

# 利用篩選之*Oscillatoria* sp. Wu1生產藻膽蛋白之研究

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## 摘要

藻膽蛋白(Phycobiliprotein)是種共價聯結的線性四吡咯發色基團(linear tetrapyrrole chromophoric group)，為水溶性的補光色素(light harvesting pigments)，其常存於藍綠菌和某些真核藻類之中，而藍綠菌會因為環境不同(例如光波長以及溫度)而調節四吡咯含量與成分。此外，其中又以藻藍蛋白(Phycocyanin)為主要成分，而這藻藍蛋白是一種天然色素，具高營養成分，可作為食品營養補充品，也可作為化妝品中的天然色素使用。除此之外，亦可應用於免疫分析、抗發炎以及抗癌藥物的使用。本研究係於台灣本島周圍海域以及離島海域篩選出適合生產藻藍蛋白之本土菌株，並針對生產條件、萃取以及純化方式進行探討。實驗結果顯示，本研究以所篩選之菌株(命名為*Oscillatoria* sp. Wu1)探討出最佳果糖濃度為1.0 g/L以及尿素濃度為0.5 g/L作為培養時的碳/氮源，並以培養基初始pH為8.5的條件下，放置於30 °C下進行培養，可獲得最佳的藻藍蛋白及異藻藍蛋白含量，分別為380 mg/g以及103 mg/g的粗萃取產量，且以400 mL的培養體積培養6天後，最終的乾細胞重量可達26.1 mg。此外，在光源種類影響的試驗結果發現，當培養基中同時含有碳/氮源時，光源種類對*Oscillatoria* sp. Wu1在生長及色素含量的影響較為明顯。相反的，當培養基中不額外添加碳/氮源時，光源種類對藍綠菌的生長及色素含量的影響則較不明顯。而欲對於利用特定光波長來培養*Oscillatoria* sp. Wu1生產藻藍蛋白時，則以紅光(630 nm)作為培養光源，可以達到最理想的效果。在繁複的純化程序中，利用Molecular gel chromatography來進行純化，其純化出的藻藍蛋白經由分光A620/A280測量出純度比為3.76，並藉由SDS-PAGE及傅立葉轉換紅外線光譜分析儀(Fourier Transform Infrared spectroscopy, FTIR)來進行分子量及其官能基之分析。

關鍵詞：藻膽蛋白、藻藍蛋白、純化

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## 參考文獻

- 1.李東農、郭寶江。1988。光照時間對螺旋藻生長的影響。海洋科學 3: 3-4。
- 2.林東明。1993。用於食品及化妝品的紅微細藻天然色素。今日經濟一月號:第79-85頁。
- 3.張成武和殷志敏。1995。藻膽蛋白的特色與利用。中國海洋藥物雜誌 3: 52-53。
- 4.黃瑞彥。1995。藍點鸚哥魚體內藻膽蛋白之轉變及顏色變化。國立台灣海洋大學食品科學系碩士論文。基隆。
- 5.蔡心涵。1995。螺旋藻藻藍蛋白對癌激發療法增敏作用的實驗研究。中國海洋藥物雜誌第一期:第15-18頁。
- 6.鄭俊明。1999。頭髮藻紅蛋白異價體性質分析研究。國立台灣大學海洋研究所博士論文。台北。
- 7.羅曉艷、沉艷、任體輝。2008。水中硝酸根分析方法的改進。化肥工業 35: 58-60。
- 8.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: 109-120.
- 9.Adir, N., Dobrovetsky, Y., Lerner, N. 2001. Structure of c-phycocyanin from thermophilic cyanobacterium *Synechococcus vulcanus* at 2.5 Å: structural implications for thermal stability in phycobilisome assembly. *J Mol Biol* 313: 71-81.
- 10.Adir, N., Vainer, R., Lerner, N. 2002. Refined structure of c-phycocyanin from the cyanobacterium *Synechococcus vulcanus* at 1.6 Å: insights into the role of solvent molecules in thermal stability and co-factor structure. *Biochim Biophys Acta* 1556: 168-174.
- 11.Arciero, D. M., Dallas, J. L. and Glazer, A. N. 1988. In vitro attachment of bilins to apophycocyanin. II. Determination of the structures of tryptic bilin peptides derived from the phycocyanobilin adduct. *J Biol Chem* 263: 18350-18357.
- 12.Ashokkumar P. and Anand N. 2010. Studies on growth and Phycobilin Pigments of the Cyanobacterium *Westiellopsis iyengarii*. *IJBB* 6: 315-323.
- 13.Ayehunie, S.M., Belay, A., Baba, T. W. and Ruprecht, R. M. 1998. Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). *JAIDS: Journal of Acquired Immune Deficiency Syndromes* 18: 7-12.
- 14.Beardall, J., Johnston, A. and Raven, J. 1998. Environmental regulation of CO<sub>2</sub>-concentrating mechanisms in microalgae. *Can J Bot* 76: 1010-1017.
- 15.Behrens, P. W. 2005. Photobioreactor and fermentors: the light and the dark sides of the growing algae. In: Andersen, R.A. (Ed.), *Algal Culturing Techniques*. Elsevier Academic Press, New York, USA, p. 189-204.
- 16.Belay, A. 2008. *Spirulina platensis* (*Arthrospira*): production and quality assurance. In: Gershwin M.E.; Belay A. (Ed.). *Spirulina in human nutrition and health*. Taylor & Francis, Portland, USA, p. 2-23.
- 17.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 the blue-green alga *Aphanizomenon flos-aquae*. *J Chromatogr B* 833: 12-18.
- 18.Benneman, J. R. 1988. *Microalgal Biotechnology: Products, Process and Opportunities*. Washington: OMEC International Inc vol. 1.
- 19.Bergman, B., Gallon, J. R., Rai, A. N. and Stal, L. J. 1997. N<sub>2</sub> fixation by nonheterocystous cyanobacteria. *FEMS Microbiol Rev* 19: 139-85.
- 20.Bezerra, R. P., Matsudo, M. C., Converti, A., Sato, S. and Carvalho, J. C. M. 2008. Influence of ammonium chloride feeding time and light intensity on the cultivation of *Spirulina* (*Arthrospira*) *platensis*. *Biotechnol Bioeng*. 100: 297-305.
- 21.Boussiba, S. and Richmond, A. E. 1979. Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*. *Arch Microbiol* 120: 155-159.
- 22.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.
- 23.Carlucci, M. J., Scolaro, L. A., Errea, M. I., Matulewicz, M. C. and Damonte, E. B. 1997. Antiviral activity of natural sulphated galactans on herpes virus multiplication in cell culture. *Planta Med* 63: 429-432.
- 24.Carmona, R., Vergara, J. J., Lahaye, M. and Niell, F. X. 1998. Light quality affects morphology and polysaccharide yield and composition of *Gelidium sesquipedale* (rhodophyceae). *J Appl Phycol* 10: 323-332.
- 25.Carvalho, J. C. M., Francisco, F. R., Almeida, K. A., Sato, S. and Converti, A. 2004. Cultivation of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyceae) by fed-batch addition of ammonium chloride at exponentially-increasing feeding rate. *J Phycol* 40: 589-597.
- 26.Chaneva, G., Furnadzhieva, S., Minkova, K. and Lukavsky, J. 2007. Effect of light and temperature on the cyanobacterium *Arthrospira africanum* - a prospective phycobiliprotein-producing strain. *J Appl Phycol* 19: 537 – 544.
- 27.Chen C.H. 1983. The growth and cellular characteristics of *spirulina platensis*. Master thesis.
- 28.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.
- 29.Chen, F., Zhang, Y. and Guo, S. 1996. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. *Biotechnol Lett* 18: 603-608.
- 30.Chen, H. B., Wu, J. Y., Wang, C. F., Fu, C. C., Shieh, C. J., Chen, C. I., Wang, C. Y. and Liu, Y. C. 2010. Modeling on chlorophyll a and phycocyanin production by *Spirulina platensis* under various light-emitting diodes. *Biochem Eng J* 53: 52-56.
- 31.Chisti, Y. and Moo-Young, M. 1986. Disruption of microbial cells for intracellular products. *Enzyme Microb Technol* 8: 194-204.
- 32.Ciferri, O. and Tiboni, O. 1985. The biochemistry and industrial potential of *Spirulina*. *Ann Rev Microbiol* 39: 503-526.
- 33.Cohen, Z., 1986. Products from microalgae. In: Richmond, A. (Ed.), *Handbook for Microalgal Mass Culture*. CRC Press, Boca Raton, FL., USA, p. 421-454.
- 34.Colla, L. M., Reinehr, C. O., Recichert, C. and Costa, J. A. V. 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresour Technol* 98: 1489-1493.
- 35.Contreras-Martel, C., Matamala, A., Bruna, C., Poo-Caamano, G., Almonacid, D., Figueroa, M., Martinez-Oyanedel, J. and Bunster, M. 2007. The structure at 2 Å resolution of phycocyanin from *Gracilaria chilensis* and the energy transfer network in a PC – PC complex. *Biophys Chem* 125: 388-396.
- 36.Converti, A.,

Scapazzoni, S., Lodi A. and Carvalho J. C. M. 2006. Ammonium and urea removal by *Spirulina platensis*. *J Ind Microbiol Biotechnol* 33: 8-16.

37. Costa, J. A. V., Cozza, K. L., Oliveira, L. and Magagnin, G. 2001. Different nitrogen sources and growth responses of *Spirulina platensis* in microenvironments. *World J Microbiol Biotechnol* 17: 43-442.

38. Dakubu, S. 1976. Cell inactivation by ultrasound. *Biotechnol and Bioeng* 18: 465-471.

39. Danesi, E. D. G., Rangel-Yagui, C. O. and Carvalho, J. C. M. 2002. An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by *Spirulina platensis*. *Biomass Bionerg* 23: 261-269.

40. Danesi, E. D. G., Rangel-Yagui, C. O., Sato, S. and de Carvalho, J. C. M. 2011. Growth and content of *Spirulina platensis* biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources. *Braz J Microbiol* 42: 362-373.

41. Del Campo, J. A., Garcia-Gonzalez, M., Guerrero, M.G. 2007. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl Microbiol Biotechnol* 74: 1163-1174.

42. Der-Vartanian, D., Espardellier, F. J. and Astier, C. 1981. Contributions of respiratory and photosynthetic pathways of a facultative photoautotrophic cyanobacterium, *Aphanocapsa* 6714. *Plant Physiol* 68: 974-978.

43. Deshniem, P., Paithoonrangarid, K., Suphatrakul, A., Meesapyodsuk, D., Tanticharoen, M. and Cheevadhanarak, S. 2000. Temperature-independent and -dependent expression of desaturase genes in filamentous cyanobacterium *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC 9438). *FEMS Microbiol Lett* 184: 207-213.

44. Desikachary, T. V. 1959. Cyanophyta. Indian council of Agricultural Research, New Delhi, India, 686.

45. Doke, J. H. 2005. An improved and efficient method for the extraction of phycocyanin from *Spirulina* sp. *Int J Food Eng* 1: 2.

46. Doulah, M. S. 1977. Mechanism of disintegration of biological cells in ultrasonic cavitation. *Biotechnol and Bioeng* 19: 649-660.

47. Engler, C. R. 1985. Disruption of Microbial Cells, in *Comprehensive Biotechnology*, M. Moo-Young, Editor., Pergamon Press: Oxford, England.

48. Fabregas, J., Garcia, D., Fernandez-Alonso, M., Rocha, A. I., Gomez-Puertas, P., Escribano, J. M., Otero, A. and Coll, J. M. 1999. In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. *Antiviral Res* 44: 67-73.

49. Faucher, O., Coupal, B. and Leudy, A. 1997. Utilization seawater-urea as culture medium for *spirulina maxima*. *Can J Microbiol* 25: 752-759.

50. Flores, E. and A. Herrero. 1994. Assimilatory nitrogen metabolism and its regulation. In: D. A. Bryant (ed.), *The molecular biology of cyanobacteria*. p. 487-517. Kluwer Academic Publishers, Dordrecht, The Netherlands.

51. Furuki, T., Maeda, S., Imajo, S., Hiroi, T., Amaya, T., Hirokawa, T., Ito, K. and Nozawa, H. 2003. Rapid and selective extraction of phycocyanin from *Spirulina platensis* with ultrasonic cell disruption. *J Appl Phycol* 15: 319-324.

52. Galland-Irmouli, A. V., Pons, L., Lucon, M., Villaume, C., Mrabet, N. T., Gueant, J. L. and Fleurence, J. 2000. One-step purification of R-phycocerythrin from the red macroalga *Palmaria palmate* using preparative polyacrylamide gel electrophoresis. *J Chromatogr B* 739: 117-123.

53. Gantt, E. and Lipschultz, C.A. 1973. Energy transfer in phycobilisomes from phycocerythrin to allophycocyanin. *Biochim Biophys Acta* 292: 858-861.

54. Gantt, E., and Lipschultz, C. A. 1974. Phycobilisomes of *Porphyridium cruentum*: Pigment analysis. *Biochemistry* 13: 2960-2966.

55. Gao, K., Li, P., Watanabe, T. and Helbling, E. W. 2008. Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta). *J Phycol* 44: 777-786.

56. Geciova1, J., Bury, D. and Jelen, P. 2002. Methods for disruption of microbial cells for potential use in the dairy industry-a review. *Int Dairy J* 12: 541-553.

57. Glazer, A. N. 1982. Phycobilisomes: Structure and Dynamics. *Annu Rev Microbiol* 36: 173-198.

58. Glazer, A. N. 1984. Phycobilisome A macromolecular complex optimized for light energy transfer. *Biochim Biophys Acta* 768: 29-51.

59. Glazer, A. N. 1988. Phycobiliproteins. *Methods Enzymol* 167: 291-303.

60. Glazer, A. N. 1989. Light guides. Directional energy transfer in a photosynthetic antenna. *J Biol Chem* 264: 1-4.

61. Godinez-Ortega J. L., Snoeijs, P., Robledo, D., Freile-Pelegrin, Y. and Pedersen, M. 2007. Growth and pigment composition in the red alga *Halymenia floresii* cultured under different light qualities. *J Appl Phycol* 20: 253-260.

62. Gonzalez, R., Rodriguez, S., Romay, C., Ancheta, O., Gonzalez, A., Armesto, J., Ramirez, D. and Merino, N. 1999. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. *Pharmacol Res* 39: 55-59.

63. Griffiths, D. J., Thresher, C. L., Street, H. E. 1960. The heterotrophic nutrition of *Chlorella vulgaris*. *Ann Bot* 24: 1-11.

64. Grossman, A., Schaefer, M., Chiang, G. and Coller, J. 1993. Environmental effects on the light-harvesting complex of cyanobacteria. *J Bacteriol* 175: 686-582.

65. Heifetz, P. B., Foster, B., Osmond, C. B., Giles, L. G. and Boynton, J. E. 2000. Effects of acetate on facultative autotrophy in *Chlamydomonas reinhardtii* assessed by photosynthetic measurements and stable isotope analyses. *Plant Physiol* 122: 1439-1445.

66. Henrikson R. 1989. In: Henrikson Robert, editor. *Earth food Spirulina*. Laguna Beach, CA: Ronore Enterprises Inc.

67. 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.

68. Hilditch, C. M., Balding, P., Jenkins, R., Smith, A. J. and Rogers, L. J. 1991. Rphycocerythrin from the macroalga *Corallina officinalis* (Rhodophyceae) and application of a derived phycofluor probe for detecting sugar-binding sites on cell membranes. *J Appl Phycol* 3: 345-354.

69. Hirata, T., Tanaka, M., Ooike, M., Tsunomura, T. and Sakaguchi, M. 200. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis*. *J Appl Phycol* 12: 435-439.

70. Holzwarth A. R. 1991. Structure-function relationships and energy transfer in phycobiliprotein antennae. *Physiol Plant* 83: 518-528.

71. Hoshino, T., Hayashi, T., Hayashi, K., Hamada, J., Lee, J. B. and Sankawa, U. 1998. An antivirally active sulfated polysaccharide from *Sargassum horneri* (TURNER) C. AGARDH. *Biol Pharm Bull* 21: 730-734.

72. Kay, R. A. 1991. Macroalgae as food and supplement. *Critical Reviews in Food Science Nutrition* 30: 555-573.

73. Kitamura, K. 1982. Re-examination of zymolase purification. *Biol Chem* 446: 963-969.

74. Krimm, S. and Bandekar, J. Vibrational spectroscopy and conformation of peptides, polypeptides and proteins. *Adv Protein Chem* 38: 181-364.

75. Kula, M. R. and Shutte, H. 1987. Purification of proteins and the disruption of microbial cells. *Biotechnol Progr* 3: 31-42.

76. Lalucat, J., Imperial, J. and Pares, R. 1984. Utilization of light for the assimilation of organic matter in *Chlorella* sp. VJ79. *Biotechnol Bioeng* 26: 677-681.

77. Lee, Y. -K. 2001. Microalgal mass culture systems and methods: their limitation and potential. *J Appl Phycol* 13, 307-315.

78. Lenton, K. J. and Greenstock, C. L. 1999. Ability of human plasma to protect against ionising radiation is inversely correlated with age. *Mech Ageing Dev* 107: 15-20.

79. Lewitus, A. J., Caron, D. A. and

Miller, K. R. 1991. Effects of light and glycerol on the organization of the photosynthetic apparatus in the facultative heterotroph *Pyrenomonas salina* (Cryptophyceae). *J Phycol* 27:578-587.

80.Li, X., Xu, H. and Wu, Q. 2007. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnol Bioeng* 98: 764-771.

81.Liu, B. H. and Lee, Y. K. 2000. Secondary carotenoids formation by the green alga *Chlorococcum* sp.. *J Appl Phycol* 12: 301-307.

82.Carmen, S. S., Teresa, P. N., Roxana, O. R., Jaime, O. L. and Rosa Olivia, C. V. 2004. Extraction and purification of phycocyanin from *Calothrix* sp. *Process Biochem* 39: 2047-2052.

83.Madhyastha, H. K. and Vatsala, T. M. 2007. Pigments production in *Spirulina fusciformis* in different photophysical conditions. *Biomol Eng* 24: 301-305.

84.Markou, G., and Georgakakis, D. 2011. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Appl Energy* 88: 3389-3401.

85.Marquez, F. J., Sasaki, K., Kakizono, T., Nishio, N. and Nagai, S. 1993. Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. *J Ferment Bioeng* 76: 408-410.

86.Martinez, F. and Orus, M. I. 1991. Interactions between glucose and inorganic carbon metabolism in *Chlorella vulgaris* strain UAM101. *Plant Physiol* 95: 1150-1155.

87.Masih, S., Sofi, M. Y. and Singh, S. G. 2011. Growth Performance of *Spirulina platensis* under Mixotrophic Culture. *RJAS* 2: 119-121.

88.Masojidek, J., Koblizek, M. and Torzillo, G. 2004. Handbook of microalgal culture: biotechnology and applied phycology, photosynthesis in microalgae. Edited by Amos Richmond, Blackwell Science, 20-39.

89.Middelberg, A. P. J. 1995. Process-scale disruption of microorganisms. *Biotechnol adv* 13: 491-551.

90.Minkova, K. M., Tchernov, A. A., Tchobadjieva, M. I., Fournadjieva, S. T., Antova, R. E. and Busheva, MCh. 2003. Purification of C-phycocyanin from *Spirulina (Arthrospira) fusiformis*. *J Biotechnol* 102: 55-59.

91.Mishra, S. K., Shrivastav, A. Pancha, I., Jain, D. and Mishra, S. 2010. Effect of preservatives for food grade C-Phycocerythrin, isolated from marine cyanobacteria *Pseudanabaena* sp.. *Int J Biol Macromol* 47: 597-602.

92.Monroe, K. and Poore, A. G. B. 2005. Light quantity and quality induce shade-avoiding plasticity in a marine macroalga. *J Evol Biol* 18: 426-435.

93.Morais, M. and Costa, J. A. V. C. 2007a. Carbon dioxide fixation by *Chlorella kessleri*, *C. vulgaris*, *Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnol Lett* 29: 1349-1352.

94.Morais, M. G. and Costa, J. A. V. C. 2007b. Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *J Biotechnol* 129: 439-445.

95.Neppiras, E. A. and Hughes, D. E. 1964. Some experiments on the disintegration of yeast by high intensity ultrasound. *Biotechnol and Bioeng* 6: 247-270.

96.Nield, J., Rizkallah, P. J., Barber, J., Chayen, N. E. 2003. The 1.45 Å three-dimensional structure of C-phycocyanin from the thermophilic cyanobacterium *Synechococcus elongatus*. *J Struct Biol* 141: 149-155.

97.Niu, J. F., Wang, G. C., Lin, X. Z., Zhou, B. C. 2007. Large-scale recovery of C-phycocyanin from *Spirulina platensis* using expanded bed adsorption chromatography. *J Chromatogr B* 850: 267-276.

98.Oesterhelt, C., Schmalzlin, E., Schmitt, J.M. and Lokstein, H. 2007. Regulation of photosynthesis in the unicellular acidophilic red alga *Galdieria sulphuraria*. *Plant J* 51: 500 – 511.

99.Oliveira, E. G., Rosa, G. S., Moraes, M. A., Pinto, L. A. A. 2008. Phycocyanin content of *Spirulina platensis* dried in spouted bed and thin layer. *J Food Process Eng* 31: 34-50.

100.Ortega-Calvo, J. J., Mazuelos, C., Hermosin, B. and Saiz-Jimenez, C. 1993. Chemical composition of spirulina and eukaryotic algae food products marketed in Spain. *J Appl Phycol* 5: 425-435.

101.Padayana, A. K., Bhat, V. B., Madyastha, K. M., Rajashankar, K. R., Ramakumar, S. 2001. Crystal structure of a light-harvesting protein C-phycocyanin from *Spirulina platensis*. *Biochem Biophys Res Comm* 282: 893-898.

102.Parsons, T. R., Maita, Y. and Lalli, C. M. 1984. A manual of chemical and biological methods for seawater analysis. 1st ed. Pergamon Press, New York, U.S.A. p. 14-17.

103.Patel, A., Mishra, S., Pawar, R. and Ghosh, P. K. 2005. Purification and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat. *Protein Expr Purif* 40: 248-255.

104.Patil, G. and Raghavarao, K. S. M. S. 2007. Aqueous two phase extraction for purification of C-phycocyanin. *Biochem Eng J* 34: 156-164.

105.Patil, G., 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.

106.Perez-Garcia, O., Escalante, F. M. E., de-Bashan, L. E. and Bashan, Y. 2011. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water research* 45: 11-36.

107.Piorreck, M., Baasch, K. and Pohl, P. 1983. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry* 23: 207-216.

108.Pulz, O., Gross, W. 2004. Valuable products from biotechnology microalgae. *Appl Microbiol Biotechnol*. 65: 635-648.

109.Rangel-Yagui, C. O., Danesi, E. D. G., Carvalho, J. C. M., Sato, S. 2004. Chlorophyll production from *Spirulina platensis*: cultivation with urea addition by fed-batch process. *Biores Technol* 92: 133-141.

110.Redlinger, T. and Gantt, E. 1982. A M(r) 95,000 polypeptide in *Porphyridium cruentum* phycobilisomes and thylakoids: Possible function in linkage of phycobilisomes to thylakoids and in energy transfer. *Proc Natl Acad Sci U. S. A.* 79: 5542-5546.

111.Reis, A., Mendes, A., Lobo, H., Fernandez, J. and Maggiolly, J. 1998. Production, extraction and purification of phycobiliproteins from *Nostoc* sp. *Biores Technol* 66: 181-187.

112.Rhie, G. and Beale, S. I. 1994. Regulation of heme oxygenase activity in *Cyanidium caldarium* by light, glucose, and phycobilin precursors. *J Biol Chem* 269: 9620-9626.

113.Richmond, A. 1986a. Cell response to environmental factors. In: Richmond, A. (Ed.), *Handbook for Microalgal Mass Culture*. CRC Press, Boca Raton, FL., USA, pp. 69-99.

114.Richmond, A. 1986b. Outdoor mass cultures of microalgae. In: Richmond, A. (Eds.), *Handbook of Algal Mass Culture*. CRC Press, Boca Raton, FL, USA, pp. 285-330.

115.Richmond, A., Karg, S., Boussiba, S. 1982. Effect of bicarbonate and carbon dioxide on the competition between *Chlorella vulgaris* and *Spirulina platensis*. *Plant Cell Physiol* 23:1411-1417.

116.Rimbau, V., Camins, A., Romay, C., Gonzalez, R. and Pallas, M. 1999. Protective effects of C-phycocyanin against kainic acid-induced neuronal damage in rat hippocampus. *Neurosci. Lett.* 276: 75-78.

117.Rito-Palomares, M., Nunez, L. and Amador, D. 2001. Practical application of aqueous two-phase systems for the development of a prototype process for C-phycocyanin recovery from *Spirulina maxima*. *J Chem Techn Biotechnol* 76: 1273-1280.

118.Robert, M. C. 2004. Allophycocyanin and energy transfer. *Biochim Biophys Acta* 1657: 73-81.

119.Rodrigues, M. S., Ferreira, L. S., Converti, A., Sato, S. and Carvalho, J. C. M. 2010. Fed-batch cultivation of *Arthrospira (Spirulina) platensis*: Potassium nitrate and ammonium chloride as simultaneous

nitrogen sources. *Bioresour Technol* 101: 4491-4498. 120. Rodriguez, H., Rivas, J., Guerrero, M. G. and Losada M. 1991. Enhancement of phycobiliprotein production in nitrogen-fixing cyanobacteria. *J Bacteriol* 20: 263-270. 121. Rogner, M., Nixon, P. J. and Diner, B. A. 1990. Purification and Characterization of Photosystem I and Photosystem II Core Complexes from Wild-type and Phycocyanin-deficient Strains of the Cyanobacterium *Synechocystis* PCC 6803. *J Biol Chem* 265: 6189-6196. 122. Romay, C., Gonzalez, R., Ledon, N., Ramirez, D. and Rimbau, V. 2003. C-Phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 4: 207-216. 123. Romay, C., Ledon, N. and Gonzalez, R. 1998. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm Res* 47: 334-338. 124. Ronda, R.R. and Lele, S.S. 2008. Culture conditions stimulating high  $\alpha$ -linolenic acid accumulation by *Spirulina platensis*. *Braz J Microbiol* 39: 693-697. 125. Rossano, R., Ungaro, N., D' Ambrosio, A., Liuzzi, G. M. and Riccio, P. 2003. Extracting and purifying R-phycoerythrin from Mediterranean red algae *Corallina elongata* Ellis & Solander. *J Biotechnol* 101: 289-293. 126. Salasbury, T. 1989. Clarification and extraction: Disruption. In E. L. V. Harris, & S. Angal (Eds.), *Protein purification methods: A practical approach*. Oxford: IRL Press at Oxford University Press. 127. Santiago-Santos, M. C., Ponce-Noyola, T., Olvera-Ramirez, R., Ortega-Lopez J. and Canizares-Villanueva R. O. 2004. Extraction and purification of phycocyanin from *Calothrix* sp. *Process Biochem* 34: 2047-2052. 128. Sarada, R., Pillai, M. G. and Ravishankar, G. A. 1999. Phycocyanin from *Spirulina* sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. *Process Biochem* 34: 795-801. 129. Sauer, T., Robinson, C. W. and Glick, B. R. F. 1989. Disruption of native and recombinant *E. coli* in a high pressure homogenizer. *Biotechnol Bioeng* 33: 1330-1342. 130. Schirmer, T., Bode, W. and Huber, R. 1987. Refined three-dimensional structures of two cyanobacterial C-phycocyanins at 2.1 and 2.5 Å resolution: A common principle of phycobilin-protein interaction. *J Mol Biol* 196: 677-695. 131. Schmidt, R. A., Wiebe, M. G. and Eriksen, N. T. 2005. Heterotrophic high cell density fed-batch cultures of the phycocyanin producing red alga *Galdieria sulphuraria*. *Biotechnol Bioeng* 90: 77-84. 132. Schneegurt, M. A., Sherman, D. M., Sherman, L. A. 1997. Growth, physiology, and ultrastructure of a diazotrophic *Cyanothece* sp. Strain ATCC 51142 in mixotrophic and chemoheterotrophic cultures. *J Phycol* 33: 632-642. 133. Shutte, H., Kroner, K. H., Hustedt, H. and Kula, M. R. 1983. Experience with a 20 litre industrial bead mill for the disruption of microorganism. *Enzyme Microb Technol* 5: 143-148. 134. Silveira, S. T., Burkert, J. F. M., Costa, J. A. V., Burkert, C. A. V. and Kalil S. J. 2007. Optimization of phycocyanin extraction from *Spirulina platensis* using factorial design. *Bioresour Technol* 98: 1629-1634. 135. Sloth, J. K., Wiebe, M. G. and Eriksen, N. T. 2006. Accumulation of phycocyanin in heterotrophic and mixotrophic cultures of the acidophilic red alga *Galdieria sulphuraria*. *Enzyme Microb Technol* 38: 168-175. 136. Soletto, D., Binaghi, L., Ferrari, L., Lodi, A., Carvalho, J. C. M., Zilli, M. and Converti, A. 2008. Effects of carbon dioxide feeding rate and light intensity on the fed-batch pulse-feeding cultivation of *Spirulina platensis* in helical photobioreactor. *Biochem Eng J* 39: 369-375. 137. Soletto, D., Binaghi, L., Lodi, A., Carvalho, J. C. M., Converti, A. 2005. Batch and fedbatch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. *Aquaculture* 243: 217-224. 138. Soni, B., Kalavadia, B., Trivedi, U. and Madamwar, D. 2006. Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata*—isolated from the rocky shores of Bet-Dwarka, Gujarat, India. *Process Biochem* 41: 2017-2023. 139. Soni, B., Trivedi, U. and Madamwar, D. 2008. A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium fragile* and its characterization for antioxidant property. *Bioresour Technol* 99: 188-194. 140. Spolaore, P., Joannis-Cassan, C. Duran, E. and Isambert, A. 2006. Commercial applications of microalgae. *J Biosci Bioeng* 102: 87-96. 141. Stanca, D. and Popovici, E. 1996. Urea as nitrogen source in modified Zarrouk medium. *Rev Roum Biol Ser Biol Veg* 41: 25-31. 142. Stec, B., Troxler, R. F., Teeter, M. M. 1999. Crystal structure of C-phycocyanin from *Cyanidium caldarium* provides a new perspective in phycobilisome assembly. *Biophys J* 76: 2912-2921. 143. Susi, H. 1972. Infrared spectroscopy conformation. *Methods Enzymol* 22: 455-472. 144. Talarico, L. and Maranzana, G. 2000. Light and adaptive responses in red macroalgae: an overview. *J Photochem Photobiol B-Biol* 56: 1-11. 145. Tan X., Yao L., Gao Q., Wang W., Qi F. and Lu X. 2011. Photosynthesis driven conversion of carbon dioxide to fatty alcohols and hydrocarbons in cyanobacteria. *Metab Eng* 13: 169-176. 146. Telford, W., Moss, M., Morsemann, J. and Allnut, T. 2001. Cryptomonad algal phycobiliproteins as fluorochromes for extracellular and intracellular antigen detection by flow cytometry. *Cytometry* 44: 16-23. 147. Tomaselli, L., Margheri, M. C. and Sacchi, A. 1995. Effects of light on pigments and photosynthetic activity in a phycoerythrin-rich strain of *Spirulina subsalsa* Aquat. *Microb. Ecol.* 9: 27-31. 148. Troxler, R. F., Ehrhardt, M. M., Brown-Mason, A. S. and Offner, G. D. 1981. Primary structure of phycocyanin from the unicellular rhodophyte *Cyanidium caldarium*. II. Complete amino acid sequence of the b subunit. *J Biol Chem* 256: 12176-12184. 149. van Liere, L., de Groot, G. J. and Muur, L. R. 1979. Pigment variation with irradiance in *Oscillatoria agardhii* Gomont in nitrogen (nitrate)-limited chemostat cultures. *FEMS Microbiol Lett* 6: 337-340. 150. Venkataraman, L. V., Becker, E. W. 1985. Algal cultivation. In: Venkataraman LV, Becker EW, editors. *Biotechnology and utilization of algae. The Indian experience*, 12-32. 151. Venugopal, V., Prasanna, R., Sood, A., Jaiswal, P. and Kaushik, B. D. 2006. Stimulation of pigment accumulation in *Anabaena azollae* strains: effect of light intensity and sugars. *Folia Microbiol* 51: 50-56. 152. Viskari, P. J. and Colyer, C. L. 2003. Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. *Anal Biochem* 319: 263-271. 153. Viskari, P. J., Kinkade, C. S. and Colyer, C. L. 2001. Determination of phycobiliproteins by capillary electrophoresis with laser-induced fluorescence detection. *Electrophoresis*. 22: 2327-2335. 154. Volkmann, H., Imianovsky, U., Oliveira, J. L. B. and Sant' Anna, E. S. S. 2008. Cultivation of *Arthrospira* (*Spirulina*) *platensis* in desalinator wastewater and salinated synthetic medium: protein content and amino acid profile. *Braz J Microbiol* 39: 98-101. 155. Vonshak, A., Chanawongse, L., Bunnag, B. and Tanticharoen, M. 1996. Light acclimation and photoinhibition in three *Spirulina platensis* (cyanobacteria) isolates. *J Appl Phycol* 8: 35-40. 156. Watson, J.D., Hopkins, N.H., Roberts, J.W., Steitz, J.A. and Weiner, A. M. 1987. *Molecular Biology of the Gene*. CA: Benjamin/Cummings Publishing Co., Inc., p. 507-507. 157. Zhang X. W., Zhang Y. M., Chen F. 1998. Kinetic models for phycocyanin

production by high cell density mixotrophic culture of the microalga *Spirulina platensis*. *J Ind Microbiol* 21: 283-288. 158.Zhang X. W., Zhang Y. M., Chen F. 1999. Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis*. *Process Biochem* 34: 477-481. 159.Zhang, S. P., Xie, J., Zhang, J. P., Zhao, J. Q. and Jiang, L. J. 1999a. Electron spin resonance studies on photosensitized formation of hydroxyl radical by C-phycoerythrin from *Spirulina platensis*. *BBA* 1426: 205-211. 160.Zhang, Y. M. and Chen, F. 1999b. A simple method for efficient separation and purification of C-phycoerythrin and allophycoerythrin from *Spirulina platensis*. *Biotechnol Tech* 13: 601-603. 161.Zhu, Y., Chen, X. B., Wang, K. B., Li, Y. X., Bai, K. Z., Kuang, T. Y. and Ji, H. B. 2007. A simple method for extracting C-phycoerythrin from *Spirulina platensis* using *Klebsiella pneumoniae*. *Appl Microbiol Biotechnol* 74: 244-248. 162.Zucchi, M. R. and Necchi, O. Jr. 2001. Effects of temperature, irradiance and photoperiod on growth and pigment content in some freshwater red algae in culture. *Phycol Res* 49: 103-114. 163.Brejč, K., Fieker, R., Huber, R. and Steinbacher, S. 1995. Isolation, crystallization, crystal structure analysis and refinement of allophycoerythrin from the cyanobacterium *Spirulina platensis* at 2.3 Å resolution. *J Mol Biol* 249: 424-440. 164.Liu, J. Y., Jiang, T., Zhang, J. P. and Liang, D. C. 1999. Crystal structure of allophycoerythrin from red algae *Porphyra yezoensis* at 2.2-Å resolution. *J Biol Chem* 274: 16945-16952. 165.Jiang, T., Zhang, J., and Liang, D. 1999. Structure and function of chromophores in R-Phycoerythrin at 1.9 Å resolution. *Proteins* 34: 224-231. 166.Reynolds, C. S. 1984: *The ecology of freshwater phytoplankton*. Cambridge University Press, Cambridge. 167.Garcia, M. C. C., Miron, A. S., Sevilla, J. M. F., Grima, E. M., Camacho, F. G. 2005. Mixotrophic growth of *Phaeodactylum tricoratum*: Influence of different nitrogen and organic carbon sources on productivity and biomass composition. *Process Biochem* 40: 297-305. 168.Luning, K. 1992. Day and night kinetics of growth rate in green, brown, and red seaweeds. *Journal of Phycology* 28: 794-803. 169.Reed, G and Nagodawithana, T. W. 1991. Wine yeast. In: *Yeast Technology*, Van Nostrand Reinhold, New York, pp. 151-224. 170.Wimpenny, J. W. T. 1967. Breakage of Micro-organisms. *Process Biochem* 2: 41-44. 171.McCullough, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. *Clin Chim Acta* 17: 297-304. 172.Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem* 28: 350-356. 173.  
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