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Original Article

In-vitro pharmacological applications of pigment producing halophilic microorganism from the marine region of India

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ABSTRACT

In the present study, two pigment producing promising strains were identified and characterized based on the biochemical properties from the halophilic environment. Based on the biochemical and physiological characteristics the strain BQ31 and BV32 were selected and both the strains exhibited showed high similarity towards the Haloarcula species. It is identified that the strains showed wide range of carbon utilization, able to tolerate different salts with multiple antimicrobial susceptibility pattern. The extracellular pigment was identified by various chromatographic techniques and the molecular weight of the pigment was characterized by MASS spectrum. Interestingly, the pigment revealed significant liver lipid peroxidase activity and haematological parameters under different body weight of albino rats. The biochemical parameter of the pigment under different body weight of Albino rats is an additional importance. Thus the pigment could be used for the various pharmaceutical applications.

Keywords: Halophilic microbes, Pigment; MS spectrum, In-vitro biological applications

INTRODUCTION

The microorganisms exist in extreme environments such as high salt, high temperature and high atmospheric pressures are defined as the extremophiles. These extremophilic microbes possess unique properties of biotechnological and commercial significance. They also possess special adaptation strategies that make them interesting not only for the fundamental research but also for the industrial applications [1]. Among the extrmophiles, the studies on ecology, physiology and taxonomy of halophiles have revealed an impressive diversity in hyper saline and alkaline lakes [2,3]. Halophiles are belonged to the order Halobacteriales and the family Halobacteriaceae [4]. Halophilic bacteria also categorized as psychrophilic, thermophilic, alkaliphilic, mesophilic, aerobic and anaerobic halophilic based on the environments [5]. They are mainly aerobic and exist in the hypersaline regions such as salterns, salt lakes, sub surface salt formation, and solar salts. Halophiles are mainly involved in the biogeochemistry of phosphorus, carbon and other elements in hypersaline environments [6]. Few reports claimed that halophiles also play in the degradation of organic pollutants as hydrocarbons, pesticides and crude oil [7]. In addition to degradation of organic pollutants, halophiles also used as a biocontrol agents against certain pathogenic fungi [8,9]. Traditionally, halophiles have been used in the food and nutraceutical industries as the fermentation of soy and fish sauces and β-carotene production, also, recently looked into many novel and unique molecules such as the compatible solutes, biopolymers or carotenoids, novel extracellular polysaccharides; exoenzymes such as cellulase, amylase, lipase, proteases and xylanase, and biodegradable plastics, bio-surfactants, bioemulsifiers and bacteriorhodopsins for molecular biotechnological applications [10,11]. Novel protein isolated from from *Halobacterium Salinarum* used in the treatment of cancer. Hypersaline environments are commonly present in the southern parts of the India. Until now, no reports on the characterization of halophilic archaeal communities from Kanyakumari district, India. The present study aimed in the isolation and identification of the halophilc bacteria from the southeast coastal region

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of India and investigates its potential application the medical field.

MATERIALS AND METHODS

Isolation of halophilic strains: Sediment samples from seawater, salt depositing area and salt disposals areas were collected and aseptically transferred in to the laboratory. One gram of the sample was mixed with sterile water and were serially diluted and plated on the nutrient agar plates supplemented with saturated level of 35 % (w/v) NaCl and other ingredients such as casaminoacids (7.5g/l); KCl (2g/l); NaCl (25 g/l); trisodium citrate (3 g/l); magnesium sulphate (20 g/l); manganese sulphate (0.05 g/l); ferrous sulphate (0.5 g/l); yeast extract (10 g/l); agar (10 g/l). The pH was adjusted to 7.4. The plates were incubated at 37ºC incubated up to developing visible colonies. The colonies were purified to obtain the single colony in the same medium, labeled and stored in the glycerol stock (-20^0 C) for long storage, whereas for the routine laboratory experiments the purified strains were cultivated in the slants and stored in the refrigerator at (4^0 C). Based on the growth characteristics, pigment production ability and antimicrobial activity, strain BQ31 and BV32 were selected for the further characterization.

Biochemical and physiological characterization of halophilic strains: To test the biochemical and physiological properties, the strains were freshly cultivated in the nutrient broth and mid log phase strains were routinely used. Phenotypic characteristics of the strains BQ31 and BV32 were evaluated by determining the Gram staining, colonial morphology, size and motility by comparing with the standard reference strains. The appearance of the individual colony appearance and pigment production capability were determined after growth for seven days. The biochemical and physiological properties of the isolate were analysed using routine methods [12-14]. API 50CHB test kits were used to characterize phenotypes [14]. The API test strips were prepared according to the manufacturer's instructions and scored after 96 h incubation at 37°C. To evaluate the starch hydrolysis, the strains were streaked onto nutrient medium with 1% (w/v) soluble starch and plates were flooded with freshly prepared iodine solution after growth was obtained. For catalse test freshly prepared H_2O_2 solution to colonies on medium. The presence of oxidase was determined with tetramethyl *p*-phenylendiamine-HCl. Casein hydrolysis was determined by observing the formation of clear zones around colonies on agar medium with 0.15% (w/v) skimmed milk powder. The results were compared with suitable positive and negative control. Each experiment was conducted in triplicate.

Antibiotic susceptibility pattern of the halophilic strains BQ31 and BV32: Antibiotic sensitivity pattern of the halophilic strains BQ31 and BV32 were determined by the disc diffusion method of Balachandren et al. (2015) [12]. Briefly, the freshly prepared strains were spread on the top of the solidified media and allowed to dry for 15 min at room temperature. Antibiotic discs, penicillin (10 µg), tetracyclin (15 µg), carbencillin (5 µg), rifampicin (15 µg), streptomycin (5 µg), erythromycin (20 µg), bacitracim (25 µg), gentamycin (5 µg), kanamycin (15 µg), ampicillin (15 µg), amoxycillin (15 µg) and chloramphenicol (10 µg) were placed on the surface of the medium and left for 30 min at room temperature for the antibiotics to diffuse. After that, the plates were incubated for 72 h at 37°C and inhibition were measured in millimetres, and the experiment was performed in triplicate.

Optimizing the parameters for the better growth and pigment production: The effect of different pH (5, 6, 7, 8 and 9), different temperature (20, 30, 40 and 50°C) and different salt concentration (5, 10, 15, 20, 25 and 30%) were evaluated for the better growth and pigment production [15]. Briefly, the freshly prepared strains (0.01 cell density) were transferred in the 250 ml flask containing DSM-97 medium and incubated for three weeks. After incubation the cells were separated by centrifuging and the cell growth was determined by taking the absorbance at 600 nm and the supernatant were investigated for the pigment level. The level of pigments was determined by calorimetric method.

Extraction and identification of the pigment: The pigments were extracted by following the method of Blig and Dyer method. To obtain a lipid extract free of retinol, the polar lipids were isolated by precipitation in ice-cold acetone. The isolated polar lipids were dissolved in chloroform and stored at -20 ºC. Further, mass spectrometric analysis was carried out for the confirmation of the pigment. The pigments were dissolved in the mixture of chloroform- methanol (1:1v/v), and The experimental conditions of the mass spectrometry were as follows: range, start (100 amu), stop (1300 amu), and scan time (4.8 s); curtain gas, 20 psi (N2); heating gas temperature, 550^0C; nebulizing gas, 50 psi; heating gas, 50 psi; ion spray voltage, 5500 V; declustering potential, 100 V; and entrance potential, 10 V.

Effect of the pigment on hematological and biochemical properties: Different concentrations

of the pigment (100, 200 and 300 mg) was mixed with the diet and fed to the Swiss albino rats weighing about 30 g. Feed was administrated orally for 4 week. Each week blood was removed from each experimental and control group of rats and hematological parameters such as hemoglobin (%), RBC content WBC content, macrophages, eosinophils, neutrophils and monocytes were determined. Then serum samples were analyzed for various biochemical parameters such as protein, cholesterol, glucose, urea, albumin etc. and the obtained results were recorded. After 4 weeks, final body weight was measured and the rats were anesthetized and sacrificed at the final day of experiment. The liver tissues were homogenized with thiobarbituric acid and the entire homogenate was used for lipid peroxidase activity. Briefly, the reaction mixture consists of 0.1 ml of liver extract, 0.2 M of tris HCl buffer of pH 7, 0.3 mM of ascorbic acid and ferrous ammonium sulphate (0.8 mM), whereas 0.1 ml of one water acts as the control. The reaction mixture was allowed to incubate for 12 h and then mixed with solution containing 0.2 ml of TCA (4 %), 2 ml of TBA (0.8%) and 0.2 ml butylated hydroxy toluene (BHT) (0.4%). This mixture was incubated for 30 minutes in a boiling water bath and then allowed to cool for 10 min under ice cold conditions and 2 ml of chloroform was added and the absorbance was measured at 532 nm.

RESULTS

Physiochemical properties of the samples: The physico-chemical characteristics of the water content of the sediment samples are presented in Table 1. The average temperature at the sampling sites was found to be 27ºC at morning and 32ºC at noon. Color of the soil collected from four sites were observed as reddish black, light brown, slightly reddish and creamy white respectively for the selected isolation sports. Interestingly the chloride content of the seawater was found to be more in all the samples. Copper content was ranged from 4.01 to 4.3 ppm ppm in all the samples.

Isolation and characterization of halophilic strains: The results indicated that the total number of extremely halophilic bacteria in saltern samples was found to be 10^{-3}CFU/g. Among the isolated strains, BQ31 and BV32 noted as the promising strains with regards to the morphology and appearances (Table 2). Therefore, the two strains were studied further for its applications. The colonial pigmentation of these strains were documented as orange red to pale pink, yellowish cream and certain colonies were irregular and spread colonies. The strain BQ31 was found to be square shaped morphology. Colonies on agar plates were small, smooth red orange coloured and entire type. It was a pleomorphic flat cell and measured between 2 µm and 3 µm in diameter (Table 3). Cells were motile by means of flagella. It was noted that the optimal growth temperature of BQ31 strain was be 52ºC and BV32 strain showed optimum temperature at 42ºC. No growth was obtained below 40 ºC and above 60ºC. Biochemical studies confirmed that the strains were secrete extra cellular enzymes such as amylase, gelatinase, cellulase, catalase, oxidase and urease. Simmilarly, the standard strains such as *H. vallismortis, H. quadarata, H. hispanica, H. japonica* and *H. marimorti* also expressed similar profile in terms of extracellular enzyme secretion. The carbohydrate fermentation efficiency of these strains was interesting. The results indicated that the strains able to ferment wide range of sugars such glucose, fructose and sucrose etc., whereas the it cannot able to ferment glycerol and acetate salts (Table 4). The growth pattern of strain BQ31 and BV32 under various salts such as Potassium dichromate, Mercuric chloride, Potassium chloride, Cobaltous chloride, Magnesium sulphate, Copper sulphate and Potassium nitrate indicated that the strain have the ability survive under various stress conditions (Table 5). In general the identified strains could be susceptible to various commercially available antibiotics. Similarly, the selected strains also revealed antimicrobial sensitivity towards various commercially available antibiotics such as penicillin, carbencillin, streptomycin and gentamycin (Table 5). Overall, the micro-morphological, physiological, biochemical and carbohydrate fermentation of the strains confirmed that the strains were belonged to the halophilic in nature. Further medical applications of these strains were studied by checking various parameters.

Optimization of growth and pigment production pattern of strains: Cell growth and pigment production status of the selected strains were optimized by cultivating the strains under different pH, temperature and salt concentrations. Among the pH, 8 and 9m were ideal for both growth and pigment production. Whereas, both the strains comparatively showed similar growth and pigment production profile under temperature 30 and 40. The growth pattern of strains under different concentration of salt is attractive. The results indicated that the strains can able to survive and withstand fewer than 30% of salt. However, the maximum cell growth and pigment production profile was detected fewer than 25% of salt concentration (Figure 1).

Characterization of pigment: The red color pigment isolated by the organic solvent method was further confirmed by MASS spectrum analysis.

The chromatographic spectrum revealed that the maximum peak values were observed at 124. The fragmentation pattern of the red color pigment was observed at 124. Therefore, it is concluded that the molecular weight of the pigment was 124.

Biological activity of the pigment: The lipid peroxidase activity was mentioned in table 7. The body weight of rats was maintained normally. There was no significant change in the body weight of rats within two weeks. There was significant rise in the body weight of rats in medium 200 mg/kg, 300 mg/kg higher sets of rats. Lipid peroxidase activity/mg of protein in control was found to be 1.31 ± 0.09 mg/protein. In the case of least (100 mg/kg), the value was found to be 0.97 ± 0.07 mg/protein. In the medium type rats (200 mg/kg), the peroxidase effect was found to be 1.43 ± 0.09 mg/protein and in the higher type rats (300 mg/kg) the effect of lipid peroxidase was found to be 1.22 ± 0.05 mg/protein comparing four results.

The results indicated that the haematological parameters like RBC, WBC, Hb, eosinophiles, neutrophiles and monocytes were increased (Table 8). In the first week control group showed haemoglobin 11.25 mg%, RBC in the range of 7.97cu/mm and WBC in the range of 10.5 cu/mm and macrophages 9.1 x 10^6 when compared with control. In the first week, all the haematological parameters were gradually increased.

The effect of the pigment of the biochemical parameters under different body weight of Albino rats were displayed in table 9. In the fourth week, nearly one month of treatment control group animal showed more value than the other three weeks. This may be due to gradual growth of the animal. But in least group animal, cholesterol level raised 142 mg/dl, glucose level raised to 128 mg/dl and the urea was found to be 42 g/dl and albumin was found to be 1.4 mg/dl. In median group animals (200 mg/kg) there was no change in protein. But cholesterol, glucose, urea and albumin were increased. In higher weight group animals the cholesterol level was higher than the other two groups of rats.

DISCUSSION

The isolated microbial strains documented different colonial pigments, including red, yellow and orange were observed. Results confirmed that most of isolates were Gram negative rods. Literature study evidenced that the strains were abundantly found in saline soil samples, concentration of salts and organic compounds enhanced the growth and reproduction of haloarchaea strains. So, the presence of salinity and other organic components were analyzed to study the growth and development of haloarchaea. The present study evidenced that the strains might be gradually concentrated by brine evaporation. So, the high content of salt and organic substances in the soil samples might support the growth of the extreme haloarchaea. The most abundant extreme halophilic organism in the saltern soil was red, yellow and brick red strains. The pleomorphic cells were predominant type. The most characteristic inhabitants of hypersaline waters are red due to carotenoids that serve as protective agents. Other retinal proteins permit complex photactic behaviour. The peculiar carotenoids of the halobacteria, bacteriorubrins act primarily as photoprotectants but other roles have not been suggested. For instance, they are involved in an energy transfer capacity that facilitates photoreactivation of DNA damaged by exposure to ultraviolet light [16]. It has been also suggested that the red colour of halobacteria act as an infrared (IR) trap increasing the heat accumulation of the water, which is favorable to these slightly thermophilic organisms [17].

In the present study, the red pigment of both strains shows resistant against certain antibiotics. And some antibiotics are sensitive to red producing strains. The strain isolate from brine pool in Sinai Peninsula (Egypt) was characterized. This strain showed sensitive to bacitracin, novobiocin and anisomycin (25 µg ml^{-1}) and resistant to pencillin, ampicillin, rifampicin, chloromphenicol, neomycin and erythromycin (all at 50 µg ml$^{-1)}$) [18]. Bacteriocins and Halocins like molecule are a diverse collection of proteinacious compounds often referred as "protein Antibiotics" or Bacteriocidal protein with molecular weight ranging from 1kDA-100 kDA. The extremely halophilic archaeon *Haloferax mediterraneii* was able to grow in a minimal medium with optimum pH 7.5 [19]. In the present study, the haloarchaeal strain (BQ31) was found to grow at pH 7. Another isolate (BV32) the pH was found to be 8.

Present study, the result showed that red pigment contains some lipid associated with it but the exact structure was not determined. The molecular weight description was identified by means of Mass spectrometric studies indicated that lipid are also necessary for the synthesis of red pigmented substance. By the use of chemical analysis chromatography, spectral analysis (including FTIR, NMR, FAB-MS and CI-MS) in determining the chemical structure of phospholipid and glycolipids in extremely halophilic archaeal bacteria is reviewed in the presence of phospholipid in halobacteria, novel bis–bis sulfated glycolipid in an extreme halophile from Japan, an unusual

alkailiphilic archaeabacteria from India [20]. It is evident that involvement of lipids in certain bacteriorhodopsin properties. Such properties include the regulation of pK for the purple to blue transition caused by deionization and the reformation of trimers from monomers after exposure of the membrane to Triton –X100 and participation of acidic amino acids, mainly asp36 and /or asp 38 analyzed [21]. So in the present study, red pigment contains 75% protein and 25% lipid and also retinol pigment. Cells of extremely halophilic bacteria, *Halobacterium cutirubrum* and *Halobacterium halobium* in a chemically defined medium (BSMK) were red due to the presence of bacteriorubrins (maxima, 370, 388, 494, and 527 nm). Adding 1% glycerol to BMSK stimulated growth but cells rapidly lost bacterioruberin becoming greyish purple in the stationary phase. Acetone extract of these cells were yellow with a broad absorption band at 360-390nm, partly attributable to retinal. In BMSK medium with or without glucose, the bacteriorubrin concentration increased until maximal growth was reached, then felt rapidly [22]. In the present study, the temperature optimum for the formation of red pigment was found to be 40ºC. The salinity for the production of red pigment in strain BQ31 and BV32 was found to be 20 and 40 respectively. It was evident from the study that, more than five bright red pigments, motile, rod shaped, extremely halophilic bacteria were isolated from saltern crystallizer ponds in Alicante (two strains) and Mallorea (three strains) Spain [23,24]. It grew optimally at 20 and 30% of salt concentrations and no growth below 15% salts. Thus, these isolates were among the most halophilic organism known within domain Bacteria. So the red pigment was potentially a valuable source for the nutrient level as well as many biotechnological applications.

CONCLUSIONS

In summary the active pigments identified from the halophilic bacteria revealed significant liver lipid peroxidase activity and haematological parameters under different body weight of albino rats. The biochemical parameter of the pigment under different body weight of Albino rats is an additional importance. Thus the pigment could be used for the various pharmaceutical applications.

Table 1. Physicochemical characteristics of solar saltern located at Thamaraikulam near Kanyakumari coast of India.

Characteristics	Sample site-1	Sample site-2	Sample site-3	Sample site-4
Colour	Reddish black	Light brown	White	Creamy white
Dissolved oxygen (mg/L)	9.04	3.68	2	5. 02
Free CO_2 (ppm)	300	230	130	130
Carbonate (ppm)	10	25	0	18
Bicarbonate (ppm)	65	65	60	56
Chloride (mg/L)	24. 82	20.28	14.51	0
Salinity (ppt)	44.83	36.63	26.22	0
Hardness (mg/L)	220	100	801	200
Electrical conductivity (ohm)	0.08	0.03	0.04	0.28
pH	7	7	8	7
Magnesium (g/kg)	1.5	1.09	1.04	0.44g
Nitrogen (ppm)	34	23	32	28
Potassium (ppm)	120	118	112	92
Iron (ppm)	3.1	5.2	5	4.3
Copper (ppm)	1	0.74	0.92	60
Zinc (ppm)	0.543	0.76	0.924	0.917
Manganese (ppm)	3.09	3.01	0.91	4.01.
Calcium (ppm)	4	4.3	2.1	1.7

Table 2. Phenotypic characteristic of haloarchaeal strains BQ31 and BV32

Characteristics	**BQ31**	**BV32**	**Reference strains**				
			Haloarcula vallismortis	***Haloarcula quadrata***	***Haloarcula hispanica***	***Haloarcula japonica***	***Haloarcula marimorti***
Colonial morphology	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colonial size	3-5	3-5	3-5	2-3	2-3	2-5	3.5
Colony edge	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Cell shape	Pleo-morphic flat	Pleo-morphic rod	Pleo-morphic square flat	Pleo-morphic triangular flat	Pleo-morphic flat disc	Pleo-morphic rod	Pleo-morphic rod
Square end	+	–	+	+	+	+	+
Gram stain	–	–	–	–	–	–	–
Motile	Motile	Motile	Motile	Motile	Motile	Motile	Motile
Pigmentation	Orange red	Brown	Brick red	Yellow	White	Red	Light yellow
pH	6.5-7.2	7-7.4	7.4-7.5	6.5-7	7	7-7.5	7-7.2
NaCl (%)	4.3-4.37	4-4.2	4-4.3	3.4-4.3	3.3-4.2	2.5-5	-7.3

Table 3. Biochemical characteristics of strains BQ31 and BV32.

Characteristics	BQ31	BV32	*H. vallismortis*	*H. quadarata*	*H. hispanica*	*H. japonica*	*H. marimorti*
Amylase	+	-	+	-	-	-	-
Gelatinase	-	-	-	-	-	+	-
Cellulase	+	-	-	+	-	-	+
Catalase	+	+	+	+	+	+	-
Oxidase	+	+	+	+	-	+	-
Urease	+	+	+	+	-	-	-
Caseinase	-	-	-	-	-	+	-
Phenylalanine deaminase	-	-	-	-	-	-	-
H_2S production	-	-	-	-	+	-	-
Indole production	-	+	+	-	+	+	-
Phenol red broth	+	+	+	+	-	-	-
Tween 20	-	-	-	-	-	-	+
Tween 80	-	-	-	-	-	-	-
Nitrate/nitrite-reduction	+	+	_	_	+	+	+
Citrate utilization test	-	-	-	-	-	-	-
Pigmentation	Orange red	Retinal	Orange red	Orange red	Retinal	White	White
ONPG test	+	+	+	+	+	+	+
Gram staining	-	-	-	-	-	-	-
Simple Staining	Rods flagellated	Rods flagellated	ND	ND	ND	ND	ND

Table 4. Utilization of carbon sources by BQ31, BV32 and known species of Haloarchaea strains.

Carbon	BQ31	BV32	*H. vallismortis*	*H. quadarata*	*H. hispanica*	*H. japonica*	*H. marimorti*
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	++	+	+
Galactose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+|
Glycerol	-	+	+	-	+	+	+
Maltose	+	+	+	+	+	-	+
Gluconate	-	-	-	-	-	-	-
Mannose	-	-	-	-	+	-	-
Ribose	+	+	-	+	+	-	+
Rhaminose	-	-	-	-	+	-	-
Xylose	+	-	-	+	-	+	-
Mannitol	-	-	-	-	-	+	+
Sorbitol	-	-	-	-	-	+	+
Acetate	-	-	-	-	-	-	-
Succinate	-	-	-	-	-	-	-
Lactose	+	-	+	-	-	-	-
Pyruvate	+	+	+	+	-	-	-

Table 5. Effect of various salts on strain BQ31 and BV32

Salts	BQ31	BV32
Potassium dichromate	+	+ +
Mercuric chloride	+++	++
Potassium chloride	+	++
Sodium tungstate	-	-
Cobaltous chloride	+	+
Magnesium sulphate	++	++
Potassium thiocyanate	-	-
Sodium molybedate	-	-
Copper sulphate	+	-
Cadmium nitrate	-	+
Potassium nitrate	+	++

Table 6. Antimicrobial activity of the strains against various pathogens.

Antibiotics	BQ 31		BV 32	
	Zone of inhibition (mm)	**Susceptibility**	**Zone of inhibition (mm)**	**Susceptibility**
Penicillin	15	S	14	R
Tetracyclin	7	S	7.5	R
Carbencillin	6	S	8	R
Rifampicin	8	S	8	R
Streptomycin	16	S	18	S
Erythromycin	16	I	18	I
Bacitracim	10	D	5	D
Gentamycin	10	R	12	R
Kanamycin	2	R	14	S
Ampicillin	-	-	15	S
Amoxycillin	3	R	4	R
Chloramphenicol	-	-	15	I

Table 7. The effect of pigment in liver lipid peroxidase activity of albino rats under different body weights.

	Average Initial body weight (g)	**Average Final body weight (g)**	**Liver peroxidase activity (mg/protein)**
Control	30 ± 3	32 ± 2	1.31 ± 0.09
100	30 ± 3	31 ± 3	0.97 ± 0.07
200	30 ± 3	34 ± 3	1.43 ± 0.09
300	30 ± 3	34 ± 2	1.22 ± 0.05

Table 8. Effect of pigment extract of Haloarchaeal strains BQ31 and BV32 on different haematological parameters under different body weight of albino rats

Weeks	Haematological parameters	Experimental diets (mg/kg)			
		Control	100	200	300
1	Haemoglobin (mg %)	11.25	12.1	11.78	12
	Erythrocytes	7.97	7.89	7.88	8.17
	WBC	10.5	10.7	10.1	10.8
	Macrophages	9.1	9.5	9.1	9.5
	Eosinophils	0.5	0.5	0.5	0.6
	Neutrophils	7.1	7.4	7.4	7.3
	Monocytes	1.3	1.2	1.2	1.4
2	Haemoglobin (mg)	12.5	12.7	12.14	13.1
	Erythrocytes	7.83	7.89	8.31	8.42
	WBC	10.7	11.06	11	10.8
	Macrophages	9.1	9.7	9.8	9.8
	Eosinophils	5	0.5	0.6	0.6
	Neutrophils	7.3	7.4	7.4	7.4
	Monocytes	1.3	1.4	1.3	1.4
3	Haemoglobin (mg)	12.5	12.9	12.6	13.5
	Erythrocytes	8.31	8.77	8.42	8.43
	WBC	10.8	11.7	10.9	11
	Macrophages	8.9	9.7	9.9	9.8
	Eosinophils	0.5	0.6	0.7	0.6
	Neutrophils	6.9	7.7	7.9	7.4
	Monocytes	1.4	1.4	1.4	1.3
4	Haemoglobin (mg)	12.7	13.6	13.1	13.8
	Erythrocytes	8.41	8.49	8.51	8.61
	WBC	10.8	11.3	10.9	10.9
	Macrophages	0.3	9.4	9.9	9.7
	Eosinophils	0.5	0.6	0.9	0.6
	Neutrophils	7	7.8	7.9	7.4
	Monocytes	12	1.3	1.4	1.4

Table 9. Effect of pigment extract of Haloarchaeal strains BQ31 and BV32 on different biochemical parameters under different body weight of Albino rats

		Experimental diets (mg/kg)			
Weeks	**Biochemical analysis**	**Control**	**100**	**200**	**300**
1	Protein (g/dl)	2.7	2.9	3.5	2.9
	Cholesterol (mg/dl)	70	81	79	80
	Glucose (mg/dl)	82	87	90	83
	Urea (mg/dl)	29	29	26	32
	Albumin (g/dl)	0.8	0.9	0.9	0.8
2	Protein (g/dl)	3.3	3.9	3.5	0.1
	Cholesterol (mg/dl)	79	85	83	90
	Glucose (mg/dl)	93	97	102	100
	Urea (mg/dl)	33	34	39	39
	Albumin (g/dl)	0.8	0.9	0.9	0.9
3	Protein (g/dl)	3.1	4.2	4.2	3.9
	Cholesterol (mg/dl)	78	91	97	89
	Glucose (mg/dl)	105	121	113	117
	Urea (mg/dl)	40	38	38	37
	Albumin (g/dl)	1	1.3	1.1	1
4	Protein (g/dl)	3.2	4.2	4.4	3.4
	Cholesterol (mg/dl)	91	142	135	139
	Glucose (mg/dl)	99	128	131	131
	Urea (mg/dl)	48	42	41	37
	Albumin (g/dl)	1.1	1.4	1.4	1.3

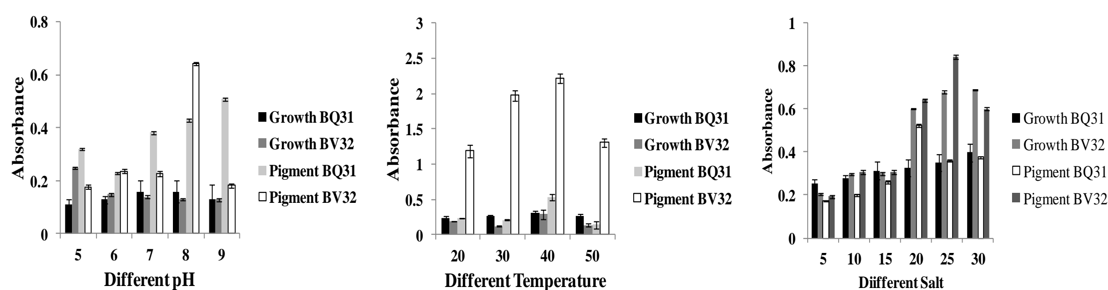

Fig.1 Cell growth and pigment production under different cultivation conditions.

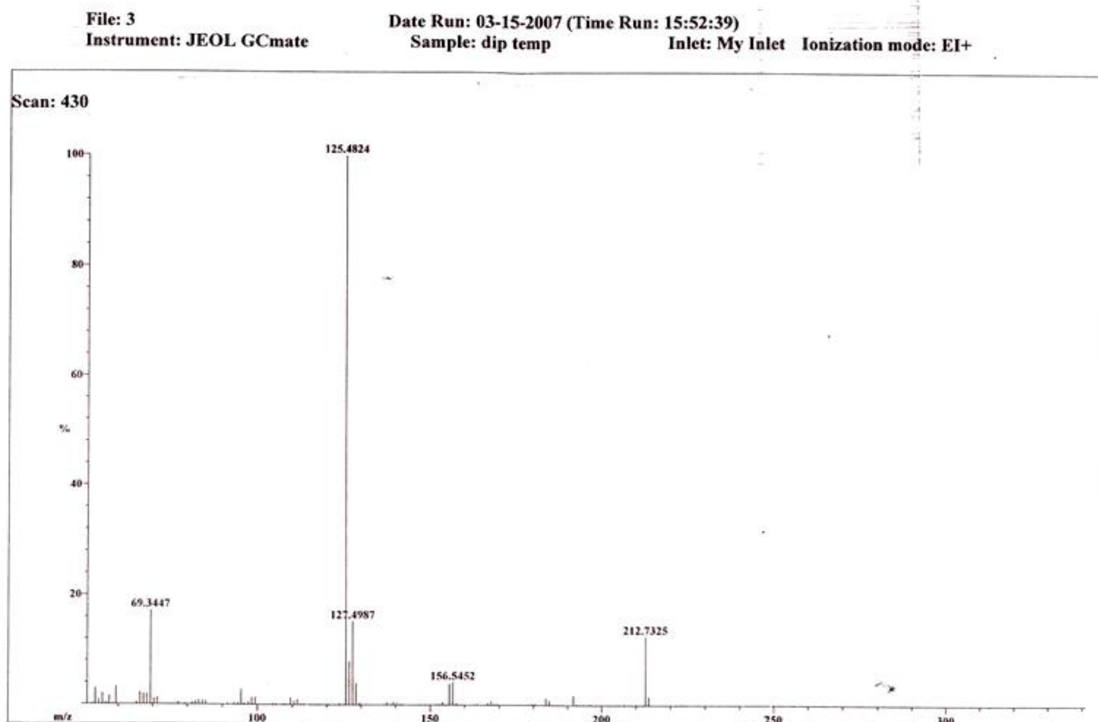

Fig.2 Mass spectrometric analysis of red pigment

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