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Application of Plackett-Burman Design to evaluate Media Components Affecting Antibacterial Activity of Alkaliphilic Cyanobacteria Isolated from Lonar Lake

[Placknett-Burman Düzenlemesinin Lonar Gölünden İzole Edilen Alkalifilik Siyanobakterilerin Antibakteriyel Aktivitesini Etkileyen Vasat Bileşenlerinin Değerlendirilmesine Uyarlanması]

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ABSTRACT

Objective: To evaluate the media components affecting the antimicrobial activity of alkaliphilic cyanobacteria using Plackett-Burman design.

Material and Methods: Seven cyanobacterial cultures were isolated from alkaline-saline lake Lonar, MS, India. Cell extract of cyanobacteria in methanol, distilled water, acetone and isopropanol was evaluated for antibacterial activity against 5 bacterial cultures using disc diffusion method. Media components affecting the antibacterial activity of cyanobacteria were evaluated with the help of Plackett-Burman design.

Results: Among cultures under test the unicellular cyanobacterium, *Synechocystis aquatilis* showed highest antibacterial activity against the test cultures. Acetone was found to be suitable extraction agent yielding maximum antibacterial activity for *Synechocystis aquatilis* From the Plackett-Burman experiment magnesium sulphate and ferric ammonium citrate were appeared to be the media components influencing antibacterial activity of *Synechocystis aquatilis*.

Conclusion: Plackett-Burman design was useful in determining the media components affecting antibacterial activity of *Synechocystis aquatilis* significantly.

Keywords Alkaliphilic cyanobacteria · Lonar Lake · antibacterial activity · Plackett-Burman

ÖZET

Amaç: Alkalifik siyanobakterilerin antibakteriyel aktivitesini etkiliyen vasat bileşenlerinin Plackett Burman düzenlemesi kullanılarak değerlendirilmesi.

Metod ve Materyal: Alkali-tuzlu Lonar gölünden (MS, Hindistan) yedi siyanobakteri kültürü izole edildi. Disk diffuzyon metodu kullanılarak metanol, distile su, aseton ve isopropanol içindeki syanobakteri hücre ekstraktının beş bakteri kültürüne karşı antibakteriyel etkisi değerlendirildi. Placknet-Burman düzenlemesi kullanılarak vasat komponetlerinin siyanobakterinin antibakteriyel aktivitesinine olan etkisi değerlendirildi.

Sonuçlar: İncelemeye alınan siyanobakteri kültürleri içinde tekhücreli *Synechocystis aquatilis* test edilen kültürlere karşı en yüksek antibakteriyel aktiviteyi gösterdi. Aseton, *Synechocystis aquatilis* için maksimum antibakteriyel aktiviteyi sağlayan en uygun ekstraksiyon ajanı olarak belirlendi. Magnesium sülfat ve ferrik amonyum sülfat Placknet-Burman deneyi ile *Synechocystis aquatilis*'in antibakteriyel aktivitesini etkilermiş gibi gözüken vasat bileşenleri olarak belirlendi.

Yorum: *Synechocystis aquatilis*'in antibakteriyel aktivitesini önemli düzeyde etkiliyen vasat bileşeninin belirlenmesinde Placknet-Burman düzenlemesi yararlı olmuştur.

Anahtar kelimeler: Alkalifik siyanobakteri, Lonar Gölü antibakteriyel aktivite, Plackett-Burman

Introduction

A characteristic feature of the majority of soda lakes is the presence of permanent or seasonal colored blooms of phototropic microorganisms (1). Extreme alkaliphiles live in soils laden with soda or in soda lakes where the pH can rise to 12, but such organisms grow poorly at neutral pH. As many as 13 alkaliphilic cyanobacteria were reported to grow under alkaline conditions. Very often, soda lakes are monospecific, inhabited by *Spirulina platensis* which serves as human food (single cell protein) of high nutritional value (2)

The alkaline lake of Lonar is situated in the outskirts of Lonar town in district Buldhana in Maharashtra, India (lat 19° 58', lag 76° 34'). The lake originated as a meteorite impact crater around 50-60 thousand years ago. It is the third largest in the world and only crater in basaltic rock (3). The lake water is highly alkaline and the alkalinity is due to high content of sodium carbonate. Craters formed by such hypervelocity impact offer unique ecological environment. The alkaline saline lakes often have cyanobacterial blooms with dominance of *Spirulina* sp. (1). Presence of *Synechocystis aquatilis* is another typical character of cyanobacterial population associated with alkaline lake. *Synechocystis aquatilis* is a unicellular cyanobacterium arranged in groups or scattered with a dull blue-green color (4).

Cyanobacteria have been screened for a variety of potentially useful bioactivities including cytotoxic, multidrug resistance reversals, antifungal and antiviral effects (5). They also provide novel and useful pharmaceuticals that are difficult to produce synthetically because of their structural complexity (6). In general isolation of bioactive compounds from cyanobacteria is done with two objectives. One is to discover new compounds for pharmaceutical, agricultural or biocontrol application. The other is to better understand the interactions of individual organisms within their natural communities (7,8). In literature various cyanobacterial strains belonging to genera *Spirulina*, *Tolypothrix*, *Anabaena*, *Cylindrospermopsis*, *Tychonema*, *Microcystis*, *Aphanizomenon*, *Oscillatoria* and *Trichodesmium* (9-12) have been reported for antibacterial activities.

Media composition and growth conditions influence the culture growth thus the antibacterial performance. Antibacterial activity of culture can be increased with proper optimized media composition. Conventional optimization may involve screening of large number of variables. The Plackett-Burman design provides an efficient way of a large number of variables and identifying the most important ones. Numerous reports have proved the applicability of Plackett-Burman design in the optimization of media components for various culture activities (9, 13-17). The aim of the present work was to study antibacterial activity of alkaliphilic cyanobacteria isolated from Lonar Lake. A statistical model viz. Plackett-Burman design was used for identification of important components in the media influencing the antibacterial activity.

Materials and Methods

Cyanobacterial cultures

Water samples were collected from Lonar Lake, District: Buldhana, MS, India. Presterilized plastic bottles were used for the collection of water samples. Alkaliphilic cyanobacterial cultures from water samples were isolated using BG-11 medium containing (g/L) $NaNO_3$, 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 and pH was adjusted to 10.36 as similar to lake water pH. Medium was amended with 1 ml trace solution of composition (g/L) H_3BO_3, 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. Axenic cultures were obtained by repeated transfers from solid to liquid and through antibiotic selection (18) at 22-24 °C illuminated with white fluorescent tube light with a light intensity of 1500-1800 lux with a 16/8 h light/dark cycle. The identification of the isolates was done on the bases of morphology according to (4, 19).

Preparation of cell extracts

Cyanobacterial biomass was harvested after 15 days of incubation by centrifugation at 5000 *g* for 15 min (Sorvall, USA, Model RC2). Water extracts were made by resuspending cyanobacterial biomass in distilled water (10 mg/L) and ultrasonicating at 20 KHz frequency (Sonics and Materials Inc., USA) for 2 min. The biomass was also extracted simultaneously with organic solvents viz. methanol, isopropanol and acetone. The cell mass was separated by centrifugation at 5000 *g* for 20 min and the extraction procedure was repeated twice. The pooled supernatants were dried at 40 °C under reduced pressure in a rotary evaporator (Roteva, India). The dried extracts were resuspended in 3 ml of each solvent and preserved at 4°C till further use in antibacterial assays.

Test organisms

Five bacterial cultures from laboratory repository viz. *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus vulgaris* were used in the study. Freshly grown 12 h old cultures in nutrient broth were used as the inoculum in antibacterial assays.

Agar diffusion assay

Antibacterial activity of cell extracts against the test organisms was done by disc diffusion assay (20). Petri plate containing 15 ml of solidified nutrient agar was spread inoculated with 100 µl of 12 h old test bacterial cultures. Presterilized Whatman No.1 paper discs (6 mm) were saturated with 50 l of cyanobacterial extracts and dried to be used in assays. The plates were kept at 4 °C for 2 h before they were incubated at 37 °C for 24 h. Anti-

bacterial activity was assessed by measuring the diameter of growth inhibition zone around the discs. Sensitivity of test organisms was also checked against commercial discs (Hi Media, India) containing standard antibiotics viz. Penicillin-G (P), Clindamycin (Cd), Erythromycin (E) Tetracycline (T) and Cephotaxime (Ce).

Plackett-Burman design

The Plackett-Burman design based on the first order model (21) was used to screen and evaluate the important media components that influence the production of antibacterial compounds. All the experiments were carried in triplicate according to designed matrix (Table 1) using the equation 1:

$$Y = \beta_0 + \Sigma \beta_i X_i \quad (i = 1, \ldots. k) \qquad \text{(Equation 1)}$$

Where, Y is the estimated target function, β_0 is a constant, β_1 *is* the regression coefficient, X is independent variable and k is number of variables. Total eight variables from BG-11 were screened include $NaNO_3$, K_2HPO_4, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, citric acid, ferric ammonium citrate, EDTA and Na_2CO_3. Each independent variable was investigated at a high (+1) and a low (-1) level which represents two different nutrient concentrations as shown in Table 1. The level of micronutrients in all experiments was kept constant as described earlier. The level of micronutrients in all experiments was kept constant as described earlier. From the experimental trials (T_1 - T_{12}) cell mass was extracted with acetone and used in antibacterial disc diffusion assay. The diameter of zone of inhibition was recorded as response value. The student's *t*-test was performed to determine the significance of each variable employed. The regression coefficients were determined by least square method.

Results and Discussion

The alkaliphiles are unique microorganisms, with great potential for microbiology and biotechnological exploitation. Cyanobacteria produce a variety of remarkable compounds collectively referred to as secondary metabolites (22). In spite of being potential producers of a wide spectrum of natural substances of vital human need, microalgae have so far been a rather underexplored source in the development of biotechnology (23). Alkaliphilic microorganisms have received the most attention for extracellular enzymes and their genetic analysis, mechanisms of membrane transport and pH regulation, and the taxonomy of alkaliphilic microorganisms (24). In present study cyanobacterial cultures were isolated from the soda lake of Lonar, which is a unique habitat in India, rich source of naturally occurring alkaliphilic microbes. One of the striking facts about soda lakes is that, despite apparently inhospitable caustic conditions, these environments are extremely productive because of high ambient temperatures, high light intensities, and effectively unlimited supplies of CO_2 via the $HCO_3^-/CO_3^{2-}/CO_2$ equilibrium (1). A characteristic feature of the majority of soda lakes is the presence of permanent or seasonal colored blooms of phototropic microorganisms. The Lonar lake remains green colored througho-

Table 1. Plackett-Burman experimental design matrix for screening composition of growth medium, BG-11 for *Synechocystis aquatilis*

Trial	Level and concentration of variable (g/L)							
	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
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)

Values in parentheses are concentrations in g/L of each variable in BG-11; Level of micronutrients in all experiments was kept constant.

ut the year due to dominance of cyanobacterial population, especially genus *Spirulina*. In addition to *Spirulina* we could isolate other cultures belonging to genera as minor partner viz. *Synechocystis* [1], *Oscillatoria* [2] and *Phormidium* [3]. An account of diversity of aerobic bacteria of the Lonar lake ecosystem has been reported by Joshi et al. (3). There are meager data available on cyanobacterial diversity of Lonar lake.

An earlier study carried out in our laboratory has pointed out the antimicrobial potentials of halotolerant cyanobacteria (20). It is evident that the selection of experimental organisms to a high degree is restricted to species which are the conventional laboratory strains. Studies have documented the importance of extremophilic cyanobacteria as producers of a wide spectrum of natural products of vital need and economic potential (20, 22). Antibacterial activities of seven alkaliphilic cyanobacterial isolates extracted with acetone, isopropanol, methanol along with aqueous extracts were tested using disc diffusion method (Table 2). In all 28 extracts were tested against 5 test cultures. Out of 140 combinations of extracts and test cultures growth inhibition of test organism was seen in 127 cases. On the basis of cumulative antibacterial effect for cultures under test, *Synechocystis aquatilis* appeared as most effective culture. Highest cumulative inhibitions were noted as 11.0, 8.5, and 7.5 cm for *Synechocystis aquatilis* extracted with acetone, methanol and isopropanol, respectively. Among the four solvents, aqueous extraction did not show highest inhibition in any of the cultures. The overall performance of solvents in terms of cumulative antibacterial activity had followed order inhibitory activity as follows; methanol (28.5%) > acetone (26.8%) > isopropanol (24.4%) > aqueous (20.3%). Based on these results acetone extract of *Synechocystis aquatilis* was chosen for further experiments. Table 3 shows the data for growth inhibition of test cultures by standard antibacterial antibiotics. The overall sensitivity of test cultures against standard antibiotics has generated sensitivity series as; *S. aureus* > *P. vulgaris* > *P. aeruginosa* > *B. subtilis* > *E. coli*. Similar pattern of culture sensitivity was also observed with cyanobacterial extracts only with reversed order for *P. vulgaris* and *P. aeruginosa*. Complete growth inhibition of *S. aureus* was seen in presence of standard antibiotics indicating highest sensitivity. Antibacterial activity was found to be dependent on type of algal species, type of solvent and test organisms.

Media composition and growth conditions influence the culture growth thus the antibacterial performance. In order to obtain higher antibacterial activities media optimization was necessary. The BG-11 medium contains eight variables thus media optimization would require 256 trials to be run. Plackett-Burman design matrix (Table 1) with eight variables was applied to find out variables influencing antibacterial activity of *Synechocystis aquatilis* Antibacterial activities of acetone extracts of *Synechocystis aquatilis* grown as per Plackett-Burman design are presented in Table 4. Since the data observed was inhibitory against all the test organisms a mean of cumulative inhibition was considered (Table 4). Highest mean of cumulative antibacterial activity was obtained for trial T_9 followed by T_{10}. The mean cumulative antibacterial effect was further processed by using the statistical software MINITAB 13.13. Significance of each variable was determined using student *t*-test. The components were screened at the confidence level of 95% on the basis of their effects. Table 5 represents the result of Plackett-Burman trials with respect to *t*-value, *p*-value and confidence level of each component. The *p*-value is probability of magnitude of contrast coefficient due to random process variability and serves as a tool for checking significance of each coefficient (14). The significant values indicate that the component was effective in antibacterial activities. Out of eight media components tested, only magnesium sulphate and ferric ammonium citrate had a significant effect on antibacterial activity of alkaliphilic cyanobacterial cultures, when cumulative effect against all test organisms was considered. Media components viz. sodium chloride, magnesium sulphate, micronutrients and iron sulphate have been reported as effective for different test cultures following Plackett-Burman design (9).

The role of magnesium and iron in increasing antibacterial activity could be correlated to change in regulation of 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 (25). The iron branch insertion of Fe^{2+} into PIX by ferrochelatase leads to the formation of protoheme, whereas in the magnesium branch magnesium catalyzes the chelation of Mg^{2+} into PIX, there by directing tetrapyrroles into Chl synthesis. Cyanobacteria and plants accumulate various tetrapyrrole species in different quantities in the cell. The influence of magnesium and iron on antibacterial activity of *Synechocystis aquatilis* has indicated to the tetrapyrolic nature of antibacterial compound. However, these results emphasize further studies on purification and characterization of antibacterial principles in *Synechocystis aquatilis.*

Several species of microalgae have been shown to produce substances with antibiotic activity (26). The antibacterial of the cyanobacteria may be fatty acids, other organic acids, peptides, polysaccharides and alcohols. Some of these substances are secreted by the cell while others depend upon the influence of cultivation factors (22). Production of these compounds is indirectly influenced by the media components. The results have indicated that Plackett-Burman design was useful in determining the media components affecting antibacterial activity of *Synechocystis aquatilis* significantly. The media further will be optimized giving only consideration to these factors. Thus the present work will certainly

Table 2. Antibacterial activity of alkaliphilic cyanobacterial extracts evaluated by disc diffusion method

Isolate	Extract	Mean diameter of zone of inhibition (cm)					
		E. coli	*P. aeruginosa*	*P. vulgaris*	*B. subtilis*	*S. aureus*	Cum.
Spirulina platensis	Ac	0.9 (0.20)*	1.1(0.15)	1.0 (0.15)	1.5 (0.25)	2.2 (0.35)	6.7
	Aq	1.3 (0.23)	1.2 (0.15)	1.3 (0.37)	1.3 (0.10)	1.0 (0.20)	6.1
	Isp	1.0 (0.20)	1.0 (0.20)	1.0 (0.25)	R	1.1 (0.26)	4.1
	Met	1.3 (0.15)	1.2 (0.20)	1.5 (0.36)	0.8 (0.15)	2.2 (0.20)	7.0
Synechocystis aquatilis	Ac	2.5 (0.25)	2.2 (0.37)	1.3 (0.37)	2.6 (0.30)	2.4 (0.45)	11.0
	Aq	1.1 (0.26)	1.2 (0.32)	0.9 (0.30)	1.2 (0.20)	0.8 (0.15)	5.2
	Isp	1.7 (0.26)	1.8 (0.25)	1.1 (0.30)	1.6 (0.35)	1.3 (0.47)	7.5
	Met	1.3 (0.35)	2.3 (0.26)	1.4 (0.40)	1.4 (0.20)	2.1 (0.37)	8.5
Oscillatoria minimus	Ac	1.9 (0.40)	1.2 (0.30)	1.1 (0.25)	1.2 (0.15)	0.9 (0.20)	6.3
	Aq	1.1 (0.36)	R	R	R	1.3 (0.26)	2.4
	Isp	1.0 (0.20)	1.5 (0.30)	1.9 (0.15)	1.0 (0.20)	1.2 (0.37)	6.6
	Met	1.4 (0.35)	1.0 (0.17)	0.9 (0.15)	0.8 (0.15)	1.5 (0.20)	5.6
Oscillatoria amphibia	Ac	1.0 (0.20)	1.8 (0.20)	1.4 (0.20)	1.2 (0.30)	1.3 (0.11)	6.7
	Aq	1.2 (0.20)	1.2 (0.35)	1.4 (0.35)	R	1.2 (0.20)	5.0
	Isp	1.2 (0.32)	0.9 (0.32)	1.2 (0.35)	1.0 (0.20)	1.0 (0.20)	5.3
	Met	1.3 (0.20)	1.5 (0.36)	1.9 (0.45)	1.2 (0.30)	1.0 (0.36)	6.9
Phormidium laminosum	Ac	R	1.1 (0.20)	1.3 (0.36)	1.2 (0.35)	1.3 (0.25)	4.9
	Aq	1.0 (0.20)	1.2 (0.30)	1.3 (0.35)	1.0 (0.32)	1.3 (0.23)	5.8
	Isp	1.2 (0.30)	1.2 (0.11)	1.2 (0.26)	1.4 (0.35)	0.9 (0.15)	5.9
	Met	1.2 (0.30)	R	1.3 (0.41)	1.2 (0.30)	1.9 (0.40)	5.6
Phormidium tenue	Ac	R	1.0 (0.15)	1.4 (0.30)	1.2 (0.35)	0.9 (0.20)	4.5
	Aq	1.3 (0.37)	1.2 (0.30)	1.3 (0.36)	1.0 (0.20)	1.3 (0.35)	6.1
	Isp	1.3 (0.15)	1.4 (0.20)	0.9 (0.30)	1.3 (0.11)	0.9 (0.11)	5.8
	Met	1.4 (0.30)	1.4 (0.35)	1.0 (0.20)	1.2 (0.41)	2.0 (0.40)	7.0
Phormidium fragile	Ac	1.0 (0.15)	0.8 (0.15)	0.9 (0.20)	R	1.4 (0.20)	4.1
	Aq	R	0.9 (0.15)	1.2 (0.30)	1.2 (0.30)	R	3.3
	Isp	1.5 (0.30)	1.3 (0.30)	R	2.3 (0.30)	R	5.1
	Met	1.2 (0.11)	1.4 (0.20)	1.0 (0.30)	1.4 (0.20)	1.2 (0.30)	6.2

* Values in parentheses are standard deviation; Ac, Acetone; Aq, Aqueous; Isp, Isopropanol; Met, Methanol; R, resistant; Cum, Cumulative

Table 3. Sensitivity of test organisms to standard antibiotics in disc diffusion assay

Antibiotic	Mean diameter of zone of inhibition (cm)				
	E. coli	*P. aeruginosa*	*P. vulgaris*	*B. subtilis*	*S. aureus*
Penicillin G (P), 10 U	2.6	3.8	1.8	1.2	HS
Clindamycin (Cd), 2 mg	1.0	1.8	2.6	2.8	HS
Erythromycin (E), 15 mg	3.0	2.6	2.2	3.2	HS
Tetracycline (T), 30 mg	2.6	2.0	4.4	3.2	HS
Cephotaxime (Ce), 30 mg	1.6	2.4	2.4	1.0	HS
Mean Inhibition	10.8	12.6	13.4	11.4	HS

HS, Highly sensitive where growth of test culture was completely inhibited.

Table 4. Effect of media components on antibacterial activity of *Synechocystis aquatilis* tested as per the Plackett-Burman design

Trial	Mean diameter of zone of inhibition (cm)					
	E. coli	*P. aeruginosa*	*P. vulgaris*	*B. subtilis*	*S. aureus*	Mean Cumulative effect
T_1	1.3 (0.41)	1.1 (0.30)	1.4 (0.20)	1.6 (0.40)	1.1 (0.30)	1.3 (0.21)
T_2	0.9 (0.11)	1.7 (0.30)	1.9 (0.11)	1.6 (0.20)	1.3 (0.30)	1.5 (0.40)
T_3	1.6 (0.40)	1.1 (0.41)	1.4 (0.20)	1.1 (0.30)	0.9 (0.11)	1.2 (0.30)
T_4	0.9 (0.11)	2.1 (0.23)	1.0 (0.20)	2.3 (0.23)	1.1 (0.23)	1.5 (0.70)
T_5	1.1 (0.20)	0.9 (0.11)	1.3 (0.30)	1.7 (0.41)	2.1 (0.11)	1.4 (0.50)
T_6	0.9 (0.23)	1.3 (0.30)	2.3 (0.30)	1.2 (0.41)	1.1 (0.30)	1.4 (0.50)
T_7	1.7 (0.41)	0.9 (0.11)	0.9 (0.20)	2.3 (0.30)	1.3 (0.41)	1.4 (0.60)
T_8	1.4 (0.20)	1.4 (0.52)	1.0 (0.20)	0.9 (0.11)	1.9 (0. 30)	1.3 (0.40)
T_9	1.2 (0.40)	2.5 (0.30)	2.0 (0.20)	1.1 (0.11)	2.1 (0.30)	1.8 (0.60)
T_{10}	1.9 (0.30)	2.1 (0.30)	1.3 (0.30)	2.2 (0.20)	1.1 (0.11)	1.7 (0.50)
T_{11}	2.4 (0.40)	1.2 (0.20)	1.1 (0.30)	1.3 (0.41)	1.3 (0.30)	1.5 (0.53)
T_{12}	1.6 (0.20)	2.1 (0.30)	0.9 (0.11)	1.6 (0.30)	0.9 (0.11)	1.3 (0.51)

Table 5. The effects and coefficients of variables estimated using Plackett-Burman design

Variable	Effect	Coefficient	SE Coefficient	*t*-value	*p*-value
Constant		1.44167	0.025	+57.67	0
X_1 ($NaNO_3$)	0.05000	0.02500	0.025	+1.00	0.391
X_2 (K_2HPO_4)	-0.08333	-0.04167	0.025	-1.67	0.194
X_3 ($MgSO_4.7H_2O$)	-0.18333	-0.09167	0.025	-3.67	0.035*
X_4($CaCl_2.2\ H_2O$)	0.08333	0.04167	0.025	+1.67	0.194
X_5(Citric acid)	0.05000	0.02500	0.025	+1.00	0.391
X_6 (Ferric ammonium citrate)	0.21667	0.10833	0.025	+4.33	0.023*
X_7 (EDTA)	-0.01667	-0.00833	0.025	-0.33	0.761
X_8 (Na_2CO_3)	0.05000	0.02500	0.025	+1.00	0.391

SE, standard error; *Significant at 95% level ($p<0.05$)

help to understand and determine the main factors controlling the production of bioactive compounds from cyanobacteria. There is a need to identify the chemical nature of these bioactive compounds and their possible effects against pathogenic bacterial strains.

Conclusion

Alkaliphilic cyanobacteria are known as potential producers of different metabolites one of them are antibacterial substances. Though the alkaline lake of Lonar is known throughout the world very less information in terms of cyanobacterial potentials has been reported from this lake. In this study the antibacterial potential of the cyanobacteria isolated from Lonar Lake has been studied. All of the cyanobacterial cultures isolated from this lake showed antibacterial activity. Plackett-Burman design was used to identify the media components influencing antibacterial property of *Synechocystis aquatilis*. Two components of the BG-11 medium viz. magnesium sulphate and ferric ammonium citrate were identified. Dependence of antibacterial activity on magnesium and iron concentration has reflected upon tetrapyrollic nature. However, the studies demand further work on purification and characterization of antibacterial principles of *Synechocystis aquatilis* and other cyanobacterial isolates of unique alkaline habitat of Lonar Lake in India.

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