CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomic classification of cyanobacteria

Taxonomic classification is a method for understanding of the biodiversity of organisms on earth based on phylogenetic background, ecological data, morphological variation and molecular biology (Komárek, 2005). The cyanobacteria, cyanoprokaryota, cyanophytes or blue green algae are fascinating organisms. They are gram – negative prokaryotic bacteria which their cells developed the plant-type photosynthetic apparatus including chlorophyll *a* and both photosystems, I and II. Thus, they are able to perform oxygenic photosynthesis (Castenhöltz, 2001a). Cyanobacteria occur in most environments. They are cosmopolitan and widely distributed in freshwater and marine environments. They are also commonly found in the soil and on rocks from the tropics to polar regions and from temperate climates to extreme arid deserts, where they sometimes participate in the formation of microbial crusts or mats (Bold and Wynne, 1985; Mazor *et al*., 1996).

Geitler (1932) reported that, classification of cyanobacteria was based on morphology and ecology information, as its most prominent representative. However, it does not reflect evolutionary relation between taxa. Moreover, the morphological diversity of cyanobacteria is the result of diverse environmental conditions on the restricted number of genotypes (Drouet, 1981). The bacterial nature of the "blue-green algae" put forward a proposal for their integration under the Bacterial Code of Nomenclature. This concept was realized in the system of Rippka (1988),

which is based on phenotypic and genotypic characters, only on those strains brought to culture. In addition, the system of cyanobacteria based on all available type of information such as morphological, ecological, genetic and ultrastructural on both cultivated and uncultivated cyanobacteria was created by Anagnostidis and Komarek, (1985, 1990). This system uses botanical code squares with the tradition of cyanobacteria systematics and that the acceptance of the bacterial code would only cause more confusion. Two cyanobacterial systematics; the system of Castenhölz, (2001b) was based on the bacteriological code and Komárek (1999, 2005) system was created according to the rules of the botanical code of nomenclature that traditionally studied by botanist is still very complex. So, Oren (2004) proposed the formation of the consensus nomenclature that would be acceptable for both bacteriologists and botanists.

2.1.1 Polyphasic taxonomy

The term polyphasic taxonomy was introduced by Colwell (1970) referred by Boone *et al*. (2001). It is a taxonomy that assembles and assimilates many levels of information, from molecular to ecological and incorporates several distinct and separable portions of information extractable from a non homogeneous system to yield a multidimensional taxonomy. Recently, polyphasic taxonomy refers to a consensus type of taxonomy and aims to utilize all the available data in delineating consensus group, decisive of the final conclusion. For the main methodological approach in cyanobacterial taxonomy, which is important for modern classification of cyanobacteria, combined with (i) ecological, ecophysiological and biogeographical studies, (ii) morphological variation limits in nature and in cultures, (iii) data about

ultrastructure including explanation of cytological structures in cyanobacterial cell, (iv) biochemical characteristics and their stability and (v) molecular analyses mainly those concerning diversity, diversification processes and speciation and phylogenetic relations (Anagnostidis and Komárek,1988; Castenhölz, 2001a; Suda *et al.*, 2002; Flechtner *et al.*, 2002; Komárek, 2006).

2.1.2 Modern approach to the classification of *Nostochopsis*

Nostochopsis belongs to

Domain Bacteria

Phylum	Cyanobacteria
Class	Cyanobacteria
Subclass	Nostocophycideae
Order	Nostocales
Family	Hapalosiphonaceae
Genus	*Nostochopsis* Wood ex Bornet and Flahault, 1886

Source: The Taxonomicon and Systema Naturae, (2000)

In the past, *Nostochopsis* belonged to Division Cyanophyta, Class Cyanobacteria, Order Stigonematales based on the morphological characters and recognized by the classic botanical taxonomy (Anagnostidis and Komárek, 1990, Peerapornpisal, 2006a). This order show filamentous heterocytous type (terminal, intercalar, lateral and intercalar in the polyseriate trichome) (Figure 2.1) and true branching (Y-branching, T-branching) (Figure 2.2). There are 9 species of *Nostochopsis* at present *i.e N. lobatus* Wood em. Geitler 1886, *N. radians*

Bharadwaja, *N. hansgirgii* Schmidle, *N. hansgirgii* var. sphaericus N.L. Gardner, *N. goetzei* Schmidle, *N. rupestris* Schmidle, *N. stagnalis* (Hansgirg) Pascher, *N. transvaalensis* Welsh and *N. wichmannii* Weber-van Bosse (Guiry and Guiry, 2013).

Subsequently, there are heterocyte forming cyanobacteria supported by molecular analyses such as 16S rDNA gene sequences (Wilmotte and Herdman, 2001; Lyra *et al*., 2001), *nif*H sequences (Gaby, 2012) and RELP and genomic fingerprinting (Lyra *et al*., 2001). *Nostochopsis* belongs to the Order Nostocales (subsection V of Bergey's Manual, Castenhölz, 2001b). The Nostocales strains which are the true branching cyanobacteria, are polyphyletic and should be separated into two groups.

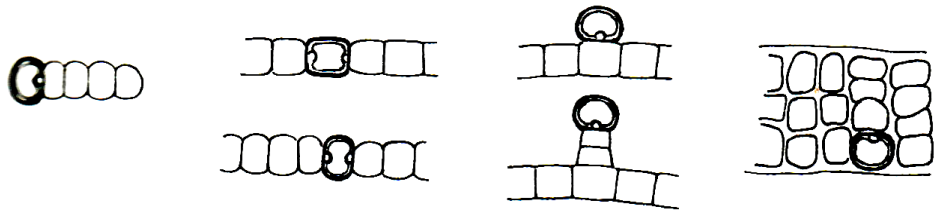


Figure 2.1 Type of heterocyte position based on branching (Anagnostidis, and Komárek,1990)
A) Terminal **B)** Intercalar **C)** Lateral **D)** Intercalar (in polyseriate trichome)

The first group is characterized by T-branching and the second group by Y-branching (Gugger and Hoffmann, 2004; Komárek and Kaštovský 2003 and Hoffmann *et al*., 2005). *Nostochopsis* belongs to the Family Hapalosiphonaceae which is characterized by intercalary bipored, more or less spherical heterocytes

which are wider than vegetative cell, or lateral unipored heterocytes, attached to the vegetative cells, forming at the ends of short branches (with 1 or few cells) (Figure 2.2).

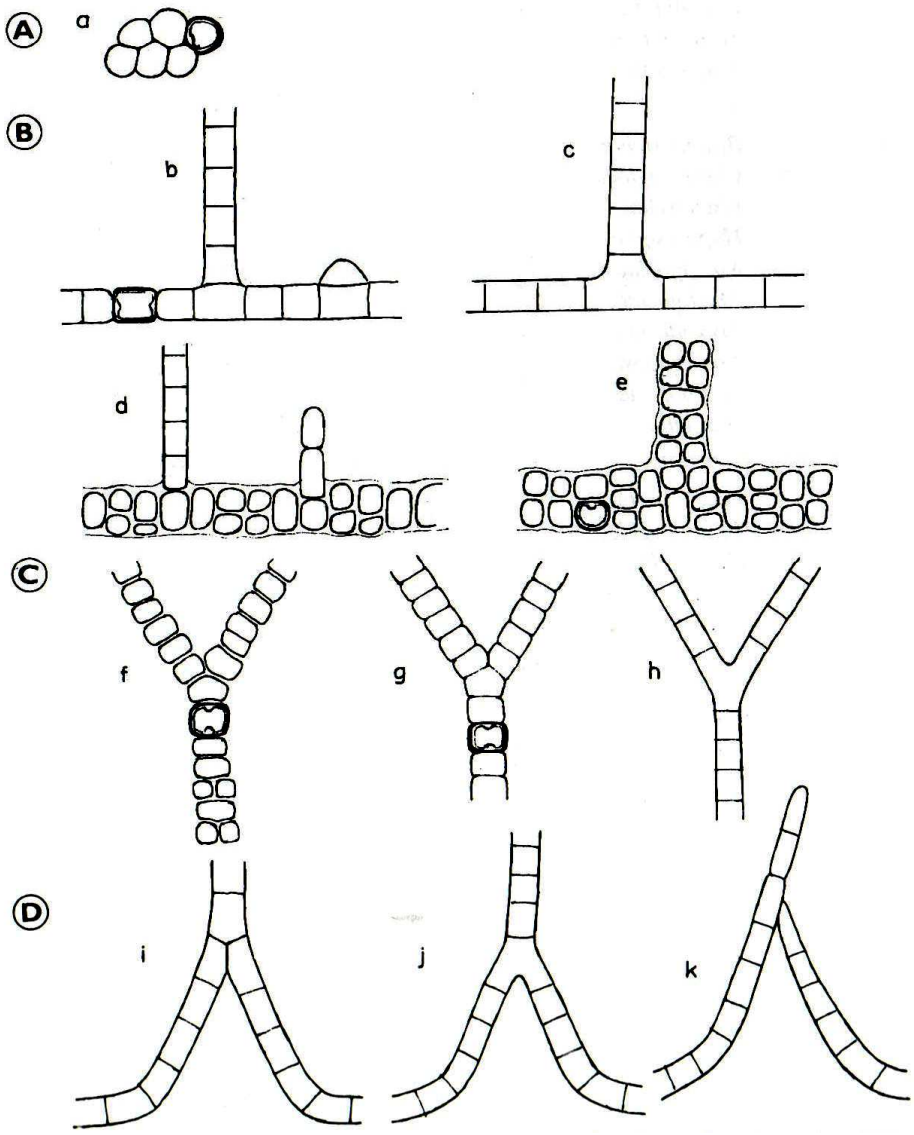


Figure 2.2 Type of true branching in stigonematalean genera (Anagnostidis and Komárek, 1990) **A**-Irregular clustering (X-branching), a-*Chlorogloeopsis*-type; **B**-Lateral (T-branching), b- *Hapalosiphon*-type, c-*Westiella*-type, d- *Fischerella*-type, e-*Stigonema*-type; **C**-pseudodichotomous (V-branching), f- *Hyphomorpha*- type, g- *Loriella*-type, h-*Mastigocoleopsis*-type; **D**- reverse (Y-branching)

Table 2.1 The modern systematic classification of cyanobacteria according to recent molecular and polyphasic approach. List of nostocalean genera which were confirmed by 16S rRNA gene sequencing are printed bold.

Nostocineae	Nostocales heterocytous, akinetes present	**Scytonemataceae** isopolar, false branching	Kyrtuthrix, Scytonematopsis, **Scytonema**, **Brasilonema**, Scytonema subg. Myochrotes, Petalonema
		**Symphyonemataceae** true branching (Y)	Brachytrichia, Handeliella, Herpyzonema, Iyengariella, Mastigocladopsis, **Symphyonemopsis**, **Symphyonema**, Spelaeopogon, **Umezakia**
		Rivulariaceae heteropolar, hairs	**Calothrix**, Dichothrix, Gardnerula, Gloeotrichia, Isactis, Rivularia, Sacconema (sine typo)
		Microchaetaceae heteropolar	Borzinema, Camptylonemopsis, **Coleodesmium**, Fortiea, **Hassallia**, **Microchaete**, **Rexia**, **Spirirestis**, Sacconema, Seguenzaea, **Tolypothrix**
		Nostocaceae isopolar, without branching	**Anabaena**—planktic (**Dolichospermum**), **Anabaena**—benthic, **Anabaenopsis**, **Aphanizomenon**, Aulosira, Capsosira, **Cuspidothrix**, **Cyanospira**, **Cylindrospermopsis**, **Cylindrospermum**, Hydrocoryne, Isocystis, Macrospermum, **Mojavia**, **Nodularia**, **Nostoc**, Raphidiopsis, Richelia, **Sphaerospermum**, Stratonostoc, **Trichormus**, Wollea
		Chlorogloeopsidaceae	**Chlorogloeopsis**
		Hapalosiphonaceae true branching (T)	Albrightia, Brachytrichiopsis, Chondrogloea, Colteronema, **Fischerella**, Geitleria, **Hapalosiphon**, Leptopogon, Loefgrenia, Loriella, **Mastigocladus**, Matteia, Mastigocoleopsis, **Mastigocoleus**, **Nostochopsis**, Parthasarathiella, Thalpophila, **Westiella**, Westiellopsis
		Stigonemataceae true branching, multiseriate	Cyanobotrys, Desmosiphon, **Doliocatella**, Homoeoptyche, Nematoplaca, Pulvinularia, Stauromatonema, **Stigonema**

Source: Komárek (2010)

2.1.3 Ecology and distribution of *Nostochopsis*

Ecology and distribution of *Nostochopsis* spp. were in fresh water. They were found in many countries (Table 2.2).

Table 2.2 Ecology and distribution of *Nostochopsis* spp.

Species	Habitat/Country	References
Nostochopsis sp.	Freshwater in southern France	Frémy and Feldmann (1934)
Nostochopsis sp.	Freshwater in Argentina	Caceres (1973)
Nostochopsis sp.	Freshwater near Bethell's Beach, Auckland, New Zealand	Sarma and Chapman (1975)
Nostochopsis sp.	At Darling Downs, Leichhardt, Moreton Moreton, North Kennedy and Wide Bay, America.	Matthews (1982)
Nostochopsis sp.	Australia and New Zealand	Day *et al*. (1995)
Nostochopsis sp.	Australia and New Zealand	Phillips (2002)

Table 2.2 (continued) Ecology and distribution of *Nostochopsis* spp.

Species	Habitat/Country	References
Nostochopsis sp. (*Mastigocladopsis jogensis*)	On submerged stone in freshwater, Corsica, France	Gugger and Hoffmann (2004)
Nostochopsis sp.	Freshwater in Argentina	Rodriguez *et al*. (2006)
Nostochopsis sp.	Freshwater in Czech Republic	Kaštovský *et al*. (2010)
Nostochopsis sp.	At Darling Downs, Leichhardt, Moreton Moreton, North Kennedy and Wide Bay, America.	Bostock and Holland (2010)
N. lobatus	A cryptoendolithic cyanobacteria in sandstone in South Africa	Gugger and Hoffmann (2004)
N. lobatus Wood em. Geitler	In running and stagnant waters, Madhabkunda waterfall area at Maulvi Bazar, Bangladesh	Azi (2008)
N. lobatus Wood em. Geitler	Freshwater in Spain	Moreno and Aboal (2010)
N. lobatus Wood em. Geitler	Freshwater in Spain	Monteagudo *et al*. (2011)
N. radians sp. nov	Grow on submerged stones in a shallow stream running in a deep shady valley in the Jog Falls region of Mysore state, India	Bharadwaja (1934)
N. wichmannii Weber van Bosse 1913	The temperate zone of the northern hemisphere from hot springs at 30 °C of Japan	Anagnostidis and Komárek (1990)

In Thailand, the first report of *Nostochopsis* spp. was by Smith, (1934), referred by Lewmanomont (1978) who also found *N. lobatus* Wood em Guitler in Chiang Mai and Chantaburi Provinces. These cyanobacterial colonies attached on rock and grew in fresh water stream or waterfall. Peerapornpisal *et al.* (2006b) and Thiamdao *et al*. (2011) found two species of *Nostochopsis*, *N. hansgrig* Schmidle and *N. lobatus* Wood em Guitler from stone substrate of Nan River. They were found in cold dry and hot dry seasons from November to April only. In rainy season, due to

strong water current and high turbidity, these cyanobacteria could not grow. These species grow in clear running water with the turbidity not exceeding 20 NTU, the velocity of 2-3 $m.s^{-1}$, the temperature of 20-27 ℃, pH 7-8 with oligotrophic (clean water quality) to mesotrophic status (moderate water quality).

2.1.4 Potential and cultivation of *Nostochopsis*

Nostochopsis spp. contain high nutritional value (Table 2.3) such as protein, carbohydrate and lipid. Besides, including high fiber, vitamins and minerals such as vitamin A, niacin, vitamin B1, B2, B6, vitamin C and selenium. In addition, they have calcium as high in content as the small fish, which are eaten whole, as calcium 6,405 $\mu g.100\ g^{-1}$ dry wt. (Peerapornpisal *et al*., 2006b; Thiamdao *et al*., 2011).

Table 2.3 Nutritional values of *Nostochopsis lobatus* Wood em. Geitler

Nutrients	Values	
Lipid	0.64	%
Protein	19.10	%
Carbohydrate	31.94	%
Fiber	2.05	%
Vitamin C	1.07	$mg.100g^{-1}$ dw
Vitamin B1	0.12	”
Vitamin B2	0.07	”
Niacin	2.48	”
Calcium	6,405	”
Sodium	136.9	”
Potassium	0.5	”
Chloride	0.3	”
Magnesium	265.4	”
Manganese	4.5	”
Iron	114.9	”
Zinc	0.65	”
Selenium	37	$\mu g.100\ g^{-1}$ dw

Source: Thiamdao *et al*. (2011)

They are rich in pigments such as chlorophyll 21.63 mg.g dry wt^{-1}, carotenoid 3.95 mg.g dry wt^{-1}, phycocyanin 98.50 mg.g dry wt^{-1}, phycoerythrin 158.0 mg.g dry wt^{-1} (Pandey and Pandey, 2008a). Besides, *Nostochopsis* spp. substantiate the nutraceutical potential. *N. lobatus* exhibited anti-gastric ulcer activity, as well as anti-inflammatory, and antioxidant activity by 1,1-dipheny l-2- picrylhydrazyl (DPPH) radical scavenging activity assay. The ethanolic extract of these cyanobacteria clearly revealed a higher antioxidant activity than the aqueous extract as shown in Table 2.4 (Thiamdao *et al*., 2011). Pandey and Pandey (2008b) reported that, it showed antioxidant activity as 140.50 μM ascorbic acid equivalent capacity g fresh wt^{-1}. Furthermore, the aqueous extract of *Nostochopsis* spp. exhibited 77% inhibition of the ethyl phenyl propiolate (EPP) induced inflammation in ear and paw edema. In addition, this cyanobacterial extract given orally to rats showed 94% of gastroprotective activity against induced gastric ulcer (Amornlerdpison *et al.*, 2011).

Table 2.4 The 50% inhibition concentration (IC50) for ABTS

Extract	ABTS IC50 ($mg.mL^{-1}$)	Maximum antioxidant capacity (μM of trolox equivalent per gram of wt)
Aqueous extract	25.79	30
Ethanolic extract	5.36	250.3

Source: Thiamdao *et al*. (2011)

Cultivation of *Nostochopsis* spp. showed maximal growth in half concentration of semi-solid BG-11 media. They produced higher amount of mucilage substance than those cultivated in the liquid media (Mungmai, 2006). Tiwari (1978) found that *N. lobatus* forms infrequent intercalary heterocyte in the main trichome

while the short lateral branches (1-4 celled) characteristically produce long lateral branches during the later stages of growth in ammonium medium. Pandey and Pandey (2008a) studied enhanced production of *N. lobatus* in a full factorial design with supplemental zinc, glutamine, and zinc + glutamine in batch culture. Production of biomass, pigments, and antioxidant capacity were higher under immobilized cell cultures in comparison to free cell cultures. Maximum biomass of 2,390 mg dry wt, delta-aminolevulinic acid (δ-ALA) 2.715 μg.mg dry $wt^{-1}.h^{-1}$ were recorded when zinc and glutamine were supplemented together in the growth medium at pH 7.8. Pandey and Pandey (2008b) also showed potentially improvement of immobilized cell cultures of *N. lobatus* when a mixture of phosphorus and ferric was supplemented. Algal biomass, pigments, nutritional value and antioxidant capacity increased. When considered separately, phosphorus appeared to be a better supplement than ferric for the production of biomass, chlorophyll and carotenoids. However, for phycocyanin, phycoerythrin, nutritional value and antioxidant , ferric appeared more effective than phosphorus. Those studies indicated that *N. lobatus* is a promising bioresource for enhanced production of nutritionally rich biomass, pigments and antioxidants.

2.2 Polysaccharides

Polysaccharides are polymeric carbohydrate structures of repeating units of either monosaccharides or disaccharidess joined together by glycosidic bonds. Polysaccharides are often heterogeneous, containing slight modifications of the repeating units. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water (Varki *et al*., 1999; 2008).

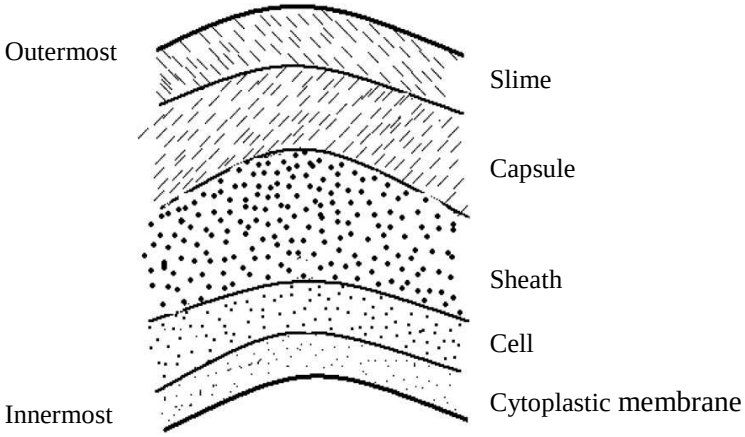
Polysaccharides have a general formula of $Cx(H_2O)y$ where x is usually a large number between 200 and 2500. The repeating units in the polymer backbone are often six-carbon monosaccharides, the general formula can also be represented as $(C_6H_{10}O_5)n$ where $40 \leqslant n \leqslant 3000$ (Varki *et al*., 1999; 2008).

They were classified according to their degree of polymerization (DP) and are divided initially into three principal groups namely sugar (DP 1-2) such as monosaccharide, disaccharide and polyols, oligosaccharide (DP 3-9) consisted of malto-oligosaccharide and other oligosaccharide and polysaccharide (DP >9) which are divided in 2 sub-groups; i) starch consisted of amylose, amylopectin and modified starches, ii) non-starch polysaccharide consisted of cellulose, hemicellulose, pectins and hydrocolloids (FAO/WHO, 1997).

2.2.1 Cyanobacterial polysaccharides

Bacteria and many other microbes, including fungi and algae, often secrete polysaccharides as an evolutionary adaptation to help them adhere to surfaces and to prevent them from drying out (Varki *et al*., 2008).

Many cyanobacteria are surrounded by mucilaginous external layers called capsule, sheath, mucilage, glycocalyx or slime (Figure 2.3). The exocellular mucilaginous material is mainly polysaccharide in nature (Li *et al*., 2001a). The capsule or slime layers of some other cyanobacterial strains have also been reported to posses hydrophobic properties (Fattom and Shilo, 1985; Kidron *et al*., 1999).



Source: Adhikary (1998)

Figure 2.3 Schematic diagram of cell surface in cyanobacteria

2.2.2 Types of cyanobacterial polysaccharides

There are three types of polysaccharide: (i) a sheath, a thin uniform structured external layer immediately next to the outer membrane, containing either concentric or radial fibres, depending on the strains. (ii) a capsule or slime (capsular polysaccharide, CPS), which is intimately associated with the cell surface and may be covalently bound with well-defined limits. In contrast, slime polysaccharide is only loosely associated with the cell surface without sharply defined limits. (iii) soluble polysaccharide (released polysaccharide, RPS), released by many cyanobacteria into the media (Bold and Wynne, 1985; Li, *et al*., 2001a).

Monosaccharide of cyanobacterial can be divided into 4 categories according to their chemical structures: (i) the hexoses such as glucose, galactose, and mannose; (ii) the pentoses such as ribose, xylose, and arabinose; (iii) the deoxyhexoses such as fucose and rhamnose; and (iv) the acidic hexoses such as glucouronic and galactoronic acids (de Philippis *et al*., 1998). In few cases, some monosaccharides

with methyl or amino functional groups have been reported, such as amino sugars e.g., N-acetyl-gluco and galactosamine, methylated sugars e.g., 3-O-methyl-pentose, 3-O-methyl deoxyhexose, 4-O-methyl hexose, and other rare sugars e.g., 4-O-[1-carboxyethyl] mannose (Morvan, 1997). Glucose is the most frequently found monosaccharide (in more than 90% of cyanobacterial polysaccharides), followed by galactose, mannose, and rhamnose, respectively and xylose is the most common pentoses (Bertocchi *et al*.,1990).

Cyanobacterial CPSs consist of various neutral monosaccharides ranging from two to nine units depending on the examined strains (Table 2.4). Most cyanobacterial CPSs also contain glucuronic and/or galacturonic acids. Plude *et al*. (1991) referred that, the slime polysaccharide of *Microcystis flos-aquae* resembles that on the plant polysaccharide pectin.

Table 2.5 Chemical composition of CPSs from cyanobacteria

Species	X	A	F	R	Ga	G	M	UA	Others
Anabaena cylindrical	+	-	t	-	+	d	+	-	
Anabaena flos-aquae	+	-	-	-	-	+	-	+	r
Anabaena sphaerica	-	-	-	d	d	d	+	-	
Anabaena WSAF	d	+	+	+	+	+	-	+	o
Cyanospira capsulata	-	+	+	-	-	+	+	d	
Nostoc sp.	-	+	+	-	-	+	-	+	
Palmella mucosa	-	+	+	-	-	+	-	+	
Scytonema hofimanni	-	-	t	-	t	d	-	-	
Fischerella muscicola	+	+	+	-	+	d	+	-	
Mastigocladus laminosus	+	t	+	+	+	d	+	+	

X, Xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid

+, present; -, absent;d, dominant; t, trace; o, osamine; r, ribose

Source: Li *et al*. (2001a)

Cyanobacterial RPS contain five or more monosaccharides (Sutherland, 1994). They are neutral sugars such as xylose, arabinose, fucose, rhamnose, galactose, glucose, mannose and uronic acids (Table 2.5). Glucuronic and/or galacturonic acids are present in most cyanobacterial RPSs. Usually, glucose is the dominant monosaccharide in the RPSs.

Table 2.6 Chemical composition of RPSs from cyanobacteria

Species	X	A	F	R	Ga	G	M	UA	Others
Anabaena ATCC 33047	d	-	-	-	+	+	+	+	pr
Anabaena flos-aquae-A-37	+	-	-	-	-	d	-	+	r
Anabaena sphaerica	-	+	-	-	d	+	+	-	
Nostoc commune DRH-1	+	-	-	-	+	d	-	+	r
Nostoc sp. PCC 7423	+	-	+	+	+	d	+	+	
Palmella mucosa	-	+	+	-	-	+	-	+	
Scytonema hofmanni	-	-	-	-	+	+	+	d	
Tolypothrix tenuis	-	+	+	+	+	d	+	-	
Chlorogloeopsis sp. 6912	-	+	+	+	+	d	+	+	
Fischerella muscicola	+	-	+	-	+	d	+	-	

X, Xylose; A, arabinose; F, fucose; R, rhamnose; Ga, galactose; G, glucose; M, mannose; UA, uronic acid

+, present; -, absent; d, dominant; t, trace; pr, protein; r,ribose

Source: Li *et al*. (2001a)

2.2.3 Biosynthesis and production of cyanobacterial polysaccharides

Different environmental, nutritional, chemical and physical parameters affect biosynthesis and production of the cyanobacterial exopolysaccharide. Moreover, exopolysaccharide production is strain dependent. Filali-Mouhim *et al*. (1993) reported that the proportion of galactose in CPSs extracted from *Spirulina platensis* cultures of different ages is significantly different. *Chroococcidiopsis* increased envelope thickness which is useful in the prevention of water loss (Grilli-Caiola *et al*.,

1996). *Phormidium* sp. J-1 and *Anabaena circularis* 6720 produced extracellular cell-bound flocculants related to the co-flocculation with suspended clay particles. In addition, *Phormidium* sp. J-1 produced a polymeric extracellular emulsifying agent when cultured with substrate (Bar-Or and Shilo, 1988). In, *Anabaena flos-aquae,* the intracellularly synthesized polysaccharide may diffuse through the cell wall into the surrounding medium (Li *et al*., 2001b). Nitrogen starvation caused an increase in total exopolysaccharide production of *Spirulina* (Filali-Mouhim *et al*., 1993). Nicolaus *et al*. (1999) reported that the dramatic decrease in polysaccharide yield occurred when *Phormidium* was grown with a light–dark cycle, in the absence of aeration and phosphorus. High NaCl concentration decreased RPS production in *Anabaena* sp. ATCC 33047. RPS production in *Anabaena* sp. ATCC 33047 was markedly enhanced by increased in temperature (Moreno *et al*., 1998). In cyanobacterial cultures, the production of polysaccharides appeared to depend on the C:N ratio. A change in light intensity, temperature, and the concentrations of sulfur, iron, phosphate and potassium also affected polysaccharide production (de Philippis, 1998).

2.2.4 Potential of cyanobacterial polysaccharides

Cyanobacteria have an influence on soil structure and fertility due to their polysaccharide-excreting capacity as well as nitrogen-fixing activity. *N. muscorum* has effect on soil physical, chemical and biological properties and improved seeding emergence (Li *et al*., 2001a). Polysaccharide-releasing cyanobacteria can tolerate high concentrations of heavy metals by binding them with living cells or dry cells, and consequently mitigating the toxicity of the heavy metals. One recent study shows that a non toxic cyanobacterium, *Gloeotheca magna*, can remove cadmium and

manganese (Mohamed *et al*., 2001). Cyanobacterial polysaccharide is suitable for forming stable gels, fibers, films, and liquid crystals; stabilizing suspensions and emulsions; enhancing viscosity of aqueous solutions; serving as flocculants and could be used in the food industry (Liu and Chen, 2006). Cyanobacterial monosaccharides and polysaccharides could be applied in medical antiviral therapy (Witvrouw *et al*., 1997; Schaeffer *et al*., 2000). Kaji *et al*. (2002) reported that, sulfated polysaccharide from *Spirulina platensis* consisting of two types of disaccharide repeating units, O-hexuronosylrhamnose (aldobiuronic acid) and O-rhamnosyl-3-O-methylrhamnos (acofriose) with sulfate groups, showed anti-atherogenic and anti-thromobogenic activities.

2.3 Prebiotics

Currently, there is an interest in the use of food which may exert a positive functional effect on health. Two of these ‘‘functional food’’ are known as probiotics and prebiotics, both of which have a favorable effect on the ‘‘good’’ bacteria that reside in the digestive system, also known as gut microflora (Gibson and Roberfroid, 1995).

2.3.1 Definition of prebiotics

A prebiotic was defined by Gibson and Roberfroid (1995) as: a non-digestive food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health. In addition, a new prebiotic definition as a selectively fermented ingredient that allows specific changes; both in the composition and/or activity in the

gastrointestinal microbiota that confers benefits upon host well-being and health (Gibson *et al*., 1995; Roberfroid, 2007). Prebiotics include carbohydrates that are not digested in the upper part of the gastrointestinal tract but selectively fermented by bacteria in the colon. This selective fermentation affects the composition of the intestinal microflora by stimulating bifidobacteria and lactobacilli, both in humans and in animals, where these bacteria have health promoting properties (Van *et al*., 1999; Flamm *et al*., 2001).

2.3.2 Prebiotic properties

According to this definition, candidate prebiotics must fulfill the following criteria which are to be proven by *in vitro* and *in vivo* tests; (i) non-digestibility such as resistance to low pH of gastric acid, enzymatic digestion and intestinal absorption; (ii) fermentation by the intestinal microbiota and (iii) selective stimulation of growth and activity of intestinal bacteria (de Vrese and Schrezenmeir, 2008).

2.3.3 Type of prebiotics

2.3.3.1 Substrate utilization in the colon

The resident gut microbiota ferments substances that cannot be digested by the host in the small gut, these include resistant starch, non-digestible carbohydrates, oligosaccharides, proteins and mucins (Gibson and Roberfroid, 1995). The two main types of fermentation that are carried out in the gut are saccharolytic and proteolytic that produced the short chain fatty acids (SCFA), acetate, propionate and butyrate. All contribute towards the host's daily energy requirements. Acetate is metabolised in systemic areas like muscle, while propionate is transported to the liver and used to

generate ATP. Butyrate is an important source of energy for the colonocytes and is thought to have anti-tumour properties (Cummings *et al*., 1989).

2.3.3.2 Fibre fermentation by gut bacteria

Foods rich in dietary fibre include vegetables, fruits, cereal grains and legumes. Dietary fibres display different degrees of solubility. Some such as pectins, hemicellulose, guar gum and inulin are readily soluble in water. It leads to the formation of gels in the gastrointestinal tract. Bacterial fermentation results in lowering colonic pH. Lower pH values impede the growth of certain pathogenic bacterial species while encouraging the growth of the bifidobacteria and lactic acid microflora. A low colonic pH may also aid in the excretion of carcinogens, which bind to dietary fibre in the colon (Gibson *et al*., 2004 referred from Rowland, 1995).

2.3.3.3 Oligosaccharides as prebiotics

Oligosaccharides have been tested using various *in vivo* methods such as animal models and human clinical trials. Oligosaccharides used as prebiotics are;

Lactulose

Lactulose is a synthetic disaccharide in the form of 4-O-β-D-galactopyranosyl-β-D-fructofuranose. Lactulose was originally used as a laxative as it is not hydrolysed or absorbed in the small intestine. Lactulose has also received attention as a bifidogenic factor and has been administered (Gibson *et al*., 2010). Tuohy *et al*. (2002) reported that, the prebiotic effects of lactulose were monitored in a human feeding study. Bifidobacteria showed a statistically significant increase during lactulose intake, whilst genetic probing showed a concomitant decrease in *Clostridia*.

Viable plate counts of lactobacilli increased when lactulose was fed, but this was not replicated by the genetic probing. The prebiotic nature of 10 $g.day^{-1}$ lactulose towards the human gut microbiota has been clearly demonstrated. Lactulose is shown to be an effective food-grade prebiotic for healthy adults particularly in sections of the community with low bifidobacterial populations.

Inulin and fructo-oligosaccharides

Inulin is a polysaccharide in the form of α-D-glucopyranosyl-[β-D-fructofuranosyl] (n-1)-D-fructofuranosides, where n>10. The structural relatives of inulin, fructo-oligosaccharides (FOS, a lower molecular weight version) have been the best documented oligosaccharides for their effect on intestinal bifidobacteria and are considered important prebiotic substrates. Human trials with FOS and inulin, include those with a controlled diet and demonstrate prebiotic activity of the substrates (Gibson, 2004). FOS have important beneficial physiological effects such as low carcinogenicity, a prebiotic effect, improved mineral absorption and decreased levels of serum cholesterol, triacylglycerols and phospholipids. Currently FOS are increasingly included in food products and infant formulas due to their prebiotic effect stimulating the growth of non pathogenic intestinal microflora (Molina *et al*., 2009).

Galacto-oligosaccharides

Galacto-oligosaccharides are galactose-containing oligosaccharides in the form of (β -D-galactopyranosyl-(1→4)-α-D-glucopyranose). They are produced from lactose syrup using the trans-galactosylase activity of β-galactosidase (Attanasio *et al*., 2011). *In vitro* experiments have demonstrated that the specific short-chain fatty

acid pattern, at a pH similar to that found in fecal samples of breastfed infants, reduces the growth of pathogens in a dose-dependent manner but does not influence the growth of bifidobacteria and lactobacilli. In an animal vaccination model, the prebiotic mixture improved the response to vaccination. In an allergy model (sensitization by ovalbumin), the allergic reaction was reduced by the prebiotic mixture (Fanaro *et al*., 2005).

Soybean oligosaccharides

Main oligosaccharides contained in soybeans are raffinose and stachyose. Yeo and Liong (2010) found that, *Lactobacillus* sp. FTDC 2113, *L. acidophilus* FTDC 8033, *L. acidophilus* ATCC 4356, *L. casei* ATCC 393, *Bifidobacterium* FTDC 8943 and *B. longum* FTDC 8643 showed viability exceeding 7 $\log_{10}$ colony-forming units mL^{-1} after 24 h in prebiotic-supplemented soymilk. Their growth was significantly ($p < 0.05$) increased on supplementation with maltodextrin, pectin, mannitol and FOS.

Gluco-oligosaccharides

The gluco-oligosaccharide from *Gluconobacter oxydans* has recently been shown to be a candidate prebiotic. There is some evidence from *in vitro* model systems that this material with its molecular weight of approximately 7.8-65.6 kDa will display better persistence to the distal colon (Wichienchot *et al*., 2006). Wichienchot *et al*. (2009) reported that, *G. oxydans* gave significantly ($p<0.01$) higher gluco-oligosaccharide yield in maltodextrin complex medium (30.4%) than using cell suspensions (19.6%). Cell concentration had a significant ($p<0.05$) effect on gluco-

oligosaccharide yields at 24 h. The optimal pH was found to be 4.5 for the cell suspension method.

Xylo-oligosaccharides

Xylo-oligosaccharides (XOS) are chains of xylose molecules linked by ß1–4 bonds and mainly consist of xylobiose, xylotriose and xylo-tertraose (Hopkins, 1998). The number of *Bifidobacterium* increased in all XOS and xylan simulations when compared to the growth observed in the baseline simulations, and increased levels of *B. lactis* were measured with the two XOS compounds that had larger distribution of the degree of polymerisation. Fermentation of XOS and xylan increased the microbial production of short chain fatty acids in the simulator vessels; especially the amounts of butyrate and acetate increased (Mäkeläinen *et al*., 2010).

2.4 Probiotic

2.4.1 Definition of probiotic

Probiotics are live strains of ‘‘good’’ bacteria, which help digestive system to work efficiently *e.g.* bifidus, lactobacillus and acidopilus. A probiotic may be defined as: a preparation or product containing viable, defined micro-organisms in sufficient numbers, which alter the microflora of the host intestine and, by that, exert beneficial health effects on the host (Schrezenmeier and De Vrese, 2001). Today, probiotics are understood to be dietary supplements or functional foods which contain potentially beneficial bacteria or yeast which can colonize the intestines and are beneficial to health. There are many different genera and species of probiotics. One of the most studied genus is *Lactobacillus,* which has many species such as: *L. acidophilus*, *L.*

brevis, *L. bulgaricus*, *L. casei*, *L. delbrueckii*, *L. fermentum*, *L. helveticus*, *L. plantarum*, *L. rhamnosus*, *L. rhamnosus* GG (LGG), *L. reuteri*, and *L. sanfranciscensis*. Other probiotics are *Bifidobacterium* spp., *Bacillus* spp. and fungi such as *Saccharomyces* spp. and *Aspergillus* spp. (Gibsion, *et al*., 1995; Gibson *et al*., 2005).

2.5 Synbiotics

2.5.1 Definition of Synbiotics

Synbiotic was described as a combination of a prebiotic and a probiotic. The addition of an appropriate prebiotic may improve survival and establishment of a probiotic organism by providing a readily available nutritional source that might not be used by competing organisms (Weese, 2002).