對螺旋藻營養成分的影響。魯東大學動物學系碩士論文。山東。27.郭志博、潘涌璋。2012。城市汙水廠汙泥脫水液培養小球藻的研究。中國科技論文在線。28.陳幸慧。2009。以藍綠菌*Thermosynechococcus* sp. CL-1固碳與生質潛能組成分析之研究。國立成功大學環境工程學系碩士論文。台南。29.陳政忻。2006。專訪「遠東生物科技股份有限公司」一千金難買「藻」知道。農業生技產業季刊(7) : 58-61。30.陳柏榮。2011。利用篩選之*Oscillatoria* sp. Wu1生產藻膽蛋白之研究。大葉大學生物產業科技學系碩士論文。彰化31.陳新美、梅興國、房偉、鐘凡。2003。培養條件對螺旋藻生長和藻膽蛋白含量的影響。氨基酸和生物資源 25(1):21-24。32.陳聲明、呂琴。微生物冷凍乾燥的抗性機理。1996。微生物學通報 23 (4) : 236-238。33.彭為民、商樹田、劉國琴等。1998。螺旋藻藻膽蛋白研究進展。農業生物技術學報 6(2) : 173-177。34.曾文爐、叢威、蔡昭鈴、歐陽藩。2002。螺旋藻的營養方式及光合作用影響因素。植物學通報19(1) : 70-77。35.游馥榕。2009。台灣溫泉菌*Thermosynechococcus elongates* TA01菌種鑑定與生理生化之研究。輔仁大學生命科學系碩士論文。新北市。36.華澤釗、任禾盛 編著。1994。低?生物醫學技術。第20-145頁。科學出版社。北京，中國。37.黃凱旋、謝雅慧、呂頌輝。2009。米氏凱倫藻對氮源的吸收利用特徵。生態環境學報18(2): 453-457。38.葉俊良。2006。在光生化反應器中以二階段策略培養微藻生產油脂之研究。國立成功大學化學工程學系碩士論文。台南。39.鄒寧、郭小燕、孫東紅、趙麗麗、賈愛榮。2005。固定化培養底棲矽藻研究。中國水產355(6) : 72-73。40.趙以軍、王旭、程凱。1998。滇池微囊藻水華藻膽蛋白?源化研究。華中師範大學學報(自然科學版) 32 (3) : 333-337。41.劉慧瑛。1984。溫度與照度對螺旋藻生長速率、生理與生化性質之影響。中華農業研究33 (3) : 276-291。42.劉慧瑛。1986。螺旋藻 *Spirulina platensis*.與*S. maxima*形態、生理與生長特性之比較。中華農業研究35 (1):63~80。43.鄭江。2002。藻膽蛋白的提取純化研究進展。食品科學 23 (11) : 159-161。44.盧雁、李向榮。2005。蛋白質變性機理與變性時的熱力學參數研究進展。化學進程17 (5) : 905-910。45.蕭茂修。2007。以海洋微藻固定CO₂並作為生質能源之研究。國立成功大學環境工程學系碩士論文。台南。46.Abalde, J., Betancourt, L., Torres, E., Cid, A. and Barwell, C. 1998. Purification and characterization of phycocyanin from the marine cyanobacterium *Synechococcus* sp. IO9201. Plant Sci. 136:109 – 20.47.Abdulquader, G., Barsanti, L. and Tredici, M. 2000. Harvest of *Arthrospira platensis* from Lake Kossorom (Chad) and its household usage among the Kanembu. J. Appl. Phycol. 12: 493 – 498.48.Adams, SM., Kao, OHW. and Berns, DS. Psychrophile c-phycocyanin. 1979. Plant Physiol. 64: 525 – 7.49.Adir, N., Dobrovetsky, Y. and Lerner, N. 2001. Structure of c-phycocyanin from thermophilic cyanobacterium *Synechococcus vulcanus* at 2.5 ?: structural implications for thermal stability in phycobilisome assembly. J Mol Biol 313: 71 – 81.50.Adir, N., Vainer, R. and Lerner, N. 2002. Refined structure of c-phycocyanin from the cyanobacterium *Synechococcus vulcanus* at 1.6 ?: insights into the role of solvent molecules in thermal stability and co-factor structure. Biochim Biophys Acta 1556:168 – 174.51.Aichi, M., Takatani, N. and Omata, T. 2001. Role of NtcB in activation of nitrate assimilation genes in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J Bacteriol 183: 5840 – 5845.52.Ajayan, K.V., Selvaraju, M., Thirugnanamoorthy, K. 2012. Enrichment of chlorophyll and phycobiliproteins in *Spirulina platensis* by the use of reflector light and nitrogen sources: An in-vitro study. Biomass and bioenergy 47: 436-441.53.Albertson, P. A. 1986. Partitioning of Cell Particles and Macromolecules, 1th ed., Wiley: New York,.54.Allen, M. M. and Smith, A. J. 1969. Nitrogen chlorosis in blue-green algae. Arch Microbiol 69:114 – 120.55.Allen, M. M. and Stanier, R.Y. 1968. Growth and division of some unicellular blue-green algae. J. Gen. Microbiol. 51: 199-202.56.Anamika, P., Sandhya, M., Richa Pawar, P. K. and Ghosh. 2005. Puri?cation and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat. Protein Expression and Puri?cation 40: 248 – 255.57.Antelo, F. S., Costa, J. A.V. and Kalil S. J. 2008. Thermal degradation kinetics of the phycocyanin from *Spirulina platensis*. Biochem Eng J 41:43 – 7.58.Arnon, D. I. 1984. The discovery of photosynthetic phosphorylation. Trends Biochem Sci 9: 258 – 262.59.Arnon, D. I. 1988. The discovery of ferredoxin: the photosynthetic path. Trends Biochem Sci 13: 30 – 33.60.Atanasiu, R., Stea, D., Mateescu. M.A., Vergely, C., Dalloz, F., Briot, F., Maupoil, V., Nadeau, R. and Rochette, L. 1998. Direct evidence of caeruloplasmin antioxidant properties. Mol Cell Biochem 189 : 127 – 135.61.Badrishi, S., Beena, K., Ujjval, T. and Datta, M. 2006. Extraction, purification and characterization of phycocyanin from *Oscillatoria uadripunctulata*—Isolated from the rocky shores of Bet-Dwarka, Gujarat, India. Process Biochemistry 41(9): 2017-2023.62.Batista, A. P., Raymundo, A., Sousa, I. and Empis, J. 2006 Rheological characterization of coloured oil-in-water food emulsions with lutein and phycocyanin added to the oil and aqueous phases. Food Hydrocoll 20: 44 – 52.63.Belay, A., Ota, Y., Miyakawa, K. and Shimamatsu, H. 1993. Current knowledge on potential health benefits of *Spirulina*. J. Appl. Phycol. 5, 235 – 241.64.Belkin, S. and Boussiba, S. 1991. High internal pH conveys ammonia resistance in *Spirulina platensis*. Bioresour Technol. 38:167 – 169.65.Belknap, W. R. and Haselkorn, R. 1987. Cloning and light regulation of expression of the phycocyanin operon of the cyanobacterium *Anabaena*. J. EMBO 6:871 – 884.66.Benedetti, S., Benvenutti, F., Pagliarani, S., Francogli, S., Scoglio, S. and Canestrari, F. 2004. Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*. Life Sci 75:2353 – 2362.67.Benedetti, S., Rinalducci, S., Benvenuti, F., Francogli, S., Pagliarani, S., Giorgi, L., Micheloni, M., D ' Amici, G. M., Zolla, L. and Canestrari, F. 2006. Purification and characterization of phycocyanin from blue-green

alga Aphanizomenon flos-aquae. J. Chromatogr B 833:12 – 18.68.Bermejo, P., Pi?ero, E. and Villar, ?. M. 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*. Food Chem 110:436 – 445.69.Berry, S., Bolychevtsvea, Y.V., R?gner, M. and Karapetyan, N.V. 2003. Photosynthetic and respiratory electron transport in the alkaliphilic cyanobacterium *Arthospira (Spirulina) platensis*. Photosynth. Res. 78, 67 – 76.70.Bhaskar, S. U., Gopalaswamy, G. and Raghu, R. 2005. A simple method for efficient extraction and purification of c-phycocyanin from *Spirulina platensis* Geitler. Indian J Exp Biol 43(3): 277 – 9.71.Bhat, V. B, Madayastha, K. M. 2000. C-phycocyanin: a potent peroxy radical scavenger in vivo and in vitro. Biochem Biophys Res Comm 275: 20 – 25.72.Blot N, Wu X. J., Thomas, J.C., Zhang, J., Garczarek, L., Bohm, S., Tu J. M., Zhou, M., Ploscher, M., Eichacker, L., Partensky, F., Scheer, H. and Zhao K.H. 2009. Phycourobilin in trichromatic phycocyanin from oceanic cyanobacteria is formed post-translationally by a phycoerythrobilin lyase-isomerase. J. Biol. Chem. 284: 9290-9298.73.Borowitzka, M. A. 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70:313 – 321.74.Borowitzka, M. A., 1992. Algal biotechnology products and processes: matching science and economics. J. Appl. Phycol 4: 267 – 279.75.Boussiba, S. and Richmond, A. E. 1979. Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*. Arch Microbiol 12:155 – 159.76.Boussiba, S. and Richmond, A. E. 1980. C-phycocyanin as a storage protein in the blue-green alga *Spirulina platensis*. Arch Microbiol 125:143 – 147.77.Bryant, D. A., Dubbs, J. M., Fields, P. I., Porter, R. D. and de Lorimier, R. 1985. Expression of phycobiliprotein genes in *Escherichia coli*. FEMS Microbiol Lett 29: 242 – 249.78.Cai, Y. A., Murphy, J. T., Wedemaye, G.J. and Glazer, A. N. 2001. Recombinant phycobiliproteins. Recombinant C-phycocyanins equipped wit affinity tags, oligomerization, and biospecific recognition domains. Anal Biochem 290:186 – 204.79.Cai, Y. and Wolk, C. P. 1997. Nitrogen deprivation of *Anabaena* sp. strain PCC 7120 elicits rapid activation of a gene cluster that is essential for uptake and utilization of nitrate. J Bacteriol 179: 258 – 266.80.Candau, P., Manzano, C. and Losada, M. 1976. Bioconversion of light energy into chemical energy through reduction with water of nitrate to ammonia. Nature 262: 715 – 717.81.Carollozzi, P. 2003. Dilution of solar radiation through “ culture ” lamination in photobioreactor rows facing South – North: a way to improve the efficiency of light utilisation of cyanobacteria (*Arthospira platensis*). Biotechnol Bioeng 81: 305 – 315.82.Chaneva, G., Furnadzhieva, S., Minkova, K. and Lukavsky, J. 2007. Effect of light and temperature on the cyanobacterium *Arthronema africanum*—a prospective phycobiliprotein-producing strain. J Appl Phycol 19: 537 – 544.83.Chang, C. J., Yang Y. H., Liang Y. C., Chiu, C. J., Chu, K. H., Chou, H. N. and Chiang B. L. 2010. A novel phycobiliprotein alleviates allergic airway inflammation by modulating immune responses. Am J Respir Crit Care Med. 183(1): 15-25.84.Chen ,T., Zheng W., Yang, F., Bai, Y., Wong, Y-S. 2006. Mixotrophic culture of high selenium-enriched *Spirulina platensis* on acetate and the enhanced production of photosynthetic pigments. Enzyme and Microbial Technology 39: 103 – 107.85.Chen, F. and Zhang, Y. 1997. High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzyme Microb Technol 20:221 – 224.86.Chen, F., Zhang, Y. and Guo, S. 1996. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol Lett 18: 603 – 608.87.Chen, T., Wong, Y-S. and Zheng, W. 2006a. Purification and characterization of selenium-containing phycocyanin from seleniumenriched *Spirulina platensis*. Phytochemistry 67: 2424 – 2430.88.Chen, T., Zheng, W., Yang, F., Bai, Y. and Wong, Y-S. 2006b. Mixotrophic culture of high selenium-enriched *Spirulina platensis* on acetate and the enhanced production of photosynthetic pigments. Enzyme Microb Technol 39:103 – 107.89.Cherng, S-C., Cheng, S-N., Tarn, A. and Chou, T-C. 2007. Anti-inflammatory activity of c-phycocyanin in lipopolysaccharide-stimulated RAW 264.7 macrophages. Life Sci 81:1431 – 1435.90.Chethana, S, Sridevi, A. S, Raghavarao, K.S.M.S. 2006. Method to obtain C-phycocyanin of high purity. J Chromatogr A 1127: 76 – 81.91.Chojnacka, K. and Noworyta, A. 2004. Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme Microb Technol 34: 461 – 465.92.Chojnacka, K. and Noworyta, A. 2004. Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme and Microbial Technology. 34: 461-465.93.Cifferi, O. 1983. *Spirulina*, the edible microorganism. Microbiol. Rev. 47: 551.94.Cisneros, M. and Rito-Palomares, M. 2004. A simplified strategy for the release and primary recovery of c-phycocyanin produced by *Spirulina maxima*. Chem Biochem Eng Q 18(4):385 – 90.95.Colaco, C., Sen, S. and Thangavelu, M., et al. 1992 Extraordinary stability of enzymes dried in trehalose : simplified molecularbiology. J . Biotechnology N Y, 10 (9) : 1007.96.Contreras-Martel, C., Matamala, A., Bruna, C., Poo-Caama?o, G., Almonacid, D., Fig.ueroa, M., Mart?nez-Oyanedel, J. and Bunster, M. 2007. The structure at 2 ? resolution of phycocyanin from *Gracilaria chilensis* and the energy transfer network in a PC – PC complex. Biophys Chem 125: 388 – 396.97.Converti, A., Scapazzoni, S., Lodi, A. and Carvalho, J.C.M. 2006. Ammonium and urea removal by *Spirulina platensis*. J and Microbiol Biotechnol. 33 : 8-16.98.Cornejo, J. and Beale, S. I. 1997. Phycobilin biosynthetic reactions in extracts of cyanobacteria. Photosynth Res 51: 223 – 230.99.Crowe, J. H., Hoekstra, F.A., Nguyen, K.H. and Crowe, L.M. 1996. Is verification involved in depression of the phase transition temperature in dry phospholipids.. J. Biochimica et Biophysica Acta 1280(2): 96-187.100.Crowe, J. H., Crowe, L. M. and Carpenter, J. F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J. and Anchordoguy, T.J. 1988. Interactions of Sugars with Membranes. J . Biochimica et Biophysica Acta 947(2): 84-367.101.Curdt, I., Singh, B.B., Jakoby, M., Hachtel, W. and Bo?hme, H. 2000. Identification of amino acid residues of nitrite reductase from *Anabaena* sp. PCC 7120 involved in ferredoxin binding. Biochim Biophys Acta 1543: 60 – 68.102.Dangeard, P. 1940. Sur une algue bleue alimentaire pour l ’ homme: *Arthospira platensis* (Nordst.) Gomont. Actes Soc. Linn. Boreaux Extr. Proces-verbaux 91: 39-41103.de Lorimier, R., Bryant, D. A., Porter, R. D., Liu, W. Y., Jay, E. and Stevens, S. E. 1984. Genes for the a and b subunits of phycocyanin. Proc Natl Acad Sci USA 81: 7946 – 7950.104.Dean, A. S. and Theodore, W. 1998. Effects of drying methods and additives on structure and function of actin : mechanisms of dehydration induced damage and its inhibition . J. Archives of Biochemistry and Biophysics . 46(1) : 171.105.Dhiab, R.B., Ouada, H.B., Boussetta, H., Franck, F., Elabed, A. and Brouers, M. 2007. Growth, fluorescence, photosynthetic O₂ production and pigment content of salt adapted cultures of *Arthospira (Spirulina) platensis*. J. Appl.

Phycol., 19: 293 – 301.106.Diamond, A. D. and Hsu, J. T. 1992. Aqueous two-phase systems for biomolecule separation. *Adv. Biochem. Eng./Biotechnol* 47: 89-135.107.Doke, J. M. 2005. An improved and efficient method for the extraction of phycocyanin from *Spirulina* sp. *Int J Food Eng* 1(5): 1556-3758..108.Edwards, M.R., Hauer, C., Stack, R. F., Eisele, L. E. and MacColl, R. 1997. Thermophilic C-phycocyanin: effect of temperature, monomer stability, and structure. *Biochim Biophys Acta* 1321:157 – 164.109.Enrique, F., Jose E., Fr? as, L. M. R. and Antonia, H. 2005. Photosynthetic nitrate assimilation in cyanobacteria. *Photosynthesis Research.* 83: 117 – 133.110.Eriksen, N.T. 2008. Production of Phycocyanin – a pigment with applications in biology, biotechnology, foods and medicine. *Appl Microbiol Biotechno* 180:1 – 14.111.Estrada, J.P.N, Besc?s, P.B, Villar del Fresno, A.M. 2001. Antioxidant activity of different fractions of *Spirulina platensis* protean extract. *II Farmaco* 56: 497 – 500.112.Falkowski, P. G. and Stone, D. P. 1975. Nitrate uptake in marine phytoplankton:energy sources and the interaction with carbon fixation. *J. Mar Biol.* 32 (1): 77-84.113.FAN, C, GLIBERT, P. M and ALEXANDER, J. 2003. Characterization of urease activity in three marine phytoplankton species, *Aureococcus anophagefferens*, *prorocentrum minimum*, and *Thalassiosira weissflogii*. *J. Marine Biology.* 142: 949-958.114.Farrar, W. V. 1966. Tecuitlatl: a glimpse of Aztec food technology. *Nature* 211: 341 – 342.115.Faucher, O., Coupal, B. and Leudy, A. 1997. Utilization seawater-urea as culture medium for *Spirulina maxima*. *Can. J. Microbiol.* 25 : 752-759.116.Flores, E. and Herrero, A. 1994. Assimilatory nitrogen metabolism and its regulation. In: Bryant DA (ed) *The Molecular Biology of Cyanobacteria*, pp 487 – 517. Kluwer Academic Publishers, Dordrecht, The Netherlands.117.Flores, E., Guerrero, M.G. and Losada, M. 1980. Short-term ammonium inhibition of nitrate utilization by *Anacystis nidulans* and other cyanobacteria. *Arch Microbiol* 128: 137 – 144.118.Flores, E., Guerrero, M.G. and Losada, M. 1983a. Photosynthetic nature of nitrate uptake and reduction in the cyanobacterium *Anacystis nidulans*. *Biochim Biophys Acta* 722: 408 – 416.119.Flores, E., Herrero, A. and Guerrero, M.G. 1987. Nitrite uptake and its regulation in the cyanobacterium *Anacystis nidulans*. *Biochim Biophys Acta* 896: 103 – 108.120.Flores, E., Romero, J.M., Guerrero, M.G. and Losada. 1983b. Regulatory interaction of photosynthetic nitrate utilization and carbon dioxide fixation in the cyanobacterium *Anacystis nidulans*. *Biochim Biophys Acta* 725: 529 – 532.121.Forchhammer, K. and Tandeau de Marsac, N. 1995. Functional analysis of the phosphoprotein PII (glnB gene product) in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Bacteriol* 177: 2033 – 2040.122.Francine, S., Antelo, Andr?ia, A., Jorge, A. V. Costa and Susana, J. Kalil. 2010. Extraction and Purification of C-phycocyanin from *Spirulina platensis* in nventional and Integrated Aqueous Two-Phase Systems. *J. Braz. Chem. Soc.*, Vol. 21, No. 5, 921-926.123.Fr? as, J. E., Flores, E. and Herrero, A. 1997. Nitrate assimilation gene cluster from the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *J Bacteriol* 179: 477 – 486124.Fr?as, J. E., Flores, E. and Herrero, A. 1994. Requirement of the regulatory protein NtcA for the expression of nitrogen assimilation and heterocyst development genes in the cyanobacterium *Anabaena* sp. PCC 7120. *Mol. Microbiol.* 14 : 823-832.125.Fuhrman, J. 2003. Genome sequences from the sea. *Nature*, 424:1001-1002.126.Fujii, M., Shinohara, N., Lim, A., Otake, T., Kumagai, K. and Yanagisawa, Y. 2003. A study on emission of phthalate esters from plastic materials using a passive flux sampler!. *J. Atmospheric Environment.* 37: 5495—5504.127.Fujiwara-Arasaki, T., Yamamoto, M. and Kakiuchi, K. 1985. C-phycocyanin from a red alga, *Porphyra tenera*: subunit structure . *Biochim. Biophys. Acta.* 828: 261-265.128.Fukui, K., Saito, T., Noguchi, Y., Kodera, Y., Matsushima, A., Nishimura H, Inada, Y. 2004. Relationship between color development and protein conformation in the phycocyanin molecule. *Dyes Pigm* 63: 89 – 94.129.Ganapathi Patil, S. Chethana, M.C. Madhusudhan, K.S.M.S. Raghavarao. 2008. Fractionation and puri?cation of the phycobiliproteins from *Spirulina platensis*. *Bioresource Technology* 99: 7393 – 7396.130.Garnier, F. and Thomas, J.C., 1993. Light regulation of phycobiliproteins in *Spirulina maxima*. In: Doumenge, F., Durand-Chastel, H., Toulemont, A. (eds.) *Spirulina Algae of Life. Spirulina Algae de Vie*. Monaco Musee Oceanographique NS 12: 41 – 48.131.Gautam, S. and Simon, L. 2006. Partitioning of ?-glucosidase from *Trichoderma reesei* in poly(ethylene glycol) and potassium phosphate aqueous two-phase systems: Influence of pH and temperature. *J Biochemical Engineering* 30: 104-108.132.Ge, B., Qin, S., Han, L., Lin, F. and Ren, Y. 2006. Antioxidant properties of recombinant allophycocyanin expressed in *Escherichia coli*. *J Photochem Photobiol B Biol* 84:175 – 180.133.Gittelson, A, Quiang, H. and Richmond, A. 1996. Photic volume in photobioreactors supporting ultrahigh population densities of the photoautotroph *Spirulina platensis*. *Appl Environ Microbiol* 62:1570 – 1573.134.Glazer, A. N .1994. Phycobiliproteins — a family of valuable, widely used fluorophores. *J Appl Phycol* 6: 105 – 112.135.Glazer, A.N. and Stryer, L. 1984. Phycofluor probes. *Trends Biochem Sci* 9: 423 – 427.136.Grant, I.R, Hitchings, E. I, McCartney, A., Ferguson, F. and Rowe, M. T. 2002. Effect of commercial-scale high-temperature, short-time pasteurization on the viability of *Mycobacterium paratuberculosis* in naturally infected cows ' milk. *Appl Environ Microbiol* ;68: 602 – 7.137.Graverholt, O. S. and Eriksen, N. T. 2007. Heterotrophic high cell-density fed-batch and continuous flow cultures of *Galdieria sulphuraria* and production of phycocyanin. *Appl Microbiol Biotechnol* 77: 69 – 75.138.Gross, W. and Schnarrenberger, C. 1995. Heterotrophic growth of two strains of the acido-thermophilic red alga *Galdieria sulphuraria*. *Plant Cell Physiol* 36: 633 – 638.139.Guan, X., Qin, S., Zhao, F., Zhang, X. and Tang, X. 2007b. Phycobilisomes linker family in cyanobacterial genomes: divergence and evolution. *Int J Biol Sci* 3:434 – 445.140.Guerrero, M. G, Manzano, C. and Losada, M. 1974. Nitrite photoreduction by a cell-free preparation of *Anacystis nidulans*. *Plant Sci Lett* 3: 273 – 278.141.Guerrero, M.G.. and Lara, C. 1987. Assimilation of inorganic nitrogen. In: *The cyanobacteria* (Fay, P. and Van Baalen, C., Eds.). p.163-186. Elsevier Science Publisher, Amsterdam :142.Guo, N., Zhang, X., Lu, Y. and Song, X. 2007. Analysis on the factors affecting start-up intensity in the upstream sequence of phycocyanin. *Biotechnology Letters* 29(3): 459-464.143.Hahn, J., K?hne, R. and Schmieder, P. 2007. Solution-state ¹⁵N NMR spectroscopic study of a-C-phycocyanin: implications for the structure of the chromophore-binding pocket of the cyanobacterial phytochrome Cph1. *ChemBioChem* 8: 2249 – 2255.144.Hasegawa, P. M., Bressan, R. A., Zhu, J.-K. and Bohnert, H. J. 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* 51: 463 – 499.145.Hattori, A. 1962. Light-induced reduction of nitrate, nitrite and hydroxylamine in a blue-green alga, *Anabaena cylindrica*. *Plant Cell Physiol* 3: 355

- 369.146.Hattori, A. and Myers, J. 1967. Reduction of nitrate and nitrite by subcellular preparations of *Anabaena cylindrica*. II. Reduction of nitrate to nitrite. *Plant Cell Physiol* 8: 327 – 337.147.Hemlata, T. F. 2009. Screening of Cyanobacteria for Phycobiliproteins and Effect of Different Environmental Stress on Its Yield. *Bull Environ Contam Toxicol.* 83:509 – 515.148.Herrera, A., Boussiba, S., Napoleone, V. and Hohlberg, A. 1989. Recovery of C-phycocyanin from the cyanobacterium *Spirulina maxima*. *J Appl Phycol* 1: 325 – 331.149.Herrera, A., Boussiba, S., Napoleone, V. and Hohlberg, A. 1989. Recovery of cphycocyanin from the cyanobacterium *Spirulina maxima*. *J. Appl. Phycol.* 1: 325 – 331.150.Herrero, A, Flores, E. and Guerrero, M.G.1981. Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans*, *Anabaena* sp. strain 7119, and *Nostoc* sp. strain 6719. *J Bacteriol* 145: 175 – 180.151.Herrero, A. and Guerrero, M.G. 1986. Regulation of nitrite reductase in the cyanobacterium *Anacystis nidulans*. *J Gen Microbiol* 132: 2463 – 2468.152.Herrero, A., Flores, E. and Guerrero, M. G. 1985. Regulation of nitrate reductase cellular levels in the cyanobacteria *Anabaena variabilis* and *Synechocystis* sp. *FEMS Microbiol Lett* 26: 21 – 25.153.Herrero, A., Flores, E. and Guerrero, M.G. 1981. Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans*, *Anabaena* sp. strain 7119, and *Nostoc* sp. strain 6719. *J Bacteriol* 145: 175 – 180.154.Hill, R. 1939. Oxygen produced by isolated chloroplasts. *Proc Royal Soc B* 127: 192 – 210.155.Hirasawa, M., Rubio, L.M., Griffin, J.L., Flores, E., Herrero, A., Li, J., Kim, S.K., Hurley, J.K., Tollin, G. and Knaff, D.B. 2004. Complex formation between ferredoxin and *Synechococcus* ferredoxin: nitrate oxidoreductase. *Biochim Biophys Acta* 1608: 155 – 162.156.Huang, F., Parmryd, I., Nilsson, F., Persson, A.L., Pakrasi, H.B., Andersson, B. and Norling, B. 2003. Proteomics of *Synechocystis* sp. strain PCC 6803. Identification of plasma membrane proteins. *Mol Cell Proteom* 1: 956 – 966.157.Huang, Z., Guo, B. J., Wong, R.N.S. and Jiang, Y. 2007. Characterization and antioxidant activity of selenium-containing phycocyanin isolated from *Spirulina platensis*. *Food Chem* 100:1137 – 1143. Isolated from the rocky shores of Bet-Dwarka, Gujarat, India. *Process Biochemistry* 41: 2017 – 2023.158.Izydorczyk, K., Tarczynska, M., Jurczak, T., Mrowczynski, J. and Zalewski, M. 2005. Measurement of phycocyanin fluorescence as an online early warning system for cyanobacteria in reservoir intake water. *Environ Tox* 20: 425 – 430.159.Jaouen, P., L?pine, B., Rossignol, N., Royer, R., Qu?m?neur, F. 1999. Clarification and concen-tration with membrane technology of a phycocyanin solution extracted from *Spirulina platensis*. *Biotechnol Tech*13: 877 – 81.160.Jeeji Bai, N. 1985, Competitive exclusion or morphological transformation? A case study with *Spirulina fusiformis*. *Arch. Hydrobiol. Suppl.* 71, *Algal. Stud* 191: 38 – 39.161.Jensen, G. S, Ginsberg, D. I. and Drapeau, C. 2001. Blue-green algae as an immuno-enhancer and biomodulator. *J Am Nutraceut Ass* 3:24 – 30.162.Jensen, G.S., Ginsberg, D.I. and Drapeau, C. 2001. Blue-green algae as an immuno-enhancer and biomodulator. *J Am Nutraceut Ass* 3:24 – 30.163.Jensen, S. and Knutsen, G. 1993. Influence of light and temperature on photoinhibition of photosynthesis in *Spirulina platensis*. *Journal of Applied Phycology* 5: 495-504.164.Jepson, B.J.N., Anderson, L.J., Rubio, L.M., Taylor, C.J., Butler, C.S, Flores, E., Herrero, A., Butt, J.N. and Richardson, D.J. 2004. Tuning a nitrate reductase for function: the first spectropotentiometric characterization of a bacterial assimilatory nitrate reductase reveals novel redox properties. *J Biol Chem* 279: 32212 – 32218.165.Jespersen, L., Str?mdahl, L. D., Olsen, K., Skibsted, L. H. 2005. Heat and light stability of three natural blue colorants for use in confectionery and beverages. *Eur Food Res Technol* 220:261 – 266.166.Jim?nez, C., Coss?o, B.R. and Niell, F.X. 2003. Relationship between physicochemical variables and productivity in open ponds for the production of Supirulina: a predictive model of algal yield. *Aquaculture*, 221: 331-345.167.Jim?nez, C., Coss?o, B.R., Labella, D. and Niell, F.X. 2003. The feasibility of industrial production of Spirulina (*Arthrospira*) in Southern Spain. *Aquaculture* 217:179 – 190.168.Kai, Y. K., Roger C. A., Kelly, C H., Youmie P., Steve T. and Kevin G. R. 2000. Strategies for maintaining the particle size peptide DNA condensates following freeze2dryng. *International Journal of Pharmaceutics* 203: 81-88.169.Kanda, J., D. A. Ziemann, Conquest, L. D. and Bienfang, P. K. 1989. Light-dependency of nitrate uptake by phytoplankton over the spring bloom in Auke Bay. Alaska. *Mar. Biol* 103: 563 - 569.170.Kao, O.H.W, Edwards, M.R. and Berns, D.S. 1975. Physical – chemical properties of c-phycocyanin isolated from an acido-thermophilic eukaryote, *Cyanidium caldarium*. *Biochem J*;147:63 – 70.171.Kaplan, A. 1981. Photoinhibition in *Spirulina platensis* : response of photosynthsis and HCO₃⁻ uptake capability to CO₂- depleted conditions. *Journal of Experimental Botany*, 32: 669-667.172.Kaplan, A., and Reinhold, L. 1999. CO₂ concentrating mechanisms in photosynthetic microorganisms. *J. Annual Review of Plant Biology*. 50: 539-570.173.Kaplan, D., Cohen, Z., and Abeliovich, A. 1986. Optimal growth conditions for *Isochrysis galbana*. *Biomass* 9: 37-48.174.Kaushik, J.K. and Bhat, R. 1999. A mechanistic analysis of the increase in the thermal stability of proteins in aqueous carboxylic acid salt solutions. *Protein Sci*;8:222 – 33.175.Kebede, E., 1997. Response of *Spirulina platensis* (=*Arthrospira fusiformis*) from Lake Chitu, Ethiopia, to salinity stress from sodium salts. *J. Appl. Phycol.*, 9: 551 – 558.176.Kikuchi, H., Aichi, M., Suzuki, I. and Omata, T. 1996. Positive regulation by nitrite of the nitrate assimilation operon in the cyanobacteria *Synechococcus* sp. strain PCC 7942 and *Plectonema boryanum*. *J Bacteriol* 178: 5822 – 5825.177.Knaff, D.B. 1996. Ferredoxin and ferredoxin-dependent enzymes. In: Ort DR and Yocom CF (eds) *Oxygenic Photosynthesis: the Light Reactions*, pp 333 – 361. Kluwer Academic Publishers, Dordrecht, The Netherlands.178.Knaff, D.B. and Hirasawa, M. 1991. Ferredoxin-dependent chloroplast enzymes. *Biochim Biophys Acta* 1056: 93 – 125.179.Kobayashi, M., Rodr??gue, R., Lara, C. and Omata, T. 1997. Involvement of the C-terminal domain of an ATP-binding subunit in the regulation of the ABC-type nitrate/nitrite transporter of the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Biol Chem* 272: 27197 – 27201.180.Koca, N., Karadeniz, F. and Burdurlu, H.S. 2006. Effect of pH on chlorophyll degradation and colour loss in blanched green peas. *Food Chem*;100:609 – 15.181.Krimm, S. and Bandekar, J. 1986. Vibrational spectroscopy and conformation of peptides, polypeptides and proteins. *Adv Protein Chem* 38: 181-364.182.Kula, M. R., Kroner, K. H. and Hustedt, H. 1982. Purification of enzymes by liquid – liquid extraction. *Adv. Biochem. Eng./Biotechnol* 24: 73-118.183.Kupka, M. and Scheer, H. 2008. Unfolding of C-phycocyanin followed by loss of non-covalent chromophore – protein interactions. 1. Equilibrium experiments. *Biochim Biophys Acta* 1777: 94 – 103.184.Kwak, J.Y, Takeshige, K., Cheung, B.S, Minakami, S. 1991. Bilirubin inhibits the activation of superoxide-producing NADPH oxidase in a neutrophil cell-free system.

Biochim Biophys Acta 1076:369 – 373.185.Kylin, H. 1910. Über Phykoerythrin and Phykocyan bei Ceramium rubrum (Huds.) Ag. Hoppe-Seyler ' s Z. Physiol. Chem. 69: 169-239.186.Kylin, H. 1912. Über Phykoerythrin and Phykocyan bei Ceramium rubrum (Huds.) Ag. Hoppe-Seyler ' s Z. Physiol. Chem. 76: 396-425.187.Lee, H. M, Flores, E., Herrero, A., Houmard, J. and Tandeau de Marsac, N. 1998. A role for the signal transduction protein PII in the control of nitrate/nitrite uptake in a cyanobacterium. FEBS Lett 427: 291 – 295.188.Lee, Y-K. 1997. Commercial production of microalgae in the Asia-Pacific rim. J Appl Phyco l 9: 403 – 411.189.Lemberg, R. 1928. Die chromoproteide der rotalgen. I. Liebigs. Ann. Chem. 461: 46-89.190.Lemberg, R. 1930. Chromoproteide der Rotalgen. II. Spaltung mit pepsin un sauren. Isolierung eines pyrrolfarbstoffs. Liebigs. Ann. Chem. 477. 195-245.191.Leonard, J. 1966 The 1964 – 1865 Belgian Trans-Saharan expedition. Nature 209: 126 – 128.192.Leonard, J. and Compare, P. 1967. Spirulina platensis (Gom) Geitl., algue bleue de grande valeur alimentaire par sa richeseen proteins. Bull. Jard. Bot.nat. Belg. 37: 3 – 23.193.Lewin, R.A., 1980, Uncoiled variants of Spirulina platensis. Arch. Hydrobiol. Suppl. 60, Algal. Stud., 26: 48 – 52.