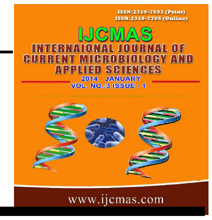

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Original Research Article

Potential producers of economical and medical important products in hot water spring Tattapani, Himachal Pradesh, India

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A B S T R A C T

Distribution of cyanobacteria in Tattapani hot water spring with season variation in species along thermal gradient was studied The water samples from hot water spring Tattapani were analysed. Results reveal that the water of spring itself is a good medium containing all essential inorganic ions which support the considerble growth of both nitrogen and non nitrogen fixing cyanobacteria. However, the water of spring used in lab for growth of cyanobacteria ,does not support their growth.Along the temperature gradient from 65^{0}C to 35^{0}C,the water showed a gradual decrease in salt residue per unit volume.This indicate that the decrease in water temperature,the salt particles get prcipitated. These changes in the quality of water and variation of temperature along the spring affected the distribution and occurrence of cyanbacterial population The cultivation and characterization of axenic thermopilic cyanobacterial strains yield a source of organisms for possible exploration of cyanbacteria for biomedical engineering purpose as Indian hot water springs have not gained enough attention in this field.

Keywords

Cyanobacteria distribution; extreme temperatures; population; thermal springs; HP; biomedical engineering of cyanobacteria.

Introduction

Cyanobacteria (blue-green algae) are one of the most interesting group among the algae from structural, functional, adaptation, distribution and economic point of view. Some of their physiological characteristics are similar to higher plants and green algae in respect of chlorophyll, biosynthesis, oxygenic photosynthesis and essential amino acids (Fogg *et al.*, 1973). They are only nitrogen fixing organisms that have oxygen generating photosynthetic system and they have atmospheric nitrogen in the form of ammonia at the expense of photosynthesis (Fogg *et al.*, 1973; Whitton and Roger, 1989), offer a unique biological material for an understanding of physiological processes operating in extreme environments (Edwards *et al.*, 1968).They share similarities in light reactions mediated by photosystem I and II, protein pigment complex (Phycobilisomes), chlorophyll and carotenoids. Their ancestors are possibly the oldest primary producer organisms common in the distant past, and they perhaps used thermal springs as refugia (Gold, 1992, 1999; Plescia *et al.,* 2001; Adhikary, 2006; Izagiurre *et al.,* 2006; Hindák, 2008).

Ecology of cyanobacteria

Cyanobacteria have been evolved in the early Precambrian era (Schopf, 1970) when the temperature of the earth was too high for the survival of life. The cyanobacteria have long been known to be exceptionally adaptable to the wide range of variations in environmental conditions to which they are exposed in nature (Vallentyne, 1963). There is an evolutionary adaption of some of the cyanobacterial forms to environmental extremes (Brock, 1978). For the survival of the thermal cyanobacteria, the temperature is one of the most important factors controlling the activities and evolution of the organisms, as at higher thermal regime the solubility of the oxygen greatly reduces, circumvents the viscosity of the water and increase ionization, there by affecting the organisms indirectly by modifying the other environmental factors. Some species of cyanobacteria successfully withstand extremes of temperature (Brock, 1967, 1970; Castenbolz, 1969a,b) and grow at varying degrees of alkaline pH. (Roger, P.A Reynaud, 1979). Hexadecenoic acids have a possibly important role in the adaptation of cyanobacteria to high temperatures and the ratio between saturated and unsaturated fatty acids (S/U ratio) decreases with decreasing temperature of a thermophilic strain of *Synechococcus* (Maslova *et al.,* 2004). In mesophilic strains cultivated at 25–32 ^{0}C the S/U ratio was increased. A change in the S/U ratio, the mechanism of adaptation of algae to high temperatures, was documented from a quite opposite spectrum of conditions. Cryoseston species of *Chloromonas*, isolated from Antarctica, had a higher proportion of unsaturated acids, enabling the functioning of cells in temperatures just above zero (Bidigare et al.,1993).

The temperature range of most of the geothermal springs all over the world varies as high as 74^{o}C where exclusively unicellular form of Synechococcus lividus occur in West-north America and below 64^{o}C in the rest of world (Brock, 1967). The non viability of these species at temperature exceeding 74^{o}C has not yet been satisfactory explained biochemically though it may attributed to the inability of these organisms to construct the functional photosynthetic membranes at higher temperature (Brock, (1967b; Brock, 1978).

In the hot spring the biochemical composition of phytoplankton keeps on changing in different growth phases (Febreges *et al.,* 1985) with different growth conditions e.g. light, temperature, pH and nutrients (Febreges *et al.,* 1985; Webb and Chu, 1982) which are invoked to be important variables in controlling phytoplankton community structure and biomass (Hutchinson, 1961). Inorganic ions, such as sodium, potassium, carbonate, silicate, sulphite and sulphate make a rich medium that support considerable growth of both nitrogen-fixing and non nitrogen-fixing cyanobacteria in hot spring water. Most of the thermal springs are alkaline in nature and three forth of hot springs of the world are alkaline (Waring, 1965). In addition to the major elements, N, P, Mg, S and K the elements, *viz,* calcium, iron, boron, molybdenum, maganese, sodium, cobalt, zinc and copper are also considered to be essential for the growth of the blue-green algae. The function of these elements are similar to their function in higher plants (Holm, O. Hanson, 1968).

Modern-day cyanobacteria include some 2000 species in 150 genera and 5 orders (Table 1), with a great variety of shapes and sizes. Ecologically, there are three major groups in the aquatic environment:mat-forming species, which form periphytic biofilms over rocks, sediments, and submerged plants; bloom-formers,which create a wide range of water quality problems and that are most common in nutrient-rich (eutrophic)lakes; and picocyanobacteria, which are extremely small cells (<3 mmin diameter) that are often abundantin clear water lakes. Additional groups include colonialnon-bloom-formers, which are common in a variety of aquatic habitats, including mesotrophic lakes, wetlands,and saline waters; metaphytic species that form aggregates that are loosely associated with emergentmacrophytes (water plants such as rushes and reeds that extend out of the water into the air above); certain species that grow as periphyton but that can also enter the plankton; and various symbiotic associations. (Vincent, 2009)**.** Among aquatic habitats, streams of hot water springs are one of the best habitats for blue-green algae (Stewart, 1970). Hot water spring occur world wide except Antarctica (23) and most of them occur in Yellow-stone Plateu of North America (Keefer, 1971), Iceland (Barth, 1950), New-Zealand and Japan (Uzamasa, 1965). In our country too, hot water springs of varied temperature regimes are concentrated in the state of Himachal Pradesh (Fig. 1) and rest of states, Haryana, Maharastra, Gujarat, Bihar, West Bengal and Orrisa (Vasishta, 1968) as shown in Fig. 2.

Although the systematic of various Indian hot springs have been studied (Prasad and Srivastava, 1965 ;Vasishta, 1968), their ecological study have been rather neglected. Among the predominant forms of cyanobactria common to all hot water springs in India are *Chroococcus yellowstonesis*, *Synchococcus elongatus. var. amphigranulatus*, *Oscillatoria jasorvensis*, *O. tenus*, *O. filiforms*, *Phormidium laminosus*, *Lyngbya nigra* and *Mastigocladus laminosus*. (Vasishta, 1968). The form of Synechococcus (*S. elongatus var. amphigranulatus* and *S. vuleanus*) are considered as strictly thermal forms as they have not been recorded from non thermal situations. In hot water spring both heterocystous and non heterocystous cyanobacteria are found. The non-eterocystous cyanobacteria are lacking N_2-fixation capacity However, unicellular non-eterocystous algae such as *Gloeocapsa* (Wyatt and Silvey, 1969) and *Aphanotheca* (Singh, 1973) are important because they fix nitrogen under aerobic conditions.

Cyanobacteria for applied purpose

Thermophilic cyanobacteria ,*Phormidium* sp. produced an anti-microbial material against G–, G+ bacteria, *Candida albicans* and *Cladosporidium resinae* (Fish, and Codd, 2004). Another *Phormidium* sp., immobilized in calcium alginate, was used for treatment of dye-rich wastewater, (Ertugrul *et al.*, 2008). Cancer drugs were produced from thermophilic cyanobacteria by (Javor, 1999). Some unusual Fe-proteins, siderophores, were identified in some thermophiles Ferredioxins from *Mastigocladus laminosus*, however, act as toxins and restrict public use exploitation of a spring in Saudi Arabia (Mohamed, 2008).

Table.1 Recognized orders of cyanobacteria in taxonomic scheme

Order	Characteristics	Illustrative genera
Chroococcales	Coccoid cells that reproduce by binary fission or budding	*Aphanocapsa, AphanotheceGloeocapsa Merismopedia, Microcystis, Synechocystis, Syanechococcus*
Pleurocapsales	Coccoid cells, aggregates or pseudo-filaments that reproduce by baeocytes	*Chroococcidiopsis, Pleurocapsa*
Oscillatoriales	Uniseriate filaments without heterocysts or akinetes	*Microcoleus Lyngbya Leptolyngbya Oscillatoria, Phormidium Planktothrix*
Nostocales	Filamentous cyanobacteria divide in only one plane heterocysts false branching Scytonema	*Anabaena Aphanizomenon, Calothrix Cylindrospermopsis, Nostoc Scytonema, Tolypothrix*
Stigonematales	Division in more than one plane; true branching and multiseriate forms; heterocysts	*Mastigocladus (Fischerella),*

Fig.1 Hot water springs of varied temperature regimes are concentrated in the state of Himachal Pradesh

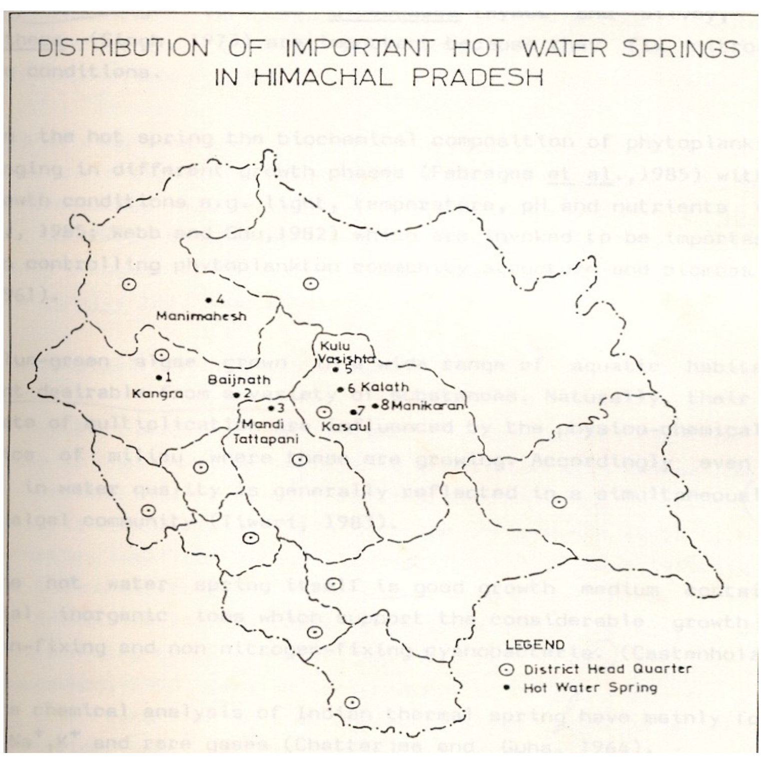
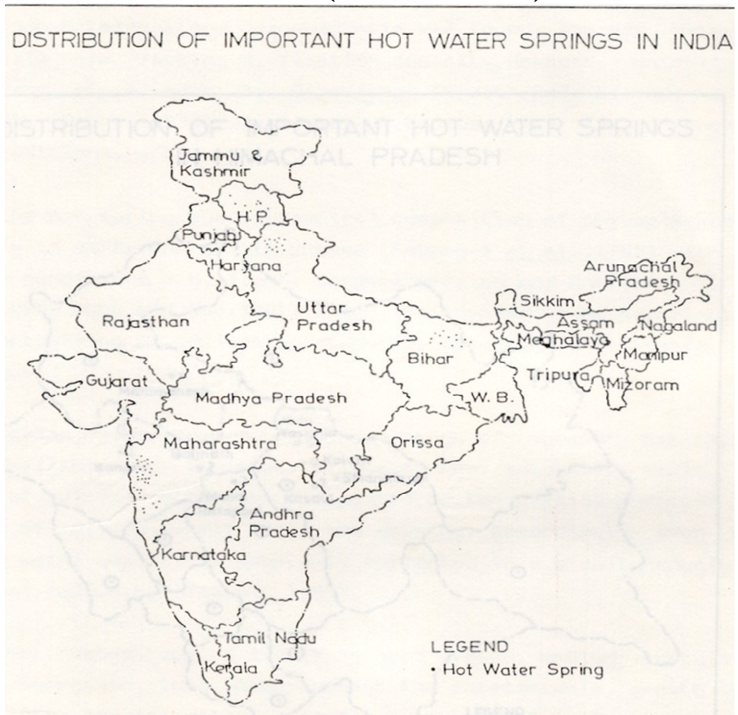


Fig.2 Shown Haryana, Maharastra, Gujarat, Bihar, West Bengal and Orrisa (Vasishta, 1968)



Thermophilic *Synechococcus* sp. is a potential producer of poly- -b-hydroxybutyrate, which is the basis of biologically degradable plastics (Miyake *et al.*, 1996). Production of hydrogen by some cyanobacteria is a promising source of energy for the future (Mitsui, 1987). The exploitation of natural hot water with a large content of CO_2 is highly profitable for algal biotechnology, e.g. production of *Spirulina* (Fournadzhieva *et al.*, 2002) and potentially for production of thermal species. Precipitation of travertine, using cultures of thermal cyanobacteria, is a promising method for capturing and sinking CO_2 of anthropogenic origin (Hayashi *et al.*, 1994; Ono and Cuello, 2007).

Thermophilic cyanobacteria also have the potential to remove nutrients from thermal effluents (.Weismann *et al.*, 1998). Gamma-linolenic acid (GLA), a nutritionally important fatty acid in human and animal diets, is not produced in oil seed crops. Many oil seed plants, however, produce significant quantities of linoleic acid, a fatty acid that could be converted to GLA by the enzyme delta 6-desaturase if it were present. As a first step to producing GLA in oil seed crops, Reddy AS and Thomas TL (Reddy, and Thomas, 1996) cloned a cyanobacterial delta 6-desaturase gene. Expression of this gene in transgenic tobacco resulted in GLA accumulation. Octadecatetraenoic acid, a highly unsaturated, industrially important fatty acid, was also found in transgenic tobacco plants expressing the cyanobacterial delta 6-desaturase. This is the first example of engineering the production of 'novel' polyunsaturated fatty acids in transgenic plants.

Cyanobacteria account for 20–30% of Earth's primary photosynthetic productivity and convert solar energy into biomass-stored chemical energy at the rate of ~450 TW [1],the electrogenic pathway of cyanobacteria might be exploited to develop light-sensitive devices or future technologies that convert solar energy into limited amounts of electricity in a self-sustainable, CO_2-free manner(Pisciotta *et al.,* 2010).

A recent study demonstrated the successful utility of thioesterase, Isolated from the cryptophycin biosynthetic pathway, in the macrocyclization of a series of linear synthetic precursors in generating 16-membered cyclic depsipeptides as potential anticancer agents (Tan, 2007).Other cyanobacterial metabolites such as curacin A, dolastatin 10 ,Symplostatin 3 and Belamide A have been in preclinical and/or clinical trials as potential anticancer drugs. In addition, these molecules served as drug leads for the development of synthetic analogues, e.g. compound 4, TZT-1027, ILX-651, and LU- 103793, usually with improved pharmacological and pharmacokinetic properties (Tan, 2007; Simmons *et al.,* 2006; Gerwick *et al.,* 2008).

Cyanobacteria in biomedical engineering

Reports show that cyanobacteria is in use for tissue engineering .Choi, Gerwick and their colleagues conducted various laboratory experiments and discovered that the seaweed (the cyanobacterium *Leptolyngbya crossbyana*) generates natural products known as honaucins with potent anti-inflammation and bacteria-controlling properties,will help ful for the development of drugs to prevent infection in patients who require catheters to deliver vital nutrients to key areas such as arteries, as well the development of new treatments for acne and other skin conditions.

The blastp results revealed that the collagen-like sequence of *Trichodesmium erythraeum* resembles various collagen sequences among all other protein sequences in the NCBI RefSeq protein database. Taking the unusual hydrophobic nature of *T. erythraeum's* collagen-like protein into consideration, some of its potential biomedical applications can be predicted. This protein can be used to make collagen membranes. These membranes can be used in dentistry for surgical procedures like guided tissue regeneration and aid in preventing 31 epidermal down-growth along the root surfaces during the initial stages of wound healing. These membranes may also be used in repairing abdominal hernias. In the form of capsules, this collagen may be used to contain any movements of the graft materials to maximize the formation of new bone around graft materials in procedures like tooth extraction or maxillary sinus elevation. This hydrophobic collagen may also be used in topical skin dressings effective for dry skin, skin burns and skin wounds as an insoluble collagen support matrix containing releasable soluble collagen. It can also be used as the base material for drug delivery systems. Several species of cyanobacteria and algae are known to produce novel compounds ,many that have promising use in drug development for human and other uses (Mario Aguilera , 2012).Chronic inflammatory conditions for which currently do not have really good medicines ,cyanobacteria produce chemical compounds that exhibit porn as anti-inflammatory and in combating bacterial infections (Mario Aguilera , 2012) In order to gauge the potential of this collagen-like gene of *Trichodesmium erythraeum* in tissue engineering, further analyses are required. (Shah and Ruchit Girishchandra, (1987).

Keeping in view of currently reported potential of cyanobacteria in tissue engineering and regenerative medicines and in use of economical and in medical applications, the present investigation was undertaken to assess the effect of physio-chemical parameters of hot water spring “Tattapani” on the distribution of thermal cyanobacteria along the thermal gradient of spring . This will helpful for cultivation and characterization of axenic thermopilic cyanobacterial strains yield a source of organisms for possible biomedical engineering exploration as cyanbacteria of Indian hot water springs have not gained enough attention This is the first report of distribution of cyanobacteria in Tattapani hot water spring with season variation in species along thermal gradient.

Materials and Methods

The physico-chemical properties of the thermal spring water collected from site No. 1 (65^0C) were analysed according to the methods described in standard methods for the examination of water and waste water: In American Public Health Association (edited book by Greenberg Arnold *et al.*, 16th ed. Inc., New York, 1985)

Algal samples were collected from the different sites of thermal spring "Tattapani" in many replicates. Pre-sterlised screw cap glass vials of 15ml capacity were used for collection purpose. Randomly selected 1cm^2 blocks of algal patch were scrapped and collected in separate vials. Remaining volume of the vials was filled with natural spring water and these were brought to laboratory for isolation. Streak plate technique was used for clonal isolation of species of *Mastigocludus***,** *Chroococcus, Lyngbya, Phormidium, Microcystis, Oscillatoria***.** and *Synechococcus.*Algal population was fragmented with sterilized awl pins on the rotator for about 5min. The algal suspension was then diluted and aliquots (a drop) of different dilutions were streaked on different petri-dishes containing Allen and Arnon nutrient agar medium (1.0% w/v) with the help of a sterilized inoculation needle. Both nitrate free as well as nitrate containing media were used for this purpose. The Petri-plates were incubated at 45^0C under continuous illumination of 2500 lux and regularly observed for development of individual colonies.

Discrete colonies seemingly free from contaminants were sucked up with the help of sterilized micropipettes and transferred separately presterilized culture tubes, each containing 10ml of nitrate free or nitrate containing medium (AA medium). These culture tubes were transferred unshaken to the incubator. After about a fortnight of incubation, many colonies were started growing in the respective liquid media. The same were observed under a microscope for purity. If contaminates were detected, the algal suspension was streaked again and the process repeated until the pure lines were established.

Taxonomic identification of algal forms collected from the different sites of hot spring Tattapani were made using standard reference works of (Desikachary, 1959). Some cyanobacterial forms were identified at National Facility for Marine Cyanobacteria, Bharathidasan University Tiruchirapalli-620024.India.

Results and Discussion

The temperature at the emergent site (Fig.3) of the spring was as high as

65^0C which gradually declined to 33^0C at the junction where hot spring water mixed with the Sutlej river water. The maximum and minimum (day/night) ambient air temperature during winter months in district Mandi, H.P. was recorded to be within the range of $22/12^0$C (maximum/minimum) temperature. The high water temperature recorded at the emergent site(65^0C) gradually equilibrates with air temperature resulting in the formation of a descending temperature gradient along the spring before it finally joins with the flow of river water. This descending temperature gradient, having a variation in the mineral composition forms small groups of different ecological niche along the stream that influence the distribution and type of cynobacterial population. At high temperature (Fig.3) at the emergent site of thermal spring, salts were dissolved due to high temperature (65^0C) which subsequently were found to be deposited on the rocks during gradual cooling of water from 65^0C to 55^0C. As a consequence, numerous rocks with salts deposited over them were found at site2(Fig.4).

Microscopic observations of algal mats showed filamentous forms of *Phormidium* sp. and free *Synechococcus elongatus* as unicellular form. Both *Phormidium*. and Synechococcus were found to be free and in floating condition at this spring. Yoneda (1952) and Sompong *et al* (2005) also reported the occurrence of *Synechococcus* and *Phormidium* relatively at high temperature as compared to other cyanobacteria . Temperature is one of the most important parameter for cyanobacterial species diversity in microbial mats of hot springs. The studies of Skirnisdottir et al. (2000), Nakagawa and Fukui (2002) and Sompong et al. (2005) revealed that cyanobacterial diversity and community complexity decreased with increasing temperature. Ferris et al. (1996b), Ferris and Ward (1997), Ward et al. (1998) showed that at thermal gradients from 50°C to 75°C, the layered mats are characterized by the presence of unicellular forms like Synechococcus. The cyanobacterial mats occurring at the lower end of thermophily ($40\text{-}50^0$C) are often dominated by morphologically defined filamentous cyanobacteria like *Phormidium, Oscillatoria, Pseudanabaena, Calothrix* and *Fischerella* (Ward, and Castenholz. 2002; Sompong *et al.*, 2005; Debnath Mandal *et al.*, 2009). However, Norris et al. (2002) have reported that at lower temperature *Synechococcous* also co-occurs with other unicellular and filamentous cyan bacteria . The present study also support this data that temperature influence is most factor efeecting the distribution of cyanobacterial species in the thermal spring Fig.5 shows the site 3. This spring starts where the water at 55^0C (site 2) sharply declines to 45^0C and is located at a distance of about 12 meters from the emergent site. Both free and submerged forms of cyanobacteria have been found in this spring. The temperature variation in this spring during the year was 42^0C to 45^0C. Fig.6 shows the site 4, where the temperature of site 3 declines to 35^0C. It is located at a distance of 15 meters from the origin. Free, floating and submerged forms of cyanobacteria were present at this site. The temperature at this site varied from 33^0C to 35^0C during the year.

Both temperature and flow of water in the four springs mentioned above had a great influence on the chemical composition of the water. At site 1, the water discharges with high speed relatively through a narrow passage and the temperature remains as high as 65^0C throughout the year. As a result, most of the salts are in a dissolved state and these conditions do not allow deposition of suspended particles over the surrounding rocks. As soon as temperature declines to 55^0C and flow rate of water decreases as a result of expansion of the stream relatively to a larger area, some of the salts get deposited over the surface of the surrounding rocks. The percentage of total dry residue per unit volume of water was found to be low as compared to the site 1.

Along the temperature gradient from 65^0C to 35^0C, the water showed a gradual decrease in salt residue per unit volume. This indicated that with the decrease in water temperature, the salt particles get precipitated. These changes in the quality of water and variations in temperature along the spring, affected the distribution and occurrence of cyanobacterial population in the springs.

Physico-chemical composition of water of Tattapani hot spring

Because most of the precipitation of dissolved salts took place at site 3 and 4, the water analysis was not conducted at these sites. Therefore in order to have the composition of water of the stream, samples were taken from site 1 "65^0C" for analysis. The water of this site was clear with smell of sulphur. The detailed physico-chemical properties are given in Table 2.

The pH of water was 7.2 which showed that the spring is slightly alkaline in nature. Total dissolved solids were10,120 mg/l which are higher than the total solid reported from other hot springs (30). The concentration of nitrate was also quite high (2.0 mg/l). In many hot water springs, studied by other workers, nitrate was found to be very low or absent (Brock and Brock, 1969a,b). In Hunter hot spring, Eastern Oregon, nitrate was undetected at one source and ranged from 0.042 to 0.14 mg/l at other source. In India many hot water springs in various states, studied by Vasishta (1969) don't contain any appreciable amount of nitrate. This may be due to the fact that nitrate estimation for the present study was done at the first or emergent site of the hot spring which showed high amounts. On the other hand, it is possible that the content is very low where cyanobacterial population was abundant (**site 3** and **4**) simply because of the precipitation of the salts. Apart from temperature some workers (Ward and Castenholz. 2002; Sompong *et al.,* 2005; Debnath Mandal *et al.*, 2009) also focus on the role of pH and combined nitrogen (especially ammonium), on the species distribution in cyanobacterial mat community below ~ 60^0C.

Ammonia was found to be another nitrogen source in the spring. The concentration was 0.2 mg/l which is higher than the earlier reports which ranged from 0.001 to 0.080 mg/l (Bharati, 1991). Ammonia was found in less than one half of the 860 hot springs analyzed in Japan. In this spring Tattapani, average value was recorded to be 1.60 mg/l. Ammonia was more abundant than nitrate at least at spring source in both alkaline and acid springs, but

Table.2 Physical and chemical characteristics of Tattapani hot water spring

Physical examination	
Appearance	Clear with some particles
Colour(Hazen Scale Unit)	Normal
Taste and odour (qualitative)	Salty taste
Turbidity(Naphthao turbidityunit)	6
Conductivity (microelements cm)	11,800
pH	7.2
Temperature(during collection)	$65^{0}C$

Chemical examination

	mg/l
Total solids dried at 1050c	10120
Free carbon dioxide as CO2	55
Phenolphathlein alkalinity as CaCO3	Nil
Methyl Orange alkalinity as CaCO3	256
Total hardness as CaCO3	430
Carbonate hardness as CaCO3	256
Non-Carbonate hardness as CaCO3	174
Free and saline ammonia as N	0.2
Nitrate as N	2.0
Nitrite as N	0.005
Chloride as Cl	3500
Sulphate as SO4	115
Sulphide as S2	8.5
sulphite as SO3	7.8
Fluoride as Fl	3.0
Iron as Fe	0.2
Calcium as Ca	164
Magnesium as Mg	4.8
Phosphate as PO4	0.5
Potassium as K	240
Sodium as Na	270
Silicate	119

Table. 3 Variation in dissolved oxygen, free CO_2 and bicarbonate alkalinity along the temperature gradient of hot water spring Tattapani

Water Temperature (^0C)	Dissolved Oxygen (mg/litre	Free CO_2 (mg/litre)	Bicarbonate alkalnity (mg/litre)
65	2.3	55.0	21.6
55	3.5	25.6	21.0
45	4.2	12.7	20.0
34	4.9	2.6	19.8

Table.4 Occurrence of cyanobacterial spp. along temperature gradient of hot water spring "Tattapani"

Site 3 (45^0C)	Site 4 (35^0C)
Aphanocapsa grevillei	Calothrix brevissima
Aphanocapsa thermalis	Chroococcus ansuyensis
Calothrix parietina	Chroococcus minar
Chroococcus ansuyensis	Chroococcus minutus
Chroococcus minor	Coelosphaerium kuetzingiana
Chroococcus yellowstonensis	Coelosphaerium dubium
Entophysalis granulose	Lynqbya calotrichicola
Leptochaete hansqirgi	Lynqbya diqueti
Lynqbya aerugino-coerulea	Lynqbya holdenii
Lynqbya diqueti	Lynqbya kuetzingiana
Lynqbya nigra	Mastigocladus laminosus
Mastigocladus laminosus	Microcytis stagnalis
Oscillatoria brevis	Oscillatoria cortiana
Oscillatoria laete-virens	Oscillatoria filiformis
Oscillatoria onimatis	Oscillatoria lativana
Oscillatoria princeps	Oscillatoria lamnosus
var.tenella	Oscillatoria onimatis
Oscillatoria proboscidea	Oscillatoria princeps
var.westii	var. tenella
Phormidium africanum	Oscillatoria proboscidea
Phormidium cebennese	Oscillatoria tenuis
Phormidium tenue	Phormidium valderianum
Plectonema notatum	Spirulina subsalasa
var. africanum	Synechococcus elonatus
Scytonema leptobasis	var.amphigranulatus
Var.thermalis	Spirulina subsalsa
Syn chococcus elongates	
Va.r amphigranulatus	

Plate1. Photograph of Tattapani hot water spring microbial mats dominated by cyanobacterial .

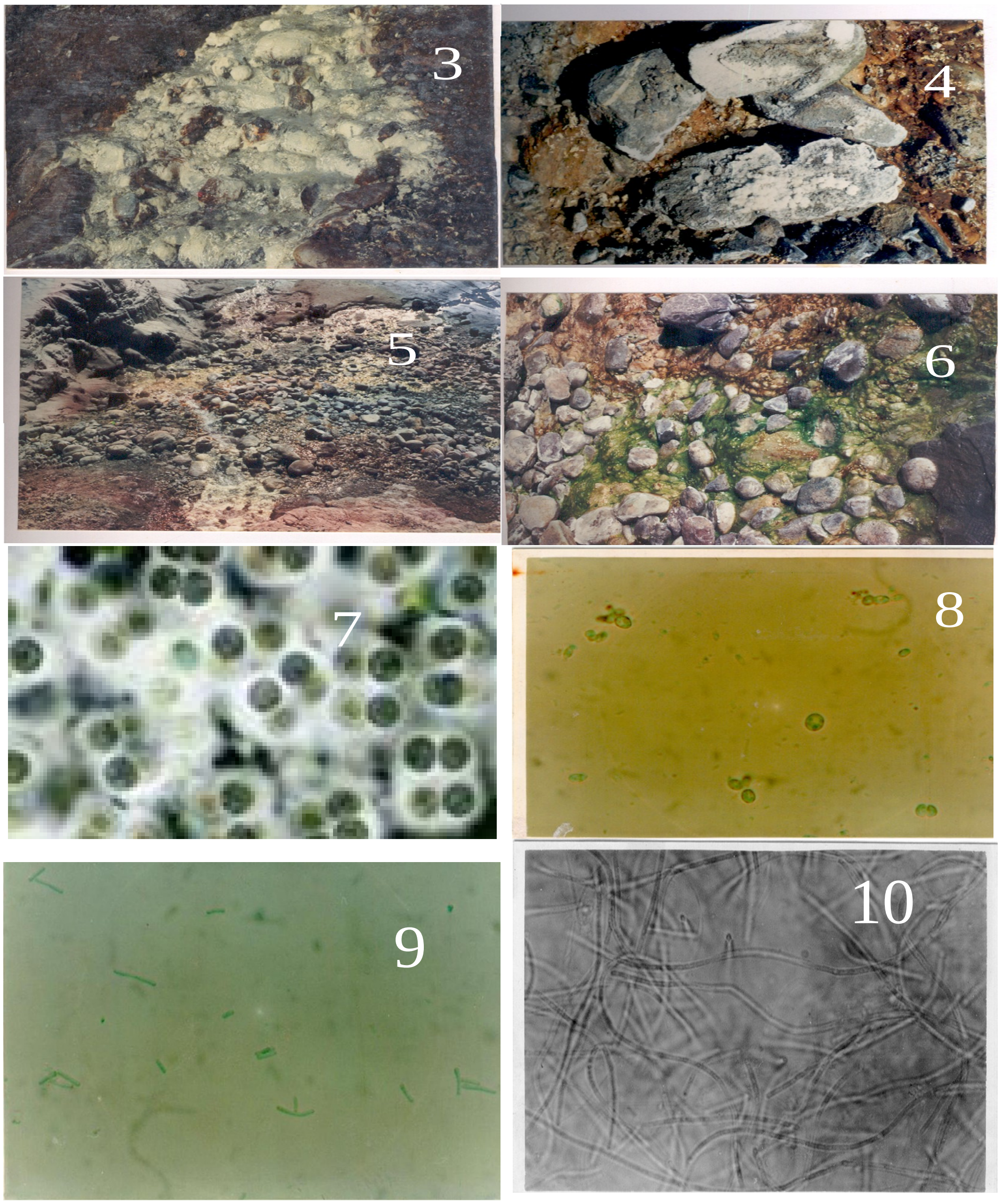


Fig.3 Site 1 65 ^{0}C the emergent site of the thermal spring; Fig 4 Site 2 55^{0}C,represent minerals depositionand occasional cyanobacterial patches over rocks; Fig.5 Site 3 45^{0}C represent numerous rocks with cyanobacterial colonies; Fig 6.Site 4 35^{0}C represents a small pool with floating filamentous cyanobacterial mats adhered to rocks; Fig.7 *Chroococcus minor*., unicellular thermal cyanobacterium; Fig. 8 Microcystis sp., unicellular thermal cyanobacterium; Fig. 9 Unicellular thermal cyanobacterium Synechococcus elongates; Fig 10 Filamentous thermal cyanobacterium Phormidium tenue Strain 1

Plate.2 Photograph of Tattapani hot water spring microbial mats dominated by cyanobacterial

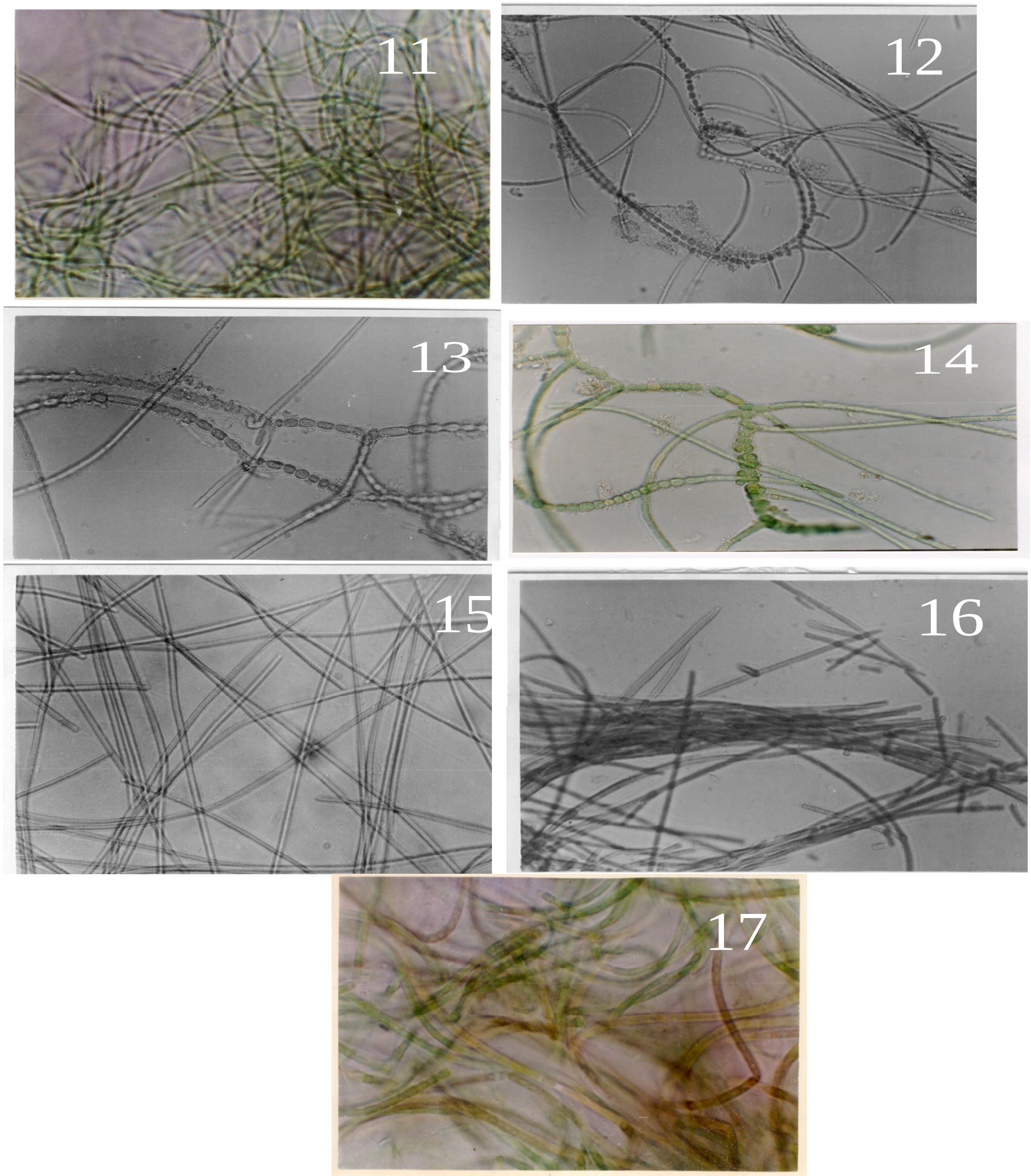


Fig 11 Filamentous thermal cyanobacterium Phormidium tenue Strain 2 Fig 12 Vegetative and branched filaments of thermal Mastigoclasdus lamionsus Low magnification.Fig 13 Filaments of thermal Mastigoclasdus lamionsus with heterocyst high magnification.Fig 14 Filaments of thermal Mastigoclasdus lamionsus with dividing cells.Fig 15 Filamentous thermal cyanobacterium Oscillatotria laete –virens.Fig 16 Filamentous thermal cyanobacterium Oscillatotria onimatis Fig 17 Filamentous thermal cyanobacterium Lyngbya digueti

particularly in case of later. Diazotrophic cyanobacteria are able to colonize springs where nitrogen levels are low to support other taxa. Conversely they may be out-competed by non diazotrophic cyanobacteria in spring with sufficient combined nitrogen (Ward and Castenholz, 2002)

There are three sources for the supply of carbon in natural water such as free CO_2, $HCO^{3'}$and $CO^{3''}$.The carbon status of water affects the growth and photosynthesis of the phytoplanktons of hot water spring. Free CO_2 was 55 mg/l at the emergent site of the thermal spring (65°C) while there was a rapid fall along the thermal gradient (Table 3).

In site 3 and 4 where the free CO_2 was less than at the emergent site, the dissolved bicarbonate had greater significance as alternative carbon source for photosynthesis at higher temperature and alkaline pH. Since no phenolphthaleine alkalinity was detected in this hot water spring, the alkalinity entirely was assumed to be due to methyl orange alkalinity. Bicarbonate hardness was 174 mg/l which showed that free CO_2 is not a limiting factor to control the growth of phytoplanktons in this spring.

Inorganic phosphate was found in large quantity in the water of the thermal spring in comparison to the surface fresh water. This is similar to what had been reported by Brock and Brock, (1969b). This spring is also characterized by high amount of silica. Faulty rocks and their dissociation may be the cause of high silicate values as also reported earlier for the Manikaran hot water spring (Singh and Sharma, 1986) .Sulphide concentration of some hot springs of North Iceland and New-Zealand has been reported to be as high as 105 mg/l, generally 1.5 mg/l in 60 percent of the absent in the remaining springs (Castenholz, 1976). In the present investigation, the total concentration of sulphide and sulphite were 8.5 and 7.8 mg/l respectively. The observed high concentration of sulphide and sulphite in this spring indicate theprevailing anaerobic and reducing conditions of the spring, which reflect a reminiscent of archaic environment of Pre-cambrian era (Castenholz, 1967). These conditions are well suited for the growth of blue-green algae (Brock, 1970), some of which might have been the direct descendant of Pre-cambrian thermophiles (Castenholz, 1967).

The physico-chemical analysis of water revealed the presence of nutrients such as nitrogen, phosphorus, sodium, potassium and others which though present in low concentration, were conducive for fair growth of the blue-green algae and thermophilic bacteria. These organisms formed compact benthetic mats in the effluent of hot springs. Such thermal environments are conducive for many photosynthetic prokaryotic algae which are known to grow at constant temperature as high as 75°C in neutral or alkaline water (Brock, 1967; Brock, 1967b) and up to 55°C in acidic water (Doemel and Brock, 1970).

Abundant blue-green flora have been found in various hot springs e.g, Tiverias hot springs of the Israel (Dor, 1967), hot springs in Greece (Anagnostidis, 1961) and the Yellowstone thermal springs (Wiegert and Mitchell, 1973). The predominant cyanobacteria found in site 3 and 4 are mentioned in Table 4.

It was interesting to note that with exception to few common species found in both site 3 and 4, the cyanobacterial flora in site 3 was largely different from the flora of site 4. The differences might be due to the thermo- tolerance of species or due to the variations in mineral composition of the spring. In addition to this, the common species found in both the sites showed a better adaptability towards the flexible environment. The cyanobacterial flora identified from the mat collected, were cultured in different media as mentioned in Table 5, in incubator at 45^0C. Few filamentous and unicellular forms of cyanobacteria were isolated and cultured in axenic forms in Allen and Arnon media with or without nitrogen source. These were *Chroococcus minor* (Fig.7), *Microcystis* (Fig. 8), *Synechococcus elongatus* (Fig.9) as unicellular forms. *Phormidium tenue* (Fig. 10 and 11) *Mastigocladus laminosus*, (Fig.12 ,13 and 14) Oscillatoria laete-virens (Fig.15) *Oscillatoria ornimatis* (Fig.16) and *Lyngbya digueti* (Fig. 17) as filamentous forms They were abundant and mostly colonized within the temperature range of 35-45^{o}C. However, among the various cyanobacterial population studied, unicellular *Synechococcus elongatus* and filamentous *Mastigocladus*. and *Phormidium* sp. were uniformly distributed at all the temperature ranges (35-65^{o}C) indicating their higher thermo tolerance nature compared to other sps.

Except *Synechococcus elongatus*, filamentous *Phormidium* and *Mastigocladus laminosus*, there was a seasonal variation in the occurrence and population density of the other species and occasionally some species were found to have disappeared completely for years. No new cyanobacterial sp. was found during intensive microscopic examination of the spring water and algal mats during this investigation. Most of them were already reported in other thermal springs in India and in rest of the world. These cyanobacterial forms were morphologically identical to their similar non-thermal sps., therefore, they were designated as thermal strains of the respective non-thermal forms in the text. Among the filamentous forms, heterocystous *Mastigocladus laminosus* was abundantly found in all the cyanobacterial mats collected from randomly selected submerged rocks at different sites of the spring. This species was found to be nitrogen fixing, having branched filaments with heterocysts and was often found in dessicated form.

Dessicated filaments revived within a few days in suitable nutrient media. By virtue of its dual physiological characteristics i.e. photosynthesis and nitrogen fixation often in the same or in different branches of the common filament, this strain of *M. laminosus* has a competitive advantage over other cyanobacteria with regards to sustaining its own growth and increasing primary productivity under limited nutrient level in the spring. *Phormidium* sp. and *Oscillatoria* have never been found in isolation, they always coexisted with *M. laminosus*. Diazotrophic cyanobacterial mats dominated by *Fischerella* and *Calothrix* usually occurred in spring with low nitrogen concentration and temperature as found in other hot spring of similar water chemistry (Wickstrom, 1980).

"Tattapani" is one of the important hot water sulphur spring located in district Mandi about 48 Km. from Shimla, Himachal Pradesh. The spring is slightly alkaline in nature (pH 7.2) and temperature ranges between 35-65^{o}C always higher than the ambient air temperature. The salinity of the spring water is very high (10120 mg dissolved solids/litre) and contains all the essential salts e.g. nitrate, phosphate, carbonate, sulphate, chloride, silicate, fluoride, sulphite, sulphide and micronutrients such as, Fe, Mn, Ca, Mg, K and Na conducive for fair growth of cyanobacteria. A large number of cyanobacteria sps. either forming a thick mat adhered to submerged rocks or floating forms were found along the thermal gradient (65-35^{o}C) in this stream. They were abundant and mostly colonized within the temperature range of 35-45^{o}C. Except *Synechococcus elongatus*, filamentous *Phormidium* and *Mastigocladus laminosus*, there was a seasonal variation in the occurrence and population density of the other species and occassionally some species were found to have disappeared completely for whole of years. No new cyanobacterial sp. was found during intensive microscopic examination of the spring water and algal mats during this investigation. Most of them were already reported in other thermal springs in India and in rest of the world. These cyanobacterial forms were morphologically identical to their similar non-thermal sps., therefore, they were designated as thermal strains of the respective non-thermal forms in the text.No growth of any of the thermal strains of cyanobacteria was obtained under laboratory conditions using spring water as nutrient medium. The reported cyanobacteria can be used for biomedical engineering purpose

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