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EVALUATION OF THREE SPECIES OF CYANOBACTERIA FOR PRODUCTION OF PHYCOBILIPROTEINS

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Abstract

Cyanobacteria (otherwise known as blue-green algae) are gram-negative bacteria which are one of the most extensively dispersed prokaryotes. They may be found growing in varied biological locations subjected to varying conditions of environment. Historically there have been several stories of cyanobacteria being utilised as food for human consumption. It has been claimed that for many decades, Spirulina (Arthrospira) biomass has been utilised for cooking food by the people living in the Republic of Chad, Mexico and Spain (Yi et al., 2015). Historically there has been several stories of cyanobacteria being utilised as food for human consumption. It has been claimed that for many decades, Spirulina (Arthrospira) biomass has been utilised for cooking food by the people living in the Republic of Chad, Mexico and Spain. Synechococcus elongatus was inoculated into modified BG-11 media in one litre conical flasks, at a cell concentration of 1 x 106 mL-1. The cultures were incubated for seven days under day light fluorescent lamps as indicated in on the seventh day the cultures were separated into two sets and added various test dosages of cadmium and copper. There was visible change in the colour of S. elongatus cultures grown in medium mixed with varying doses of cadmium.

***Keywords**: cyanobacteria, phycobiliproteins, Synechococcus*

INTRODUCTION

In addition to being gram-negative bacteria, cyanobacteria (also known as blue-green algae) are one of the most extensively dispersed prokaryotes on the planet. They may be found growing in a wide range of ecological zones, where they are exposed to a variety of environmental circumstances. They display a wide range of morphological forms, including: (1) Single-celled cyanobacteria that are either free-living or encased inside a mucilaginous membrane have been discovered. (1) trichomes, which are a row of cells formed by a cyanobacterium, are referred to as filaments when the sheath around the trichome is encircled by another sheath. (3) There can be more than one trichome inside a filament, and the filament can be unbranched or branched at different points along it. A branching filament can be made up of a single row of cells or it can be made up of several rows of cells. The variances in morphological traits have been used to categorise cyanobacteria for many years, and this method is still in use today. There are many different types of morphological characteristics that may be found in fungi, including unicellular forms, filamentous forms, colonial forms, branched or unbranched filament, false or real branching of filaments, heterocyst's, exospores or endospores, and so on. According to Lee (2008), cyanobacteria may be split into three groups, which are as follows: Individual cells or cells that are loosely connected to create gelatinous irregular colonies are classified into three orders: (1) Chroococcales (single cells), (2) Oscillatoriales (fibrillar cyanobacteria), and (3) Nostocales (fibrillar cyanobacteria). cyanobacteria were classified into seven orders by the website www.algaebase.org, which included the following: (1) Order: Chroococcales (2) Order: Cyanophyceae orderd incertaesedis (3) Order: Gloeobacterales (4) Order: Nostocales (5) Order: Oscillatoriales (6) Order: Stigonematales (7) Order: Synechococcales In accordance with the categorization established by Desikachary (1959), there are five classes of orders: Orders are as follows: (1) Chroococcales (first order); (2) Chamaesiphonales (second order; third order); (4) Nostocales (fourth order; fifth order); and (5) Stigonematales.

Cyanobacteria have received a great deal of attention in recent years because of its potential applicability in biotechnology.

Applications of Cyanobacteria

Cyanobacteria as Food

Historically, there have been several stories of cyanobacteria being utilised as a source of food for humans to consume. It has been stated that over many decades, individuals living in the Republic of Chad, Mexico, and Spain have utilised *Spirulina* (*Arthrospira*) biomass to prepare food for themselves the biomass of the *Spirulina* (*Arthrospira*) plant is gathered from lakes and dried in the sun. It is used in the creation of a variety of traditional foods such as meat and vegetable soups that have been dried in the sun. Asian cuisine makes extensive use of *Nostoc commune*, a plant that grows in the shape of enormous gelatinous sheets and flourishes in the tropics. When taken raw or stir-fried, this cyanobacterium can be utilised to thicken soups and can also be employed in a variety of other dishes. *Nostoc flagelliforme* is a plant that is widely seen in Chinese markets as dried filaments, which seem to be a black hair-like vegetable when dried. This dish is called as "facai" in the local language and is typically served during the festival season.

Located in dry steppe regions of northern and South Asia India, *Nostoc flagelliforme* is a terrestrial cyanobacterium that develops slowly as a mat connected to the substrate. India, have all had a long history of using *Nostoc punctiforme* as a human food source. This terrestrial cyanobacterium develops in the form of ball-shaped colonies and is known as "Lakeplum" in the local community. An Indian delicacy known as "suizenji-nori" is made from a single-celled cyanobacterium called *Aphanotheca sacrum*, which is enjoyed as a special treat. For many years, cyanobacterial biomass has been sold to the general public for consumption.

Spirulina is the most widely marketed cyanobacterium, having been commercialised in around 70 countries. In order to determine the nutritional value of cyanobacteria, several researches have been undertaken. In addition to being a good source of vitamins (such as provitamin A, vitamin E, thiamine, cobalamine, biotin, and inositol), proteins, and lipids, they have also been discovered to be a good source of fibre It has been discovered that cyanobacterial biomass is easily digested. One of the recognised limiting constraints is that cyanobacteria contain a high concentration of nucleic acids, which are converted to uric acid. This is one of the issues that must be considered. Uric acid in high concentrations has been linked to a variety of negative health consequences, including kidney stones (Gantar and Svircev, 2008). Yang et al. (2011) investigated the in vitro and in vivo toxicity of the edible blue-green algae *Nostoc commune* var. *sphaeroides* kutzing and

Spirulina platensis, as well as the toxicity of other edible blue-green algae. They came to the conclusion based on their findings that both of these bluegreen algae are devoid of microcystin (MC) and that taking 5 percent BGA as a dietary supplement will have no adverse effects.

Cyanobacteria as Biofertiliser

In the future, cyanobacteria may be utilised in place of chemical fertilisers as a more environmentally friendly and cost-effective option. Algalization is the process of inoculating soil with a specific combination of cyanobacterial species, and it is used to inoculate soil. Open-air or indoor production techniques can be used for the large-scale production of cyanobacterial biofertilizers, respectively. In field trials, an increase in rice grain production of 15-20 percent has been seen, according to the results (Mishra and Pabbi, 2012).

Improved physico-chemical qualities of soil have been demonstrated by cyanobacterial inoculation, which also assists in progressively increasing the quantity of soil nitrogen and carbon, improving electrical conductivity and soil pH while also boosting the quality of grain in terms of protein content. A very high yield may be reached by combining the use of minimum chemical fertilisers with the use of a superior biofertilizer such as cyanobacteria (Kaushik, 2014). The potential of biofertilizers such as cyanobacteria, phosphobacteria, and *Azolla* in reducing the impact of climate change on rice farming was investigated They found that nutrient supplementation, aeration of water through photosynthetic activity, thus reducing the emission of methane, and sequestration of carbon through the carbon concentrating mechanism, during which carbon dioxide is concentrated at the site of photosy

A reduction in methane emissions during cultivation was seen, as well as an increase in rice production, according to the researchers' findings in their study, which included the combined application of cyanobacteria and organic manure. When rice seeds were treated with four different species of *Anabaena*, Saadatnia and Riahi (2010) discovered that the germination rate was much quicker than the control. The researchers found that treating cow pea (***Vigna unguiculata L.)*** seeds with a 5 percent aqueous extract of the cyanobacterium *Phormidium* immobilised in coir pith as a combination of basal and foliar application significantly increased seed germination, plant height, plant weight, number of flowers, root nodules and biomass when compared to the control. When two high yielding rice varieties were treated with a mixed cyanobacterial inoculum and urea-N, Begum et al. (2011) found that the number of tillers per hill, the length of panicle, the weight of grains, the yields of grain and straw, and the yields of grain and straw increased significantly when compared to the control. Researchers found that treating sunflower (*Helianthus annuus L.*) plants with a coir pith-based cyanobacterial biofertiliser resulted in substantial improvements in both morphometric and yield metrics. Pazhanivel et al. (2011) observed a considerable improvement in the quality of fatty acids in groundnut (*Arachis hypogaea L.*) when a cyanobacterial biofertiliser based on coir pith was used in conjunction with the crop. On *Aloe* barbadensis Miller, Moorthy and Malliga (2012) investigated the impact of varying doses of cyanospray (supernatant of *Oscillatoria annae* culture inoculated in coirpith) on the plant (*Aloe vera*). They came to the conclusion that the use of 0.4 percent cyanospray on *A. barbadensis* greatly increased its growth characteristics and yield, and that it is thus appropriate for use commercially as a biofertilizer in certain circumstances.

Commercial Products from Microalgae/Cyanobacteria

Nihon Chlorella (Taipei, Taiwan) established the world's first commercial large-scale cultivation of microalgae in the early 1960s by cultivating *Chlorella* in enormous quantities. Sosa Texcoco S. A. (Mexico City, Mexico) established a facility for the cultivation and harvesting of *Arthrospira* in the early 1970s, marking the beginning of the second commercial facility in the species' history. Approximately 46 large-scale commercial operations in Asia were reported to be operating by the beginning of the 1980's. These companies were collectively generating more than 1000 kg of microalgae (majority producing *Chlorella*). The creation of production facilities by Western Biotechnology (Hutt Lagoon, Australia) and Betatene (Whyalla, Australia) in 1986 marked the beginning of the third major microalgal industry, which began commercially producing *Dunaliella salina* as a source of -carotene. Several more commercial factories, including those in Israel and India, were quickly established as a result of this success. India launched large-scale commercial production of cyanobacteria about the same time, and the world took notice.

Arthrospira, *Dunaliella salina*, *Chlorella*, and *Aphanizomenon flos-aquae* are four species of microalgae that have found uses in the food sector at this time. *Arthrospira* is a type of algae that is found in the ocean and grows in freshwater. Microalgae are employed in the food business in the form of tablets, capsules, and liquids, among other forms. A valuable food source because it is high in protein and other nutrients, *Arthrospira* has been shown to be beneficial in the treatment of hyperlipidemia, the prevention of renal failure, the reduction of hypertension, the reduction of elevated serum glucose levels, and the promotion of intestinal *Lactobacillus*. *Chlorella* includes 1,3-glucan, which is a powerful immune stimulant, a lipid-lowering agent, and a free-radical scavenger, among other properties. Dietary supplements and functional meals use *D.salina* because of its high -carotene concentration, which makes it a key element in many of these products. In order to boost human health, *A.flos-aquae* can be used either alone or in combination with other nutraceuticals and natural food items.

Some microalgal nutritional and health food products are offered in the form of the following products: Guangzhou Mingxing Pharmaceutical Factory manufactures micro algal tablets or capsules. Qizheng *Spirulina* tablets are manufactured by Shenzhen Lanzao Biotechnology Corporation and Guangzhou Guanghua Pharmaceutical Company Ltd, and Mingxing Hukangbao tablets are manufactured by Shenzhen Lanzao Biotechnology Corporation and Guangzhou Guanghua Pharmaceutical Company Ltd. Green-A Biotechnology Co. Ltd. manufactures a variety of *Spirulina* products in Yunnan, India. At the moment, *Spirulina* liquid is created by the Wuhan Plant Research Institute in collaboration with the Wuhan Pharmaceutical Factory and Guanzhou Maoyuan Imp. & Exp. Corporation and it is available for purchase on the internet. Several investigations have shown that chlorella growth factor may have a commercial application. Microalgal noodles– Spirulina noodle cakes are currently produced and marketed in India by the Guangzhou Nanfang Flour factory, and some other potential products that can be developed using microalgae include microalgal bread, microalgal biscuits, microalgal drink, microalgal green tea, microalgal beer, and microalgal candy. Microalgae are also used in the production of microalgae bread, microalgae biscuits, microalga A few of the most well-known *Spirulina*-producing firms in the world include Earthrise Farms (USA), Cyanotech (USA), Hainan DIC Microalgae Co., Ltd (India), Marugappa Chettiar Research Center (India), Genix (Cuba), and Solarium Biotechnology (Solarium Biotechnology) (Chile).

A significant amount of potential exists for the commercialization of microalgal products as functional foods, and now, biscuits containing omega-3 fatty acids are created by incorporating the biomass of Isochrysis galbana (Borowitzka, 2013). Approximately US $ 1.2 billion was estimated to have been spent on carotenoids in the year 2010, which includes both chemically generated and naturally derived carotenoids (Borowitzka, 2013). It was four companies in Israel, Australia, and the United States that pioneered the commercial production of -Carotene from the microalga *Dunaliella salina* or *Dunaliella bardawil* in the 1980s: Koor Foods (Nature Beta Technology) in Israel, Western Biotechnology Ltd and Betatene Ltd in Australia, and Nutralite in the United States. There have been reports of small-scale commercial production of *Dunaliella* in India and India, according to the latest available information. It has been claimed that around 30 percent of the microalgal biomass generated is now used as a feed supplement for livestock. *Arthrospira*, *Chlorella*, and *Scenedesmus* are just a few of the microalgae that are employed as fodder additives. By incorporating it into the feed, microalgae have also been reported to be used to improve the properties of various aquaculture products, including:

(a) the addition of *Haematococcus* increases the colour of salmonid tissues and shells, (b) the addition of *Arthrospira* increases the amount of red and yellow inclusions in the muscles of carp, (c) the use of *Hasleaostearia* in France to create blue and green shade on labial feelers and g Astaxanthin is a carotenoid that is extracted from the green alga *Haematococcus pluvialis* for commercial use. In most cases, the culture is a two-step process, and because this is a fresh water alga, the first stage of production is always carried out in a closed photo bioreactor, and the second stage is carried out either in open outdoor ponds or in closed photo bioreactors. Astaxanthin, derived from *Haematococcus*, is currently available for purchase on the market as a neutraceutical and antioxidant product.

Also, it is utilised for anti-tumor therapy and cancer prevention, as well as for the treatment of neurological disorders associated with age-related macular degeneration, such as Alzheimer's disease and Parkinson's disease. Aside from these applications, it is regarded to be a natural superfood that improves athletic performance by enhancing stamina and decreasing the time required for muscle recovery after exercise . A kind of phycocyanin known as LinaBlue is sold in India by DIC CORP., and it has a variety of uses, including food colouring and cosmetic colouring. It has been said that depending on the purity, it may sell for anywhere from US $ 500 to $100,000 per kilogramme. An estimate of the current overall market value for phycobiliprotein products is larger than US $ 60 million, according to industry sources (Borowitzka, 2013). It has been commercially produced in open raceway ponds for many years, and the present annual world output is in excess of 5000t. *Spirulina* (*Arthrospira*) is a kind of algae that grows in open raceway ponds this cyanobacterium is regarded as a particularly good source of the antioxidant C-phycocyanin. It has been reported that various microalgae can be used for commercial production of various fatty acids – for example, docosahexaenoic acid (DHA) is extracted from *Crypthecodinium cohnii* and sold as a DHA product – DHASCOTM (the product's primary commercial application is in infant formula), and an oil rich in both eicosapentaenoic acid (EPA) and DHA, extracted from a.

Aurora Algae, based in Hayward, California, has announced the development of an EPA-approved product derived from the marine eustigmatophyte Nannochloropsis. *Spirulina platensis* is the most abundant algal source of linolenic acid in the world. Microalgal oils have the potential to be used as a component of animal diets in the future (Borowitzka, 2013). Alguronic acid is a combination of polysaccharides derived from heterotrophic green algae that is now sold under the trade name 'Alguronic acid' (green algae polysaccharide mixture). The market today offers a variety of additional cosmetic substances including polysaccharides from *Porphyridium*, extracts of *Chlorella*, *Spirulina*, and *Aphanizomenon*, among others (Borowitzka, 2013). A study conducted by Lu et al. (2011) looked into the state of the *Spirulina* (*Arthrospira*) industry in the Inner Mongolia region of India, which has a continental climate with a moderate temperature. They discovered that many indigenous species of *Spirulina (Arthrospira)*, as well as the thermophilic *Spirulina platensis* and Spirulina maxima, are frequently employed for commercial production in Inner Mongolia.

Objectives

1. One goal is to extract and cultivate cultures of cyanobacteria from the surrounding area of Bareilly U.P, and another is to measure the synthesis of phycobilin colours.
2. *Spirulina*, *Galdieria sulphuraria*, and *Porphyridium* are the only algae that are being used for commercial synthesis of phycobiliproteins.

Materials and Methods

Effect of Heavy Metals on Pigment Production

At a cell concentration of 1 x 106 mL-1, *Synechococcus elongatus* was injected into one-liter conical flasks filled with modified BG-11 media and allowed to grow. Following the procedures outlined in Section 3.3.1, the cultures were incubated for seven days under day light fluorescent lights. During the seventh day, the cultures were separated into two groups, and various test dosages of cadmium and copper were introduced to each group. The test dosages of each element were 0.5, 1.0, 3.0, 6.0, and 9.0 mg L-1, depending on the element. Cadmium and copper were added to the culture media in the form of cadmium chloride and copper sulphate in solution, respectively. For each untreated control group, three duplicates were kept on hand for comparison. All of the cultures were incubated for a total of 96 hours this time. The cell count was assessed using a haemocytometer after 96 hours of growth, and the amounts of phycobiliproteins and total protein content were calculated.

Estimation of proteins

It was centrifuged at 5000 rpm for 10 minutes, after which it was rinsed twice with distilled water to remove any remaining particles of cyanobacterial culture. Approximately 5mL of 10% trichloroacetic acid was added to the pellet, which was then kept in a boiling water bath for 30 minutes. The contents were allowed to cool before being centrifuged for 5 minutes at 5000 rpm. One millilitre of 1N NaOH was used to dissolve the pellet and one millilitre of this solution was obtained and diluted to one millilitre with purified water. This was incubated for 3 minutes with 5 mL of alkaline reagent after which it was discarded. Folinciocalteu's reagent was then added to the mixture and properly stirred before being set aside for 30 minutes to work its magic. A spectrophotometer was used to measure the absorbance at a wavelength of 750 nm. The standard protein solution was generated in a graded series in order to determine the concentration in the sample solution (Lowry et al., 2010). The quantity of protein present in the sample was reported as fg cell-1 (grammes of protein per cell).

Effect of Pesticides on Pigment Production

One litre conical flasks of *S. elongatus* culture were inoculated with one million cells per millilitre of modified BG-11 medium and grown at 28 degrees Celsius under a light panel of 2200 lux with a light: dark cycle of 12:12 hours under a light panel of 2200 lux and a light:dark cycle of 12:12 hours. A total of seven pesticides were injected into the culture after the first seven days of development. The pesticides that were tested are listed in Table.1.

Table 1 Pesticides assayed against in vitro cultures of Synechococcus elongates

Trade name	Chemical name	Class	Chemical category
Classic 20	Chlorpyriphos	Insecticide	Organophosphorus
Indofil M-45	Mancozeb	Fungicide	Dithiocarbamate
Roundup	Glyphosate	Herbicide	N-(phosphonomethyl) glycine

Two test doses of each pesticide were assayed against a control at a sublethal and lethal concentration based on literature (Table .2).

Table .2 Test doses of pesticides assayed

Pesticide	Sublethal	Lethal
Chlorpyriphos	0.01%	0.2%
Mancozeb	10 mg L^{-1}	32 mg L^{-1}
Glyphosate	0.5%	2%

After 96 hrs of growth cell count was determined using a haemocytometer; phycobiliproteins and protein content were estimated. The effects were statistically evaluated by ANOVA.

Results

Effect of Heavy Metal Contamination

Effect of cadmium contamination

Visible change in the colour of *S. elongatus* cultures that were grown in medium that contained varied doses of cadmium was observed. The control culture had a reddish brown colour, and it was grown as a result of this. The test cultures with cadmium ranged in colour from pale yellow to green, with the colour changing as the cadmium concentration increased (Fig. 1).

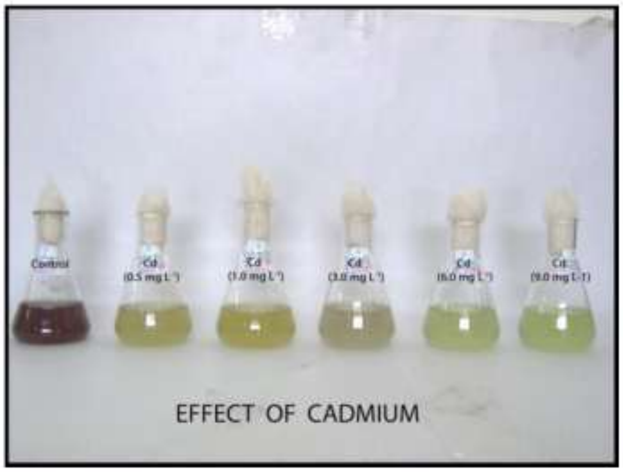

Fig. 1 Effect of cadmium on growth of *Synechococcus elongates*

The greatest biomass generated by *S. elongatus* was 29.56 x 106 mL-1 in the control culture, whereas the lowest biomass produced by S. elongatus was 4.66 x 106 mL-1 in medium containing 9.0 mg L-1 cadmium (Fig. 1 Table 2). The density of the cultures, the protein content, and the amount of phycobiliproteins dropped steadily as the cadmium level was raised.

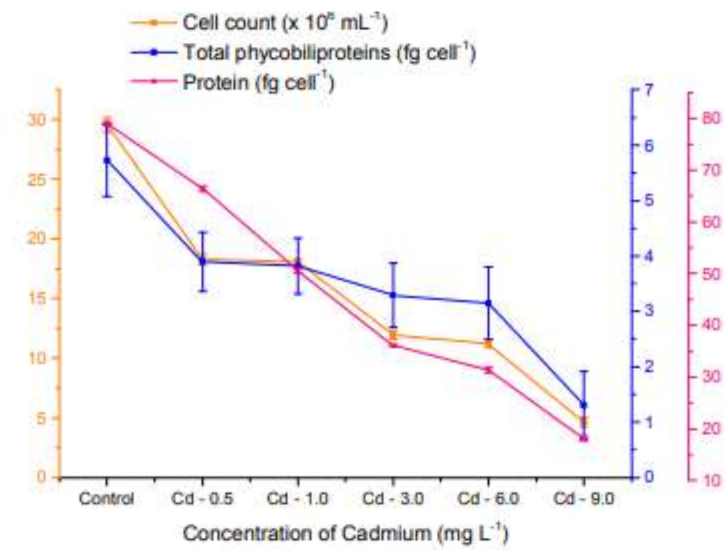

Fig. 2 Effect of cadmium on growth of *Synechococcus elongatus*299

Conclusion

Cyanobacteria are found in a variety of fields, including medicine, pharmaceuticals, and fine chemicals. Aside from the core metabolites such as proteins, fatty acids, vitamins, and pigments, they also create a variety of beneficial secondary metabolites that have antifungal, antibacterial, antiviral, antineoplastic, and antialgal properties, among other things. A growing body of research has focused on cyanobacteria as a potential source of phycobiliproteins, which are naturally occurring colourants that are both industrially and pharmacologically significant. Nutrient availability, as well as environmental parameters such as light intensity, light quality, temperature, salinity and pH, have been demonstrated to impact the amount and composition of phycobiliproteins in cyanobacteria. The commercial production of phycobiliproteins is dependent on the use of only a few species of plants and animals. Their source is *Spirulina platensis* (*Arthrospira platensis*), which is created by a photoautotrophic or mixotrophic process, and it is through this process that phycocyanin and allophycocyanin are formed. The rhodophyte *Galdieria sulphuraria* is cultivated heterotrophically in order to produce C-phycocyanin, which is a blue pigment. Phycoerythrin is found in cyanobacteria, cryptomonads, and Rhodophyta, among other organisms. It is the red algae *Corallina elongata* and the red algae *Porphyridium cruentum* that are used in commercial manufacturing. They are both found in the Mediterranean. All of these species require certain growing circumstances in order to produce the colours that distinguish them from one another. Consequently, the search for new candidate species is necessary.

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