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# A Review: Influence of *Spirulina platensis* on the Gut Microfloral Diversity and its Potential as Probiotic

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***Abstract***

*The autotrophic cyanobacteria Arthrospira sp. is generally known as Spirulina sp. The unique nature of the cell is its high protein synthesis capacity. The protein content can reach up to 70% of the total cell mass. Spirulina sp. is a multicellular and filamentous cyanobacterium. They appear blue-green due to the presence of phycocyanin pigment. The cells arranged in cylindrical unbranched filamentous forms appear as helicoidal trichomes. Spirulina sp. are really noteworthy and they are explored to a great extent due to its antioxidant property. These microscopic plants show the major biological activities, including antioxidant, anticancer, antidiabetic, and anti- obesity. Though they are under-exploited for its biotic potential in the phytoplankton groups as probiotic and role in enhancing the gut microflora. This paper reviews the outlines of our understanding of Spirulina sp. and its immunomodulatory effects.*

## Introduction

*Spirulina sp*., is a blue-green algae that belongs to the class Cyanophyceae. Blue-green algae lack the nuclear membrane thus incorporated the prokaryote Kingdom by Stanier and Van Neil in 1962 and proposed to call these microorganisms as cyanobacteria. This designation was accepted and first published in 1974 in the Bergey's Manual of Determinative Bacteriology (Guglielmi et.al.,1993). They are found in a wide variety of environments such as in warm, alkaline, salty, and brackish water bodies as marine and fresh water species (Fogg et al.,1973). *Spirulina sp.* is characterized by its dark bluish green colour due to the presence of a pigment protein complex (Miranda et al.,1998). They create extensive algal blooms in all water bodies and are identified with bluish green color change in the water bodies (Zhang et al.,2015).

## Morphology and Structure

*Spirulina sp*. is a filamentous cyanobacterium and consisted of cylindrical cells arranged in unbranched filamentous, helicoidal trichomes. The regularly spaced cross walls divide the trichomes into cells connected by plasmodesmata. These filaments are motile and gliding movements. The characteristic features of the cells include helical shaped trichome and the gas filled vacuoles. The helical parameters vary with species and changes with environmental conditions such as growth temperature. The presence of vacuoles helps them to float on aquatic bodies as floating mats. The nitrogen fixing structures, heterocysts are absent. *Spirulina sp*. shows great plasticity in the morphological features and are due to the environmental factors.

The cyanobacteria are comparable to gram-negative bacteria due to the presence of a electron- dense heteropolymer peptidoglycan, that maintains the shape and osmotic protection to the cell (Van Eykelenburg, 1978). *Spirulina platensis* and *Spirulina maxima* are the larger species with irregularity of capsule around the filament such have a granular cytoplasm containing gas vacuoles and easily visible septa (Ciferri, 1983). The electron microscopic study of *Spirulina sp.* reveals the presence of capsule and four layered cell wall (Vonshak & Tomaselli, 2000). Just below the cell wall there is plasma membrane enclosing cytoplasm, which is rich in cytoplasmic inclusions (Fogg et al., 1973). The extreme outer membrane layer composed of materials

arranged linearly in parallel with the trichomes axis and is analogous to that of the present cell wall of the gram- negative bacteria. Trichomes are surrounded by a thin diffluent sheath and the sheath is about 0.5 μm thick and has a fibrillar net like structure. The sheath material excreted through the pores situated on the cell wall has been thought to be involved in the filament motion (Van Eykelenburg, 1978). Third layer composed of protein fibrils that wound helically around the trichomes. The second layer composed of peptidoglycan, folds towards inside of the filament along with fibrillar inner layer. Cyanophycin granules are abundant organelles occupying up to 18% of the cell volume. Carboxysomes, polyhedral bodies were present in S. platensis were the storage bodies for ribulose-1, 5-bisphosphatecarboxylase and other proteins (Pankrantz & Bowen, 1963). The gas vesicles are responsible for cell buoyancy and helps in the distribution of organism along the water bodies (Walsby, 1977). The vesicle membrane consists of coils of protein molecules arranged in ribs separated by 4 to 5 nm apart.

The prominent cytoplasmic structure, thylakoids appear to be arranged in concentric whorls in adult cells. Phycobilisomes appears to be attached to the thylakoids (Van Eykelenburg, 1979). Phycobilisomes acts as light harvesting antennae and possess photosynthetic pigment, phycobilins. Phycocyanins are attached to the thylakoids and their major function is photo protection. *Spirulina sp.* protoplast can be obtained with high efficiency by lysozyme treatment at the exponential phase of algal growth at 30°C (Abo-Shady et al., 1992).

The ultrastructural cell organization of Spirulina sp. is typical of prokaryotic organism,being devoid of morphologically limited nucleus, plastids and lack of membrane-bound organelles (Vonshak and Tomaselli, 2000) but displaying an outer gram-negative type envelope called cell wall (Marty and Busson, 1970). The thin cell wall has four layers, with an easily detectable electron-dense layer corresponding to the peptidoglycan layer (Van Eykelenburg, 1978). The trichome regularly spaced crosswalls divide the cells and are connected by plasmodesmata. Trichomes are surrounded by a thin diffluent sheath and the sheath is about 0.5 μm thick and has a fibrillar net like structure. The sheath material excreted through the pores situated on the cell wall has been thought to be involved in the filament motion (Van Eykelenburg, 1978). The most outer membrane layer (layer four) is composed of material arranged linearly in parallel with the trichomes axis and is considered analogous to that of the present cell wall of the gram-negative bacteria. Layer three is composed of protein fibrils wound helically around the trichomes, whereas the peptidoglycan layer (layer two) folds towards the inside of the filament, together with a putative fibrillar inner layer one, to the septum separating the cells and the septum separating the cells would be composed of the peptidoglycan layer only. The septum is thin disk in appearance and folded in part. This fold covers a portion of the septum surface and its extent seems to be related to the pitch of the trichome, the larger pitch, smaller the folded area and vice versa. Whereas in S. platensis the folds cover 5% of the total septum area, in S. laxissima is characterized by a much larger pitch and the fold covers a 3% of the septum area (Van Eykelenburg, 1979). Just below the cell wall there is plasma membrane enclosing cytoplasm, which is rich in cytoplasmic inclusions (Fogg et.al., 1973). The prominent cytoplasmic structure, thylakoids appear to be arranged in concentric whorls especially evident in adult cells. Phycobilisomes appears to be attached to the thylakoids (Van Eykelenburg, 1978).

The low electron dense areas thylakoid free areas are filled with ribosome and fibrils of DNA. The reserved material cyanophycin granules a reserve material composed of copolymers of amino acids. Cyanophycin granules are abundant organelles occupying up to 18% of the cell volume. Carboxysomes, polyhedral bodies were present in S. platensis were the storage bodies for ribulode-1, 5-bisphosphate carboxylase and other proteins (Pankrantz and Bowen, 1963). Gas vesicles in the shape of the hollow cylinders with cone-shaped ends with a diameter of 65 nm and a length of 1 μm. The vesicle membrane consists of coils of protein molecules arranged in ribs space 4 to 5 nm apart. The organelles are responsible of cell buoyancy and thus help in the distribution of organism along the waterbodies (Walsby, 1978).

**Ecology of Cyanobacteria**

*Spirulina sp.* serve as a unique, sustainable and alternative source of carotenoids (Safafar, et.al., 2015). As compared to the field crops, single cell cultivation of the microalgae are characterized by fast growth rate with high protein content. They require less water and land and are independent of climate. They can be genetically modified for desirable characters such as amino acid composition and temperature tolerance. Among the various microorganisms used as sources of SCP, the microalgae, *Spirulina sp.* is considered as the best source. The cell composition includes high protein content, low nucleic acids content, high concentrations of vitamins, growth factors. The presence of cell wall that is more easily digestible than that of other microbes,

indicates *Spirulina sp.* as a promising source of food.

**Records from the Indian Ocean**

The ecology of Spirulina sp. from the Indian Ocean includes detailed studies from and the specific locations of the Arabian Sea and the Bay of Bengal (Liu et al., 2018). Central Arabian Sea and Bay of Bengal are some of the plankton-geographical regions that have dominance of cyanobacteria (Badrish et al., 2005). Northern Arabian Sea has records on microalgae from sediment trap studies as well as on ecological tolerances. The studies from the northern Indian Ocean have reported the presence of cyanobacterial species. The ecological aspects such as their contribution to the overall productivity of the region, the importance in maintaining the carbon chemistry of the oceans, Spirulina as a primary producer and its contribution to the productivity as a primary trophic organism and influence on particulate inorganic carbon are not fully known (Rost & Riebesell, 2004).). Low primary productivity is generally seen in lower latitudes and high productivity in upwelling areas (Jyothibabu et al., 2004). Ryther et al., (1962) has experimentally proved that the Western Indian Ocean is one of the most productive oceanic regions around the world. In general, all the findings indicate that the distribution of Spirulina in Indian waters is scarcely studied and temperature is a determining factor in Spirulina growth and production. Regions between the Tropic of Cancer and the Tropic of Capricorn provide suitable temperature and are suitable for the Spirulina production (Antonio piccolo, 2012). More studies have carried out on Cyanobacterial distribution and its ecological significance in Indian waters (Mohan & Biju, 2020).

**Carotenoids**

Carotenoids are tetraterpenes (C 40 ) belongs to the class of secondary metabolites that have coloring property and are widely used in various industries and thus leads it market. The distinct colors of the organisms were constituted by two major classes of molecules such as carotenes (hydrocarbons) and xanthophylls (similar to carotenes but contain oxygen) (Lorenz & Cysewski, 2000). Carotenoids are widespread among living organisms, including both plants and animals, but they are found in greater concentration in certain microalgae. Carotenoids are abundant in green plants, but the presence is masked by green chlorophyll. Therefore, in recent years, the industrial interest towards the production of natural carotenoids from microalgae has considerably increased as they have advantages over terrestrial plants. Microalgae containing carotenoids are classified into two groups, xanthophylls (lutein, violaxanthin, neoxanthin, astaxanthin, flavoxanthin and zeaxanthin) and carotenes (alpha carotene, beta carotene and lycopene). Both types of carotenoids have antioxidant property and are commercially important (Cha, et.al., 2010). Based on the metabolism and function, the microalgae carotenoids can be classified into two, primary and secondary carotenoids. Primary carotenoids are structural and functional components which take direct part in photosynthesis whereas secondary carotenoids are pigments produced as a result to specific environmental stimuli (Krinsky, et.al., 2004). Primary and secondary carotenoids placed an important role as natural colorants as well as their potential in human health. Specifically, they possess a wide range of distinctive biological activities, including antioxidant, anticancer, antidiabetic, and anti-obesity, which have been recently reviewed (Zhang, et.al., 2014). The studies show that carotenoids from microalgae Spirulina have the ability to enhance immunity and are considered as a powerful tonic to boost immune system (Gordon & Pesti, 1971). It enhances the distribution of gut microbiota, its therapeutic values, diseases susceptibility and the metabolic outputs of the host health (Flint et.al., 2012).

**Effect of dietary carotenoids**

Mice models are extensively being used to study the role and functioning of the gut microbiota and its association with diseases. In research, mice models are widely used of its physiological and anatomical similarities to humans (Nguyen et.al., 2015). Many metabolic disorders in human is linked to the gut microbiota composition and its diversity and it implies the significance of maintaining a diverse and healthy gut microbiota (Russell et.al., 2012). The consistent and systematic introduction of dietary carotenoids from cyanobacteria photo protective pigments induced changes in the gut flora. The composition of the human gut microbiota has been investigated in several studies 16S rDNA sequencing approaches (Arumugam et.al., 2011). Mice faecal pellets were collected for DNA sequencing and analyzing the bacterial diversity with and without dietary carotenoids.

Mice models are powerful tool to perform experiments. The human gut microbiota and the mice models have 99% similarly. These models offer opportunities to experiment and find out the possibilities to enhance the gut microbiota in health and disease. Such studies are extremely valuable for understanding the underlying disease mechanisms and causative agents, and thus for getting closer to developing preventive or therapeutic treatments. The intestine of all organisms are colonized by functionally stable climax community of microbes, referred to as the gut microbiota. The presence of metabolically active microbial population that has a substantial impact on the host health. A close relationship exists between the host and its gut inhabitants, comprising microbial modulation of epithelial gene expression and signal transduction pathways, and an extensive host microbe co-metabolism (Li et.al. 2008).

The importance of the gut microbiota to overall host biology, that variation of gut microbiota and its diversity can have a obvious effect on metabolic and immunologic responses of the mammalian host (Yap et.al. 2008). In this study, evidence is provided that dietary supplementation of blue green algae, Spirulina platensis leads to alterations in the gut microbiota in mice. The gut microbiota has a profound impact on the physiology, immunology, metabolism and health of the host and recent studies have shown that even subtle changes in the gut bacterial community have an impact on host phenotypes (Rohde et.al. 2007).

The findings of this studies have permitted to determine the influence of blue-green algae consumption on the human gut microbiota. The findings further warrant investigations on how alteration of the gut microbiota induced by blue green algae, and impact the host. The variations of the gut microbiota should be considered when the pharmaceutical characteristics of blue-green algae are studied. Putative pharmaceutical effects of micro algal species in animals and humans might be attributed the alteration of the gastrointestinal microbiota. To sustain or restore the intestinal bacterial homeostasis in healthy individuals or disease states, several approaches have been implemented like oral supplementation of probiotics (Verdu,2009).

The nutraceuticals derived from the food source used as oral supplementation has been widely used helps in disease prevention. They are capable in disease control due to its potential capacity of promoting the growth of commensal gut microorganisms (Wu, et.al., 2011; Spor, et.al., 2011). *Spirulina* sp. is a commonly consumed microalga. Its potential benefits and therapeutic properties as a food supplements has been widely recognized in human health (Flint, et.al., 2012.,Geller, et.al., 2017). Recent studies in animal models have shown that *Spirulina sp*. can modify the composition of gut microbiota thus may link to improved health status. The unique characteristics of microalgae have prompted researchers and scientists to think about their potential efficiency around the globe (Rasmussen, et.al., 2009; Yusuf, et.al., 2016). The findings in this review pave the way for developing an effective platform to use Spirulina carotenoids in several applications including food supplements and pharmaceutical drugs. Spirulina sp has been demonstrated as a safe food for production of natural edible carotenoids. It In this review paper the beneficial effects of Spirulina carotenoid as nutritional and dietary supplements with special focus on its extraction techniques, immunomodulatory and antioxidant effects to mitigate gut dysbiosis.

**Recent observations**

The existing environmental circumstances, changes in the food stuff, physical and mental stress are the common risk associated with humans and enhance the death rate. Due to these reasons, new trends are being extensively developed in modern medicine and biotechnology to treat and prevent various diseases. Biotechnology associates cyanobacteria, *Spirulina sp*., which have been widely used as food and feed additives in agriculture, food industry, pharmaceuticals and medicine (Ebrahim, 2020). The unique characteristics of blue-green algae have prompted researchers and scientists to think about their potential efficiency around the globe. The findings in this review revealed background knowledge about Spirulina. It is identified that the Spirulina is a potential autotrophic primary producer for several applications including environmental implications, sustainable agriculture practice and pharmaceutical drugs (Aondohemba, et.al., 2020). Microalgae, Spirulina production occupies only a small amount of footprints with considerable efficiencies and energy consumption. Its production is suitable in saline and alkaline conditions and the presence of easily digestible proteins with anti-carcinogenic, antioxidative, anti-hypertensive and hepatoprotective activity (Akshita et.al.,2019).

## Conclusion

Spirulina is easy culture in aquatic bodies and economical to maintain and harvest and are also processed easily. Thus, Spirulina gained considerable amount of popularity in the food industry by as a dietary supplement. Even with all these unique features, the research in the domain of blue- green algae is still ongoing. However, the results are promising hence Spirulina supplement can be added to regulate diet to protect health now and in the future.

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