



A Mini Review on *Spirulina*

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Abstract

Spirulina is photosynthetic, filamentous, spiral-shaped, multicellular and green-blue microalga. The two most important species of which are *Spirulina maxima* and *Spirulina platensis*. Since its chemical composition includes proteins, carbohydrates, vitamins, minerals, essential fatty acids and pigments like chlorophyll *a*, carotenes and phycocyanin, it is considered an excellent food and feed. It also used in medicine and cosmetic industries. Because of its nutritional, medical and biological value, genetic studies on *Spirulina* have been increased all over the world to develop new strains gained new properties.

Key Words: *Spirulina*; Molecular genetics; Industrial uses; Systematic

INTRODUCTION

In the early 1960s, *Spirulina*, a filamentous blue-green (cyanobacteria) algae attracted the interest of researchers. The biochemical components supply the marketing value to *Spirulina*. *Spirulina* is especially rich in protein of which amount to 60-70 percent of its dry weight. *Spirulina* contains up to 20 percent of phycocyanin, a water-soluble blue pigment [1] and green pigment chlorophyll *a*. *Spirulina* contains a relatively high content of cyanocobalamin (vitamin B₁₂). It was also found to contain β-carotene, provitamin A, vitamin C and E (Table 1).

Spirulina is the source of essential fatty acid, γ-linolenic acid (GLA) claimed to have medicinal properties. *Spirulina* has also minerals, iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium and zinc [2].

Nutrients such as phosphorus and nitrogen present in agro-industrial effluents as well as in domestic wastewater may cause serious eutrophication in any water body. But these nutrients can be used to increase plant growth, such as phytoplankton, which can be utilized as natural fish food or for pharmaceutical purpose. *Spirulina* is one of the most promising microalgae for culture due to its high nutritional values [3]. Although many studies have been carried out about the physiology of *Spirulina* in microalgal biotechnology, the studies about the genetic isn't enough. Genetic of cyanobacteria was known generally, however, there is need for new improved strains for particular aims and methods gene transfer system on *Spirulina*.

Table 1. Vitamin in *Spirulina* powder [2].

Vitamins	mg 100/g
Provitamin A	2.330x10 ³ IU/kg
(β-carotene)	140
Vitamin E	100 α-tocopherol equiv.
Thiamin B ₁	3.5
Riboflavin B ₂	4.0
Niacin B ₃	14.0
Vitamin B ₆	0.8
Vitamin B ₁₂	0.32
Biotin	0.005
Folic acid	0.01
Pantothenic acid	0.1
Vitamin K	2.2

Table 2. Minerals in *Spirulina* powder [2].

Mineral	mg 100 g ⁻¹
Calcium	700
Chromium	0.28
Copper	1.2
Iron	100
Magnesium	400
Manganese	5.0
Phosphorus	800
Potassium	1400
Sodium	900
Zinc	3.0

SYSTEMATIC

Spirulina is the oxygenic photosynthetic bacteria belongs to *Cyanobacteria* and *Prochlorales* according to the classification in Bargey's Manual of Determinative Bacteriology. In this classification, sequence of the rRNA sub-unit 16S is considered. In 1989, these microorganisms were classified into two genera, according to suggestion by Gomont in 1892 [4]. This classification is accepted currently [5,6]. At the present time, there are some discrepancies about classification of *Spirulina*. Botanists are identify this microorganism as microalgae because of they are photosynthetic organisms. On the other hand, bacteriologists are include this microorganisms into bacteria after the determined the main difference as a phospholipidic membrane between prokaryotes and eukaryotes.

Industrial Uses

The filamentous cyanobacterium *Spirulina* appears to be a ubiquitous component of the phytoplankton growing in ocean and seawater [7]. Because of its attractive nutritional value, production and trade of *Spirulina* and its products have developed industrially. In China, total annual production capacity of *Spirulina* dry powder in 10⁶ m² total cultivation area is up to 400 t [8].

At that time, *Spirulina* is used in food, feed, medicine and cosmetic industries. As a food, *Spirulina* is include high quality protein, vitamins, minerals and many biologically active substances [9]. Its cell wall consists of polysaccharide which has a digestibility of 86%, and could be easily absorbed by human body [8, 10].

Pills and capsules made from *Spirulina* have been used as a supplement food. *Spirulina* is also used in noodles, stylish noodles, nutritious, beverages, candies, cookies, natural coloring in chewing gums etc. [8, 11].

Spirulina is used to promote the growth of livestock, poultry, prawn, carp, canaries and exotic birds [8, 12, 13]. Fishmeal, groundnut meal and soybean meal can be partially replaced by spirulina in the preparation of diets of fish, poultry, cattle and domestic animals [14,15]. Adding to silage, in cattle and horse, the quantity of sperms in males and the fertility in females are increased [10]. *Spirulina* increases the mononuclear phagocyte system function thereby enhancing their disease resistance in chickens [16]. Using in poultry diet provides yellowness and redness of broiler flesh [9, 17].

Spirulina is also used to reduce the cultivation time and mortality and increase shell thickness in scallop, to promote the immunity and viability of prawns, to improve the survival rate of abalone, aquarium fish food etc.

Spirulina capsule has proved effective in lowering blood lipid level, and in decreasing white blood corpuscles after radiotherapy and chemotherapy [8, 18, 19]. It has also anti-arthritis affect due to the anti-inflammatory and antioxidative properties of phycocyanin [20], anti-atherogenic property [21], tumor burden inhibition [22] and cell degeneration [23].

Molecular Biology and Genetics

Because of its benefits for human, animal and ecologic environment (photosynthesis), studies on cyanobacteria have been increased all over the world. Especially during the past half century, cyanobacteria has been used in photosynthesis and its genetic control, photoregulation of genetic expression, cell differentiation and N₂ fixation, metabolism of nitrogen, carbon

and hydrogen, resistance to environmental stress and molecular evolution [24]. Many cloning vectors and other genetic tools developed for cyanobacteria. Transformation, electroporation and conjugation techniques are also used for gene transfer studies. Mutant strains for specific genes have been developed by mutagenesis also. Complete genomic sequence of some strains have been obtained and some genomic sequence projects are under way.

Although *Spirulina* expected to be a suitable organism for producing recombinant proteins, there are only few researches on gene transfer studies on *Spirulina platensis*. Toyomizu et al. [25] transferred the plasmid pHSG399 into *Spirulina* using electroporation technique and suggested the best electroporation efficiency condition is 5.0 ms pulse duration with an electric field of 4-8 kV cm⁻¹. On the other hand Kawata et al. [26] transferred Tn5 transposon, transposase and cation liposome complex into *Spirulina* and suggested 5.0 ms pulse duration with an electric field of 7.5 kV cm⁻¹. Nowadays gene transfer studies on *Spirulina platensis* are maintaining in various laboratories.

Besides this, several genes obtained from *Spirulina platensis* were cloned in other organisms by several researches. Tiboni et al. [27] cloned the genes for the large and small subunits of ribulose-1,5-bisphosphate carboxylase from *S. platensis* in *E. coli*. Sanangelantoni et al. [28] cloned the gene for ribosomal protein S2 and part of the gene for the elongation factor Ts (EF-Ts) from *S. platensis* in *E. coli*. Riccardi et al. [29] constructed the genomic library of *S. platensis* DNA using lambda EMBL3 vector. After that, they cloned the acetohydroxy acid synthase gene from this recombinant library in *E. coli*.

Bini et al. [30] cloned the gene for β -isopropylmalate dehydrogenase of *S. platensis* (*leuB*) from a λ EMBL3 genomic library by heterologous hybridization using the *Nostoc* UCD 7801 *leuB* gene as a probe. Salvi et al. [31] identified, cloned, characterized and expressed the gene encoding serin esterase from *S. platensis* in *E. coli*. Kawata et al. [32] presented an efficient and simple method for constructing a genomic DNA library by using a TA cloning vector. In their studies, the researchers cloned the gene coding phytoene synthase from *S. platensis* in *Synechococcus* and *Synechocystis*. Lui et al. [33] cloned C-phycocyanin operon of *Arthrospira platensis* into pMD18-T and showed that the operon consists of 427 bp *ussB*, 519 bp *cpcB* gene, 111 bp *igsB-A* region, 489 bp *cpcA* gene, 184 bp *ussH* region and 357 bp *cpcH* gene. Zhang et al. [34] cloned and characterized the partial *hoxH* genes encoding large subunit of nickel-iron hydrogenase of 2 cyanobacterial genera, including 5 strains of *Arthrospira* and 2 strains of *Spirulina* in *E. coli*.

Several investigations have been studied on the acyl-lipid desaturases genes. Meesapyodsuk et al. [35] cloned the *desC* gene from *S. platensis* in baker's yeast and thus, the cyanobacterial gene product appeared to be functional in yeast. Apiradee et al. [36] cloned and successfully expressed for the first time the genes encoding the acyl-lipid desaturases (*desC*, *desA*, *desD*) involved in γ -linolenic (GLA) synthesis in *E. coli*. Finally, Kurdrid et al. [37] cloned, expressed and characterized the *desD* gene in *Saccharomyces cerevisiae*.

Buttarelli et al. [38] sequenced the 5.3 kb DNA segment containing the *str* operon of *S. platensis*. Same as Kasahara et al. [39] sequenced the *cyaC* gene encoding an adenylate cyclase of *S. platensis*. Kawamura et al. [40] three restriction endonucleases *SplI*, *SplII* and *SplIII* have purified from *S. platensis* subspecies *siamese* and named. Milano et al. [41]

demonstrated the two isoenzymatic forms of the enzyme acetohydroxy acid synthase (AHS), which catalyse the first common step in the biosynthesis of isoleucine, leucine and valine in *S. platensis* and they sequenced the genes *ilvX* and *ilvW* encoding these two enzymes. Tanioka et al. [42] were characterized cobalamin-dependent methionine synthase (MS) to clarify the physiological function of pseudovitamin B12 (or adenylcobamide; AdeCba) in *Spirulina platensis* NIES-39. Then they cloned the full-length *Spirulina* MS. Linjawi [43] investigated the protective effect of *Spirulina* against Mitomycin C (MMC)-Induced genotoxic damage in male rats and suggested that SP exerts its anti-mutagenic properties by inhibiting alterations in the gene expression and the MnPCEs formation in the hepatic tissues and bone marrow cells of male rats exposed to MMC.

CONCLUSION

Because of its non-toxicity, nutritious value and other some properties for humans, animals and bovines (against viral attacks, anemia and tumoral growth, low prostaglandins production, sexual maturation and fertility factor), *Spirulina platensis* is one of the most commercially important species of microalgae. Especially it is reach source of protein, carbohydrates, minerals, chlorophyll *a*, phycocyanin, vitamin B₁₂, β -carotene and essential fatty acids like gamma-linoleic acid for human and animal nutrition. So its production and consumption are increasing every year. The production is over 2000 tons per year. Some countries such as China, South Africa, Japan, Mexico, Australia, Chile are leader countries on production of *Spirulina*.

In recent years, some studies have done and some are still going on its genetic. But most of these studies are on carrying out of its genetic structure. In these studies, some of its genes have been cloned and identified. Nowadays some genetic researches have been started to increase its nutritional and commercial value. So, some different properties will have been gained in near future to *Spirulina*.

REFERENCES

- [1] Ciferri O, 1983. Mutants, genes and phylogeny of *Spirulina platensis*, algae of life. Bulletin de L'Institut Oceanographique, no: 12, Monaco.
- [2] Belay A, 1997. Mass culture of *Spirulina* outdoors, the earthrise farms experience. In: Vonshak A. (ed.), *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology*. Taylor and Francis, London, pp: 131-158.
- [3] Toyub MA, Uddina MZ, Miahb MI and Habib MAB, 2011. Growth performance and nutritional analysis of *Spirulina platensis* in different concentrations of papaya skin powder media. In: Bangladesh Journal of Scientific and Industrial Research. 46(3): 333-338.
- [4] Castenholz RW, Waterbury JB, 1989. Oxygenic photosynthetic bacteria. In: Staley, J.T., Bryant, MP, Pfennig N, Holt JG (eds.), *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins Co, Baltimore, USA, 19: 1710-1806.
- [5] Tomaselli L, Palandri M, Tredici M, 1996. On the correct use of *Spirulina* designation. Algological Studies, 83: 539-548.
- [6] Vonshak A, Tomaselli L, 2000. *Arthrospira (Spirulina): Systematics and ecophysiology*. In: Whitton, A., Potts, M. (Eds.). *The Ecology of Cyanobacteria*. Kluwer Academic Publishers, The Netherlands, pp. 505-522.
- [7] Campanella L, Crescentini G, Avino P, 1999. Chemical composition and nutritional evaluation of some natural and commercial food products based on *Spirulina*. *Analisis*, 27: 533-540.
- [8] Li DM, Qi YZ, 1997. *Spirulina* industry in China: present status and future prospects. *Journal of Applied Phycology*, 9: 25-28.
- [9] FAO, 2008. A Review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish. Food and Organization of the United Nations, FAO Fisheries and Aquaculture Circular No. 1034, Rome.
- [10] Pugh N, Ross SA, Elsohly HN, Elsohly MA, Pasco DS, 2001. Isolation of three weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. *Planta Medica*, 67: 737-742.
- [11] Henrikson R, 1994. Microalgae *Spirulina*, superalimento del futuro. Ronore Enterprises. 2^a ed., Ediciones Urano, Barcelona, Espana, pp: 222.
- [12] Nandeesh MC, Gangadhara B, Manisseriy JK, Venkataraman LV, 2001. Growth performance to Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels on *Spirulina platensis*. *Bioresource Technology*, 80: 117-120.
- [13] Saxena PN, Ahmad MR, Shyan R, Amla DV, 1983. Cultivation of *Spirulina* in sewage for poultry feed. *Experientia*, 39: 1077-1083.
- [14] Venkataraman LV, Somasekaran T and Becker EW, 1994. Replacement value of blue-green alga (*Spirulina platensis*) for fishmeal and a vitamin-mineral premix for broiler chicks. *British Poultry Science*, 35: 371-381.
- [15] El-Sayed AM, 1994. Evaluation of soybean meal, spirulina meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. *Aquaculture*, 127: 169-176.
- [16] Al-Batshan HA, Al-Myfarrej SI, Al-Homaidan AA, Qureshi MA., 2001. Enhancement of chicken macrophage phagocytoc function and nitrite production by dietary *Spirulina platensis*. *Immunopharmacol Immunotoxicol*, 23: 281-289.
- [17] Toyomizu M, Sato K, Taroda H, Kato T, Akiba Y, 2001a. Effect of dietary *Spirulina* on meat colour in muscle of broiler chickens. *British Poultry Science*, 42: 197-202.
- [18] Ruan JS, Long CS, Guo BJ, 1988. *Spirulina* prevented damage included by radiation. *Journal of Genetics*, 10: 27-30.
- [19] Ruan JS, Guo BJ, Shu LH, 1990. Effect of *Spirulina* polysaccharides on changes in white blood corpuscles induced by radiation in mice. *Journal of Radiation Research Technology*, 8: 210-213.
- [20] Ramirez D, Gonzalez R, Merino N, Rodriguez S, Ancheta O, 2002. Inhibitory effects of *Spirulina* in zymozan-induced arthritis in mice. *Mediators of Inflammation*, 11: 75-79.
- [21] Kaji T, Fujiwara Y, Inomata Y, Hamada C, Yamamoto C, Shimada S, Lee JB, Hayashi T, 2002. Repair of wounded

- monolayers of cultures bovine aortic endothelial cells is inhibited by calcium spirulan, a novel sulfated polysaccharide isolated from *Spirulina platensis*. Life Sciences, 70: 1841-1848.
- [22] Dasgupta T, Banejee S, Yadav PK, Rao AR, 2001. Chemomodulation of carcinogen metabolizing enzymes, antioxidant profiles and skin and fore stomach papillomagenesis by *Spirulina platensis*. Molecular and Cellular Biochemistry, 226: 27-38.
- [23] Bulik C, 1993. How the *Spirulina*, a green-blue alga preserves de cell from degeneration, and extends youth and human lifespan. In: Doumenga, F., Durand-Chastel, H., Toulemont, A. (eds.), *Spiruline Algue De Vie*. Musee Oceanographique. Bulletin de l'Institut Oceanographique Monaco, Numero Special, 12: 121-131.
- [24] Koksharova OA, Wolk CP, 2002. Genetic tools for cyanobacteria. Applied and Environmental Microbiology, 58: 123-137.
- [25] Toyomizu M, Suzuki K, Kawata Y, Kojima H, Akiba Y, 2001b. Effective transformation of the cyanobacterium *Spirulina platensis* using electroporation. Journal of Applied Phycology, 13: 209-214.
- [26] Kawata Y, Yano S, Kojima H, Toyomizu M, 2004. Transformation of *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC9438) with Tn5 transposase-transposon DNA-cation liposome complex. Marine Biotechnology, 6: 355-363.
- [27] Tiboni O, Pasquale G, Ciferri O, 1984. Cloning and expression of the genes for ribulose-1,5- bisphosphate carboxylase from *Spirulina platensis*. Biochimica et Biophysica Acta, 783: 258-264.
- [28] Sanangelantoni AM, Calogero RC, Buttarelli FR, Gualerzi CO, Tiboni O, 1990. Organization and nucleotide sequence of the genes for ribosomal protein S2 and elongation factor Ts in *Spirulina platensis*. FEMS Microbiology Letters, 66: 141-146.
- [29] Riccardi G, Rossi E, Milano A, Forlani G, Felice M, 1991. Molecular cloning and expression of *Spirulina platensis* acetohydroxy acid synthase genes in *Escherichia coli*. Archives of Microbiology, 155: 360-365.
- [30] Bini F, Rossi E, Barbierato L, Riccardi G, 1992. Molecular cloning and squencing of the β -isopropylmalate dehydrogenase gene from cyanobacterium *Spirulina platensis*. Journal of General Microbiology, 138: 493-498.
- [31] Salvi S, Trinei M, Lanfaloni L, Pon CL, 1994. Cloning and characterization of the gene encoding an esterase from *Spirulina platensis*. Molecular and General Genetics, 243: 124-126.
- [32] Kawata Y, Yano S, Kojima H, 1998. Efficient library construction with a TA vector and its application to cloning of the phytoene synthase gene from the cyanobacterium *Spirulina platensis*. Current Microbiology, 37: 289-291.
- [33] Lui J, Zhang X, Sui Z, Zhang X, Mao Y., 2005. Cloning and characterization of c-phycoyanin operon from the cyanobacterium *Arthrospira platensis* FACHB341. Journal of Applied Phycology, 17: 181-185.
- [34] Zhang X, Zhang X, Shiraiwa Y, Mao Y, Sui Z, Liu J, 2005. Cloning and characterization of *hoxH* genes from *Arthrospira* and *Spirulina* and application in phylogenetic study. Marine Biotechnology, 7: 287-296.
- [35] Meesapyodsuk D, Reed DW, Cheevadhanarak S, Deshniun P, Covello PS, 2001. Probing the mechanism of a cyanobacterial $\Delta 9$ fatty acid desaturase from *Spirulina platensis* C1 (*Arthrospira* sp. PCC 9438). Comparative Biochemistry and Physiology, Part B, 129: 831-835.
- [36] Apiradee H, Kalyanee P, Pongsathon P, Patcharaporn D, Matura S, Sanjukta S, Supapon C, Morakot T, 2004. The expression of three desaturase genes of *Spirulina platensis* in *Escherichia coli* DH5 α . Molecular Biology Reports, 31: 177-189.
- [37] Kurdrid P, Subudhi S, Hongsthong A, Ruengjitchachawalya M, Tanticharoen M, 2005. Functional expression of *Spirulina*- Δ^6 desaturase gene in yeast, *Saccharomyces cerevisiae*. Molecular Biology Reports, 32: 215-226.
- [38] Buttarelli FR, Calogero RA, Tiboni O, Gualerzi CO, Pon CL, 1989. Characterization of the str operon genes from *Spirulina platensis* and their evolutionary relationship to those of other prokaryotes. Molecular and General Genetics, 217: 97-104.
- [39] Kasahara M, Yashiro K, Sakamoto T, Ohmori M, 1997. The *Spirulina platensis* adenylate cyclase gene, *cyaC*, encodes a novel signal transduction protein. Plant and Cell Physiology, 38: 828-836.
- [40] Kawamura M, Sakakibara M, Watanabe T, Kita K, Obayashi A, Takagi M, Yano K, 1986. A new restriction endonuclease from *Spirulina platensis*. Nucleic Acids Research, 14: 1985-1989.
- [41] Milano A, Rossi E, Zanaria E, Barbierato L, Ciferri O, Riccardi G, 1992. Molecular characterization of the genes encoding acetohydroxy acid synthase in the cyanobacterium *Spirulina platensis*. Journal of General Microbiology, 138: 1399-1408.
- [42] Tanioka Y, Miyamoto E, Yabuta Y, Ohnishi K, Fujita T, Yamaji R, Misono H, Shigeoka S, Nakano Y, Inui H, Watanabe F, 2010. Methyladeninylcobamide functions as the cofactor of methionine synthase in a Cyanobacterium, *Spirulina platensis* NIES-39. FEBS Letters, 584: 3223-3226.
- [43] Linjawi SA, 2011. Protective effect of spirulina against mitomycin c-induced genotoxic damage in male rats. Journal of American Science, 7: 922-931.