

# Rice field cyanoprokaryota as a potential source of dietary nutrients

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## Abstract

Looking for effective food supplement to meet the demand of today's busy society is a major concern among modern nutritionists. This study takes 25 purified cyanoprokaryotes in order to find out their nutritional status in terms of carbohydrates, lipids, proteins, vitamins, minerals etc. Results show that some species of *Nostoc*, *Anabaena* and *Spirulina* have good food value. These organisms are protein and carbohydrate rich with more antioxidants. Some species are rich source of vitamins. So, rice field cyanoprokaryotes can be used as a major source of nutrition to mankind in future.

**Keywords:** Cyanoprokaryotes, antioxidants, vitamins, carbohydrates, protein.

## Introduction

Food is one of the basic needs of human life. Healthy food and healthy lifestyle are prerequisites for longer lifespan. But today, modern man busy lifestyle does not allow him to achieve the good health and wellness that he aspires through good nutrition and exercise. Due to their busy schedules, people do not find enough time to exercise or to cook healthy food although nutrition plays an important role for fundamental function the body. Today quick food alternatives like junk food, health drinks which are often deprived of the essential nutrients like protein, vitamins, minerals and other micronutrients have replaced take healthy homemade food. The food industry is under pressure to provide healthy foods which are convenient to cook, instantly prepared or ready to put on the table. Nutritionists today focus on naturally occurring components in the food that provide both health and medical affects.

Cyanobacteria are a diverse group of autotrophic organisms that have the ability to grow rapidly by efficiently using light energy, fix atmospheric CO<sub>2</sub>, and produce more biomass per acre than vascular plants (Shay, 1993). Blue green alga has been used as a food source and for treatment of various ailments for over two

thousand years ( Gao, 1998). Numerous compounds are found in cyanobacteria that are intensively exploited and are currently used in nutraceuticals. Different species of algae, specifically microalgae, that are more prevalent as food supplements and nutraceuticals are *Spirulina*, *Nostoc*, *Anabaena*, *Chlamydomonas*, *Botryococcus*, *Scenedesmus*, *Synechococcus*, etc. because of their ability of producing necessary vitamins including, VitA (Retinol), VitB1 (Thiamine), VitB2 (Riboflavin), VitB3 (Niacin), VitB6 (Pyridoxine), VitB9 (Folic acid), VitB12 (Cobalamin), Vit C (L-Ascorbic acid), VitD, VitE (Tocopherol), and VitH (Biotin) (Bishop and Zubeck, 2012). These organisms are also rich source of essential elements including: Potassium, Zinc, Iodine, Selenium, Iron, Manganese, Copper, Phosphorus, Sodium, Nitrogen, Magnesium, Cobalt, Molybdenum, Sulfur and Calcium. Cyanobacteria are rich source of several bioactive compounds. Bioactive compounds are molecules from synthetic or natural sources that have been biologically assayed for different therapeutic activities (Kris *et al.*, 2002).The activity of these compounds is to provide good health for many years, and it appears that food components can influence the health outcomes by altering the genetic expression of host cells (Milner, 2004) by providing beneficial antioxidant molecules or enzyme regulating activities (Huang, 1994).

The cellular carbohydrate serves to facilitate the buoyancy in the bloom forming genus *M. aeruginosa* (Kromkamp and Mur, 1984). Cyanobacteria have the ability to secrete extracellular carbohydrates and proteins. These molecules show their potential role in metal removal (Shah *et al.*, 2000). Watanbe (1951) reported the presence of aspartic acid, glutamic acid and alanine, the free amino acid in the culture of *Calothrix brevissima*. The liberation of these amino acids has also been reported in *Nostoc sp* and *Anabaena azollae* (Venkataraman *et al.*, 1963) production of amino acids like aspartic acids, glutamic acid, proline and valine from *Aulosira fertilissima* and *Anacystis nidulans*. Maximum liberation of amino acids has been found during lag and stationary phase of algal growth (Singh and Trehan, 1973; Misra and Kaushik, 1989). The presence of threonine, glutamic acid, proline and valine are found in the growth medium of *Nostoc muscorum*. In addition

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to this glycine, serine is also found; amino acids like lysine, cysteine and isoleucine was reported to be found in *Haplosiphon fontinalis*. Gribovskaya *et al.* (2009), reported that protein, carbohydrates and lipids were found in the *Oscillatoria sp.* Mishra *et al.*, (2004), reported the presence of protein and carbohydrates in *Anabaena sp.* and *Calothrix sp.* Keeping in view the above mentioned facts, this investigation makes an attempt has to find out the nutraceutical potential of locally available rice field cyanoprokaryotes collected from Rice fields of Sambalpur districts (21°28'04.1"N 84°01'56.7"E) (Bhattacharyya *et al.*, 2014).

## Materials and Methods

### *Determination of carbohydrate (Dubois et al., 1956)*

Sample was homogenized and harvested by centrifugation, 1gm of pellet was taken and washed once with distilled water and was homogenized. Then it was centrifuged and supernatant collected. 4ml of anthrone reagent was added to the supernatant and shaken thoroughly. The test tubes were kept in boiling water bath for 15 min and cooled under running tap. Absorbance was recorded at 620nm against appropriate blank. Standard curve was prepared by taking different concentration of glucose. The carbohydrate content was calculated as  $\mu\text{g}$  of carbohydrate/ gm of algal suspension.

### *Determination of Total protein (Lowry et al., 1951)*

Pellet (1gm) was taken and 6% TCA was added to it and kept at 60°C water bath for 1 minute. It was centrifuged for about 10 minutes. The residue left was dissolved in 5ml of protein reagent, incubated at room temperature for 30 minutes and then kept in water bath at 100°C for 5 minutes. It was again centrifuged at 3500rpm for 15min. Supernatant was collected and 0.5ml of Folin's reagent was added and kept for 10-20min at room temperature. Absorbance was recorded at 750nm against blank. Standard curve was prepared by taking different concentration of Bovine Serum Albumin (BSA). The protein content was calculated as  $\mu\text{g}$  of protein/gm of algal suspension.

### *Determination of Total lipid (Sato, 1988)*

Algal biomass (1gm) of was taken and homogenized with chloroform: methanol (2:1). Then filtered in fat free filter paper (Whatman No.1) and collected in glass vessel. Volume of filtrate was adjusted to 20ml by adding the solvent (chloroform: methanol). To the crude extract, 4ml water was added and allowed to separate into 2 phases. Upper phase was removed by siphoning and methanol was added to the lower phase. Then evaporated to dryness in a water bath. The lower phase contains all lipid substances. The gm % of lipid content in the sample was calculated by subtracting the final weight from the initial weight.

### *Determination of Ascorbic Acid (Jiang, 2015)*

1gm of pellet was homogenized with distilled water. 2.5ml of 8% TCA was added followed by 2 drops of bromine water. It was centrifuged for 5 min and supernatant collected. To 2ml of supernatant, 1ml of 2-4-Dinitro phenyl hydrazine thiourea reagent was added and incubated for 1hr. at (37-57)°C. To each test tube 5ml of conc. H<sub>2</sub>SO<sub>4</sub> was added and kept in ice bucket. The absorbance was measured at 540nm against blank. Standard curve was prepared by taking different concentration of ascorbic acid. The ascorbic acid content was calculated as  $\mu\text{g}$  of ascorbic acid/ mg of algal suspension.

### *Determination of Vitamins B*

The B-complex vitamins comprising thiamin, riboflavin and niacin were determined as per Okwu and Emenike, 2006.

### *Determination of Thiamin*

1g of the algal biomass was homogenized with ethanolic sodium hydroxide and then filtered. Potassium dichromate (1:1) was added to the filtrate for colour development and absorbance was taken at 360 nm. A standard solution of 100 ppm thiamin acid was prepared and serial dilutions of 0.0, 0.2, 0.4, 0.6, and 0.8 ppm were made and the calibration curve was plotted using the values.

### *Determination of Riboflavin*

1g of the algal biomass was extracted with 100 ml of 50% ethanol and filtered. To the extract, 5% potassium permanganate and 30% H<sub>2</sub>O<sub>2</sub> were added (1:1:1) and kept in a hot water bath for about 30 minutes. 2 ml of 40% Na<sub>2</sub>SO<sub>4</sub> solution was added and a yellowish pale color was formed. This was made up to 50 ml and the absorbance was measured at 510 nm in a spectrophotometer.

### *Determination of Niacin*

1g of the algal biomass were treated with sulphuric acid and shaken for 30 minutes. 3 drops of ammonia solution was added to the sample and filtered. 10 ml of the filtrate was pipetted into a 50 ml volumetric flask and 5 ml of potassium cyanide was added. This was acidified with 5 ml of 0.02N H<sub>2</sub>SO<sub>4</sub> and absorbance was measured in the spectrophotometer at 470 nm wavelength and the value was used to plot the calibration curve.

### *Estimation of Minerals*

For the estimation of minerals, 0.5 gm of algal biomass was wet ashed using a mixture of 18 M sulphuric acids, 12 M perchloric acid and 16 M nitric acid (0.5: 1.0:0.5 v/v/v). After proper dilution the concentration of iron, calcium, magnesium, sodium and potassium were estimated by the following procedures

### *Determination of Sodium (Ward et al., 1962)*

Liquid ammonia was added into the digested sample to adjust the pH 7 and the volume was made up to 100 ml by using dist. water. Then filtered through Whatman no 40 and the filtrate were stored in clean glass bottles. The analysis was done by flame photometer at 589nm. From the standard curve the concentration was noted.

### *Determination of Potassium (Ward et al., 1962)*

An aliquot of ash solution was diluted and sufficient HCl is added so that the concentration of the acid is same as that in the standard solution. The dilute extract was atomized in a calibrated flame photometer at 768 nm wavelength and 100% transmittance for the standard solution of potassium. From the standard curve the concentration was noted.

### *Determination of Calcium (Kirk and Sawyer, 199)*

Water was added to wet ash solution then 10 ml of saturated ammonium oxalate solution and 2 drops of methyl red

indicator was added. The solution was made slightly alkaline by addition of dilute ammonia followed by few drops of acetic acid till the colour becomes faint pink (pH 5.0). Then heated to the boiling point and allowed to stand for fortnight. This solution was filtered and washed with water to make it oxalate free. The precipitate obtained is first washed with hot dilute H<sub>2</sub>SO<sub>4</sub> followed by hot water and titrated while hot with 0.01 N KMnO<sub>4</sub> till the first permanent pink colour appeared.

#### Determination of Iron (Wong, 1928)

The iron in food is determined by converting the iron to ferric form using oxidizing agents like potassium persulphate or hydrogen peroxide and treating thereafter with potassium thiocyanate to form the red ferric thiocyanate which is measured colorimetrically at 480 nm.

#### Determination of Magnesium (Ward et al., 1962)

One drop of methyl red indicator was added to 10ml of ash solution and neutralized with NH<sub>4</sub>OH. Then 1 ml of ammonium oxalate was added and was allowed to mix and kept overnight. Next day it was centrifuged and 1 ml of supernatant was taken and 3 ml of water was added, to it 1 ml of dilute NH<sub>4</sub>OH was added and centrifuged for 7 minutes and the precipitate collected by placing the tubes in hot waterbath. To the residue 1 ml of dilute HCl and 5 ml of water was added to dissolve the precipitate. 1 ml of molybdc acid solution, 0.5 ml hydroquinone and 0.5 ml sodium sulphite solution was added, mixed thoroughly and allowed to stand for 30 min. The absorbance was taken using a red filter.

## Results and Discussion

Biomolecules like carbohydrates, fats and proteins are required in large amounts for all metabolic activities of the body and are considered as macronutrients. Likewise vitamins and minerals are needed in small amounts and are placed as micronutrients. All these nutrients are needed for physiological and biochemical activities of human body and for getting everything we consume varieties of food. The requirements of various nutrients vary with age and physiological state. Cyanobacteria are easily cultivated and in various study, it has been reported that it also have antimicrobial (Bhattacharyya et al., 2013, Ozdemir et al., 2004, Ramamurthy et al., 2009, Mayer et al., 2005, El-Sheekh et al., 2006), cytotoxic activities (Gul and Hamam, 2005) and other biological activities (Zeeshan et al., 2010). The nutraceutical values of 25 species belonging to genus *Anabaena*, *Nostoc*, *Calothrix*, *Chroococcus*, *Aulosira*, *Oscillatoria*, *Scytonema*, *Spirulina* and *Tolypothrix* was analysed in this study.

#### Carbohydrate contents

Carbohydrate plays an important role for providing energy in different body function and 70-80% of total energy requirement are coming from carbohydrates present in plant foods like cereals and millets (RDA, 2011). It has been reported that 1 gm of carbohydrate yields 4 k cals of energy. Glucose and glycogen serve as important source of energy for vital activities (Berg, 2012). Some carbohydrates also have specific function. Blue green algae are autotrophic and by the process of photosynthesis, produce carbohydrates. It was found, that the total sugar content is more in *A. variabilis*, *Calothrix clavatoidea*, *Nostoc carneum*, *N. commune*, *N. linkia*, *Phormidium*, *Scytonema*, *Spirulina*, *Tolypothrix*, and *W. prolifica* in comparison to other genus (Table 1).

#### Total protein

The proteins are of primary importance as structural and functional component of living cells. They are also the complement of enzymes involved in metabolism during growth and development. The essential amino acids, one has to obtain from proteins as human body cannot synthesize them. Plant sources like cereals, legumes, milk, egg are rich sources of protein. 1 gm of protein yields 4 k cals of energy (RDA, 2011). Total protein quantification showed that *A. circinalis*, *A. variabilis*, *A. torulosa*, *Aulosira sp.*, *Desmonostoc muscorum*, *N. carneum*, *N. commune*, *N. linkia*, *Phormidium sp.* and *Spirulina sp.* has higher protein content than other species (Table. 1) and it almost fulfill the requirement of daily protein content of an adults.

#### Lipid content

Lipids are more useful in animal body. They are essential components of cell membrane, source of metabolic energy for cell maintenance, and reproduction (Arrese et al., 2010). Fat serves as efficient source of energy and also serve as a vehicle for fat soluble vitamins like vitamin A, D, E and K and carotenes and their promote their absorption. 1 gm of lipid yields 9 k cals of energy (RDA, 2011). Dietary fats help in the absorption of fat soluble vitamins. Lipoproteins are also important cellular constituents (Yancey et al., 2000). Total lipid quantification shows that organisms like *Nostoc spongiformae*, *Anabaena circinalis*, *Calothrix clavatoidea*, *Phormidium sp.*, *Spirulina sp.*, *Nostoc carneum*, and *Anabaena torulosa* have higher amount of lipid content then the rest organisms (Table. 1).

#### Minerals content

Minerals are inorganic substance required by the organism in very small amount for growth and maintenance of different body parts. Food and vegetables are the important source of mineral for human beings and exist in foods they are present in as organic and in inorganic combinations as salt (Pennington, 1996). They combined with organic compound, e.g. iron in haemoglobin. Minerals are required for the teeth and bone formation (Soetan et al., 2010). Minute amount of mineral elements are constituent of various regulatory compounds such as, vitamins, enzymes and hormones. For example, some enzymes require calcium for their activity such as lipases and succinate dehydrogenases. Iron requiring enzymes are ferredoxin reductase, indophenol oxidase, aldehyde oxidase etc. The mineral elements present in the intra and extra cellular fluid maintained water and acid-base balance. They regulate transmission of impulses and contraction of muscles. The deficiencies of minerals cause many diseases in human beings. The amount of Ca, Mg, K, Na, Fe present in 25 different rice field cyanobacterial strains are shown in Table 1. *A. circinalis*, *C. javanica*, *D. muscorum*, *N. carneum*, *N. commune*, *N. spongiformae*, *N. linkia* and *Spirulina sp.* are rich in sodium contents. *A. circinalis*, *C. javanica*, *C. clavatoidea*, *Chroococcus sp.*, *N. carneum*, *N. spongiformae* and *Spirulina sp.* are rich in potassium contents. *Fischerella* and *Gloeocapsa* are rich in calcium. Magnesium content is found to be more in *A. circinalis*, *Aulosira sp.*, *N. commune*, *N. punctiformae* and *Spirulina sp.* Iron content was found to be more in *Spirulina* and *Nostocopsis lobatus*.

## Vitamins

Vitamins are micronutrients and these must be present in the diet as human body cannot synthesize. These are highly essential for various body processes. *Spirulina*, *N. punctiforme*, *N. linkia* and *Anabaena torulosa* are rich in vitamin C. Few cyanobacterial strains are also rich source of Vitamin B. The B-complex vitamins comprising thiamin, riboflavin and niacin were found to be more in almost all the cyanobacterial strains except *Chroococcus sp.*, *Fischerella sp.*, *Nostocopsis lobatus* and *Scytonema sp.* (Table.1).

Table 1: Dietary nutritional value of 25 rice field cyanobacterial biomass (10gm)

Locally available Cyanobacterial strains	Carbohydrates(mg)	Total Protein(mg)	Total Lipid (mg)	Ascorbic acid (mcg)	Vitamin B			Minerals				
					Thiamin(mcg)	Niacin (mcg)	Riboflavin(mcg)	Sodium (mg)	Potassium (mg)	Calcium (mg)	Iron (mg)	Magnesium
<i>Anabaena circinalis</i>	24	49	62	50	1.6	24	1.6	28	32	19	14	38
<i>Anabaena fertilissima</i>	26	41	34	30	1.1	26	0.9	20	26	11	09	32
<i>Anabaena variabilis</i>	32.4	46.2	42	25	1.8	25	1.8	13	23	14	20	12
<i>Anabaena torulosa</i>	34	48	56	55	1.4	20	1.1	18	28	12	14	33
<i>Aulosira sp</i>	24	51	46	41	1.8	21	1.3	12	30	11	12	35
<i>Calothrix clavatoides</i>	35	44	60	15	1.4	26	1.2	10	42	15	14	10
<i>Calothrix javanica</i>	26	32	41	40	1.6	22	1.8	26	38	12	10	32
<i>Chroococcus sp</i>	24	28	26	26	0.4	12	0.4	21	42	7	13	22
<i>Desmonostoc muscorum</i>	15	48	32	44	1.2	16	1.4	24	32	8	10	24
<i>Fischerella sp.</i>	10	36	22	26	0.4	15	1.6	14	22	30	11	15
<i>Gloeocapsa sp</i>	20	30	40	21	1.8	24	1.4	1.8	20	30	9	14
<i>Hapalosiphon sp</i>	24	42	50	50	1.4	13	1.2	13	30	8	4	21
<i>Lyngbya sp</i>	18	44	52	23	1.1	16	1.3	20	24	9	13	32
<i>Nostoc carneum</i>	39.7	54	58	26	1.7	25	1.6	8	6	1	4	4
<i>Nostoc commune</i>	34	52	35	28	1.4	18	1.2	32	41	12	11	30
<i>Nostoc spongiformae</i>	15	44	68	45	1.8	15	1.3	36	48	10	12	34
<i>Nostoc linkia</i>	30	52	38	51	2.0	16	1.8	24	23	11	15	32
<i>Nostoc punctiforme</i>	16	32	48	53	1.4	13	1.6	21	30	9	13	38
<i>Nostocopsis lobatus</i>	10	14	21	32	0.6	10	0.9	10	21	10	19	46
<i>Oscillatoria princeps</i>	15	32	49	44	1.1	17	1.4	14	32	12	14	32
<i>Phormidium</i>	34	52	58	41	1.6	14	2.0	10	20	7	14	31
<i>Scytonema</i>	30	40	28	15	0.8	16	1.2	7	10	4	95	23
<i>Spirulina sp</i>	32	51	58	58	1.4	20	1.7	23	36	10	17	38
<i>Tolypothix</i>	31	46	17	38	1.2	15	1.1	16	24	8	11	32
<i>Westiellopsis prolifica</i>	35	40	15	32	1.8	10	1.7	20	31	5	8	21

## Conclusions

Providing balanced nutrition to growing population now-a-days has become a great challenge. Various plants and cyanobacteria have been used as an important food source by the peoples from ancient time. The level of the dietary nutrients tested in the present study indicates the presence of high quality nutrients. Nutraceuticals obtained from cyanobacteria are highly nutritious and is less expensive which can be a boon for underdeveloped countries. Laboratory cultures of cyanobacteria do not have any pungent smell or bitter taste. So, it can be used as a food supplement for children and people suffering from malnutrition in underdeveloped areas and countries. In fact, cultivation of cyanobacteria could prove more valuable and cost effective than some of the established crops as a source of complete protein, minerals, carbohydrate, and lipid for human and animal diet. Cyanobacteria are ideal organisms which suits perfectly to the demand of food industry due to its faster growth rate, easy to handle and has pharmacological properties. The nutraceutical industries should research more on this wonderful organism to reap the maximum benefits from them and use it for the production of a complete food supplement.

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