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Optimization of media component affecting phycocyanin production from *Microcystsis* sp. Isolated from Salim ali lake, Aurangabad.

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Abstract:

The Genus *Microcystis* has always been considered as a toxin producing cyanobacteria. But apart from toxin production it is also an efficient candidate for phycocyanin production. Water samples containing bloom were collected from Salim ali lake from Aurangabad city. The *Microcystis sp* was isolated using BG-11 medium and identified based on morphological characters. A 12 run Plackett burman design was used to identify the component of BG-11 affecting the production of phycoycnain from the cyanobacterium. Two media components, magnesium sulphate and ferric ammonium citrate were found to the effective components affective the production of phycocyanin. These components were thought to be regulating the tetrapyrole synthesis and thus phycocyanin production.

Key words- Cyanobacteria, Salim ali Lake, Plackett- Burman design.

Introduction

The Salim Ali lake named after the famous Indian ornithologist Salim ali, earlier known as Delhi gate talab is situated near Himayat bagh at Longitude 75° 30' and Latitude - 19° 55' in the historical city Aurangabad on Aurangabad –Jalgaon – Agra - highway in Maharashtra State. It is an important wetland habitat in Aurangabad city, its shape is roughly rectangular and having an area about 54 acres.(Patil *et al*, 2008) . The lake is green throughout the year and always has a bloom of cyanobacteria. Cyanobacteria are the dominant phytoplankton group in eutrophic freshwater bodies (Davidson, F.F 1959.; Negri *et al*, 1995). Microcystis aeruginosa is the most commonly reported species causing hepatotoxicity and odor problems in lakes and water supplies (Sivonen et al 1990.,; (Azevedo et al 1994)

Cyanobacteria has four basic types of biliproteins i.e.; phycocyanin, allophycocyanin, phycoerythrin and phycoerythrocyanin (Sekar and Chandramohan , 2008). Allophycocyanin and C-phycocyanin have a bilin called as phycocyanobilin, and C-phycoerythrin has a bilin called phycoerythrobilin (Samsonoff and MacColl, 2001), Cyanobacterial phycobiliproteins have gained importance in the commercial sector, as they have several applications. They are used as the colorants in chewing gum, ice sherbets, popsicles, candies, soft drinks, dairy products and cosmetics like lipstick and eyeliners. In addition, phycobiliproteins are widely used in clinical and immunological research laboratories (Spolaore et al, 2006.). It's the Phycocyanin that gives many cyanobacteria their bluish colour and why these cyanobacteria are also known as blue-green algae. (Niels, 2008).
The viability of product produced from micro-organism depends on cost of production and net yield of the product.(Deshmukh and Puranik, 2012). The production of this accessory pigment phycocyanin is influenced by the media composition and growth conditions as well as growth rate. In order to avoid conventional optimization, statistical models Fractional Factorial Design (FFD) and Central Composite Design (CCD) have been proved to be effective (Hong and Lee, 2008, (Xiao et al, 2007). In the present study we have used two level factorial Plackett Burman design for increasing the production of phyocycnin isolated from Salim ali lake.

Materials and Methods

Isolation and cultivation

The blue green algae *Microcystis* sp. used in this study was isolated from the water sample of Salim ali lake. The identification was done as per Desikachary (Desikachary T.V. 1959). The cyanobacterium was maintained on BG-11 media containing (g/L)

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$NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; EDTA, 0.001; Na_2CO_3, 0.02. The medium was amended with 1 ml trace solution of composition (g/L) H3BO3, 2.86; $MnCl_2$, 1.81; $ZnSO_4.7H_2O$, 0.222; $Na_2MoO_4.2\ H_2O$, 0.39; $CuSO_4.5\ H_2O$, 0.079; and $Co(NO_3)_2.6H_2O$, 0.0494. (Kaushik, B.D. 1987). In 250 ml capacity Erlenmeyer flasks 100 ml culture medium was taken and the initial cell density was maintained at 10^6 cells/ml. The flasks were exposed to a light intensity of 75 µmol photons.m^{-2}.s^{-1} with a light/dark cycle of 16/8 h at 24 ± 2°C.

Extraction of phycocynin

After incubation of 12 days the cells were harvested by centrifugation at 5000 × *g* for 10 min at 5°C (REMI, C24). The pellet was washed with distilled water and resuspended in 5 ml of 0.05 M phosphate buffer. The content was sonicated (Sonics and Materials Inc., USA) with 30 Hz

frequency at a pulse of 10/5 min. Freezing-thawing of the sonicated content was done at least for two times and cell rupture was confirmed microscopically. The extract was centrifuged at 10000 × *g* for 10 min at 5°C. The supernatant was collected and the concentration of phycocyanin was

measured spectrophotometrically at 615 and 652 nm using following Equation (1) with 0.05 M phosphate buffer as blank (Kaushik, B.D. (1987).

Phycocyanin (PC) mg/ml = {*A*615 - (0.474 × *A*652)}/5.34 (1)

Statistical Analysis

A 12-run Plackett-Burman design (Plackett and Burman, 1946). was used to screen the eight major nutrients in BG-11 growth medium influencing the production of phycocyanin. The level of micronutrients for each flask was kept constant. A method of least squares was used to analyze the experimental response fit using the following first-order model, Equation (2):

$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 \quad (2)$$

where, $\hat{Y}$ was the predicted response (Phycocyanin production), β_0, β_1, β_2, β_3, β_4, β_5, β_6, β_7 and β_8 were the regression coefficients, and X_1, X_2, X_3, X_4, X_5, X_6, X_7 and X_8 were the coded levels of the independent variables. All the statistical analysis was done using MINITAB 13.31 statistical software.

Results and discussion

Natural colorants, such as phycobiliproteins, chlorophylls, β-carotene, are gaining importance over synthetic ones, as they are nontoxic and non-carcinogenic.(Ganka et al, 2007). Commercial production of phycocyanin has been done from a long time with market leaders such as Cyanotech and Dainippon Ink producing phycocyanin at around US $ 5,000-33,000 g-1(Sekar, S.; Chandramohan, M. 2008). , (Yamaguchi , 1997). The *Plackett-Burman* design (PBD) has been frequently used for screening process variables that make the greatest impact on a process (Plackett and Burman, 1946). It is a set of small and efficient experimental design, which is very powerful, widely applicable and especially well suited for biotechnology research and development (Haaland , 1989)

Several experimental design models could be employed to reduce the number of experiments

under different conditions. If it is desired to screen a large number of factors, experimental designs for first-order models, such as the factorial design or Plackett–Burman design (Plackett and Burman 1946), can be used.(Yin et al, 2007) In the present study considering the eight macronutrients of BG-11 influencing the production of phycocyanin a 12 run Plackett Burman design was used as shown in table 1.

Each row represents the 12 different experiments to evaluate their effect on phycocyanin production and each column represents a different variable. Each independent variable was investigated at a high (+1) and a low (-1) level which represents two different nutrient concentrations.(Deshmukh and Puranik, 2012). The *t* test and regression analysis was done to determine significant levels of each variable (Table 2.).

The table 2 represents the results of Plackett Burman trials with respect to *t*- value, *p*-value and the confidence level of each component. The significant value indicates that the component was influencing the production of phyocyanin. Out of the eight media component tested in this model, only two media component X_3 i.e $MgSO_4.7H_2O$ and X_6 i.e Ferric ammonium citrate were 96.1% and 97.7% significant.

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Table 1 The Plackett-Burman experimental design matrix for screening medium composition of BG-11

Trial	Level and concentration of variable (g L^{-1})								
	X_1 $NaNO_3$	X_2 K_2HPO_4	X_3 $MgSO_4.7H_2O$	X_4 $CaCl_2.2H_2O$	X_5 Citric acid	X_6 Ferric ammonium citrate	X_7 EDTA	X_8 Na_2CO_3	Exptl.
T_1	+1 (2.25)*	-1 (0.02)	+1 (0.1125)	-1 (0.018)	-1 (0.003)	-1 (0.003)	+1 (0.0015)	+1 (0.03)	0.480
T_2	+1 (2.25)	+1 (0.06)	-1 (0.0375)	+1 (0.054)	-1 (0.003)	-1 (0.003)	-1 (0.0005)	+1 (0.03)	0.363
T_3	-1 (0.75)	+1 (0.06)	+1 (0.1125)	-1 (0.018)	+1 (0.009)	-1 (0.003)	-1 (0.0005)	-1 (0.01)	0.030
T_4	+1 (2.25)	-1 (0.02)	+1 (0.1125)	+1 (0.054)	-1 (0.003)	+1 (0.009)	-1 (0.0005)	-1 (0.01)	0.002
T_5	+1 (2.25)	+1 (0.06)	-1 (0.0375)	+1 (0.054)	+1 (0.009)	-1 (0.003)	+1 (0.0015)	-1 (0.01)	0.161
T_6	+1 (2.25)	+1 (0.06)	+1 (0.1125)	-1 (0.018)	+1 (0.009)	+1 (0.009)	-1 (0.0005)	+1 (0.03)	0.042
T_7	-1 (0.75)	+1 (0.06)	+1 (0.1125)	+1 (0.054)	-1 (0.003)	+1 (0.009)	+1 (0.0015)	-1 (0.01)	0.003
T_8	-1 (0.75)	-1 (0.02)	+1 (0.1125)	+1 (0.054)	+1 (0.009)	-1 (0.003)	+1 (0.0015)	+1 (0.03)	0.138
T_9	-1 (0.75)	-1 (0.02)	-1 (0.0375)	+1 (0.054)	+1 (0.009)	+1 (0.009)	-1 (0.0005)	+1 (0.03)	0.102
T_{10}	+1 (2.25)	-1 (0.02)	-1 (0.0375)	-1 (0.018)	+1 (0.009)	+1 (0.009)	+1 (0.0015)	-1 (0.01)	0.243
T_{11}	-1 (0.75)	+1 (0.06)	-1 (0.0375)	-1 (0.018)	-1 (0.003)	+1 (0.009)	+1 (0.0015)	+1 (0.03)	0.215
T_{12}	-1 (0.75)	-1 (0.02)	-1 (0.0375)	-1 (0.018)	-1 (0.003)	-1 (0.003)	-1 (0.0005)	-1 (0.01)	0.384

*Values in parentheses are concentrations in g L^{-1} of each variable in BG-11; Level of micronutrients in all experiments was kept constant

Table 2 The effects and coefficient of variables estimated using Plackett- Burman design

variable	Effect	Standard error	*t*- value	*p*- value	Confidence level (%)
Constant		0.01826	9.87	0.002	
X_1 ($NaNO_3$)	0.06983	0.01826	1.91	0.152	84.9
X_2 (K_2HPO_4)	-0.08917	0.01826	-2.44	0.092	90.8
X_3 ($MgSO_4.7H_2O$)	-0.12883	0.01826	-3.53	0.039	96.1*
X_4 ($CaCl_2.2H_2O$)	-0.10417	0.01826	-2.85	0.065	93.5
X_5 (Citric acid)	-0.12183	0.01826	-3.34	0.055	94.5
X_6 (Ferric ammonium citrate)	-0.15817	0.01826	-4.33	0.023	97.7*
X_7 (EDTA)	0.05283	0.01826	1.45	0.244	75.6
X_8 ($Na_2CO_3$0	0.08617	0.01826	2.36	0.099	90.1

* significant at 95% level ($p< 0.05$)

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One possible way by which these significant component influence the production of phycocyanin is that they are correlated in the regulation of the branched metabolic pathway for tetrapyrole compounds in cyanobacteria. The three major tetrapyrrole end products chlorophyll (Chl), 3 heme, and phycobilins are synthesized in a branched metabolic pathway with protoporphyrin IX (PIX) as the last common precursor (Sobotka et al, 2008), In the magnesium branch magnesium catalyzes the chelation of Mg in PIX ,while the iron branch insertion of Fe^{2+} into PIX by ferrochelatase leads to the formation of protoheme, there by directing tetrapyrroles into Chl synthesis. Cyanobacteria and plants accumulate various tetrapyrrole species in different quantities in the cell.

Thus the present work will certainly help to understand and determine the main factors controlling the production of phycocyanin from *Microcystsis* sp isolated from Salim Ali lake , Aurangabad.

Conclusion

Cyanobacteria have been explored for different metabolite useful to humans, one of them is phycocyanin. Because of its wide application in different field the pigment is of high demand. The salim ali lake Aurnagabad is a well known lake, but very little information regarding the cyanobacteria and its potentials is available. In this study the phycocyanin producing capability if Microcystis sp isolated from Salim ali lake has been done. The Plackett- Burman design has been used to evaluate the components of BG-11, affecting the production of phycocyanin. Maganesium sulphate and Ferric ammonium citrate were found to be the effective components affecting the phycocyanin production.

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