Comparative studies on calothrix isolates from Nepalese rice-fields

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COMPARATIVE STUDIES ON *CALOTHRIX* ISOLATES FROM NEPALESE RICE-FIELDS

by

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This thesis is entirely the result of my own work. It has not been accepted for any other degree and is not being submitted for any other degree.

To my children

ABSTRACT

Algal samples were collected from three rice-fields in Nepal: A) Parwanipur (100 m), B) Khumaltar (1336 m) and C) Kakani (2064 m); the rice-field algal flora from 1982, 1983 and 1986 samples was recorded.

Three Calothrix strains were isolated from 1986 samples, one from each of the three sites, as representatives of the rice-field flora. A study was carried out on morphology of these Calothrix isolates, in particular, the influence of phosphorus deficiency on morphology. Starting with hormogonium production, morphological changes were followed in one set of cultures without any further addition of phosphate and in another set, 10 mg l^{-1} P was added to P-limited cultures. With increase in phosphorus deficiency, synthesis of hormogonia ceased in all the strains, with obvious increase in cyanophycin granules and decrease in polyphosphate granules. Formation of intercalary heterocysts in Calothrix D794 and D795 and separation discs in Calothrix D796 was followed by the formation of intra-thylakoidal vacuoles and false-branches in all, but no hair cells formed in any strain. The addition of further phosphate to P-limited cultures led to renewed synthesis of hormogonia within 18 h in all strains.

The effect of light flux and nutrient concentration on morphology of three strains was tested. The effect of change in light flux on gas vacuole formation was examined in Calothrix D794. Gas vacuoles continued to form but at a lower rate when the alga was shifted to 170 µmol photon m^{-2} s^{-1} after initial period of incubation in dark. Gas-vacuolated hormogonia formation, release and disappearance of gas vacuoles in free hormogonia occurred within two hours of exposure to 170 µmol photo m^{-2} s^{-1}.

ABBREVIATIONS

°C	degrees Celcius
t	time
s	second
min	minute
h	hour
d	day
wk	week
µm	micrometre
mm	millimetre
cm	centimetre
m	meter
km	kilometre
ha	hectare
µg	microgram
mg	milligram
g	gram
ml	millilitre
l	litre
temp	temperature
PAR	photosynthetically active radiation
µmol	micromole
n	number of measurements
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
PIPES	(piperazine-N, N'- bis [2-ethanesulphonic acid])
to	tonne

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CONTENTS

		Page
	ABSTRACT	4
	ABBREVIATIONS	5
	ACKNOWLEDGEMENTS	6
	CONTENTS	7
	LIST OF TABLES	11
	LIST OF FIGURES	14
1.	**INTRODUCTION**	18
1.1	**GENERAL INTRODUCTION**	18
1.2	**PHYSICAL ENVIRONMENT OF NEPAL**	19
1.3	**RICE CULTIVATION IN NEPAL**	19
1.4	**ALGAL COMMUNITIES IN RICE-FIELDS**	20
1.5	**BLUE-GREEN ALGAE AND RICE-FIELDS**	21
1.6	**LIGHT AND TEMPERATURE**	22
1.61	Light	22
1.62	Temperature	23
1.7	**PHOSPHORUS**	23
1.71	Phosphorus in rice-field soil	23
1.72	Influence of phosphorus on morphology	24
1.8	**MORPHOLOGY**	24
1.81	Morphology of Blue-green algae	24
1.82	Morphology of Rivulariaceae	24
1.83	Hormogonia	25
1.84	Heterocysts	25
1.85	Trichome development	25
1.86	Hairs	26
1.87	Cell structure	26
1.871	Polyphosphate bodies	26
1.872	Cyanophycin granules	27
1.9	**FACTORS INFLUENCING GAS VACUOLE FORMATION**	27
1.10	**BIOLOGICAL IMPORTANCE OF GAS VACUOLE IN BLUE-GREEN ALGAE**	28
1.11	**AIMS**	29

		Page
2	**MATERIALS AND METHODS**	30
2.1	**CULTURE TECHNIQUES**	30
2.12	Cleaning of apparatus	30
2.13	Sterilization	30
2.2	**CHEMICALS**	30
2.3	**MEDIA**	31
2.31	Stock solution	31
2.32	Standard medium	31
2.33	Solid bacterial media	34
2.34	Liquid bacterial medium	35
2.35	Experimental media	35
2.4	**CULTURE CONDITIONS**	36
2.41	Incubation chambers	36
2.42	Light and temperature	37
2.5	**BLUE-GREEN ALGAL SAMPLES**	37
2.51	Preserved samples	37
2.52	Soil and dried samples	37
2.53	Live samples	38
2.6	**ISOLATION OF AXENIC CULTURES**	38
2.61	Isolation and purification	38
2.62	Axenic cultures	40
2.63	Test for purity	40
2.7	**MAINTENANCE AND SUBCULTURING**	41
2.71	Stock cultures	41
2.72	Inoculum	41
2.73	Preservation	42
2.8	**MORPHOLOGY AND TAXONOMY**	43
2.81	Microscopy and photomicrography	43
2.82	Scoring of morphological characters	44
2.83	Statistical analysis	44
2.84	Taxonomy	45
2.85	Cytological observation	45
3	**STUDY AREAS AND ALGAL SAMPLES**	46
3.1	**STUDY AREAS**	46
3.2	**DESCRIPTION OF SITES**	47
3.21	Parwanipur	47
3.22	Khumaltar	47
3.23	Kakani	48
3.3	**SAMPLING PROGRAMME**	56

4	RICE-FIELD ALGAL FLORA OF NEPAL	57
4.1	GENERAL COMMENT	57
4.2	ALGAL FLORA OF RICE-FIELDS	57
5	DESCRIPTION OF *CALOTHRIX* ISOLATES	62
5.1	GENERAL COMMENT	62
5.2	*CALOTHRIX* D794	62
5.3	*CALOTHRIX* D795	62
5.4	*CALOTHRIX* D796	63
6	INFLUENCE OF PHOSPHORUS DEFICIENCY ON MORPHOLOGY OF *Calothrix* D794, D795 and D796	74
6.1	GENERAL COMMENT	74
6.2	GROWTH STAGES	74
6.21	Stage I	74
6.22	Stage II	78
6.23	Stage III	78
6.24	Stage IV	85
6.241	Changes on morphology of strains without addition of phosphorus to P-limited cultures	86
6.242	Changes on morphology of strains with addition of 10 mg l^{-1} P to P-limited cultures	95
7	INFLUENCE OF ENVIRONMENTAL FACTORS ON MORPHOLOGY OF *CALOTHRIX* D794, D795 and D796	109
7.1	INFLUENCE OF LIGHT FLUX ON MORPHOLOGY OF 3 STRAINS	109
7.11	*Calothrix* D794	109
7.12	*Calothrix* D795	109
7.13	*Calothrix* D796	110
7.2	INFLUENCE OF NUTRIENT CONCENTRATION ON MORPHOLOGY OF 3 *CALOTHRIX* STRAINS	114
8	EFFECT OF SHIFT OF LIGHT ON GAS VACUOLE FORMATION IN *CALOTHRIX* D794	116
9	DISCUSSION	137
9.1	ALGAL FLORA OF NEPAL	137
9.2	MORPHOLOGY OF *CALOTHRIX* ISOLATES	138
9.3	INFLUENCE OF PHOSPHORUS ON MORPHOLOGY OF 3 STRAINS	138

9.4	EFFECT OF LIGHT FLUX AND NUTRIENT CONCENTRATION ON THREE STRAINS	140
9.5	EFFECT OF LIGHT ON GAS VACUOLE FORMATION ON *CALOTHRIX* D794	141
SUMMARY		144
REFERENCES		148
APPENDIX I	LAND USE OF NEPAL	152
APPENDIX 2	RAIN FALL, AVERAGE MINIMUM AND MAXIMUM TEMPERATURE IN PARWANIPUR IN 1986	152
APPENDIX 3	TABLES REPLACED BY FIGS IN TEXT (FOUR DIFFERENT TYPES OF FILAMENTS, EACH FILAMENT EXPRESSED AS PERCENTAGE OF TOTAL FILAMENTS AND RELEASED GAS-VACUOLATED HORMOGONIA AS PERCENTAGE OF TOTAL RELEASED HORMOGONIA.	153
APPENDIX 4	CULTURE COLLECTION RECORDS	157

LIST OF TABLES

Table		Page
1.1	The area of rice cultivation, rice production and yield of rice grain per unit area in different regions of Nepal	20
2.1	Specification and suppliers of chemicals	31
2.2	Concentration of mineral salts used in Chu 10D-N	33
2.3	Final concentration of elements used in Chu 10D-N	34
2.4	Concentration of chemicals used in solid bacterial medium	35
2.5	Light conditions in various growth chambers	37
2.6	Clonal and axenic strains of blue-green algae with their sites and Durham culture number	39
2.7	Organisms used in experiments with their culture and origin	40
3.1	Details of sites and cultural practices	55
4.1	Algal flora found in field samples (preserved) from sites A, B and C	58
4.2	Algal flora found in cultured samples from site B during September, 1982 and May, 1983 as presence (+) and with relative abundance on a scale of 1-5 based on subjective estimates from sites A, B and C in the samples collected during Aug-Sept, 1986	60
5.1	Comparison of morphological characters of Calothrix D794, D795 and D796 in standard medium	68
5.2	Mean dimensions (μm) of morphological characters of Calothrix D794, D795 and D796 in standard medium	69
6.1	Morphological characters of Calothrix D794 during batch culture in 0.1 mg l^{-1} P medium	102
6.2	Morphological characters of Calothrix D794 during batch culture in Chu 10D-N medium	102
6.3	Morphological characters of Calothrix D795 during batch culture in 0.1 mg l^{-1} P medium	103
6.4	Morphological characters of Calothrix D795 during batch culture in Chu 10D-N medium	103

6.5 Morphological characters of Calothrix D796 during batch culture in 0.1 mg l^{-1} P medium 104

6.6 Morphological characters of Calothrix D796 during batch culture in Chu 10D-N medium 104

6.7 Polyphosphate bodies as estimated percentage of the cell profile which was stained and the filaments with hormogonial production as percentage of total filaments in Calothrix D794 after addition of phosphorus to P-limited culture 105

6.8 Polyphosphate as estimated percentage of the cell profile which was stained and filaments with hormogonial production as percentage of total filaments in Calothrix D795 after addition of phosphorus to P-limited culture 105

6.9 Polyphosphate bodies as estimated percentage of the cell profile which was stained and the filaments with with hormogonial production as percentage of total filaments in Calothrix D796 after addition of phosphorus to P-limited culture 105

6.10 Dimensions of trichome and vegatative cells in Calothrix D794 grown in Chu 10D-N and 0.1 mg l^{-1} P medium 106

6.11 Dimensions of trichome and vegatative cells in Calothrix D795 grown in Chu 10D-N and 0.1 mg l^{-1} P medium from 0-10 d 106

6.12 Dimensions of trichome and vegetative cells in Calothrix D796 grown in Chu 10D-N and 0.1 mg l^{-1} P medium 107

6.14 Dimensions of cells in homogonia of Calothrix D794, D795 and D796 in 0.1 mg l^{-1} P medium 107

6.15 Dimensions of heterocysts (basal only) of Calothrix D794, D795 and D796 107

6.16 Blue heterocysts (basal only) in Calothrix D794, D795 and D796 in 1.78 mg l^{-1} P and in 0.1 mg l^{-1} P medium 108

7.1 Gas-vacuolated hormogonia released expressed as a percentage of the total released gas-vacuolated hormogonia in 3 strains at 4, 10, 110 and 170 µmol photon m^{-2} s^{-1} 110

8.1 Time course morphological events in Calothrix D794 in dark, 10 and 170 µmol photon m^{-2} s^{-1} 119

8.2 Time course morphological events in Calothrix D794 when shifted to 170 µmol photon m^{-2} s^{-1} after 4, 8 and 10 h of initial period of incubation in dark 120

8.3 Ratio of total filaments with gas vacuoles : total filaments without gas vacuoles and ratio of total gas-vacuolated hormogonia : total non-gas-vacuolated hormogonia when cultures were shifted from 170-170 μmol photon m^{-2} s^{-1}, 170-10 μmol photon m^{-2} s^{-1} and 170-dark 121

8.4 Ratio of total filaments with gas vacuoles : total filaments without gas-vacuoles and ratio of **gas-vacuolated hormogonia : non-gas-vacuolated hormogonia when culture was shifted back to 170 μmol photon m^{-2} s^{-1} after initial period of 4 h, 8 h and 10 h incubation in dark.** 122

LIST OF FIGURES

Figure		Page
3.1	Map of Nepal showing Rice Research Centres and selected sites ⊙ denotes rice research centres ❁ denotes selected sites	46
3.2	Parwanipur: 1) general view 2) selected field	50 50 50
3.3	Khumaltar: 1) general view 2) selected field	52 52 52
3.4	Kakani: 1) general view 2) selected field for sampling	54 54 54
5.1	Optical micrographs of 3 Calothrix strains grown in Chu 10D-N medium showing mature filaments 1) Calothrix D794 2) Calothrix D795 3) Calothrix D796	65 65 65 65
5.2	Optical micrograph of 3 Calothrix strains showing hormogonia 1) D794 2) D795 3) D796	67 67 67 67
5.3	Optical micrograph of Calothrix D794 and D795 in stock culture showing intra-thylakoidal vacuole, false-branches and thick sheath.	71
5.4	Optical micrograph of Calothrix D796 in stock culture showing twisting of filaments and collapsed heterocysts 1) collapsed heterocysts 2) twisting of filaments	73
6.1	Growth stages of Calothrix D794, D795 and D796 grown in Chu 10D-N medium a) hormogonium b) asymmetrical hormogonium c) formation of heterocyst d) young trichome e) mature trichome	77

6.2 Optical micrographs of Calothrix D794 in Chu 10D-N showing morphological change 80
1) mature trichome with elongated apical cell
2) false-branch
3) loop formation

6.3 Optical micrographs of Calothrix D795 in Chu 10D-N showing 82
1) mature filaments with blue heterocyst
2) gas-vacuolate hormogonium attached to the trichome
3) young trichomes

6.4 Optical micrograph of Calothrix D796 grown in Chu 10D-N showing 84
1) extended sheath and necridium
2) hormogonium, vegetative cell and sheath
3) rope formation of filaments

6.5 Comparative filament morphology of Calothrix D794, D795 and D796 88
a) hormogonium production in Chu 10D-N medium
b) cyanophycin granules in 0.1 mg l^{-1} P medium

6.6 Calothrix D794 in 0.1 mg l^{-1} P medium 90
1) rope formation of hormogonia
2) young trichomes
3) clump colony
4) clump colony (x 10)

6.7 Calothrix D794, D795 and D796 showing cyanophycin granules, separation discs and false-branches 92
a-b) Calothrix D794
c-d) Calothrix D795
e-i) Calothrix D796

6.8 Calothrix D796 with increasing P-deficiency showing
1) trichomes with necridium
2) clumped colony 94
3) false-branch

6.9 Calothrix D794, D795 and D796 showing accumulation of polyphosphate bodies after addition of phosphate to P-limited cultures 97
a) trichome prior to phosphate addition
b) trichome 30 min after phosphate addition
c) trichome 1 h after phosphate addition
d) trichome 3 d after phosphate addition

6.10 Optical micrograph of Calothrix D794 and D795 after addition of phosphate to P-limited culture 99

6.11 Optical micrograph of Calothrix D796 after addition of phosphate to P-limited culture 101

7.1 Calothrix D795 grown at 4, 110 and 170 µmol photon m^{-2} s^{-1} showing
a-b) highly gas-vacuolated hormogonia at 110 µmol photon m^{-2} s^{-1}
c-d) intra-thylakoidal vacuoles and disappearance of gas vacuoles in hormogonium attached to the filament and in released hormogonium
e-g) formation of separation discs (necridium) and intercalary heterocysts at 4 µmol photon m^{-2} s^{-1} 113

7.2 Influence of nutrient concentration on gas vacuole formation in Calothrix D794 and D795 grown at 10 and 110 µmol photon m^{-2} s^{-1} 115

8.1 Influence of environmental factors on gas vacuole formation in Calothrix D794. The alga was grown at 170 µmol photon m^{-2} s^{-1} for 5 d, subcultured and incubated at 10, 170 µmol photon m^{-2} s^{-1} and in dark 124

8.2-8.4 Influence of shift of light on gas vacuole formation in Calothrix D794. The alga was grown in standard medium at 170 µmol photon m^{-2} s^{-1} for 5 d, sub-cultured and incubated in dark. The culture was shifted back to 170 µmol photon after initial period of growth in dark for 4 h, 8 h and 10 h

8.2 4 h in dark 124

8.3 8 h in dark 125

8.4 10 h in dark 126

8.5-8.6 Influence of shift of light on gas vacuole formation in Calothrix D794. The alga was grown in standard medium at 170 µmol photon for 5 d, sub-cultured and incubated at 10 µmol photon. The culture was shifted back to 170 µmol photon m^{-2} s^{-1} after an initial period 4 h and 10 h incubation in 10 µmol photon m^{-2} s^{-1}

8.5 4 h at 10 µmol photon 127

8.6 10 h at 10 µmol photon 128

8.7 Calothrix D794 showing locations of gas vacuoles in cells (under optical microscope) at low and high light and swelling of gas-vacuolated hormogonia when the culture was shifted to 170 µmol photon m^{-2} s^{-1} after 10 h incubation in dark 132

8.8 Optical micrograph of Calothrix D794 showing gas vacuole formation in tapered part of trichomes and gas-vacuolated hormogonia
1) gas vacuole formation at 10 µmol photon m^{-2} s^{-1}
2) released gas-vacuolated hormogonia
3) gas vacuole formation in tapered part of trichomes in colony in dark grown culture
4) releasing gas-vacuolated hormogonia 134

8.9 Calothrix D794 showing gas vacuole formation when the alga was grown at 170 µmol photon for 5 d, subcultured and incubated at 10, 170 µmol photon m^{-2} s^{-1} and in dark
a-c) 170-170 µmol photon m^{-2} s^{-1}
d-f) 170-10 µmol photon m^{-2} s^{-1}
g-i) 170-dark 136

1 INTRODUCTION

1.1 GENERAL INTRODUCTION

Nepal lies on the southern slopes of the Himalayas. The country's economy is predominantly agricultural and 90% of the total population of over 16 million relies on agriculture which takes place on only 16% of the total area (appendix 1). Rice is the major crop of Nepal, cultivated on 55% of the cultivable land, and contributes 59% of the total grain production.

The production of rice year after year without application of fertilizer in Nepalese rice-fields has been demonstrated in long term experiments (1975-1977) at Parwanipur (Deo and Shah, 1978). In Bangladesh, part of this natural fertility has been attributed to nitrogen-fixing blue-green algae (Brammer, 1983). It is possible that blue-green algae in Nepal may play a similar role. As the rice plants and the algae represent the two principal components in this ecosystem, a proper understanding of their various interrelationships is important (Roger and Kulasooriya, 1980), however, studies on blue-green algae from Nepalese rice-fields are meagre.

The present study was therefore planned to give a brief account of the algal flora from three different rice-fields (at 100, 1350 and 2064 m altitudes), to isolate Calothrix, one from each of the three sites (site description in Chapter 3) and to study their morphology with respect to nutrient deficiency, mainly phosphorus. In addition the factors affecting the development of gas vacuoles have been explored with the hope of understanding their biological importance for microorganisms to adapt in rice-field environment.

1.2 PHYSICAL ENVIRONMENT OF NEPAL

Nepal occupies 145305 km^2 and is situated between 26° 20' to 30° 10' N and 80° 15'to 88° 15 E. It is about 800 km long and 160 km broad. The flat Tarai in the south, the central hills and the high Himalayas in the north divide the country into three areas (Fig. 3.1)

The temperature reflects changes in altitude and decreases as one moves from the Tarai through mid-hills and high-hills to higher Himalayan ranges. At Kathmandu, 1337 m above sea level, average monthly temperatures are 10°C in January and 23°C in May. In the highest Himalaya, air temperatures are always below freezing point. Rainfall ranges from 1000-1500 mm over most of the country.

Soils of Nepal are categorized as having low to medium organic matter content, high available phosphorus content, medium available potassium content, and deficient in nitrogen (Deo et al., 1978). The pH of most soils lies between 5.9 and 7.6.

1.3 RICE CULTIVATION IN NEPAL

Rice is cultivated from the Tarai (100 m) to the hilly districts (2465 m). The maximum rice per unit area are obtained in the mid-hills (Table 1.1) because of timely inputs, low night temperature and better management.

In Nepal, 16% of the rice land is irrigated and the rest is rain-fed. Upland rice (9%) is grown on both flat and sloping fields that are seeded under dry conditions whilst rain-fed low land rice is bounded to give a water depth of up to 50 cm.

Table 1.1 The area of rice cultivation, rice production and yield of rice grain per unit area in different regions of Nepal in 1976 (Mallick, 1982).

Regions	Area of rice (ha)	Total production (t_o)	Average yield $t_o ha^{-1}$
high hills	25,733	57,260	2.25
mid-hills	194,445	502,818	2.58
Tarai	1041,441	1,826,194	1.75

Land preparation varies among rice producing areas. In the Tarai, rice is grown on land prepared with animal-drawn implements, in some areas, by tractor-drawn impliment. Direct seeding and transplanting are the two methods of planting rice. Rice is usually cultivated as a monocrop. Two or four crops are harvested each year in irrigated areas. Due to moisture stress, soil condition and rain fall pattern, however, a winter crop is not grown.

1.4 ALGAL COMMUNITIES IN RICE-FIELDS

Rice-fields constitute an artificial biotope of a particular character, which can involve communities more typical of water and ones more typical of soil. The life-cycle of organisms in rice-field water is, however, of a short duration, extending over three to six months. After harvest the majority of the aquatic microorganisms either die or form dormant structures (Venkataraman, 1972).

The algal flora of rice-fields has attracted the attention of many workers in temperate and tropical regions (Singh, 1961; Venkataraman, 1972; Roger and Kulasooriya, 1982). In temperate regions, filamentous chlorophyta are generally dominant and they are developed throughout the vegetative period of the rice plant; they are followed by Xanthophyta and Cyanophyta (Tsangridis,

1982). In the tropics, in contrast to the temperate region, Cyanophyta dominate over other algae (Gupta, 1966; Pandey, 1965a; Sharma and Gaur, 1981).

1.5 BLUE-GREEN ALGAE AND RICE-FIELDS

Blue-green algae are oxygen-evolving photosynthetic microorganisms that inhabit various fresh-water environments, including the water-logged soils of rice-fields (De, 1939; Watanabe, 1959; Materassic and Balloni, 1965; Kobayashi et al.,1967). Rice-fields provide an environment favourable for the growth of blue-green algae with respect to their requirements of light, water, high temperature and nutrient availability (Singh, 1961; Fogg et al., 1967; Venkataraman, 1972). This also accounts for the greater abundance of blue-green algae in the rice-fields than in other cultivated areas (Watanabe and Yamamoto, 1971).

Abundant growth of blue-green algae has been reported under widely different climatic conditions in India (Mitra, 1951), Japan (Okuda and Yamaguchi,1952; 1956), and the Ukraine (Prikhod'kova, 1971). Over 70% of the algal species in Indian paddy soils are comprised of blue-green algae (Pandey, 1965). In Southeast Iraq, blue-green algae constituted upto 86% of the total algal flora (Alkaisi, 1976). 21 of 41 species from deep-water rice-fields in Bangladesh were heterocystous blue-greens (Martinez and Catling, 1982). The presence of blue-green algae in the rice fields of Nepal has been reported in Parwanipur and Bara district (Maskey and Bhattarai, 1984).

Algal succession may be characteristic of particular geographic zones. The growth of a heterocystous algal bloom has been reported in a paddy field in Mali in early stage, in Sri Lanka. Experiments indicated that heterocystous forms were present throughout the cultivation cycle and some species occurred only during the latter part (Roger and Kulasooriya, 1980).

Blue-green algae grow in the rice-field water as plankton, epiphytes on rice plants and on the soil surface. Free- living blue-green algae are abundant

in tropical and sub-tropical soils than in temperate soils (Watanabe and Yamatomo, 1971; Fogg et al., 1973; Sinclair and Whitton, 1975; Stewart, 1977). Temperature, light intensity, salinity, combined nitrogen and phosphorus availability are the major environmental factors affecting the growth of blue-green algal communities in rice-field. Under natural conditions, blue-green algae grow preferentially in environments that are neutral to alkaline (Roger and Reynaud, 1979). There are reports on the presence of certain blue-green algal strains in soils with pH values between 5-6. In India, it was inferred that blue-green algae preferred a neutral or near neutral pH 6.5-7.5, but others were also capable of thriving over a wider range 5.5-8.5 (Prasad et al., 1978). Aulosira fertilissima and Calothrix brevissima have been reported to be ubiquitous in Kerala rice-fields where the pH ranged from 3.5 to 6.5 (Aiyer, 1971). Weeding, water management, the nature and the quantity of fertilizers as well as application techniques have a considerable influence on blue-green algae flora (Roger and Kulasooriya, 1980). Thus different farming practices may influence their development (Whitton and Roger, 1989).

1.6 LIGHT AND TEMPERATURE

1.61 Light

In the paddies blue-green algae occur especially as surface scum, as a bloom, or as crust-forming aggregates at the soil water interface. During day time, vertical migration of algae occurs in the water in relation to O_2 production by photosynthesis. In submerged soils, light availability depends upon the season and latitude, the cloud cover, and the turbidity of water. In rice-fields, the light-screening effect of the crop canopy appears to cause a rapid decrease of light. Light tolerance differs between algal species and may be roughly correlated with taxonomic groups (Roger and Kulasooriya, 1980).

1.62 Temperature

The optimal temperature for the growth of blue-green algae is about 30-35°C. Temperature extremes inhabiting their growth are beyond the range within which rice grows, thus, rarely is temperature a limiting factor for blue-green algae in paddy fields (Roger and Reynaud, 1978).

1.7 PHOSPHORUS

1.71 Phosphorus in rice-field soil

Phosphorus is present in the rice-field soil, organic matter and fertilizers. De Datta (1970) reported that the rice-field soil which is under upland condition and shows unavailable phosphate in soil test, shows availability of phosphorus after few days of flooding. Okuda and Yamaguchi (1952) found that the growth of blue-green algae in submerged soil is closely related to the available phosphorus. The P levels within algal cells may fluctuate widely depending on whether or not the algae are growing under P-limited conditions. Blue-green algae assimilate more P than they require and store the excess as polyphosphate, which can be used under P-deficient conditions (Roger and Reynaud, 1979).

Phosphorus availability has often been implicated as a factor limiting the growth of algae (Kuhl, 1962), particularly nitrogen-fixing forms (Stewart and Alexander, 1971; Fogg et al., 1973). Nitrogen-fixation prevents blue-green algae from becoming nitrogen deficient thus allowing phosphorus deficiency to develop further (Healey, 1982). A number of subsequent studies have indicated that the phosphorus status of the environment may be a particularly important factor in the growth of Rivulariaceae (Whitton, 1987). For this reason the phosphorus status of rice-field blue-green algae is important.

1.72 Influence of phosphorus on morphology

The morphological variability of blue-green algae as a response to environmental conditions is well known and, among these, the Rivulariaceae show a particularly marked response (Livingstone and Whitton,1983). The concentration of cellular phosphorus appears to be a key factors influencing morphology in this family (Sinclair and Whitton, 1977a). The morphological changes occuring in strains of Calothrix parietina have been described (Livingstone and Whitton, 1983; Wood et al., 1986). Under P-rich conditions, the apical region of the trichome continuously gives rise to hormogonia, but with increasing P-deficiency a multicellular colourless hair is formed instead (Livingstone and Whitton, 1983).

1.8 MORPHOLOGY

1.81 Morphology of blue-green algae

Blue-green algae display a wide variety of morphology from unicells to a branched filament. Morphological variation in blue-green algae in response to changes in the environment is well known (Sinclair and Whitton, 1977; Stam and Holleman, 1979; Jeeji-Bai and Seshadri, 1980; Rosencheck and Mikhailo, 1980; Seki et al., 1981; Meffert et al., 1981).

1.82 Morphology of Rivulariaceae

It is clear from the floristic descriptions of Rivulariaceae (Desikachary, 1959; Kirky and Whitton, 1976; Whitton 1987) that species in this family are often variable. Details of their growth are given in Sinclair (1977), Wood (1984) and Whitton (1987). The Rivulariaceae are capable of forming hormogonia, which, under certain conditions differentiate into trichomes which are tapered at one or both ends (Livingstone and Whitton, 1983). Many, but not all, Rivulariaceae can form heterocysts when grown in the absence of combined nitrogen (Sinclair and Whitton, 1977a). Brief descriptions of the morphological features and their known variability are given below:

1.83 Hormogonia

Hormogonia are short trichomes, produced at the tapered part of the parent trichome. After their release from the parent trichome, few, if any, cell divisions occur before further differentiation of the trichome (Whitton, 1987). However, Maxwell (1974) noted elongation of hormogonia and their subsequent fragmentation by separation discs in a natural population of Gloeotrichia. Gas-vacuolated hormogonia have been reported in Gloeotrichia (Singh and Tiwari, 1970), Calothrix parietina (Livingstone and Whitton, 1983) and in Calothrix (Wood, 1984). In Calothrix gas-vacuoles only appear in hormogonia which tend to float once they are released.

1.84 Heterocysts

Heterocysts are easily distinguishable from the rest of the cells by their walls and homogeneous pale yellow appearance. A feature which is perhaps quite widespread among Rivulariaceae is the presence of some heterocysts which are green or blue-green e.g. in Calothrix and Gloeotrichia from rice-fields both in nature and in the laboratory. The terminal heterocyst differentiates from the cell which was nearest the mother trichome before the hormogonia was released (Whitton, 1987). Intercalary heterocysts occur occasionally in some Calothrix species (Geitler, 1932; Jeeji-Bai, 1977; Rai et al., 1978). Heterocysts are generally produced when combined nitrogen is in short supply or absent.

Heterocysts are specialised cells for nitrogen fixation.

1.85 Trichome development

Once the hormogonium differentiates into a heterocyst and vegetative cells, growth is typically meristematic with cell division restricted to the basal end of the trichome.

1.86 Hairs

The blue-green algal hairs are defined as a region of the trichome where the cells are narrow, elongated, highly vacuolated and usually apparently colourless (Sinclair and Whitton, 1977). In Rivulariaceae, the tapered trichomes often terminate into a thin colourless hair which are probably produced in response to nutrient deficiency. As many members of this group can fix nitrogen there are few cases of nitrogen deficiency leading to hair formation, thought Sinclair (1977) found no hair production in a Calothrix grown in an atmosphere devoid of nitrogen. Kirkby (1975) noticed that hairs were more common when the external phosphorus concentration was low and Sinclair (1977) reported that, in cultures of 13 strains grown to elemental deficiency, hairs were more common under phosphorus deficiency.

1.87 Cell structure

1.871 Polyphosphate bodies

Polyphosphate bodies contain stored phosphate, the bodies being absent in young growing cells or cells grown in a P-deficient medium (Tischer, 1957). Polyphosphate granules accumulate in the stationary phase or when phosphate is added to a culture (Stewart, 1977; Lawry and Jensen, 1975). Such reserves disappear during phosphate starvation and are only able to support a limited amount of growth (Stewart and Alexander, 1971).

General studies of polyphosphate granules have been carried out by various authors. Formation of polyphosphate granules was observed in the basal cells of Calothrix parietina 10 min after the addition of phosphate to P-limited culture (Livingstone et al.,1983).

1.872 Cyanophycin granules

Cyanophycin granules, the nitrogen storage structures are highly refractile granules in the cells (Borzi, 1886). The presence or absence of these inclusions may vary from species to species, and from one environmental condition to another. The level of cyanophycin varies during growth, it is low in the exponential phase and increases during the stationary phase (Simon, 1973). Cyanophycin granules are degraded rapidly when cells are transferred into fresh medium.

1.9 FACTORS INFLUENCING GAS VACUOLE FORMATION

Gas vacuoles appear as irregular and indefinite reddish structures under the light microscope (Fogg, 1972; Walsby and Broker, 1980: Smith and Peat, 1987). Changes in environmental factors such as light intensity and nutrient availability have been shown to pronounce formation of gas-vacuolate hormogonia (Tandeau de Marsac, 1983; Armstrong et al.,1983; Rippka and Herdman, 1985). At low light intensity gas vacuole formation is most active while at high light intensity they disappear (Walsby, 1967; Fogg, 1972; Walsby and Booker, 1980).

Other factors have been shown to influence gas vacuole formation including availability of nitrogen (Klemer, 1978), inorganic carbon (Booker and Walsby, 1981) and phosphate (Booker and Walsby, 1981). The disappearance of gas vacuoles at high light is caused by a rise in cell turgor pressure generated partly by an increase in the concentration of organic products (Sinsdale and Walsby, 1972; Grant and Walsby, 1977) and partly by light-stimulated accumulation of potassium ions from the suspending medium (Allison and Walsby, 1981).

1.10 BIOLOGICAL IMPORTANCE OF GAS VACUOLE FORMATION IN BLUE-GREEN ALGAE

Gas vacuoles and their function have been investigated extensively by Walsby and co-workers. Gas vacuoles are an effective buoyancy aid (Walsby, 1987), which is a considerable biological advantage to planktonic blue-green algae. Gas vacuoles also act as a screen against strong light and scatter light away from light sensitive regions (Walsby, 1969b; Dubelaar visser and Donze, 1987). Gas vacuoles provide a buoyancy regulating mechanism (Walsby and Booker, 1980) rather than light shielding (van Liere and Walsby, 1982). Some non-planktonic blue-green algae produce gas-vacuolated hormogonia, perhaps as an aid to dispersal in conditions of flooding (Walsby, 1972).

Mechanisms of buoyancy regulation have been reviewed by Walsby (1987, 1988). Studies done in Anabaena flos-aquae (Walsby, 1971; Dinsdale and Walsby, 1972) have been shown that the alga floats when incubated at low light intensity and sinks when shifted to high light intensity. In some instances the reverse has been demonstrated, Organisms regain bouyancy when shifted back to low light intensity (Dinsdale and Walsby, 1972) or darkness (Kromkamp and Mur, 1984). A buoyancy decrease at high light intensity may be due to disappearance of gas vacuoles or regulation of gas vacuole formation. Such responses to light intensity would enable these organisms either to stratify on a vertically decreasing light intensity gradient or to migrate up and down during night and day (Reynolds and Walsby, 1975) in the natural environment. This could be of biological advantage in allowing the cells to absorb nutrients effectively by their movement through the water.

1.11 AIMS

The aims of this study are:

1) to collect samples from three different rice-field ecosystems of Nepal and to give a brief account of rice-field algal flora of each site.

2) to isolate one Calothrix from each of the three sites of different altitude.

3) to compare the morphology of Calothrix isolates in particular, the influence of phosphorus deficiency and factors affecting gas-vacuole development.

2 MATERIALS AND METHODS

2.1 CULTURE TECHNIQUES

2.11 Cleaning of apparatus

All glassware was soaked in hot tap water and detergent for 30 min, rinsed thoroughly with hot tap water and soaked in acid for 20 min; then it was rinsed with distilled water six times. Finally it was rinsed with Milli-Q water and dried at 105°C. Silicon bungs were cleaned by soaking in a 2% solution of phosphate-free detergent (Decon 90, Decon Laboratories Ltd., England) for 15 min and rinsed six times in distilled water and dried at 40°C.

2.12 Sterilization

Culture medium and utensils were sterilized by autoclaving at 121°C for 20 min. pressure. To avoid contamination all sub-culturing and isolation was carried out in a Microflow Pathfinder laminar flow cabinet. Before starting work, the cabinet was sprayed with absolute alcohol, the fan switched on and left for 5 min. Wire loops were pre-sterilized in a bunsen flame. Isolation needles were dipped in alcohol and flamed briefly.

2.2 CHEMICALS

All chemicals used in the preparation of media were AnalaR grade except for those listed in Table 2.1. Chemicals were weighed on either a Mettler H51 5-place balance or a 2-place top-pan balance.

Table 2.1 Specification and suppliers of chemicals other than Analar grade in the study.

Chemicals	Specification	Supplier
agar		Difco Lab.,U.K.
peptone		Difco, U.S.A.
nutrient agar		Oxoid, U.K.
tryptone		Oxoid, U.K.
HEPES	Sigma grade	Sigma chemical Co., U.K.
Casamino acid		Difco, U.S.A.

2.3 MEDIA

2.31 Stock solution

Stock solutions were stored in 250-ml Pyrex reagent bottles. Macronutrients and micronutrients for stock solutions are listed in Table 2.2.

2.32 Standard medium (Chu 10D-N)

Standard medium is a nitrogen-free modification of the no. 10 formula of Chu (1942).

The liquid medium was prepared according to a recipe using concentrated stock solution as listed in Table 2.22. The elemental composition of medium are listed in Table 2.3

Approximately 500 ml of distilled water containing 0.6 g HEPES was adjusted to pH 7.0 with NaOH (1.0 M). pH was measured using an E 12 model 7050 direct reading pH meter fitted with a combination electrode (Electronic Instrument Ltd. Surrey, England). The buffered distilled water was poured into a volumetric flask and then nutrient stocks were added in amounts and orders and made up to 1-litre with distilled water.

The solid medium was prepared by mixing a mineral liquid medium with 1% agar (Allen, 1968). After autoclaving and cooling to about 40-45°C, media were poured into sterilized Petri-dishes in a laminar flow cabinet. When solid, they were kept in a refrigerator at 4°C. The concentration of chemicals in solid bacterial media are listed in Table 2.4.

Table 2.2 Concentration of mineral salt stock solutions used in Chu 10D-N (pH 7.0 + HEPES)

Salts	mg l^{-1}
$MgSO_4.7H_2O$	25.0
$CaCl_2.2H_2O$	35.83
$NaHCO_3$	15.85
$Na_2SiO_3.5H_2O$	10.9
KH_2PO_4	7.8
Micro-element solution	
H_3BO_3	0.715
$MnCl_2.4H_2O$	0.045
$ZnSO_4.7H_2O$	0.056
$CuSO_4.5H_2O$	0.019
$CoSO_4.7H_2O$	0.01
$Na_2MoO_4.4H_2O$	0.007
FeEDTA solution	
$FeCl_3.6H_2O$	2.413
Na_2EDTA	3.175

Table 2.3 Final concentration of elements used in Chu 10D-N (pH 7.0 + HEPES)

Elements	mg l^{-1}
B	0.0125
Na	14.006
Mg	2.43
Si	1.43
P	1.78
S	3.254
Cl	35.21
K	2.24
Ca	9.47
Mn	0.012
Fe	0.5
Co	0.002
Cu	0.0012
Zn	0.0134
Mo	0.002

2.33 Solid bacterial media

Chemicals required for bacterial media were transfered to a 2-l flask and made up to 1-l with distilled water. 1% of agar was added in all medium. After autoclaving the medium was allowed to cool to 45-50°C before pouring. Petridishes were stored in refrigerator as in 2.311 (b).

Table 2.4 Concentration of chemicals used in solid bacterial medium

Medium	g l^{-1}
SST	
Glucose	10
Tryptone	10
Yeast	5
Nutrient Broth	25
Yeast	
Peptone-Glucose	
Glucose	1
Peptone	1

2.34 Liquid bacterial medium

This medium was enriched with 10 mg l^{-1} NH_4-N, 0.02% casamino acids and 0.5% glucose to promote bacterial growth. 10 ml of liquid bacterial medium was dispensed into each test tube (10 ml x 20 test tubes) and inoculated with a small amount of alga and kept in the dark at 32°C.

2.35 Experimental media

The standard medium was generally invariably the control for experiments investigating nutrient deficiencies. The ionic background of each treatment was kept as near constant as possible by using substitute salt solutions to supply the complementary ions presence in the omitted salt stocks.

2.4 CULTURE CONDITIONS

2.41 Incubation chambers

Stock cultures and cultures used for isolation and purification were grown in thermostatically controlled growth rooms. Flasks were shaken by hand every other day. Experiments were carried out in thermostatically controlled tanks of distilled water. A shaking mechanism moved the flasks through a horizontal distance of about 60 mm approximately 65 times a minute.

2.42 Light and temperature

In the growth rooms illumination was supplied from above by cool white fluorescent tubes. In the shaking tank, illumination was supplied from beneath by a series of warm white fluorescent tubes. The temperature in the growth room and the tank was maintained at 32°C.

Light conditions in various growth chambers are given in Table 2.5. Light flux was measured using by Macam Radiometer/Photometer (model 0101) and expressed as μmol photon m^{-2} s^{-1} PAR. Light attenuation was achieved by wrapping the flasks with neutral density papers. Green and red light was obtained by using the respective filters (Lee Filters Ltd.).
For dark incubation, the flasks were covered by two layers of aluminium foil followed by a layer of black polythene and bungs were covered loosely by aluminium foil.

Table 2.5 Light conditions in various growth chambers

Growth chambers	Type of fluorescent tubes	Position of tubes	Photon flux density (μmol photon m^{-2} s^{-1} PAR)
Growth room			
continuous light	white	above	16-65
dark/light	white	above	16-60
Shaking tanks			
full light	white	below	110-180
half light	white	below	50-120

2.5 BLUE-GREEN ALGAL SAMPLES

2.51 Preserved samples

Samples were preserved in two types of preservative separately

I) 4% Formalin solution (buffered)

II) KI + I solution

Presevatives were diluted to two and half percent by sample water

Ten preserved samples from each site were taken.

2.52 Soil and dried samples

Two dried samples and two soil samples were taken from each site. These were spread on sterile filter paper in Petri dishes and covered with sheets of filter paper. They were then allowed to dry at room temperature. Once air dry, the samples were packed into the paper bags and kept until required for culturing.

2.53 Live samples

Five live samples (5 ml alga + 5 ml field water) were collected from each site and kept in good condition by loosening the caps of the tubes to permit gaseous exchange. Field water was added into the tubes to maintain the original level.

2.6 ISOLATION OF AXENIC CULTURES

After returning to England, both live and dried samples were suspended in liquid medium and incubated in the growth room at 32°C. All strains used for experimental purposes were isolated from the samples collected from Nepalese rice-fields. The organisms used for experiments with their origin are given in Table 2.6.

2.61 Isolation and purification

All algae growing in samples were identified and recorded. Cycloheximide was added to cultures dominated with eukaryotes. Suppression of these organisms was usually successful by the next subculture. Repeated subculturing was done to establish unialgal cloned cultures. It was planned to isolate one strain, one from each of the three sites. Repeated subculturing resulted in unialgal cultures of Calothrix from sites A, B and C.

Table 2.6 Clonal and axenic strains of blue-green algae with their sites Durham culture number (see appendix 4 for details). +, yes; -, no.

Durham culture no.	Strain	Clonal	Axenic	site and isolated from
D655	Anabaena	+	-	Khumaltar rice-field, mixed with Azolla
D661	Anabaena	+	-	"
D666	Anabaena	+	-	Parwanipur rice-field, with water plant
D660	Calothrix	+	+	Khumaltar rice-field, soil sample
D794	Calothrix	+	+	Parwanipur rice-field, floating sample
D795	Calothrix	+	+	Khumaltar rice-field, vertically suspended up sample
D796	Calothrix	+	+	Kakani rice-field, dried sample
D662	Gloeotrichia	+	-	Khumaltar, sample attached to rice plant
D656	Gloeotrichia	+	-	"
D663	Gloeotrichia	+	-	"
D657	Nostoc	+	-	"
D658	Nostoc	+	-	"
D797	Synechococcus	+	+	Kakani rice-field,

Table 2.7 Strains used in experiments

Strains	Date of collection	Clonal on	Axenic on
Calothrix D794	28/08/86	19/01/87	02/03/87
Calothrix D795	11/09/86	19/01/87	02/03/87
Calothrix D796	21/09/86	02/01/87	02/03/87

Details of sites of Calothrix isolates are in Table 2.6 and Appendix 3.

2.62 Axenic culture

Unialgal strains of Calothrix from sites A, B and C were streaked on solid agar medium and plates were incubated in the growth room at 16 μmol photon m^{-2} s^{-1} PAR at 32°C. On day 5, there was a zone of hormogonia around the inoculum, some moving away from the centre. With the help of a binocular microscope, a single hormogonium was picked with isolation needle and transfered on to a fresh agar plate. After successive transfer on agar plates, a hormogonium was transferred into a series of flasks of liquid culture medium. 50 ml of medium was used in each 100-ml flask. These flasks were incubated in shaking tank so that the hormogonium would grow rapidly. The first attempt to obtain an axenic culture failed, so the least contaminated liquid culture derived from a single hormogonium was restreaked again on solid medium in order to achieve purification in a further attempts. Calothrix cultures presumed to be free from bacterial contamination were then tested for purity.

2.63 Test for purity

The three strains of Calothrix were examined after the initial purification before each sub-culturing and before use as experimental inocula.

Three different test were carried out:

(I) Algal material was examined under the microscope

(II) If free from obvious contamination it was tested in Y, SST, NB, and PG plates (see Table 2.4). Plates were incubated in dark at 32°C for three weeks, but also observed after 24 h, 48 h and 10 d.

If no bacterial growth was observed on plate,

(111) then the final test in liquid bacterial medium enriched with NH_4- N (10 mg l^{-1}), sucrose (0.2%) and Casamino acid was performed. 10 ml of medium was dispensed into each test tube (10 mls x 20 test tubes), and inoculated with small amounts of alga and kept in the dark at 32°C. These test tubes were observed after 24 h, 48 h and 10 d, the liquid did not turn cloudy or milky indicating the cultures free of bacteria. Finally Calothrix stocks from the growth room were examined under the microscope for any obvious signs of bacteria.

2.7 MAINTENENCE AND SUBCULTURING

2.71 Stock cultures

Axenic Calothrix strains were sub-cultured into fresh medium (Chu 10D-N) in straight-necked 100-ml culturing flasks capped with silicon rubber stoppers (type c-30, Sanko Plastics Co.). These stock cultures were incubated in dim light (16 μmol m^{-2} s^{-1}) in the growth room at 32°C.

2.72 Inoculum

For routine sub-culturing, clumps of algae were transferred using a sterile wire loop. For batch cultures, the homogenous inoculum was prepared by adding exponentially growing algal material to a known volume of sterile

medium. The organism was grown under physical conditions similar to those of experiments.

Strains D794 and D795 formed thin mats and could be broken easily by a wire loop. Strain D795 always formed a thick mat which was difficult to break, hence it was difficult to obtain a uniform inoculum (Kirkby, 1975). An attempt was made to break the mat into pieces by passing it through a syringe without a needle. A loopful of algae was used as inoculum, however an effort was made to transfer pieces of algal material that were of uniform size (Kirkby, 1975). For the statistical analysis of gas vacuole formation, a lightly homogenized inoculum was used. This was obtained by passing the culture through a syringe first without needle and then with a needle (19 G, 51 mm long) and finally with a fine needle (21 G, 51 mm long) respectively. A large inoculum 5-ml was used.

2.73 Preservation

I) liquid nitrogen

For long-term preservation, axenic Calothrix strains were preserved in liquid nitrogen. 5% dimethylsulphoxide was added as a cryoprotectant. Two weeks old cultures were used. Aliquots were placed in a small (5 ml) plastic ampoules (3 replicates) that were stored immediatly in liquid nitrogen.

II) Preservative

A difficulty was experienced during counting in experiments involved with gas vacuole formation. Thus the organism was tested with different fixatives and characters were scored before and after preservation. The most suitable preservative was found to be a mixture prepared as follows:

0.05 M PIPES

2.5% glutaraldehyde

1.5% formaldehyde

1.5 g of paraformaldehyde was dissolved in 20 ml of distilled water at 60°C in the fume hood. The mixture formed a precipitate and a few drops of M NaOH were added until the clear solution was obtained. Then the solution was transfered to a 100 ml measuring cylinder and 1.512 g of PIPES buffer was added to the formaldehyde with more NaOH if the PIPES refused to dissolve. Finally, the pH was adjusted to 7.0, 10 ml of 25% glutaraldehyde was added and the solution made up to 100 ml with distilled water.

III) Preservation of dried Calothrix isolates

Calothrix D794, D795 and D796 from 1986 samples and Calothrix D660 from 1982 samples were stored in dry condition which could be used in future studies and could be compared the samples of different times. Calothrix isolates were grown in Chu 10D-N with different substates (cotton wool, cotton thread, piece of filter paper and piece of writing paper) and at 32°C for 7 d and each type of substrates with algal growth were dried in incubator at 32°C for 2-3 d. These dried substrates were put inside the specimen vial and stored in dark at 15°C. These Calothrix isolates have an ability to survive after being dried and stored one year.

2.8 MORPHOLOGY AND TAXONOMY

2.81 Microscopy and photomicrography

Samples for microscopy were mounted in a drop of medium, teased apart with a needle and covered with cover slip (Chance 1.5 mm) for examination under light microscope (Nikon Fluophot) fitted with Nikon (M-350) cameras. Kodak 2415 (50 ASA) film was used for black & white pictures and Kodak Ektochrome professional(50 ASA or Fuji HK 100 ASA) for colour prints.

2.82 Scoring of morphological characters

The following criteria were used to differentiate different features of the alga.

Hormogonium production = clear differentiation of hormogonium at the tapered upper portion of the filament.

Basal = few cells next to the heterocysts

Apical = few cells below terminal cells

\+ = presence

All experiments were conducted in the shaking tank at 32°C for morphological studies. Samples were usually taken from the surface of the liquid medium with a wire loop after 1-5 d growth. Each value is the mean of 50 filaments.

2.83 Statistical analysis (influence of light on gas vacuole formation)

The flask was lightly shaken and sample was mounted on the slide with a micro-pipette and the coverslip gently placed on without pressure. In each spot, four different types of filaments and two types of hormogonia, (each and every organism in that spot) were counted. Then the slide was moved to another spot and the counting process was repeated until total no. of filaments = 500 and total hormogonia = 250. Some times more slides were prepared to make the required count, if necessary.

The following criteria were used to describe different types of filament present in the sample:

I) non-hormogonial filaments (with gas-vacuole formation at tapered portion but no clear differentiation of hormogonia)

II) Non-hormogonial filaments (without gas vacuole at the tapered portion and no clear differentiation of hormogonia)

III) Hormogonial (with clear differentiation of gas-vacuolate

hormogonia but still inside the parent sheath)

IV) Hormogonial (with clear differentiation of non gas-vacuolate hormogonia but still inside the parent sheath)

Released hormogonia (free from parent sheath)

2.84 Taxonomy

Algae were identified with the help of Desikachary, (1959); Starmach, (1966) and Islam and Uddin, (1973).

2.85 Cytological observation

Polyphosphate granules were stained using the method of Ebel et al. (1958), as modified by Fuhs (1973) using fresh material. Staining was carried out in a fume hood. Fresh material was washed in distilled water and then soaked in a 10% solution of $Pb(NO_3)_2$ for 15 min. After thorough washing with distilled water in a series of snap cap bottles, the material was transfered to another bottle with 10% $(NH_4)_2S$ and left for 30 s. It was then washed with distilled water in a series of bottles at least four times. Polyphosphate granules stained dark-brown to black when present. The extent of polyphosphate granulation was expressed in terms of estimated percentage of the cell profile which was stained.

Cyanophycin granules were identified by their characteristic refractive appearance (Fuhs, 1973).

Gas-vacuoles were identified by their irregular indefinite reddish structure under the light microscope (Smith & Peat, 1967; Fogg, 1972).

3 STUDY AREAS AND ALGAL SAMPLES

3.1 STUDY AREAS

Parwanipur, Khumaltar and Kakani rice-fields are all from the central part of Nepal (Fig. 3.1), but located in different regions and altitudes (Table 3.1). Details of the 3 study sites are in 3.1, 3.2 and 3.3. Sites A and B are major Rice Research Centres whilst site C is a terraced rice-field owned by a farmer and these sites could be used for long-term studies. In Nepal, many rice-fields have been replaced by houses and buildings as there was no definite planning for housing until 1985.

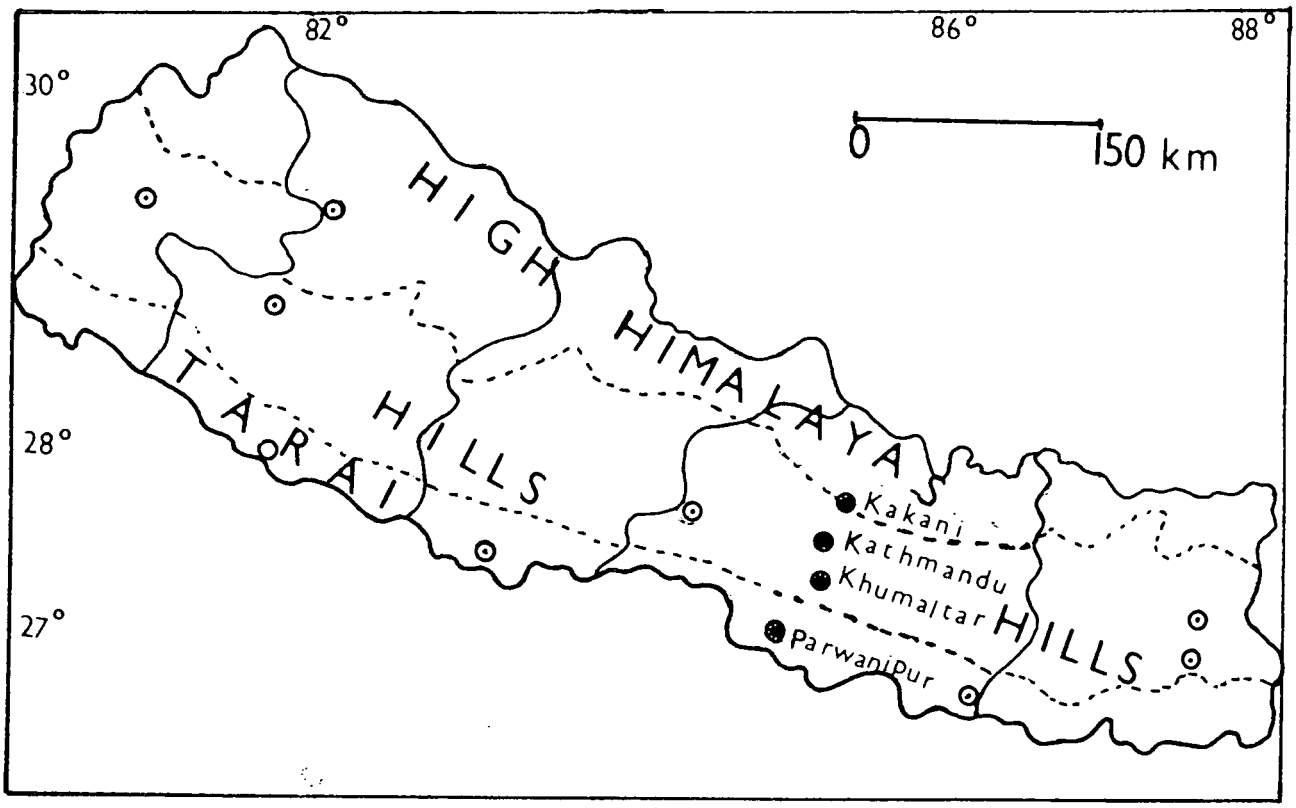


Fig. 3.1 Map of Nepal showing Rice Research Centres and selected sites
⊙ denotes rice research centres
● denotes selected sites

3.2 DESCRIPTION OF SITES

3.21 Parwanipur

Parwanipur is situated in Bara district and falls under the tropical ecological zone. This is the Central Research Station of rice for Tarai region and was established in 1947. National Rice Improvement started in 1972. The monthly average of daily mean temperature ranges from 18°C to 32°C.

The soil is very deep (138-184 cm), yellow-brown to dark-brown and well drained sandy clay-sandy clay loam. It has a slightly acidic to neutral soil reaction, pH (5.65 - 6.7), low organic matter content, very low nitrogen content, medium to high available phosphorus and medium available potash content (Mallick, 1982).

Popular cultivars include Chandani, Luxmi, Janaki, Durga and Sabitri developed at this station. The samples were collected from the field with the cultivar Janaki.

The yield of rice grains per unit area here is low due to warm day and high night temperature, high humidity and low light intensity.

3.22 Khumaltar

Khumaltar lies within the Kathmandu valley in Lalitpur (Patan) district and was established in 1965. It falls under the temperate ecological zone. Major rice research for hills is conducted here. Soil type is clay, silty loam with pH between 5.65-6.7, very low nitrogen content (0.03-0.07%), medium to high available phosphorus (30-80 kg ha^{-1} P) and potassium content (120-200 kg ha^{-1} P) as reported by Deo and Shah (1977). It is also reported that the respon to nitrogen in rice grain yield is higher at Khumaltar than at Parwanipur.

Local cultivars include Pokhareli masinu, Marsi, Thapachinia, Tauli, Rate dhan. The yield per unit area is higher (Table 3.1) in this area due to the

use of improved seeds, use of maximum fertilizer per unit area, better management, ~~has~~ lower night temperature and higher rainfall.

3.23 Kakani

Kakani lies 27 km North West of Kathmandu City in the high hill area (mountain) of Nepal overlooking the North Western Himalayan ranges. Soil is sandy/sandy loam and unlevelled. Cultivation here is in terrace fields. Upland cold-tolerance rice varieties are grown here. The selected site was one of the farmer's fields, so the information about the cultural practice is based on an interview with the land owner. Organic fertilizer (animal dung) is applied before planting and and urea granules added on the day of transplanting. Weeding was carried out twice. The cultivar, Marshy, was direct seeded in April, transplanted in June and expected period of ripening in october.

Other detailed informations of three sites are in Tables 3.1

Fig. 3.2 Parwanipur

1) general view

2) selected field


1


2

Fig. 3.3 Khumaltar

1) general view

2) selected field


1


2

Fig. 3.4 Kakani

1) general view

2) selected field for sampling

1

2

Table 3.1 Details of sites and cultural practice (-, not recorded)

site	A	B	C
location	Parwanipur	Khumaltar	Kakani
region	plain	mid-hill	high-hill
altitude (m)	100	1350	2065
annual rainfall (mm)	1293	1295	1495
rice area (ha)	82	7.75	-
date of visit	28.8.86	11.9.86	18.9.86
air temperature	32°C	25°C	26°C
weather	raining	raining	sunny
mean max. temp. (°C)	32.0	25.0	-
mean min. temp. (°C)	26.0	18.0	-
soil type	clay, loam	silty, loam	sandy
water management	rainfall and river channel	rainfall	rainfall
fertilizer used N:P:K ha^{-1}	80:30:30	80:30:20	animal dung and urea
method of planting	transplanting	transplanting	direct seeding
total yield, $t_o ha^{-1}$	2.4	3.1	2.25
crops yr^{-1}	3	2	2
crops rotation	rice + rice + wheat.	rice + lentil	rice + wheat

3.3 SAMPLING PROGRAMME

Visits were made to the selected sites between August and September, 1986. Site B was also visited in 1982 (H. M.Vaidya) and 1983 (B. A. Whitton). The location of sampling field at sites A, B and C are in Figs 3.2, 3.3 and 3.4. During the visit, in 1986, most of the rice-fields at Parwanipur were muddy, Khumaltar with standing water and Kakani rice-fields were dried but samples were chosen from the rice-fields with standing water in each site and collected from sub-merged, floating and epiphytes to the rice plants. Samples from weeds and soils were also taken. Photographs of Khumaltar (fig.3.3) were taken in 1982 when a part of field was muddy with coverage of algae. Photographs taken at this site in 1986 were not successful.

4 RICE-FIELD ALGAL FLORA OF NEPAL

4.1 GENERAL COMMENT

The algal flora of rice-fields has been studied mainly in paddy soils (see 1.11) and blue-green algae have been reported as the dominant algal flora from different rice-field localities (1.4). A study was planned to record the algal flora from three different rice-field ecosystems (two paddies and one terraced) as seen in the field samples and also in cultures.

Field samples (preserved) were observed under the optical microscope and the algal flora was recorded. Live, soil and dried samples were grown in medium free of combined nitrogen as in 2.7. The algal flora at site B in 1982 and 1983 is listed as presence or absence; that for all three sites (A, B, C) in 1986 is scored on a scale of 1-5 based on subjective estimates as in Tables 4.1 and 4.2. The list of clonal and axenic strains of blue-green algae from Nepal is given in Table 4.3 and details of isolation of strains are given in Appendix 4.

4.2 ALGAL FLORA OF RICE-FIELDS

During the field visit between August and September (1986), blue-green algae were visually the most obvious algae at sites A and B, whereas only green algae were so at site C. However blue-green algae were present in the mixed samples from all three sites. Chroococcus and Synechococcus were present in all sites. Aphanothece, not observed in field material, appeared in culture at all sites. Among heterocystous types, Aulosira and Anabaena were most abundant at sites A and B whereas at site C, Tolypothrix. Calothrix was found in most of the samples from sites A and B, whereas from site C, it was found only in dried sample. Cylindrospermum was present only at site B. Calothrix was absent in 1983 samples. Diatoms were also noted at all sites. Spirogyra, Oedogonium and Ulothrix were the common green algae

at site C.

Out of 25 taxa recorded from field and laboratory samples, 13 were blue-greens, 6 greens and 6 diatoms. The clonal and axenic strains of blue-green algae isolated (see 2.72 and 2.73) are in Table 4.3.

Table 4.1 Algal flora found in field samples (preserved) from sites A, B and C. Abundance is scored on a scale of 1-5 based on subjective estimates (1, rare; 2, occasional; 3, frequent; 4, abundant; 5, very abundant).

Algal flora	Sept 1982	Aug-Sept 1986		
	B	A	B	C
Cyanophyta				
Anabaena >2 ≤4 μm	4	4	5	
Anabaena >4 ≤8 μm		4		1
Aulosira		5	2	
Calothrix I	2			
Calothrix II		1		
Calothrix III			2	
Calothrix IV				1
Chroococcus	1	4	3	3
Cylindrospermum	3		2	
Gloeotrichia	1			
Lyngbya ≤2 μm	4	3	1	
Lyngbya >4 ≤8 μm		1	3	
Nostoc	3	3	3	1

Table 4.1 Contd.

Oscillatoria		4	3	
Phormidium ≥2 µm	1	3	1	
Phormidium >2 ≤4 µm		2	2	1
Plectonema		2	2	
Synechococcus		2	2	1
Tolypothrix >4 ≤8 µm		3	2	4
Tolypothrix >8 ≤12		1		5
Chlorophyta				
Cladophora	2			
Hydrodictyon	3			
Mougeotia	1	3		
Oedogonium	2		3	4
Spirogyra >8 ≤12 µm	2			4
Spirogyra >24 ≤36 µm				5
Ulothrix			2	4
Bacillariophyta				
Cymbella	2			
Diatoma		1	2	2
Navicula	2	1	2	2
Navicula	2			
Synedra		2	2	2
Fragilaria	1			3
Surirella	1	2		

Table 4.2 Algal flora found in cultured samples from site B during September, 1982 and may, 1983 as presence (+) and with relative abundance on a scale of 1-5 based on subjective estimates from sites A, B and C in the samples collected during Aug-sep, 1986 (1, rare; 2, occasional; 3, frequent; 4, abundant; 5, very abundant). The conventions used for coding certain genera within particular ranges of filament width (Whitton et al., 1981).

Algal flora	Sept 1982	May 1983	Aug-sept 1986		
	B	B	A	B	C
Cyanophyceae					
Anabaena >2 ⩽4 µm		+	4	3	2
Anabaena >4 ⩽8 µm	+	+	4	2	2
Aphanothece			2	1	2
Aulosira	+	+	3		
Calothrix I	+				
Calothrix II			3		
Calothrix III				3	
Calothrix IV					1
Chroococcus			2	2	1
Cylindrospermum		+			
Gloeotrichia		+	1	1	
Gloeotrichia			1		
Lyngbya ⩽2 µm	+	+	3	4	
Lyngbya >2 ⩽4 µm	+	+	4	3	3
Nostoc	+	+	4	3	1
Nostoc	+	+	3	3	
Oscillatoria	+	+	3	2	

Phormidium	+		
Plectonema		1	1
Synechococcus	3		3
Tolypothrix	2		4

The algal flora at sites A and B are similar and dominated by blue-green algae. The algal flora at site C differs from the other two and was dominated by green algae, however, Tolypothrix was abundantly present and it was also the only one of three sites at which Azolla pinnata (Fig. 3.4) was present (1986 visit). The algal flora at site B also similar in 1982, 1983 and 1986 samples, but more green algae were found in 1982 samples (green algae not recorded from 1983 samples). More than one form of Anabaena, Calothrix (I, II, III, IV) and Tolypothrix were observed in the samples.

5 DESCRIPTION OF CALOTHRIX ISOLATES

5.1 GENERAL COMMENT

This chapter contains a brief account of characteristic features of the three Calothrix isolates in culture. Calothrix D794, D795 and D796 were grown in standard medium (see 2.611) and incubated in tank at 110 µmol photon m^{-2} s^{-1} PAR at 32°C. Observations were made on day + 6. A comparison is given in Table 5.1 and morphological dimensions in Table 5.2. Strains differ each other in filament structure, growth form and other morphological features. Growth stages of three strains are given in next chapter (6.2). They resemble each other in having some blue heterocysts. Some of the features are summarized below:

5.2 CALOTHRIX D794

This strain was isolated from floating sample at Parwanipur rice-field. The organism is light-green, grows as small crust-like colonies on the bottom of the flask and forms a thin mat on the surface. It has the slowest growth rate of the three strains (based on visual observation). The trichome is tapered with a spherical basal heterocyst and firm sheath (Fig. 5.1 no. 1). The basal vegetative cells are longer than broad and slightly constricted at the cross walls. The apical cell is narrow or conical. Some intercalary heterocysts are present in all strains.

5.3 CALOTHRIX D795

This strain was isolated from vertically suspended sample from Khumaltar rice-field. The organism is dark-brown in colour, grows very fast and forms a thick brown mat on the surface. The trichomes are tapered with spherical and hemispherical basal heterocysts and firm sheath Fig. 5.1 no. 2). Basal

vegetative cells are broader than long and constricted and apical cell is blunt. This strain has highly gas-vacuolated hormogonia.

5.4 CALOTHRIX D796

This strain was isolated from dried piece of rice leaf from Kakani rice-field. The organism is blue-green in colour and grows very slowly. The trichomes are tapered with spherical or conical basal heterocysts and have firm sheath (Fig. 5.1 no. 3). The few basal cells are usually swollen and broader than long. The sheath in this strain extends well below the heterocyst. The hormogonium is non-gas-vacuolated and highly motile. The newly released hormogonia remain attached to the parent material (inoculum) at the beginning forming frilled structures through out the culture flask. Later, they cover the bottom of the flask and then form the thin mat on the surface.

Fig. 5.1 Optical micrographs of 3 *Calothrix* strains grown in Chu 10D-N medium showing mature filaments

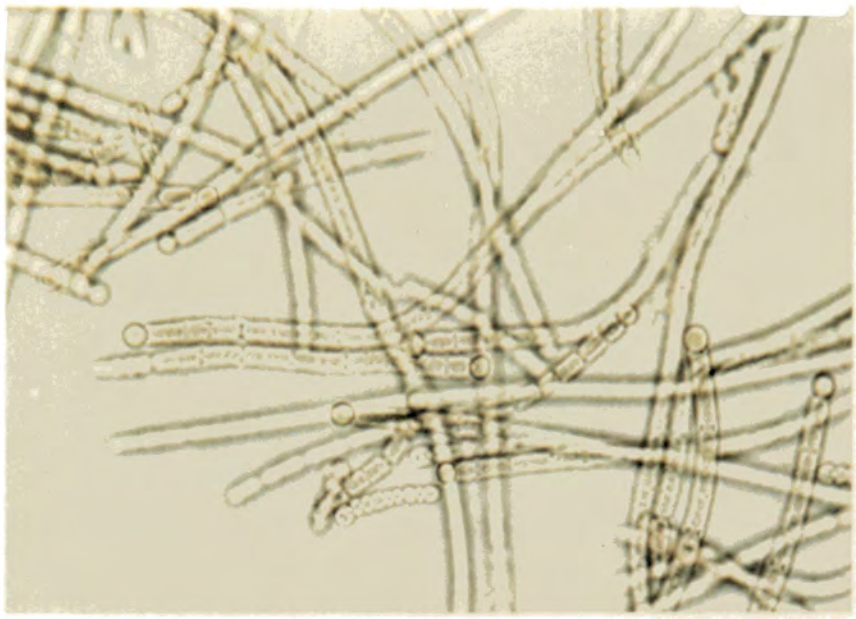
1) *Calothrix* D794

2) *Calothrix* D795

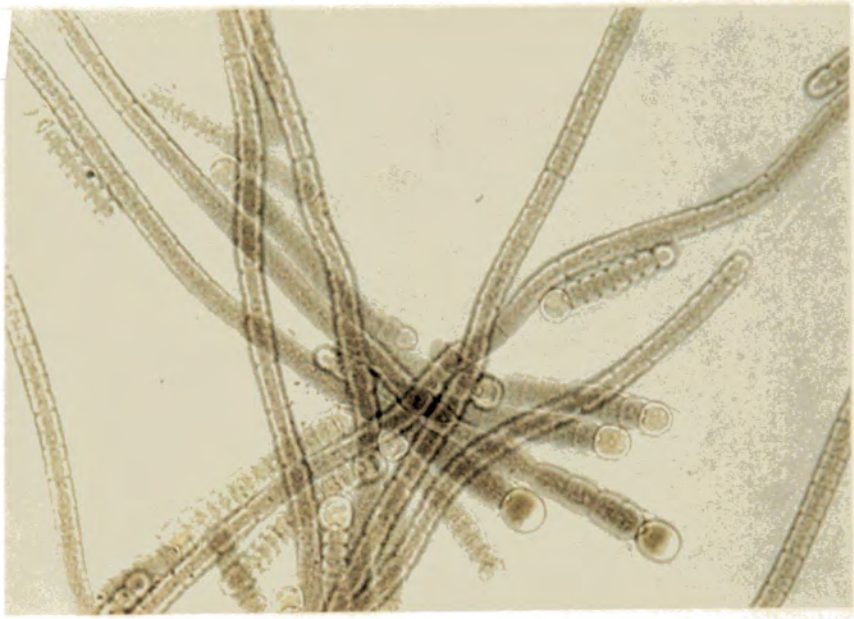
3) *Calothrix* D796

Scale bar = 10 μm

1



2



3

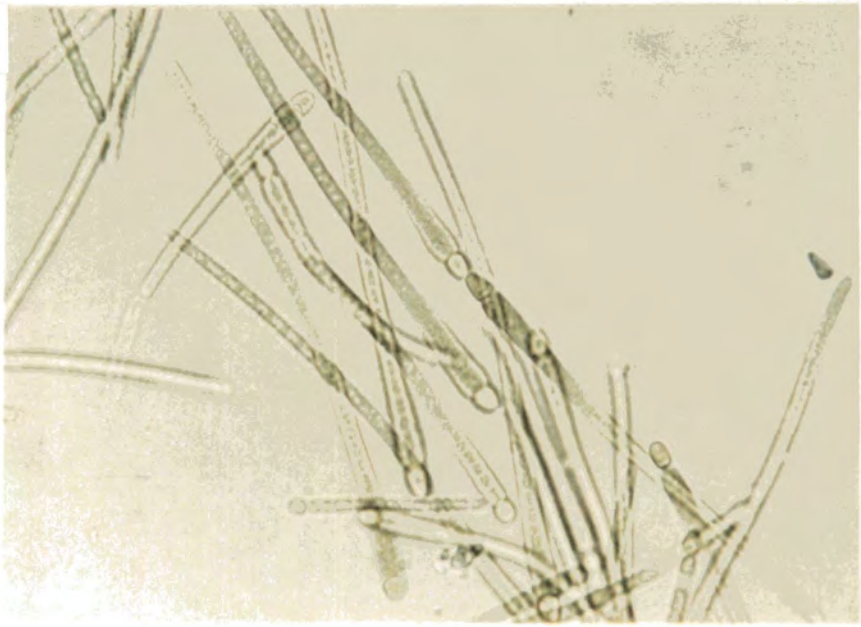


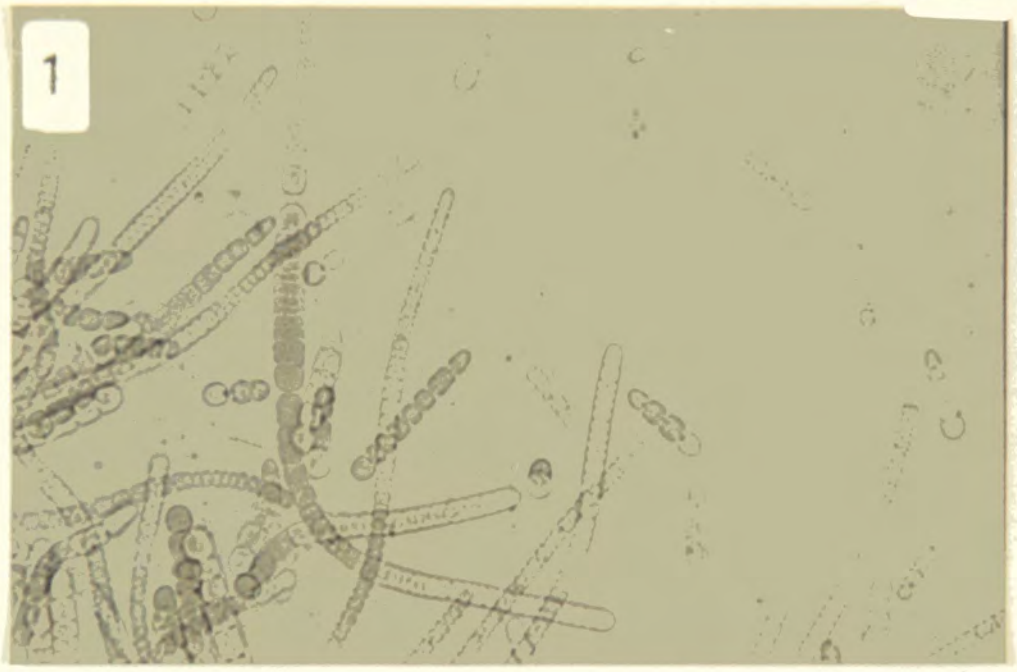
Fig. 5.2 Optical micrograph of 3 *Calothrix* showing hormogonia

1) *Calothrix* D794

2) *Calothrix* D795

3) *Calothrix* D796

Scale bar = 10 μmol


1


2


3

Table 5.1 Comparision of morphological characters of *Calothrix* D794, D795 and D796 in standard medium

Character	*Calothrix* D794	*Calothrix* D795	*Calothrix* D796
colour	light-green	dark-brown	blue-green
growth score	+	+++	++
filament structure	bent or straight	bent or straight	curved or straight
apical cell	narrow, conical	blunt	elongated
basal cell	not swollen, longer than broad	not swollen, broader than long	swollen
sheath	firm and close to the trichome, enclosed heterocyst partially	firm not close to the trichome, enclosed heterocyst partially	firm and close to the trichome, enclosed heterocyst well below
heterocyst	spherical	hemispherical,	conical, hemispherical or cylindrical
intercalary heterocyst	present	present	not
cell content	light green	reddish-brown	blue-green
hormogonium	gas-vacuolate flexible	gas-vacuolate, slow motile	non gas-vacuolate highly motile
granulation	not distinct	distinct	not distinct
hair	not formed	not formed	not formed

Table 5.2 Mean dimensions (μm) of morphological character of Calothrix D794, D795 and D796 in standard medium on day + 6 (n=50)

Calothrix	Trichome	Basal cell		Apical cell		Heterocyst	
	length	length	width	length	width	length	width
D794	375.0	14.0	8.5	10.0	6.5	10.0	10.0
D795	250.0	5.5	10.5	10.5	7.5	10.0	12.5
D796	280.5	6.25	9.8	3.5	3.0	7.5	5.0

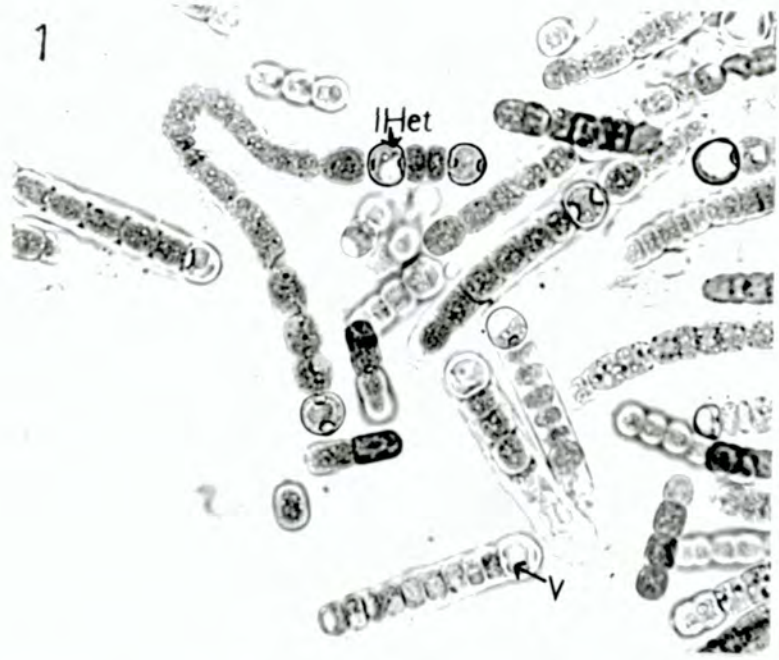
The results given in Table 5.1 are based on observation made on day + 6. On day + 14, most of the filaments in all strains were full of cyanophycin granules. Intercalary heterocysts were obvious in Calothrix D794 and D795 whilst separation discs (necridia) were obvious in Calothrix D796. Intra-thylakoidal vacuoles and false-branches were observed in all three strains but no hair was formed in any of the strains. The culture medium changedinto light-brown colour in three weeks old cultures of Calothrix D794, dark-brown in Calothrix D795 and no changes was observed in Calothrix D796. 12 wk old cultures from stock solution were observed. A thick sheath and false-branches were obvious in all three strains. Fragments of filaments with intercalary heterocyst, intra-thylakoidal vacuoles in vegetative cells and in heterocysts were observed in Calothrix D795 (Fig. 5.3). A number of collapsed secondary heterocysts and few twisted filaments (less than 1%) were observed in Calothrix D796 (Fig. 5.4).

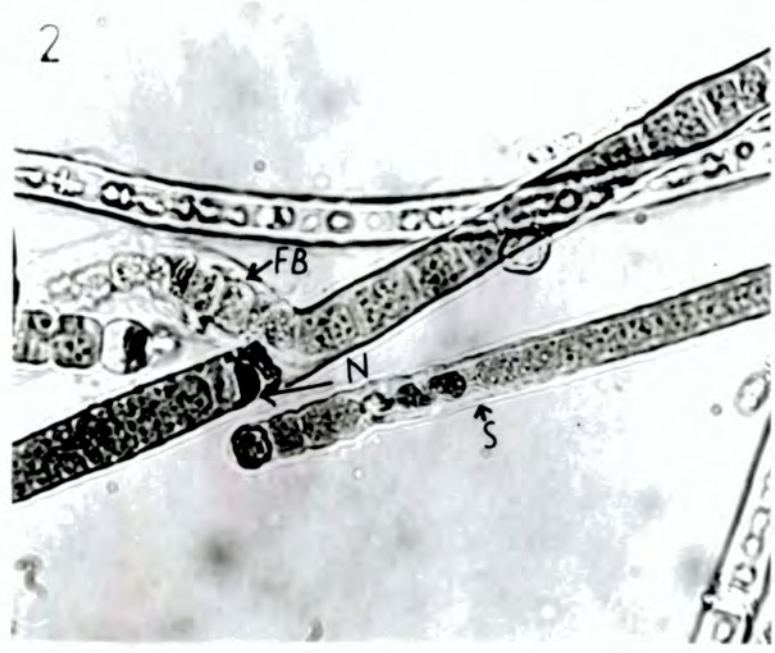
Fig. 5.3 Optical micrographs of Calothrix D794 and D795 in stock culture showing intra-thylakoidal vacuole, false-brances and thick sheath

1-2) Calothrix D795 shows intercalary heterocyst (I Het), false-branch (FB), sheath (S), necridium (N), Vacuole (V)

3) Calothrix D794 shows intercalary heterocyst (I Het), empty sheath (S)

Scale bar = 10 µmol


1
IHet
V


2
FB
N
S


3
ES
IHet

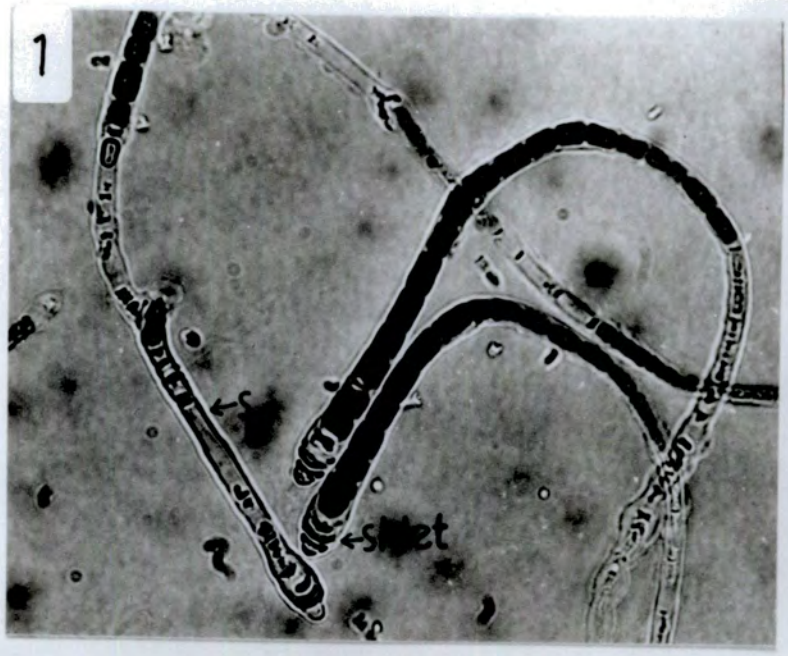
Fig. 5.4 Optical micrograph of Calothrix D796 in stock culture showing

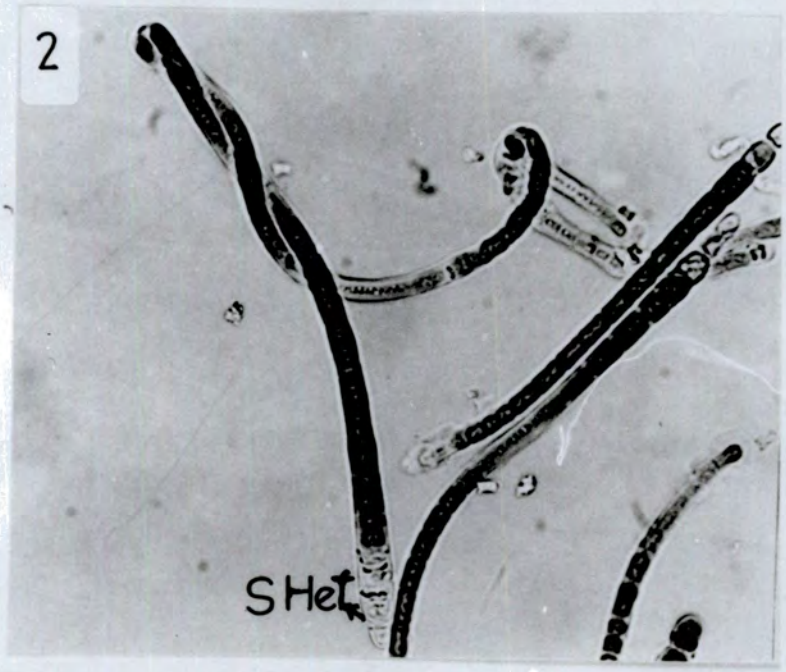
1) collapsed secondary heterocyst (S Het)

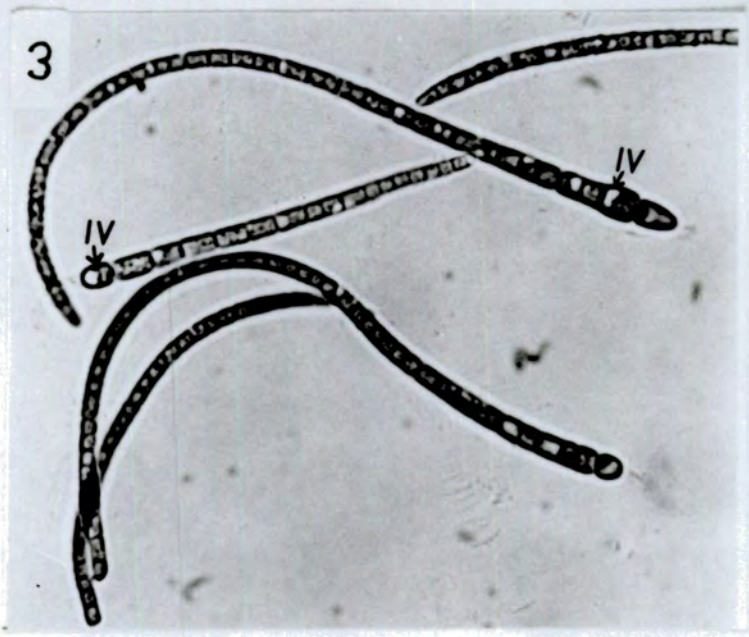
2) twisting of filaments

3) intra-thylakoidal vacuole (IV), sheath (S)

Scale bar = 10 μm


1


2
SHet


3
IV
IV

6 INFLUENCE OF PHOSPHORUS DEFICIENCY ON MORPHOLOGY OF CALOTHRIX STRAINS D794, D795 and D796

6.1 GENERAL COMMENT

The concentration of cellular phosphorus appears to be a key factors influencing morphology in the Rivulariaceae family (1.52). A comparison was therefore made of the effects of phosphorus deficiency on morphology of the three Calothrix strains and to find out whether this led to hair formation.

Axenic strains were grown in Chu 10D-N with low phosphorus concentration (0.1 mg l^{-1} P) and in standard medium (1.78 mg l^{-1} P). In low phosphorus medium, KCl was used as the complementary ion (2.314). The concentration of iron was increased (2 mg l^{-1}) in order to avoid interaction between Fe- and P-limitation. Morphological changes were observed over time. Additional observations were made on the behaviour of hormogonia by conducting a short-term experiment with 0.1 mg l^{-1} P. Results are given in Table 6.1-6.6. Cytological observations were made as described in 2.814. Changes in polyphosphate bodies and hormogonial release in strains after addition of phosphorus (10 mg l^{-1} P) to P-limited cultures are given in Tables 6.7-6.9.

Morphological changes were followed in one set of cultures without any further addition of phosphate and in another set, 10 mg l^{-1} P was added to P-limited cultures of each strain. The results are described below.

6.2 Growth stages (fig. 6.1)

6.21 Stage I

Hormogonia production was highest between 24-48 h of growth in 1.78 mg l^{-1} P whilst production was lower in 0.1 mg l^{-1} P medium. Hormogonia in all strains were released without a mucilaginous sheath (fig. 6.1) and began gliding as soon as they were released. The length of hormogonia was extremely variable (Table 6.13).

Calothrix D794

Calothrix D794

Length of hormogonium = 17.0 to 300 µm

Width " " = 3.0 to 7.5 µm

Hormogonia lacked distinct granulation and were motile and flexible. The percentage of released gas-vacuolated hormogonia was less than 1% in 0.1 mg l^{-1} P medium whilst 15% of gas-vacuolate hormogonia were released in 1.78 mg l^{-1} P medium after 24 h incubation. No observation was made before 24 h.

Calothrix D795

Length of hormogonium = 15.0 to 250.0 µm

Width " " = 5.0 to 7.5 µm

Hormogonia were highly gas-vacuolated (Fig. 6.3), contained cyanophycin and polyphosphate granules and glided slowly in both 1.78 mg l^{-1} P and 0.1 mg l^{-1} P. A sheath was formed soon after release. The hormogonia lost their gas vacuoles within 24 h of their release.

Calothrix **D796**

Length of hormogonium = 37.5 to 200.0 µm

Width " " = 3.5 to 5.0 µm

No gas-vacuoles was found in hormogonia. Cyanophycin granules were not observed. Hormogonia in this strain were highly motile and formed a mucilaginous sheath before differentiation of cells. The sheath was always extended on one end which is typical of this strain (Fig. 6.3).

Fig. 6.1 Growth stages of Calothrix D794, D795 and D796 grown in Chu 10D-N medium

a) hormogonium

b) asymmetrical hormogonium

c) formation of heterocyst

d) young trichome

e) mature trichome

10 µm

D 794

a) 12 µm

b) 12 µm

c) 20 µm

d) 69 µm

e) 160 µm

D 795

a) 10 µm

b) 10 µm

c)

d) 88 µm

e) 115 µm

D 796

a) 28 µm

b) 30 µm

c) 40 µm

d) 23 µm

e) 110 µm

6.22 Stage II

Within 24-48 h, hormogonia in Calothrix D794 and D796 became asymmetrical as apical cells tapered and the basal cell i.e. that towards the parent trichome differentiated into a heterocyst. In Calothrix D795, heterocyst developed without obvious tapering of apical cell. The heterocyst differentiation in D796 was very clear as the basal cell towards the longer sheath always differentiated into a heterocyst.

Cell division was observed after heterocyst formation and the filament increased in length in all strains. In D795, however, some of the filaments did not seem to elongate and the basal heterocyst developed vacuoles after 3 d of growth in 0.1 mg P in contrast to its behaviour in 1.78 mg l^{-1} P medium.

6.23 Stage III

Most of the filaments in all strains were in a matured state. Polyphosphate granules disappeared distally and cyanophycin granules increased. Filaments became longer in all strains and most of the filaments stopped forming hormogonia in low phosphorus medium whereas in standard medium almost all filaments releasing hormogonia in all strains (Figs 6.5a and 6.5b). Sheaths became thicker as the culture grew older in both media.

Dimensions of trichomes and cells of all strains in both media are given in Tables 6.10 - 6.14.

Filaments of Calothrix D794 got clumped and formed colonies with the heterocysts in the middle and thin long filaments towards the periphery.

Fig. 6.2 Optical micrographs of Calothrix D794 in Chu 10D-N showing morphological changes

1) mature trichome with elongated apical cell (AC)

2) false-branch (FB), sheath (S)

3) loop formation

Scale bar = 10 μm


1
AC


2
FB
S

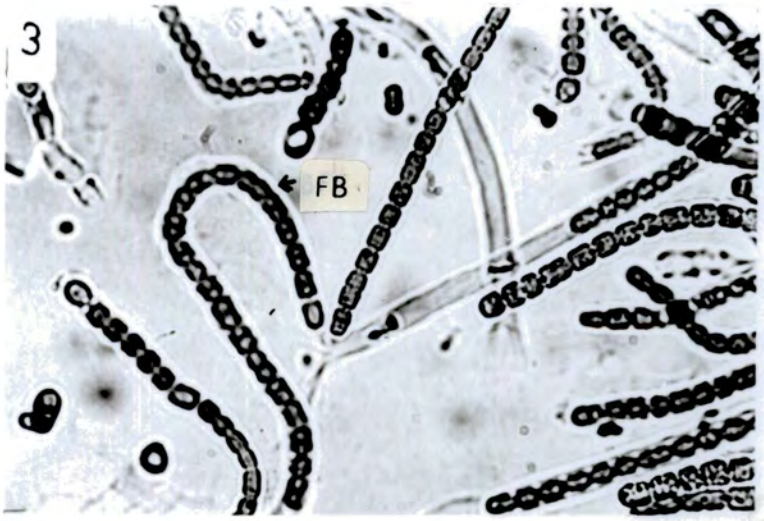

3
FB

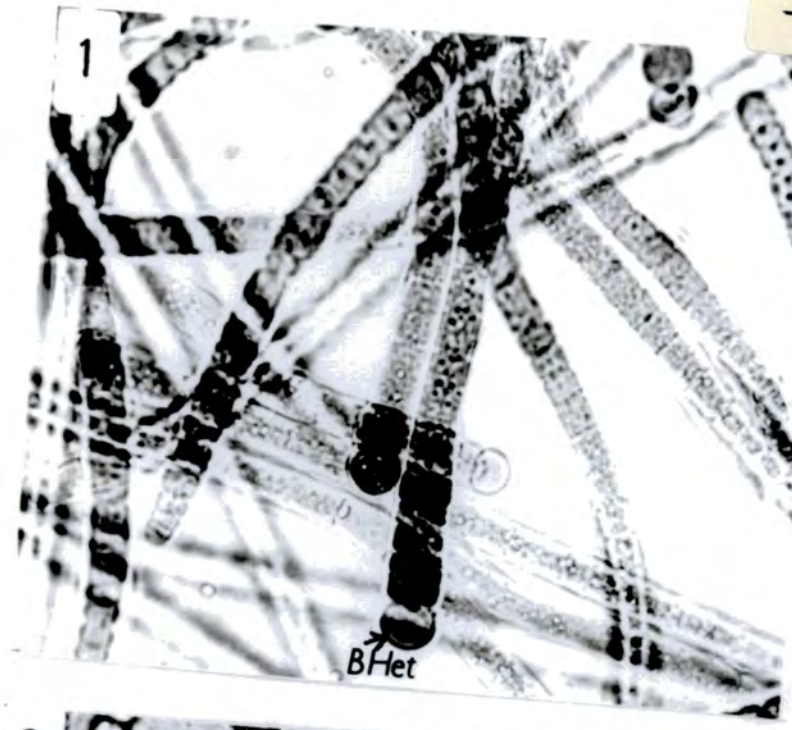
Fig. 6.3 Optical micrographs of Calothrix D795 in Chu 10D-N showing

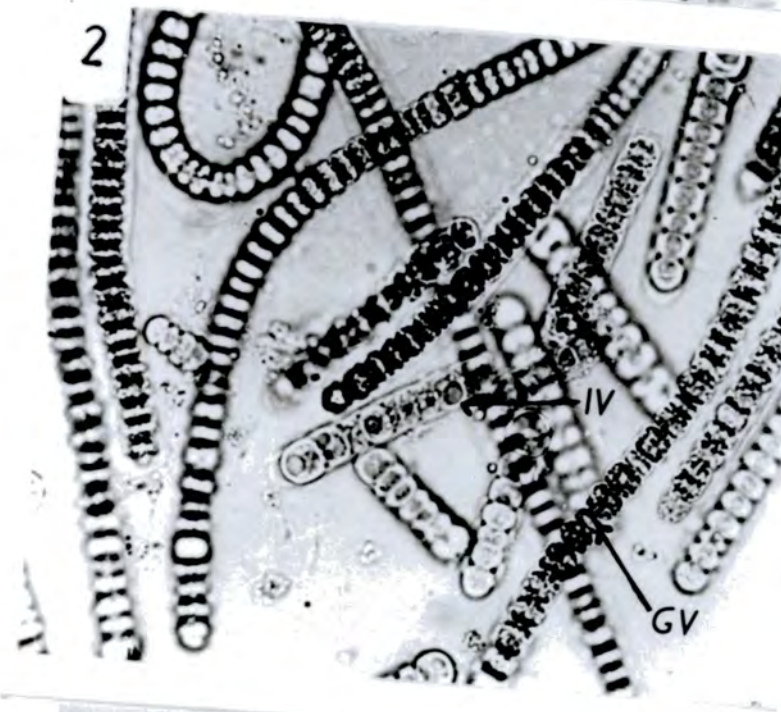
1) mature filaments with blue heterocys (B Het)

2) gas-vacuolated hormogonium (GV)

3) young trichomes

Scale bar = 10 μm


1
BHet


2
IV
GV

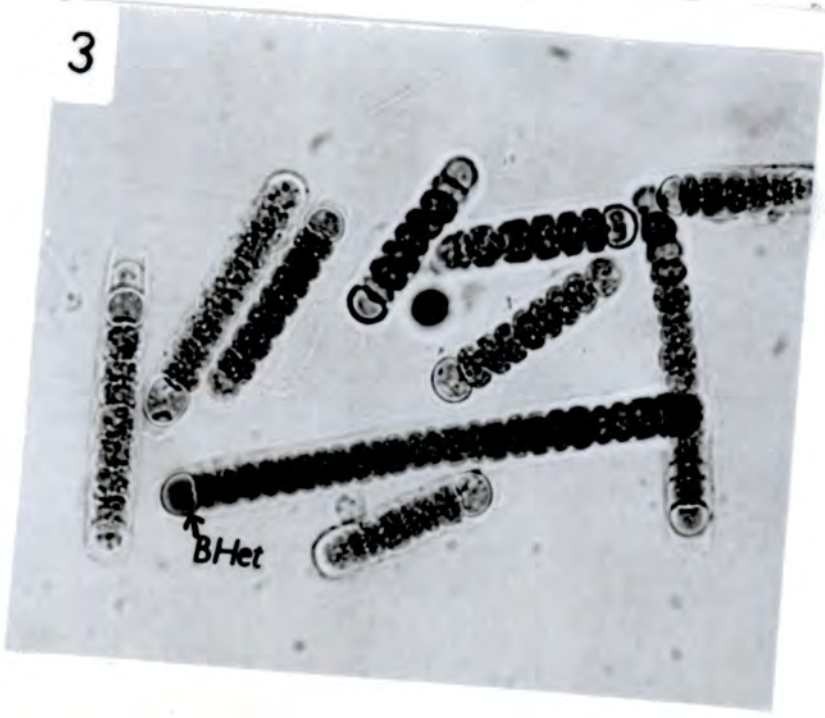

3
BHet

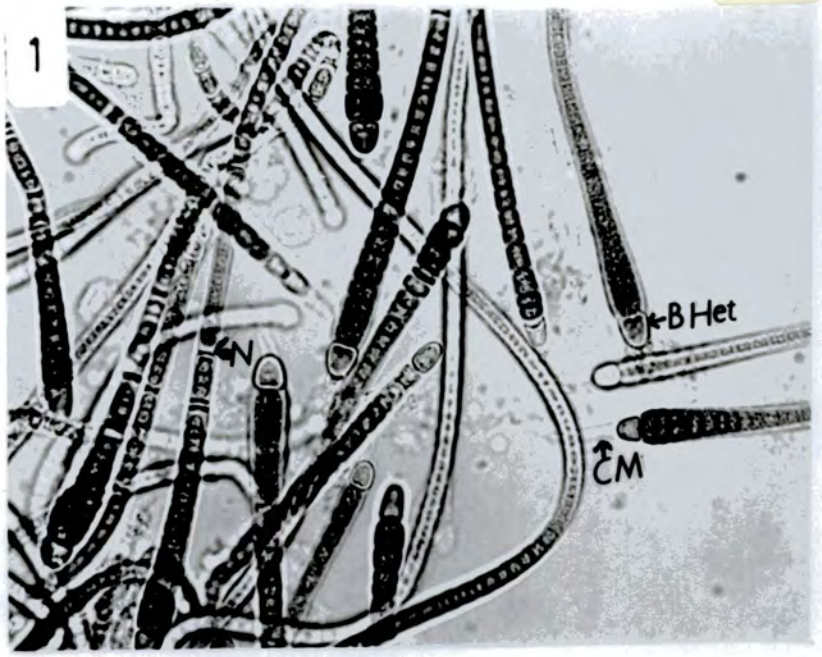
Fig. 6.4 Optical micrographs of Calothrix D796 in Chu 10D-N showing

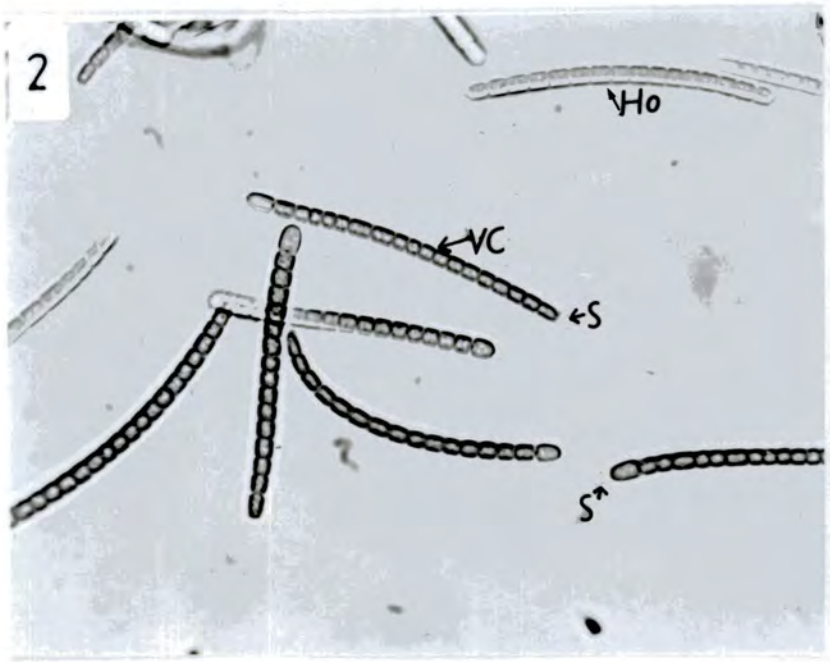
1) filaments with long sheath (S) and separation discs (N)

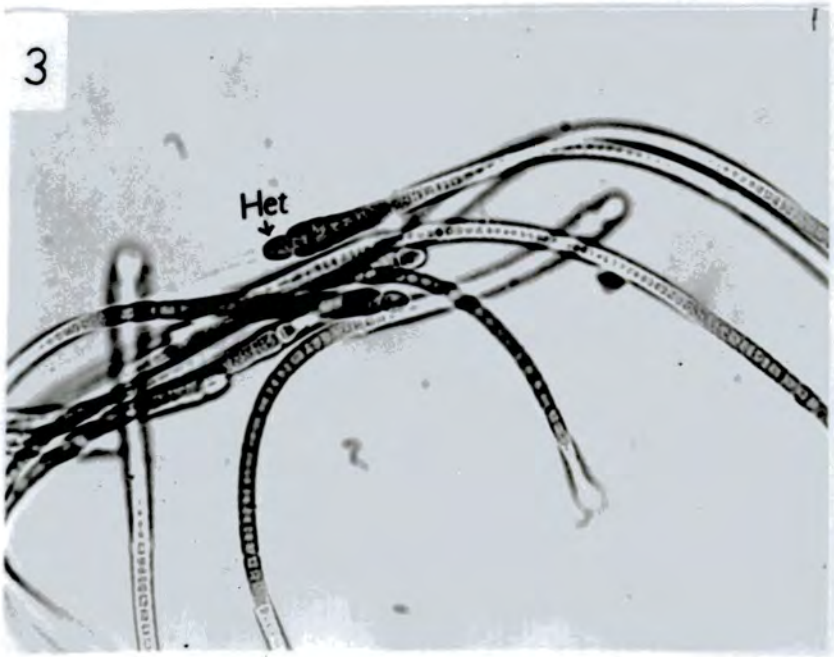
2) hormogonium (Ho), vegetative cell (VC), sheath (S)

3) rope formation of filaments

Scale bar = 10 μm


1
N
B Het
M


2
Ho
VC
S
S


3
Het

Calothrix D795 also formed colonies, however, there was no definite pattern (heterocyst not always at the centre). The filaments in D796 attached each other and formed a rope-like structure as in Fig.6.4 (3).

After stage III, many morphological changes occurred with increase in cyanophycin granules and decrease in polyphosphate granules. At the same time, cross walls between the cells started to disappeared. Other changes were:

a) Separation discs **(necridia)**

The formation of the separation discs was the most prominent morphological change. These biconcave cells (Fig. 6.9) developed in D796 on day + 3 with 0.1 mg l^{-1} P whereas in 1.78 mg l^{-1} medium, these did not appear until 7 d. The separation discs were brighter blue in colour at the beginning, later they became colourless (lysis) where false-branches occurred. Separation discs in D795 & D794 in 0.1 mg l^{-1} P medium developed after 8 d of incubation.

b) Vacuole formation

Vacuole formation in cells occurred in all three strains as they grew older, but earlier in D795 than other two strains. Vacuoles were very obvious on the upper portion of the filament mostly started from the apical cells (Fig. 6.8). These were formed in the heterocysts of some of the trichomes of D795 just after 3 d incubation in 0.1 mg l^{-1} P medium.

c) Hairs

No hairs were formed in any strains in either medium.

6.24 Stage IV

Interestingly on day + 3, the strains in 0.1 mg l^{-1} medium accumulated polyphosphate granules (confirmed by staining) without addition of phosphorus to the cultures. After 4 d, the level of polyphosphate granules disappeared distally and finally became absent whereas in standard medium, on the other

hand, the polyphosphate granules became absent only on 8 d. These cultures were confirmed to be P-limited by staining.

6.241 Changes in morphology of strains without addition of phosphorus to P-limited cultures:

There were slight reductions in basal and apical dimensions of Calothrix D794 & D795 whereas a few basal cells in Calothrix D796 were swollen in 0.1 mg l^{-1} P compared to the cultures in standard medium. The apical cell in Calothrix D794 and D796 was elongated and enlarged, no changes were observed in apical cells of D795. False-branches were very obvious in Calothrix D796 and higher in percentage in comparison to other two. Almost all filaments were with false-branches at the upper portion after 13 d of incubation in 0.1 mg l^{-1} P. Repeated false-branches were observed in Calothrix D794 and D796 (Fig. 6.2 and 6.8). The false-branches had a declining tendency in diameter every time ~~it~~ they produced branchlets. Both a single and a double false-branch were observed in Calothrix D794 and D795. After 15 d all cultures were yellowish to colourless.

At this point, 20% of filament in D794, 6% in D795 and 40% in D796 were survived in 0.1 mg l^{-1} P medium whereas in 1.78 mg l^{-1} P, the cultures were growing. After 18 d incubation, the culture medium in D794 and D796 became slimy (gelatinous) whereas the culture medium in D795 became brown colour in the culture flask.

Fig. 6.5 Comparative filament morphology of Calothrix D794, D795 and D796

a) hormogonium production in Chu 10D-N medium

b) cyanophycin granules in 0.1 mg l^{-1} P medium

Hormogonium (Ho). Cyanophycin granules (Cy)

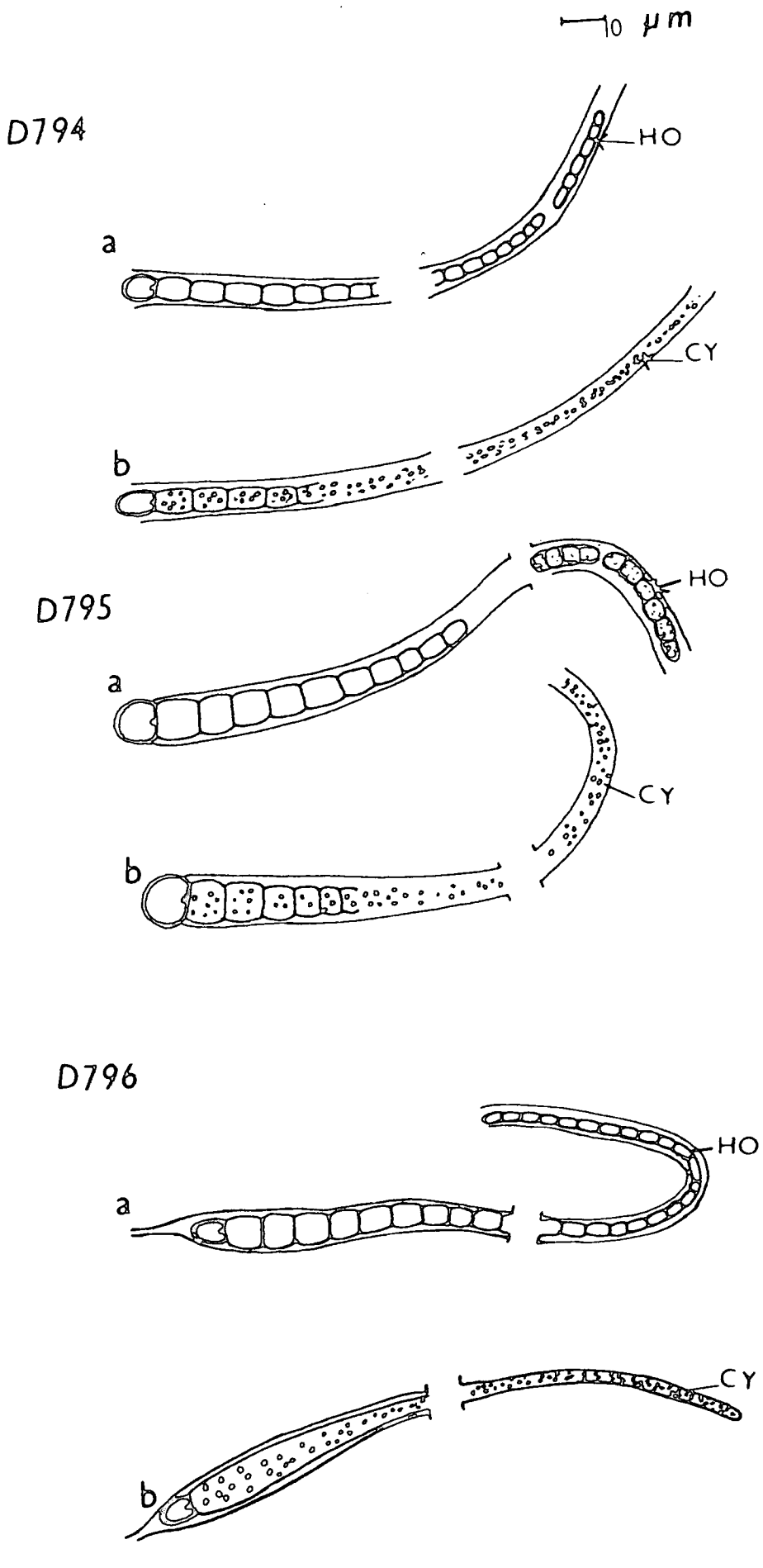

10 μm
D794
HO
a
CY
b
D795
HO
a
CY
b
D796
HO
a
CY
b

Fig. 6.6 Calothrix D794 in 0.1 mg l^{-1} mg P medium

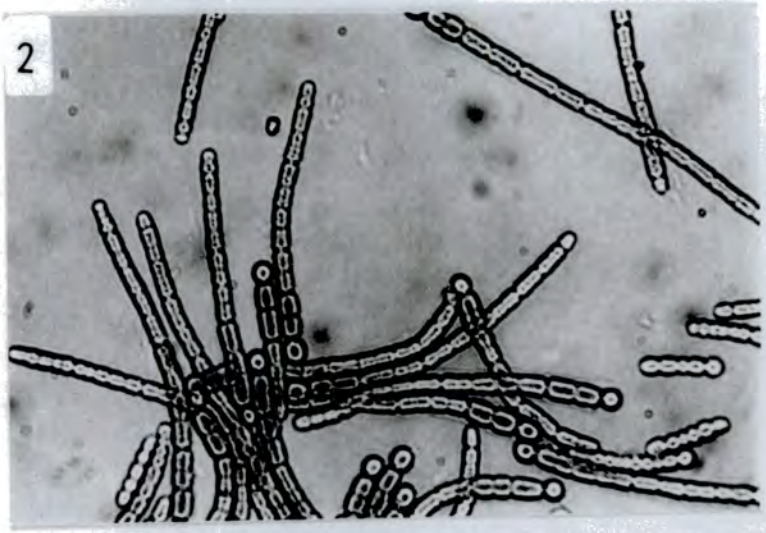
1) rope formation of hormogonia

2) young trichome

3) clump colony

4) clump colony (x 10)

Scale bar = 10 μm



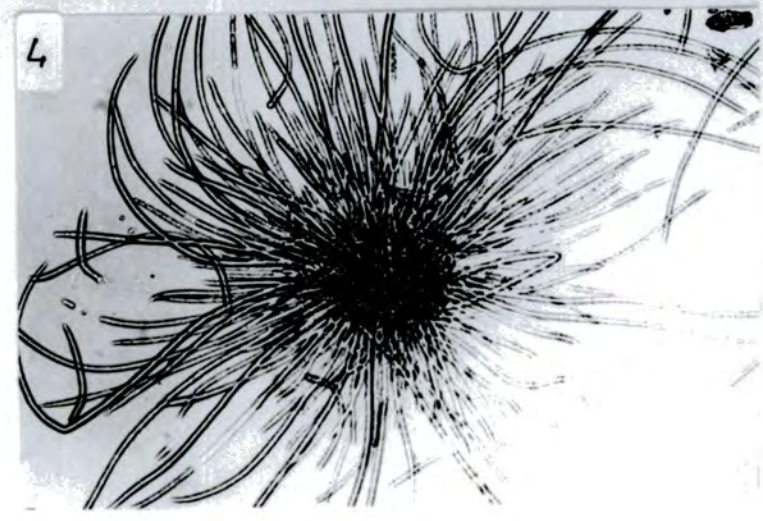


Fig. 6.7 Calothrix D794, D795 and D796 showing cyanophycin granules separation discs and false-branches.

a-b) Calothrix D794

c-d) Calothrix D795

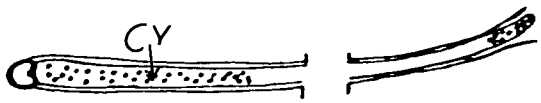
e-i) Calothrix D796

False-branch (FB). Cyanophycin granules (Cy). Necridium (N) Intra-thylakoidal vacuole (IV). Intercalary heterocyst (IHet)

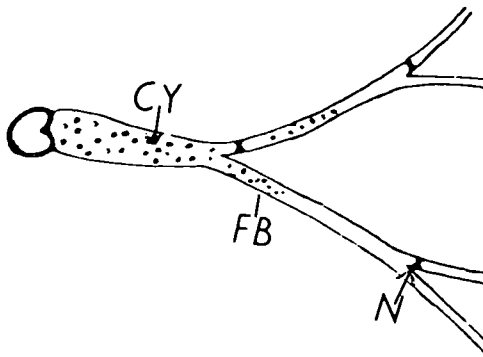
10 µm

D794

a

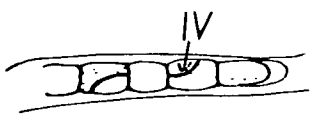


b

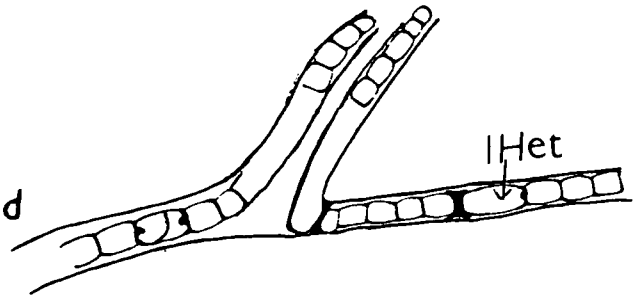


D795

c

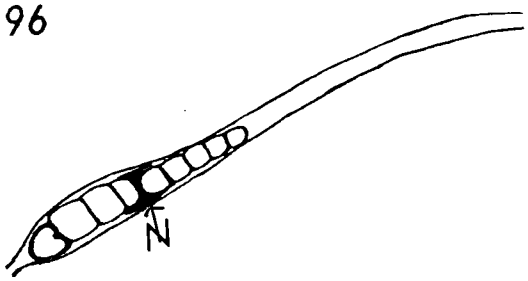


d

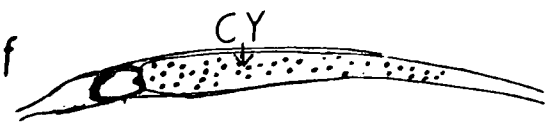


D796

e



f



g

h

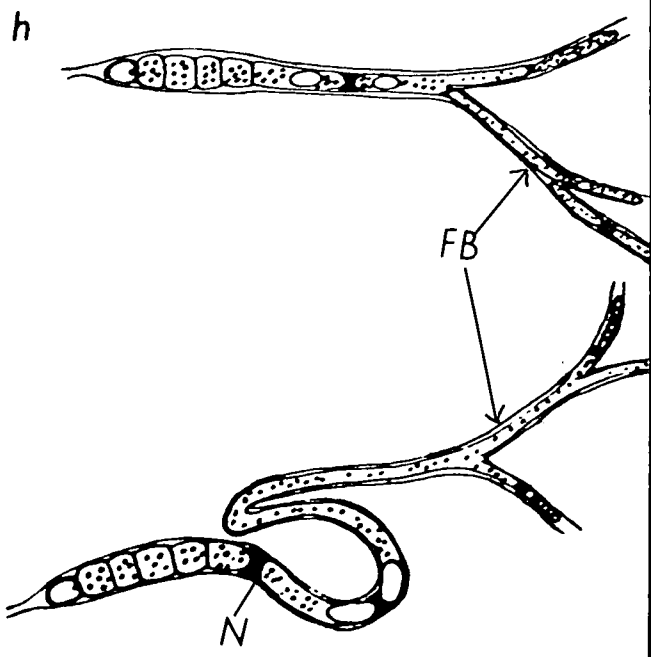


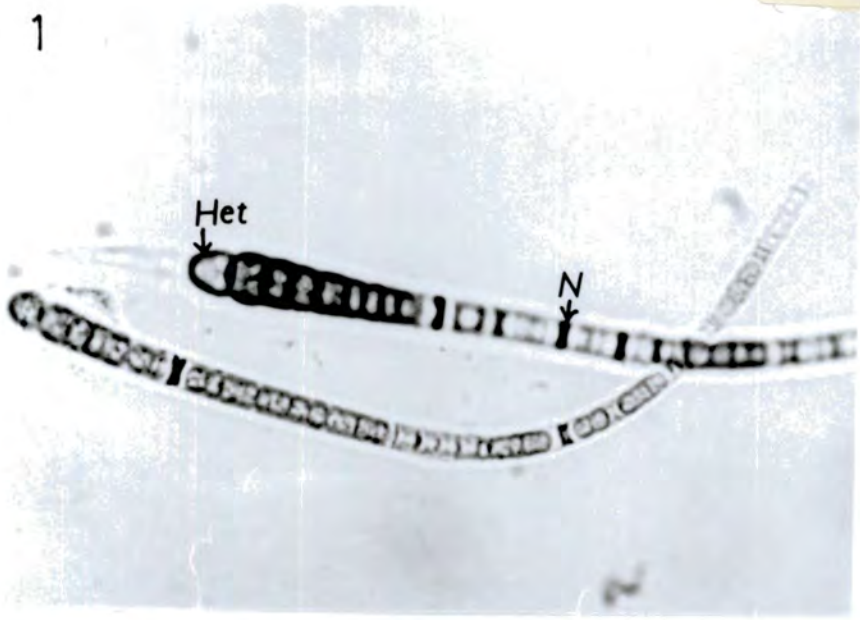
Fig. 6.8 Calothrix D796 with increasing P-deficiency showing

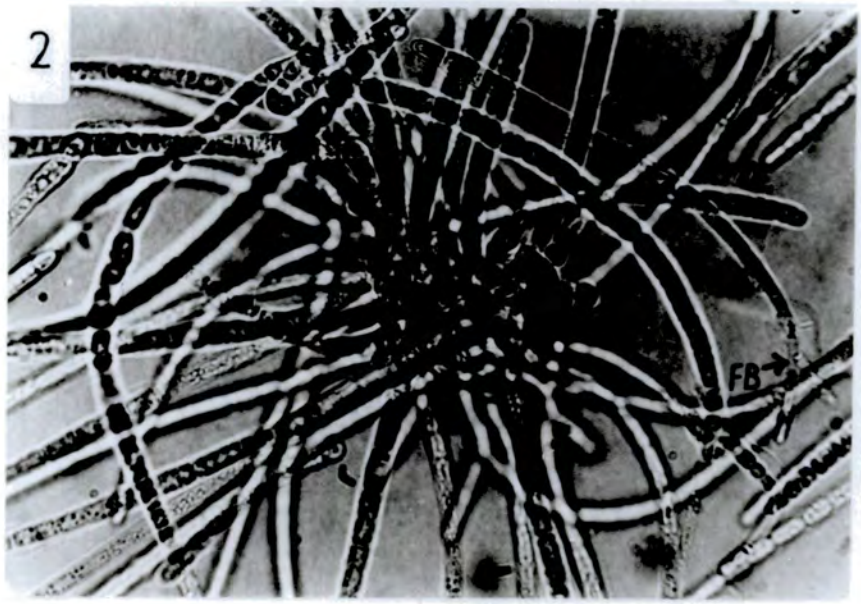
1) trichome with necridium (N), heterocyst (Het)

2) clumped colony with heterocysts in centre (Het)

3) false-branch (FB)

Scale bar = 10 μm


1
Het
N


2
FB


3
FB

5.242 Changes with addition of 10 mg l^{-1} P to P-limited cultures

10 mg l^{-1} P was added to each P-limited culture (confirmed by staining) and the percentage of polyphosphate granules at different time after the addition of phosphorus, are given in the Table 5.3. A few comments are given below:

There was a marked response to phosphorus addition. All three strains accumulated polyphosphate granules very quickly within 10 min after the addition of phosphorus, but the amount accumulated, the nature of granules and their gradients in the filaments varied between strains. The granules were accumulated centrally in cells in D796 whereas no definite gradients were observed in the other two strains (Figs 6.10, 6.11, 6.12). Within 30 min., the granules had increased in size in all strains with the largest granules in basal cells and smallest in apical cells. During the next 18 h 24 h, a massive release of hormogonia occurred in all three strains. The higher the percentage of granules, the greater hormogonial release (Table 6.7, 6.8 and 6.9). The hormogonia in Calothrix D794 and D795 were with gas-vacuoles. The level of polyphosphate granules started to decreased slowly distally and on day + 4, no granules were observed in the apical cells. The other interesting changes were occurrence of blue heterocysts in some filaments of all three strains.

Fig. 6.9 Calothrix D794, D795 and D796 showing accumulation of polyphosphate bodies after addition of phosphate to P-limited cultures

a) trichome prior to phosphate addition

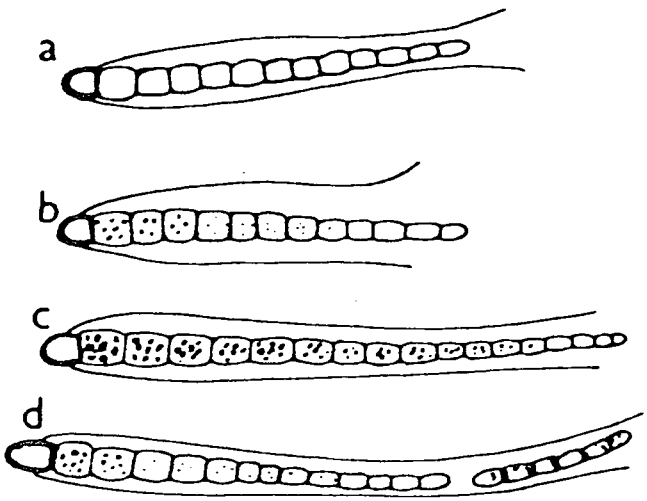
b) trichome 10 min after phosphate addition

c) trichome 1 h after phosphate addition

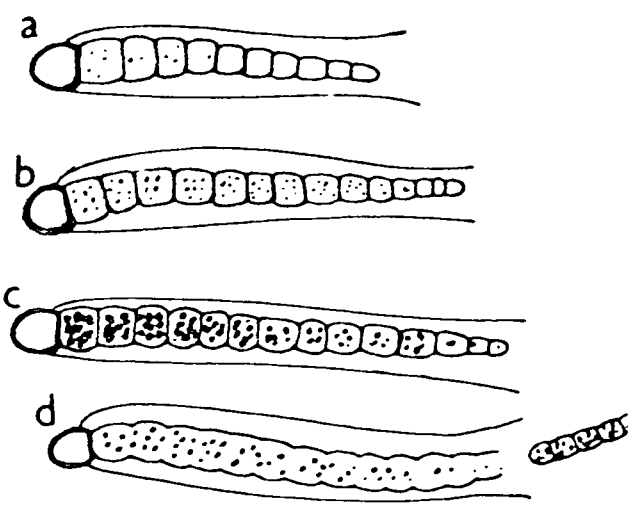
d) trichome 3 d after phosphate addition

10 µm

D 794



D795



D 796

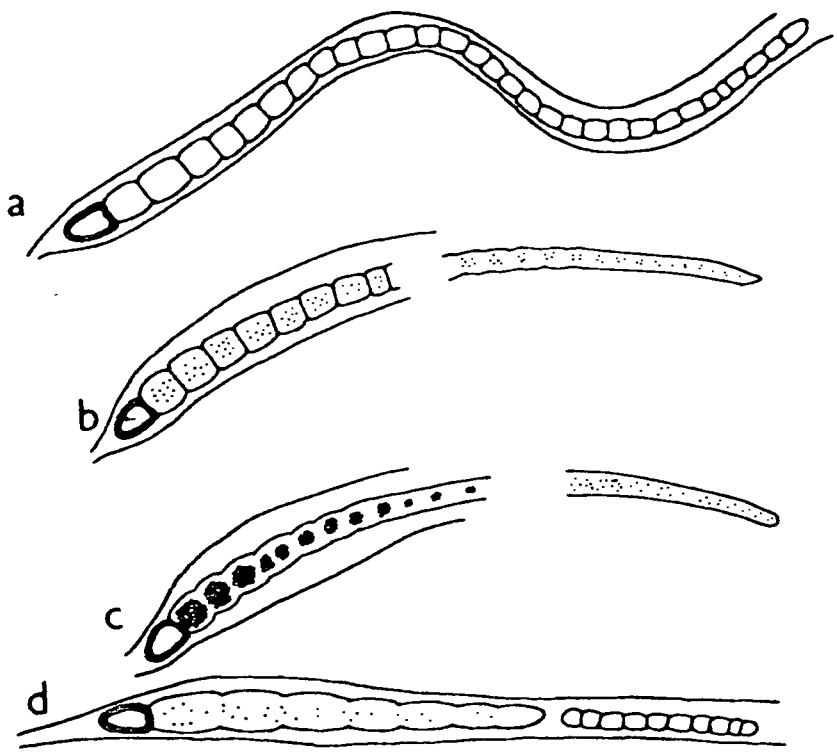


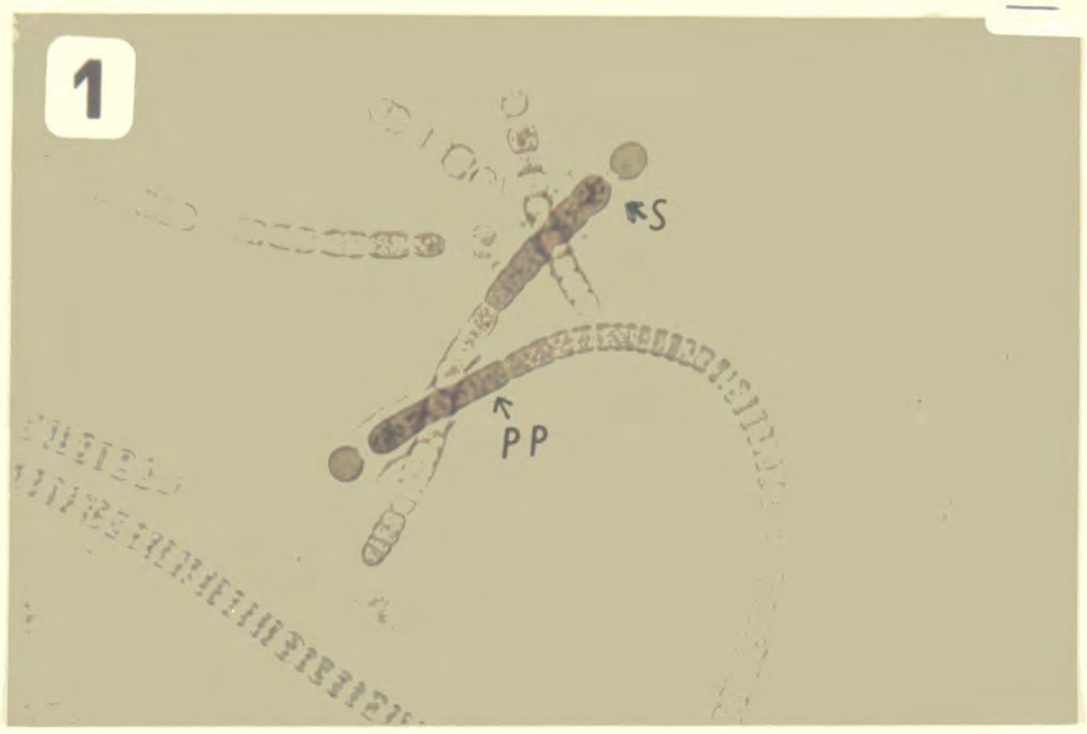
Fig. 6.10 Optical micrograph of Calothrix D794 and D795 after addition of phosphate to P-limited cultures

1) Calothrix D794

2) Calothrix D795

Polyphosphate granules (PP), Sheath (S)

Scale bar = 10 μm


1
S
PP

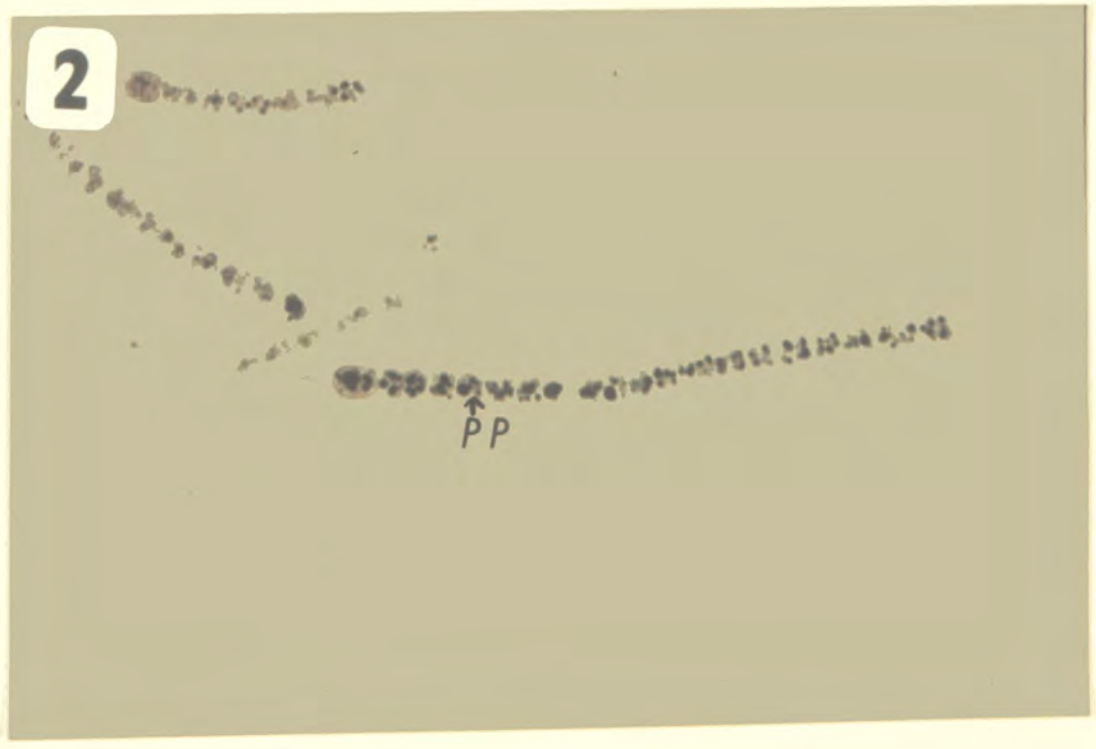

2
PP

Fig. 6.11 Optical micrograph of Calothrix D796 after addition of phosphate to P-limited culture

Polyphosphate granules (PP). Sheath (S)

Scale bar = 10 μm

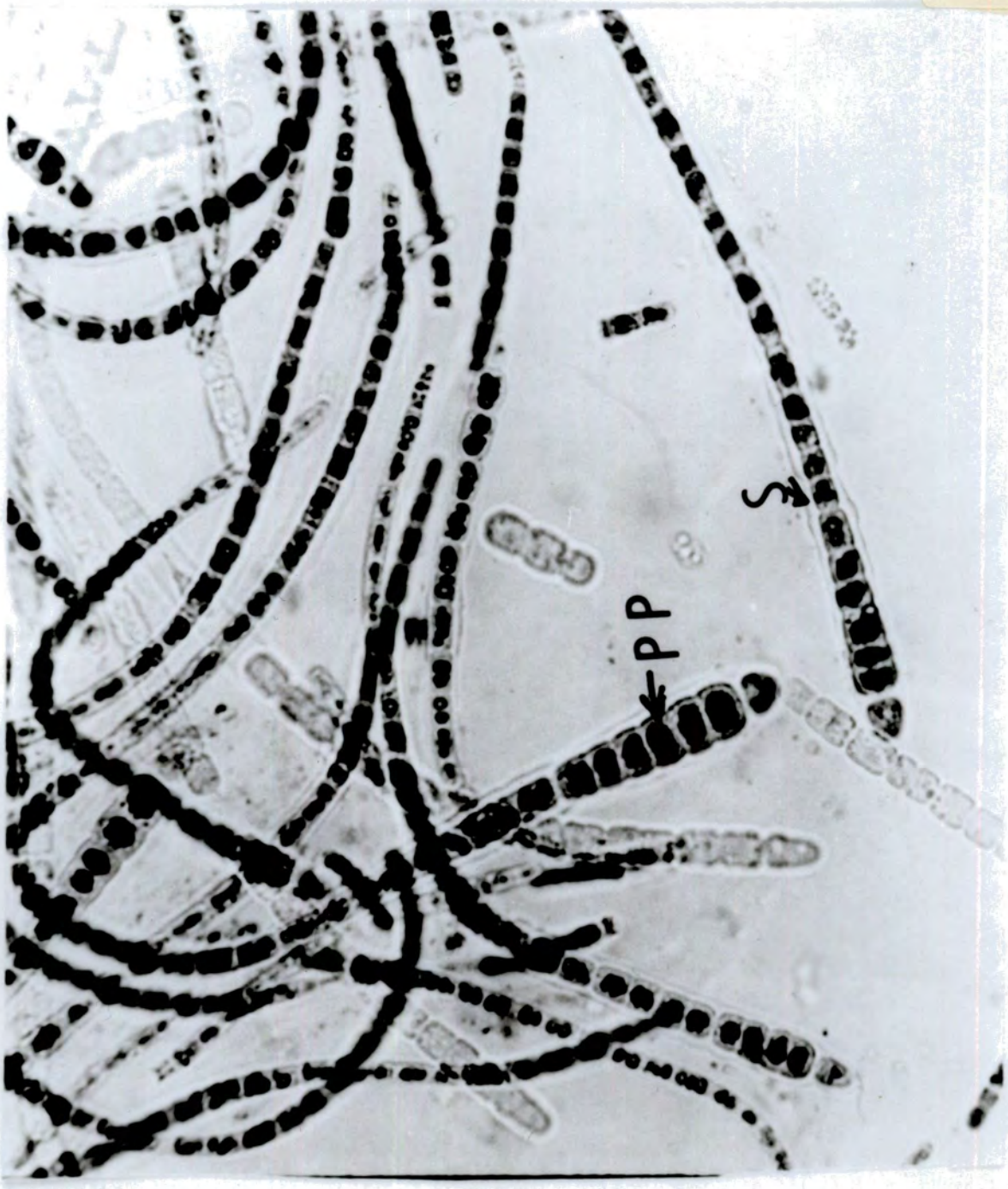

PP

Table 6.1 Morphology of Calothrix D794 during batch culture in 0.1 mg l^{-1} P medium

Key: Bl = blue, Bs = basal, Int = intercalary, +gv = gas-vacuolate, -gv = non gas-vacuolated
A = apical, Sep = separation, F = false, + = presence, - = absence, P = polyphosphate,
C = cyanophycin

Day	Heterocyst			Sheath	Hormogonia		P.Granules		C.granules		Sep.disc	F.branch	Vacuole	Hair
	Bl	Bs	Int		+gv	-gv	Bs	A	Bs	A				
0	+	+	-	+	+	-	-	-	+	+	-	-	-	-
1	+	+	-	+	+	-	+	-	-	-	-	-	-	-
2	+	+	-	+	+	-	+	-	-	-	-	-	-	-
3	-	+	-	+	-	-	+	-	-	+	-	-	-	-
4	-	+	+	+	-	-	+	-	-	+	-	-	-	-
5	-	+	+	+	-	-	+	-	+	+	-	-	-	-
6	-	+	+	+	-	-	-	-	+	+	+	-	-	-
7	-	+	+	+	-	-	-	-	+	+	+	-	-	-
8	-	+	+	+	-	-	-	-	+	+	+	-	-	-
10	-	+	+	+	-	-	-	-	+	+	+	-	-	-
12	-	+	+	+	-	-	-	-	+	+	+	-	+	-
14	-	+	+	+	-	-	-	-	+	+	+	+	+	-

Table 6.2 Morphology of Calothrix D794 during batch culture in Chu 10D-N medium
See Table 6.1 for abbreviations.

Day	Heterocysts			Sheath	Hormogonia		P. granules		C. granules		Sep. disc	F.branch	Vacuoles	Hair
	Bl	Bs	Int		+gv	-gv	Bs	A	Bs	A				
0	+	+	-	+	+	-	+	-	+	+	-	-	-	-
1	+	+	-	+	+	-	+	-	-	-	-	-	-	
2	+	+	-	+	+	-	+	+	-	-	-	-	-	-
3	+	+	-	+	+	-	+	-	-	+	-	-	-	-
4	+	+	-	+	+	-	+	-	+	+	-	-	-	-
5	+	+	-	+	-	-	+	-	+	+	-	-	-	-
6	+	+	+	+	-	-	+	-	+	+	-	-	-	-
7	+	+	+	+	-	-	+	-	+	+	-	-	-	-
8	-	+	+	+	-	-	+	-	+	+	-	-	-	-
10	-	+	+	+	-	-	-	-	+	+	+	-	-	-
12	-	+	+	+	-	-	-	-	+	+	+	-	-	-
14	-	+	+	+	-	-	-	-	+	+	+	+	-	-

Table 6.3 Morphology of Calothrix D795 during batch culture in 0.1 mg l^{-1} P
See Table 6.1 for abbreviations.

Day	Heterocyst			Sheath	Hormogonia		P. granules		C. granules		Sep. disc	F. branch	Vacuoles	Hair
	Bl	Bs	Int		+gv	-gv	Bs	A	Bs	A				
0	+	+	+	+	+	+	+	-	+	+	-	-	-	-
1	-	-	-	+	+	-	+	-	-	-	-	-	-	-
2	+	+	-	+	+	+	+	-	-	+	-	-	+	-
3	+	+	+	+	+	+	+	+	-	+	-	-	+	-
4	+	+	+	+	+	+	+	+	+	+	-	-	+	-
5	+	+	+	+	-	+	+	-	+	+	+	+	+	-
6	+	+	+	+	-	-	+	-	+	+	+	+	+	-
7	-	+	+	+	-	-	-	-	+	+	+	+	+	-
8	-	+	+	+	-	-	-	-	+	+	+	+	+	-
10	-	+	+	+	-	-	-	-	+	+	+	+	+	-
12	-	+	+	+	-	-	-	-	+	+	+	+	+	-
14	-	+	+	+	-	-	-	-	+	+	+	+	+	

Table 6.4 Morphology of Calothrix D795 during batch culture in Chu 10D-N medium
See Table 6.1 for abbreviations.

Day	Heterocyst			Sheath	Hormogonia		P. granules		C. granules		Sep. disc	F. branch	Vacuoles	Hair
	Bl	Bs	Int		+gv	-gv	Bs	A	Bs	A				
0	+	+	-	+	+	+	+	-	+	+	-	-	-	-
1	-	-	-	+	+	-	+	+	-	-	-	-	-	-
2	+	+	-	+	+	+	+	+	-	-	-	-	-	-
3	+	+	-	+	+	+	+	+	-	-	-	-	-	-
4	+	+	+	+	+	+	+	-	+	+	-	-	-	-
5	+	+	+	+	+	+	+	-	+	+	+	-	-	-
6	+	+	+	+	+	+	+	-	+	+	+	-	-	-
7	+	+	+	+	-	+	-	-	+	+	+	-	-	-
8	+	+	+	+	-	+	-	-	+	+	+	-	-	-
10	-	+	+	+	-	-	-	-	+	+	+	-	-	-
12	-	+	+	+	-	-	-	-	+	+	+	+	+	-
14	-	+	+	+	-	-	-	-	+	+	+	+	+	-

Table 6.5 Morphology of Calothrix D796 during batch culture in 0.1 mg l^{-1} P medium
See Table 6.1 for abbreviations.

Day	Heterocyst			Sheath	Hormogonia		P. granules		C. granules		Sep.disc	F. branch	Vacuoles	Hair
	Bl	Bs	Int		+gv	-gv	Bs	A	Bs	A				
0	+	+	-	+	-	+	+	-	-	+	-	-	-	-
1	-	+	-	+	-	+	+	+	-	-	-	-	-	-
2	+	+	-	+	-	+	+	+	-	-	-	-	-	-
3	+	+	-	+	-	+	+	-	+	+	+	-	-	-
4	-	+	+	+	-	+	+	-	+	+	+	-	+	-
5	-	+	+	+	-	-	-	-	+	+	+	+	+	-
6	-	+	+	+	-	-	-	-	+	+	+	+	+	-
7	-	+	+	+	-	-	-	-	+	+	+	+	+	-
10	-	+	+	+	-	-	-	-	+	+	+	+	+	-
12	-	+	+	+	-	-	-	-	+	+	+	+	+	-
14	-	+	+	+	-	-	-	-	+	+	+	+	+	-

Table 6.6 Morphology of Calothrix D796 in batch culture in Chu 10D-N medium
See Table 6.1 for abbreviations.

Day	Heterocyst			Fm. sheath	Hormogonia		P. granules		C. granules		Sep. disc	F. branch	Vacuoles	Hair
	Bl	Bs	Int		+gv	-gv	Bs	A	Bs	A				
0	+	+	-	+	-	+	+	-	-	+	-	-	-	-
1	-	-	-	+	-	+	+	+	-	-	-	-	-	-
2	+	+	-	+	-	+	+	+	-	-	-	-	-	-
3	+	+	-	+	-	+	+	+	-	+	+	-	-	-
4	+	+	-	+	-	+	+	-	+	+	+	-	-	-
5	+	+	+	+	-	+	+	-	+	+	+	-	-	-
6	-	+	+	+	-	+	+	-	+	+	+	-	-	-
7	-	+	+	+	-	-	-	-	+	+	+	-	-	-
8	-	+	+	+	-	-	-	-	+	+	+	+	-	-
10	-	+	+	+	-	-	-	-	+	+	+	+	-	-
12	-	+	+	+	-	-	-	-	+	+	+	+	-	-
14	-	+	+	+	-	-	-	-	+	+	+	+	-	-

Table 6.7 Polyphosphate bodies as estimate percentage of the cell profile stained and the filaments with hormogonial production as percentage of total filaments in Calothrix D794 after addition of Phosphorus (10 mg l^{-1} p) to P-limited culture. (Blank, undetectable under light microscope).

Time	Polyphosphate granules		Hormogonial
	Basal	Apical	production
	%	%	%
0 min			
30 min	20.0		
1 h	30.0	2.0	
24 h	50.0	5.0	30.0
2 d	60.0	5.0	80.0
3 d	50.0	5.0	60.0
4 d	30.0	2.0	20.0
5 d	20.0	0.0	10.0

Table 6.8 Polyphosphate bodies as percentage of estimate cell profile stained and filaments with hormogonial production as percentage of total filaments in Calothrix D795 after addition of Phosphorus (10 mg l^{-1} P) to P-limited culture.
See Table 6.7 for abbreviation.

Time	Polyphosphate granules		Hormogonial
	basal	apical	production
	%	%	%
0 min	1		
30 min	30.0	1.0	
1 h	40.0	2.0	
24 h	50.0	5.0	40.0
2 d	60.0	10.0	80.0
3 d	50.0	10.0	60.0
4 d	30.0	5.0	20.0
5 d	25.0	5.0	4.0

Table 6.9 Polyphosphate bodies as percentage of estimated cell profile stained and filaments with hormogonial production as percentage of total filaments in Calothrix D796 after addition of Phosphorus (10 mg l^{-1} P) to P-limited culture.
See Table 6.7 for abbreviation.

Time	Polyphosphate granules		Hormogonial
	basal	apical	release
	%	%	%
0 min			
30 min	30.0	1.0	
1 h	60.0	2.0	
24 h	70.0	5.0	60.0
2 d	70.0	10.0	80.0
3 d	40.0	5.0	30.0
4 d	30.0	5.0	10.0
5 d	20.0		

Table 6.10 Dimensions of trichome and cell in D794 grown in Chu 10D-N and 0.1 mg l^{-1} p medium from 0-10 d of incubation period in shaking tank at 32°C (n = 50).

Calothrix D794 in Chu 10D-N medium:

Incubation time (d)	Trichome length µm	Vegetative cell width basal µm	apical µm
0	90 - 250	5.25 - 6.5	3.0 - 5.5
3	40 - 85	4.25 - 6.0	3.0 - 4.5
5	85 - 175	5.25 - 6.5	3.0 - 5.5
7	100 - 200	5.5 - 6.5	3.0 - 5.5
9	150 - 290	5.0 - 6.5	3.0 - 5.5
10	200 - 400	5.0 - 5.5	3.0 - 5.5

Calothrix D794 in 0.1 mg l^{-1} P medium

0	90 - 250	5.25 - 6.5	3.0 - 5.5
3	40 - 80	4.25 - 6.5	3.0 - 4.5
5	80 - 175	5.25 - 6.5	3.0 - 5.0
7	100 - 250	5.50 - 6.5	3.0 - 5.5
9	225 - 390	5.50 - 8.5	3.0 - 5.5
10	300 - 500	5.50 - 5.9	3.0 - 3.5

Table 6.11 Dimensions of trichome, vegetative cell in Calothrix D794 grown in controlled and 0.1 mg l^{-1} P medium from 0-10 d of incubation period in shaking tank at 32°C.

a) Calothrix D795 in Chu 10D-N

Incubation time (d)	Trichome length µm	Vegetative cell width: basal µm	apical µm
0	90 - 150	10 - 12	5 - 5.5
3	50 - 100	6 - 8	4 - 5.0
5	70 - 250	8 - 10	5 - 5.5
7	100 - 250	10 - 12	5 - 5.5
8	125 - 300	10 - 12	5 - 5.5
10	125 - 300	9 - 10	5 - 5.5

b) Calothrix D795 in 0.1 mg l^{-1} p medium

0	90 - 150	10 - 12	5 - 5.5
3	50 - 20	6 - 8	4 - 5.0
5	90 - 130	7 - 10	5 - 5.5
7	100 - 250	7 - 10	5 - 5.5
10	150 - 400	9 - 10.5	5 - 5.5

Table 6.12 Dimensions of trichome, vegetative cell in Calothrix D796 grown in chu 10D-N and 0.1 mg l^{-1} P medium from 0-10 d of incubation period in shaking tank at 32°C.

a) D796 in Chu 10D-N

Incubation time (d)	Trichome length µm	Vegetative cell width: basal µm	apical µm
0	100 - 250	6 - 8.5	3.7 - 5.0
3	50 - 90	5 - 5.5	3.0 - 4.5
5	100 - 120	5 - 6.5	3.7 - 5.0
7	100 - 225	6 - 8.0	3.0 - 5.0
9	200 - 300	6 - 8.0	3.0 - 5.0
10	200 - 390	6 - 7.0	3.0 - 5.0

b) Calothrix D796 in 0.1 mg l^{-1} P medium

0	100 - 250	6 - 8.5	3.7 - 5.0
3	50 - 80	5 - 5.5	3.0 - 4.5
5	150 - 200	6 - 7.0	3.0 - 7.5
7	200 - 312	6 - 7.5	2.5 - 3.0
9	400 - 500	6 - 7.5	2.5 - 3.0
10	300 - 500	6 - 7.5	2.5 - 3.0

Table 6.13 Dimensions of cells in hormogonia in 0.1 mg l^{-1} P medium (n = 50)

Strain	Length µm	Width µm
D794	17.0 - 300	4.75 5.0
D795	15.0 - 250	4.5 - 5.5
D796	37.5 - 200	3.5 - 3.75

Table 6.14 Dimensions of heterocysts (basal only) in 7 d old culture grown in 0.1 mg l^{-1} p medium.

Strain	Basal het. width µm	Basal het. length µm
D794	5 - 7.5	5.0 - 10.0
D785	10 - 12.5	9.5 - 10.0
D796	5 - 5.5	6.0 - 7.0

Table 6.15 Blue heterocysts (only basal) in three Calothrix strains as percentage in standard (1.78 mg l^{-1} P) and P-deficient (0.1 mg l^{-1} P) medium. (n = 50). Bl.het., blue heterocyst, blank, not observed.

Time		Standard medium			P-deficient medium		
(d)	Calothrix:	D794	D795	D796	D794	D795	D796
	Bl.het.:	%	%	%	%	%	%
0			4			4	
2		25	40	20	15	20	8
4		6	12	4	2	2	0
6		4	4	0	0	0	0
8		0	2	0	0	0	0

7 INFLUENCE OF ENVIRONMENTAL FACTORS ON MORPHOLOGY OF CALOTHRIX D794, D795 AND D796

7.1 INFLUENCE OF LIGHT FLUX ON MORPHOLOGY OF 3 STRAINS

Calothrix strains were grown in standard medium and incubated at a range of light fluxes (4, 10, 110 and 170 µmol photon m^{-2} s^{-1} PAR). Gas-vacuolated hormogonia and associated morphological changes were observed. Gas-vacuolated hormogonia release expressed as a percentage of total released hormogonia in Calothrix D794, D795 and D796 under different light regimes are in Table 7.1. The results are summarized below:

7.11 Calothrix D794

At 110 and 170 µmol photon m^{-2} s^{-1}, the culture aggregated into thin net-like mats submerged just below the surface of the medium. In low light (4 and 10 µmol photon m^{-2} s^{-1}), it formed scattered small colonies. The growth rate was slowest at 4 µmol photon m^{-2} s^{-1}, but most of the trichomes continued to release hormogonia leaving empty filaments (Fig.7.1)
On day +10, less than 30% trichomes appeared healthy.

The percentage of gas-vacuolated hormogonia was highest at 10 µmol photon m^{-2} s^{-1} and suppressed at 170 µmol photon m^{-2} s^{-1}. After 24 h incubation at 170 µmol, there was no further production of gas-vacuolated hormogonia. Rope like formations of hormogonia were found under all light conditions. On long exposure to 170 µmol photon m^{-2} s^{-1} this strain grew very well although a few basal cells began to elongate (Fig.7.3).

7.12 Calothrix D795

At 170 µmol photon m^{-2} s^{-1}, a vertically suspended mass was formed with a portion attached to the bottom of the flask whilst at 110 µmol photon m^{-2} s^{-1},

the algal mass was attached to one side. This strain had a fast growth rate in all light conditions above 4 µmol photon m^{-2} s^{-1}.

This strain produced a highly gas-vacuolated hormogonia in all light conditions. At 170 µmol photon m^{-2} s^{-1}, the gas vacuoles disappeared whilst the hormogonia were still attached to trichomes within 24 h (Fig. 7.1 c) On day + 5, intercalary heterocysts and vacuoles developed in undifferentiated cells. By day + 10, the strain had lost its pigment and died. At 4 «mol photon m^{-2} s^{-1} the growth was limited and most of the filaments developed separation discs in series (Fig. 7.1 e-g).

7.13 Calothrix D796

The algal mass was attached to one side of the flask at 110 and 170 µmol photon m^{-2} s^{-1} and remained scattered at 4 and 10 «mol photon m^{-2} s^{-1}. The initial growth rate was slow for 24 h in all light conditions.

At 170 «mol photon m^{-2} s^{-1}, colour changed from blue-green to light green initially but regained the original colour within a few days and there were no obvious changes in colour at 4, 10 and 110 «mol photon m^{-2} s^{-1}.

No gas vacuoles formed under any light conditions however non gas-vacuolated hormogonia were released and rope formations of hormogonia were found in all light conditions. The sheath became indistinct at 4 «mol photon m^{-2} s^{-1}. Reduction in trichome width and formation of separation discs were observed at 4 and 10 «mol photon m^{-2} s^{-1}. Though initial growth was slow, this strain tolerated both very low and very high light.

Table 7.1 Gas-vacuolated hormogonia released expressed as a percentage of the total released hormogonia in Calothrix D794, D795 at 4, 10, 110 and 170 µmol photon m^{-2} s^{-1}. Calothrix D796 did not form gas vacuolated hormogonia at any light conditions. n = 250, blank = below detection level

strain	time	light (µmol photon m^{-2} s^{-1}):			
	(d)	4	10	110	170
		%	%	%	%
	0	6.8	6.8	6.8	6.8
D794	1	20.0	45.0	12.0	2.0
	3	35.5	30.0	5.0	1.0
	6	50.0	20.0	2.0	
	10	30.0	10.0		
	0	8.5	8.5	8.5	8.5
D795	1	20.0	80.0	50.0	60.0
	3	35.0	40.0	30.0	10.0
	6	50.0	15.0	10.0	2.0
	10	10.0	4.0	2.0	

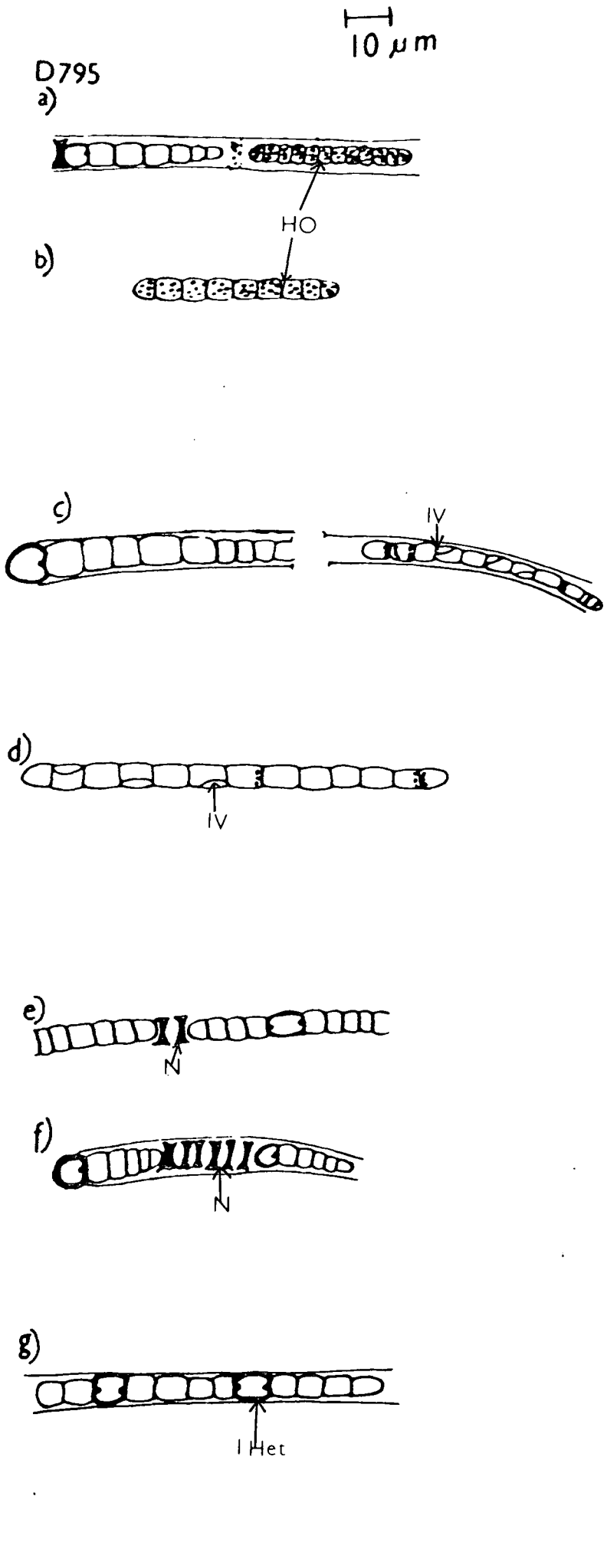
Fig. 7.1 Calothrix D795 showing

a-b) highly gas-vacuolated hormogonia at 110 µmol photon m^{-2} s^{-1}

c-d) intra-thylakoidal vacuoles and disappearance of gas vacuoles in hormogonium attached to the filament and in released hormogonium

e-g) formation of separation discs (necridium) and intercalary heterocysts at 4 µmol photon m^{-2} s^{-1}

Hormogonium (Ho). Intra-thylakoidal vacuole (IV). Intercalary heterocyst (IHet). Necridium (N)

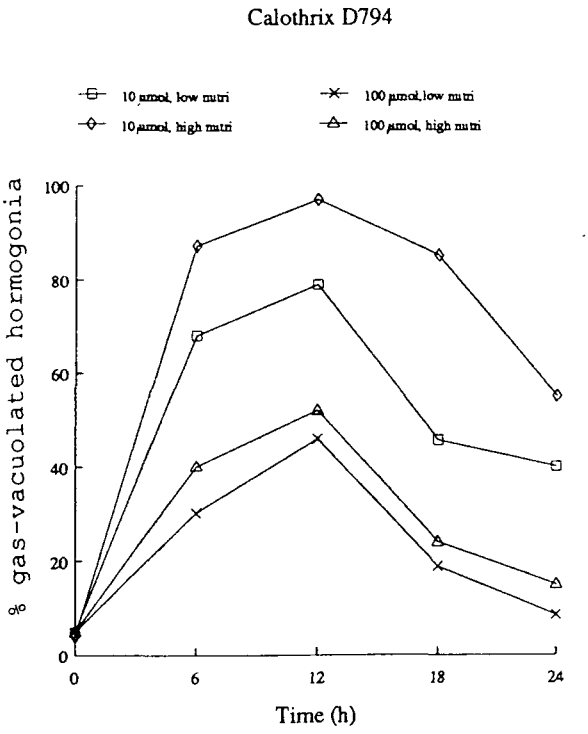

10 µm
D795
a)
HO
b)
c)
IV
d)
IV
e)
N
f)
N
g)
I Het

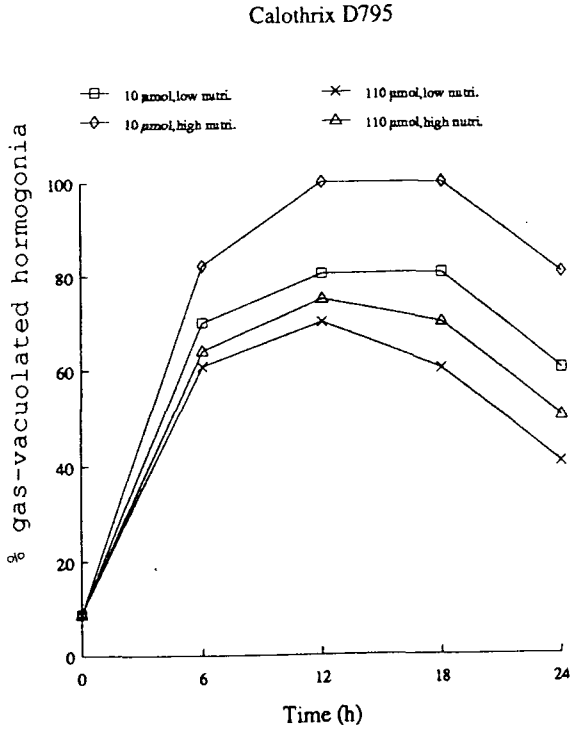
7.2 INFLUENCE OF NUTRIENT CONCENTRATION ON MORPHOLOGY OF 3 *Calothrix* STRAINS

A further study was carried out to see if gas vacuoles in *Calothrix* D794, D795 and D796 could be stimulated by nutrient composition. Mid-exponential phase cultures were grown in Chu 10D-N medium with two phosphorus concentration (1 and 10 mg l^{-1} P) and incubated at 10 and 110 μmol photon m^{-2} s^{-1} PAR.

Released gas-vacuolated hormogonia expressed as percentage of total released hormogonia in *Calothrix* D794 and D795 in 1 mg l^{-1} P and 10 mg l^{-1} P media are given in Fig. 7.1. This shows the percentage of gas vacuoles in *Calothrix* D794 and D795 was stimulated by high nutrient medium and low light whilst there was no effect in *Calothrix* D796 in formation of gas vacuoles, but higher percentage of non-gas-vacuolated hormogonia were released.

Fig. 7.2 Influence of nutrient concentration on gas vacuole formation in Calothrix D794 and D795. The algae were grown in Chu 10D-N medium with two phosphorus concentration (1 mg l^{-1} P and 10 mg l^{-1} P) and incubated at 10 and 110 µmol photon m^{-2} s^{-1}. Released gas-vacuolated hormogonia are expressed as percentage of total hormogonia released.





8 EFFECT OF SHIFT OF LIGHT ON GAS VACUOLE FORMATION IN CALOTHRIX D794

Previous experiments indicated that gas vacuole formation in Calothrix D794 was stimulated at 10 µmol photon m^{-2} s^{-1} PAR and was virtually suppressed at 170 µmol photon m^{-2} s^{-1} PAR (7.1). Thus an experiment was planned to see the effect of changes in light flux on gas vacuole formation in Calothrix D794.

The alga was grown in standard medium and incubated at 170 µmol photon m^{-2} s^{-1} for 5 d. Then it was sub-cultured into fresh medium and incubated in two environmental conditions (20 flasks in each condition):

a) Dark

b) 10 µmol photon m^{-2} s^{-1}

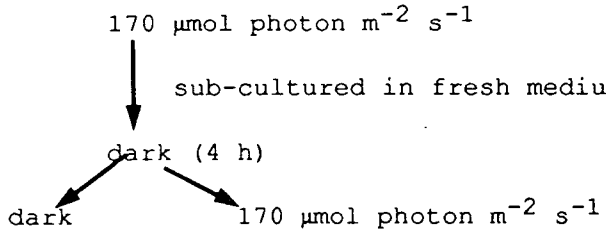
A control was set up at 170 µmol photon m^{-2} s^{-1} .

Cultures grown in these conditions were observed at 2 h intervals up to 18 h for the formation of gas-vacuolated hormogonia (attached and free). 6 replicates of culture flasks were shifted back to 170 µmol photon m^{-2} s^{-1} after an initial period of 4 h, 8 h and 10 h incubation in dark as shown in experiment sets 1, 2 and 3.

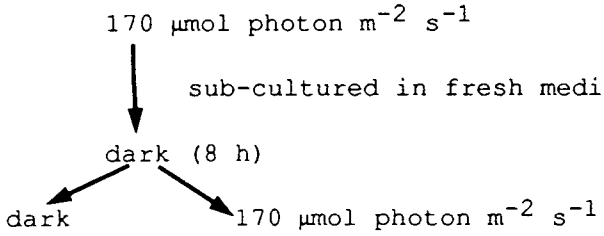
The experiment was also conducted by transferring alga to 170 µmol photon m^{-2} s^{-1} after initial period of 4 and 10 h incubation at 10 µmol photon m^{-2} s^{-1} as shown in experiment sets 4 and 5. Samples were preserved (2.8) for statistical analysis (2.93). Observations, few instances, on gas vacuole formation was also made on fresh medium.

Experiments

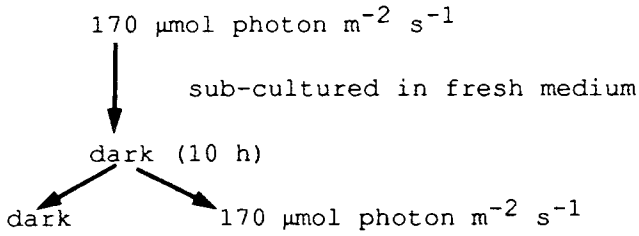
Set 1



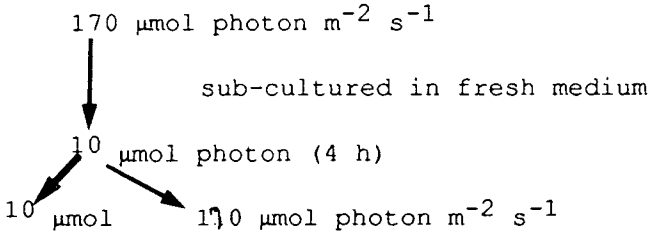
Set 2



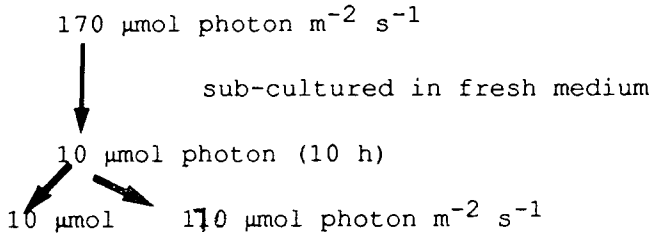
Set 3



Set 4



Set 5



The gas vacuolated hormogonia released in dark, at 10 and 170 µmol photon m^{-2} s^{-1} expressed as percentage of total released hormogonia are shown in Fig. 8.1. and the associated morphological changes are shown in Table 8.1. The similar observations were made after shifting the dark and low light (10 µmol photon m^{-2} s^{-1}) grown culture to 170 µmol photon m^{-2} s^{-1} and are given in Table 8.2. The four different types of filaments (see 2.93) are expressed separately as percentage of total filaments and released gas-vacuolated hormogonia expressed as a percentage of total released hormogonia are given in Appendix 3 (Table a-b). From these data, percentage of non-hormogonial filaments with gas vacuole formation in the tapered end, percentage of gas-vacuolated hormogonial filaments and released gas-vacuolated hormogonia are replaced by Figs 8.2, 8.3, 8.4, 8.5, 8.6. Ratio of filaments with gas vacuole formation : filaments without gas vacuole formation and ratio of released gas-vacuolated hormogonia : hormogonia without gas vacuoles are given in Table 8.3.

The experiment was also repeated by growing the alga at 110 µmol photon for 6 d instead of 170 µmol photon m^{-2} s^{-1}. It was sub-cultured and incubated in dark. Then the replicates of cultures were shifted back to 110 µmol photon m^{-2} s^{-1} after an initial period of 4 h, 8h and 10 h incubation in dark. The results are in Appendix 3 (Table i - m).

Table 8.1 Time course of morphological events in Calothrix D794 in dark, 10 and 170 µmol photon m^{-2} s^{-1}. g-v, gas-vacuolated; gv, gas vacuoles

Time	Environmental conditions		
(h)	Dark	10 µmol photon m^{-2} s^{-1}	170 µmol photon m^{-2} s^{-1}
0	gas vacuoles		
2	occupy 1/4th length of the most trichome	clear differentiation of g-v hormogonia at the tapered end	gv start to form at the tapered end of the trichome
4	occupy 1/2th length	gv occupy 3/4th length	1/2th length of most trichome
6	differentiation of g-v hormogonia and release	occupy in some filament length except few basal cell	release of hormogonia
8	continuous release of g-v hormogonia leaving a short trichome	release of a long single hormogonium leaving a few basal cells	heterocyst formation
10	g-v continue to form hormogonia	continue to form g-v hormogonia	only in a very few trichome
12	swelling of g-v hormogonia	swelling of g-v hormogonia	short length g-v hormogonia
14	g-v started to disappear in free hormogonia	g-v hormogonia continue to form	aggregation of g-v hormogonia
16-18	4.8% trichome with g-v hormogonia at the tapered end	6.6% trichome with g-v hormogonia at the tapered end	1.0% trichome with g-v hormogonia at the tapered end.

Table 8.2 Time course of morphological events in Calothrix D794 when shifted to 170 µmol photon m^{-2} s^{-1} after 4, 8 and 10 h of initial incubation in dark. (DL, dark to light)

Time (h)	4D-L	8D-L	10D-L
6	gas vacuoles occupy 4/5th length and clear differentiation of hormogonia		
8	release of a long g-v hormogonia (112-250 µm)		
10	Formation of gas vacuoles started to form at the tapered end.	g-v hormogonia forming	not observed
12-14	few forming gas vacuoles	release of g-v hormogonia	aggregation of g-v hormogonia outside the colony
16-18	stop forming	swelling of g-v hormogonia	entangled g-v hormogonia in between the filaments

Table 8.3 Ratio of total filaments with gas vacuoles : total filaments without gas vacuoles and ratio of total gas-vacuolated hormogonia : total non-gas-vacuolated hormogonia

Growth condition	Time (h)	Total filaments + gv :	Total filaments - gv	Released hormogonia + gv : - gv
170 µmol ↓	0		34.7	15.7
	2		14.10	5.6
	4		3.2	2.3
	6		16.6	54.1
	8		20.7	9.0
	10		24.0	11.5
	12		17.6	12.9
	14		25.3	26.8
170 µmol	16		40.7	34.86
	18		32.3	9.0
170 µmol ↓	0		34.7	15.7
	2		4.6	1.6
	4		3.1	1.9
	6		2.5	0.9
	8		1.5	0.7
	10		5.0	1.2
	12		9.0	4.3
	14		16.9	4.6
10 µmol	16		9.2	2.9
	18		7.28	3.6
170 µmol ↓	0		34.71	15.7
	2		3.3	7.3
	4		6.8	1.03
	6		8.7	2.7
	8		3.8	2.9
	10		7.9	4.3
	12		6.9	7.6
	14		15.7	14.6
dark	16		25.3	19.8
	18		44.4	30.2

Table 8.4 Ratio of total filaments with gas vacuoles : total filaments without gas vacuoles and ratio of gas-vacuolated hormogonia : non-gas-vacuolated hormogonia when cultures were shifted back to 170 µmol photon m^{-2} s^{-1} after initial period of 4, 8 and 10 h incubation in dark.

Growth condition	Time (h)	Total filaments + gv : blank, below detection level	Total filaments - gv	Released hormogonia + gv : -gv
170 µmol	4		6.8	1.03
↓	6		19.0	14.02
	8		32.3	9.4
dark (4 h)	10		34.7	6.1
↓	12		61.5	3.3
	14			5.3
170 µmol	16			14.6
	18			24.0
170 µmol	8		3.8	2.9
↓	10		61.5	5.6
dark (8 h)	12		17.2	4.3
↓	14		9.9	2.1
170 µmol	16		16.4	0.8
	18		9.9	1.8
170 µmol	10		7.9	4.3
↓	12		6.9	9.8
dark (10 h)	14			3.4
↓	16			3.7
170 µmol	18			0.7

Fig. 8.1 Influence of environmental factors on gas vacuole formation in Calothrix D794. The alga was grown in standard medium at 170 µmol photon m^{-2} s^{-1} for 5 d, sub-cultured in fresh medium and incubated at 10, 170 µmol photon m^{-2} s^{-1} and in dark

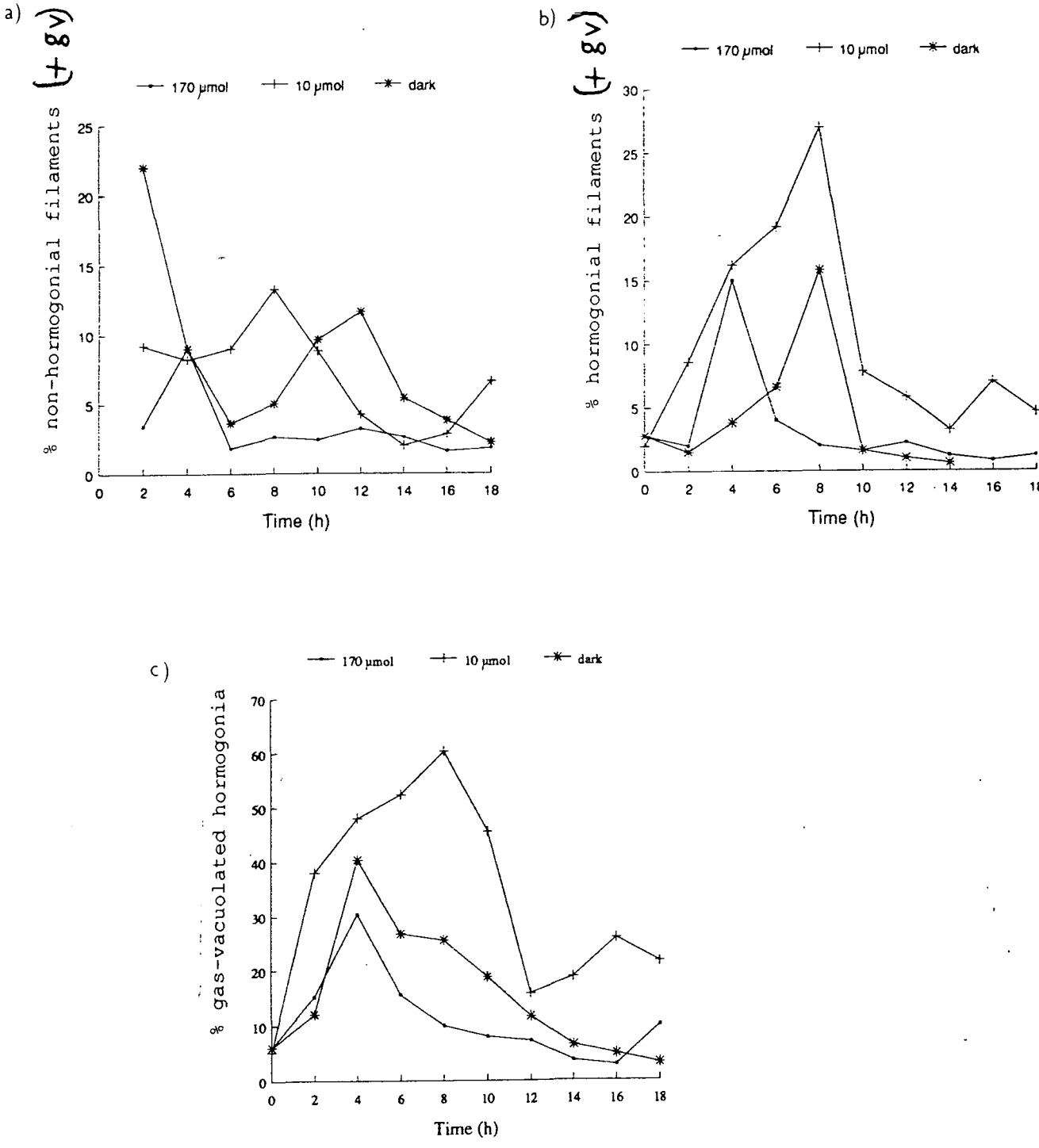


Fig. 8.2 Influence of shift of light on gas vacuole formation in Calothrix D794. The alga was grown in 170 µmol photon m^{-2} s^{-1} for 5 d and sub-cultured in fresh medium and incubated in dark. The culture was shifted back to 170 µmol photon after 4 h incubation in dark

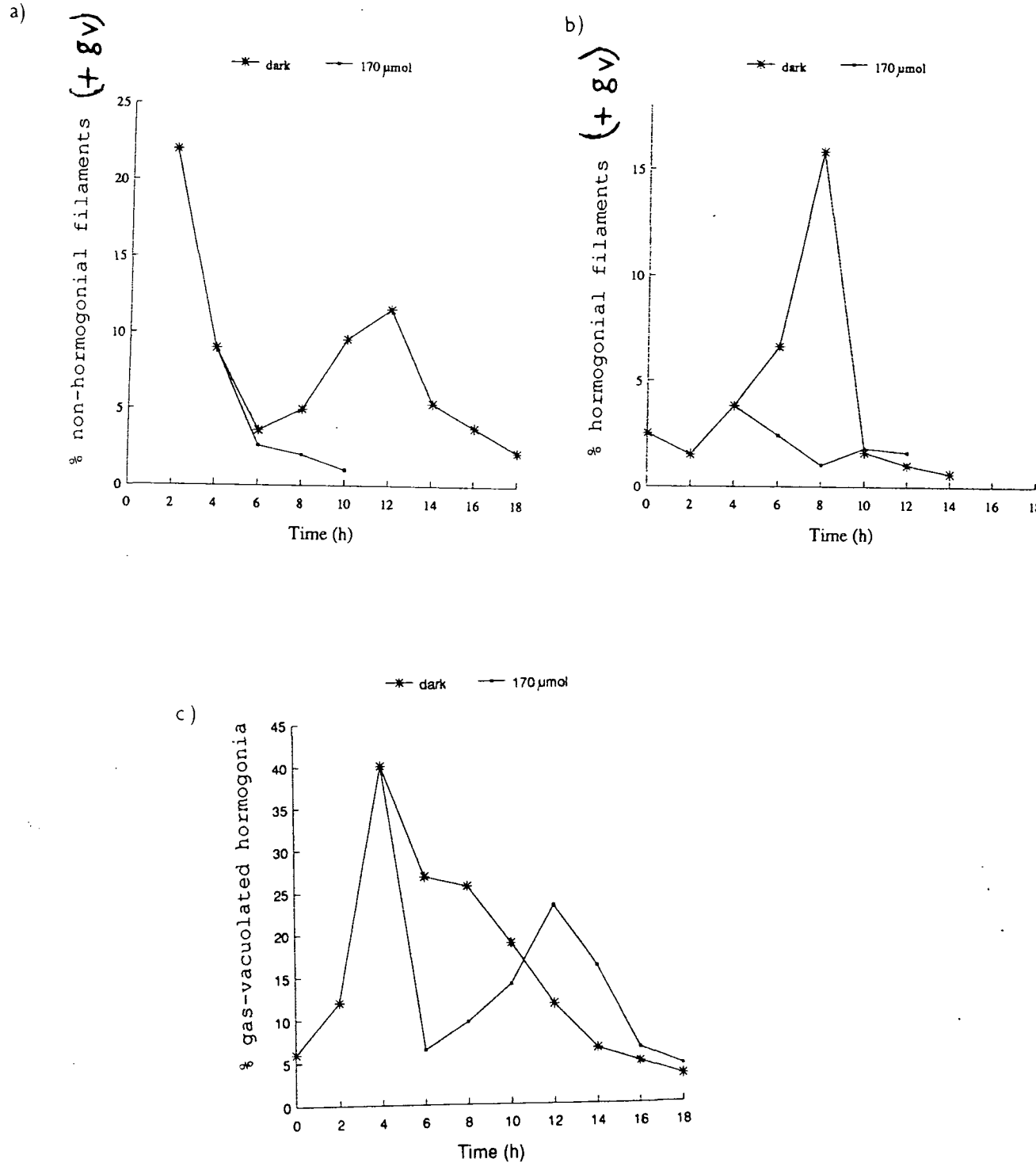


Fig. 8.3 Influence of shift of light on gas vacuole formation in Calothrix D794. The alga was grown in standard medium at 170 µmol for 5 d, sub-cultured in fresh medium and incubated in dark. The culture was shifted to 170 µmol photon m^{-2} s^{-1} after 8 h incubation in dark

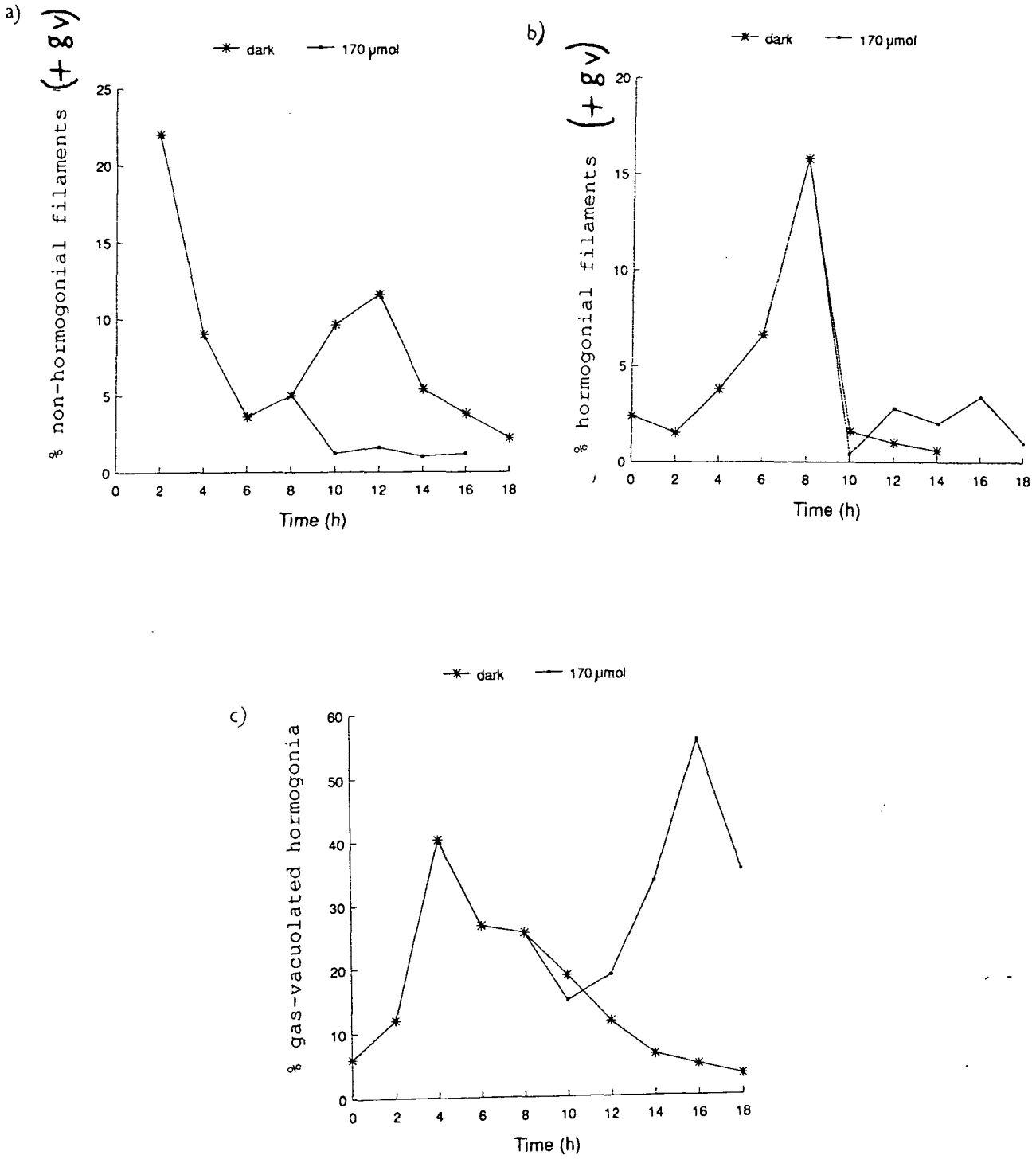


Fig. 8.4 Influence of shift of light on gas vacuole formation in Calothrix D794. The alga was grown in standard medium at 170 µmol photon m^{-2} s^{-1} for 5 d, sub-cultured and incubated in dark. The culture was shifted back to 170 µmol photon m^{-2} s^{-1} after 10 h incubation in dark

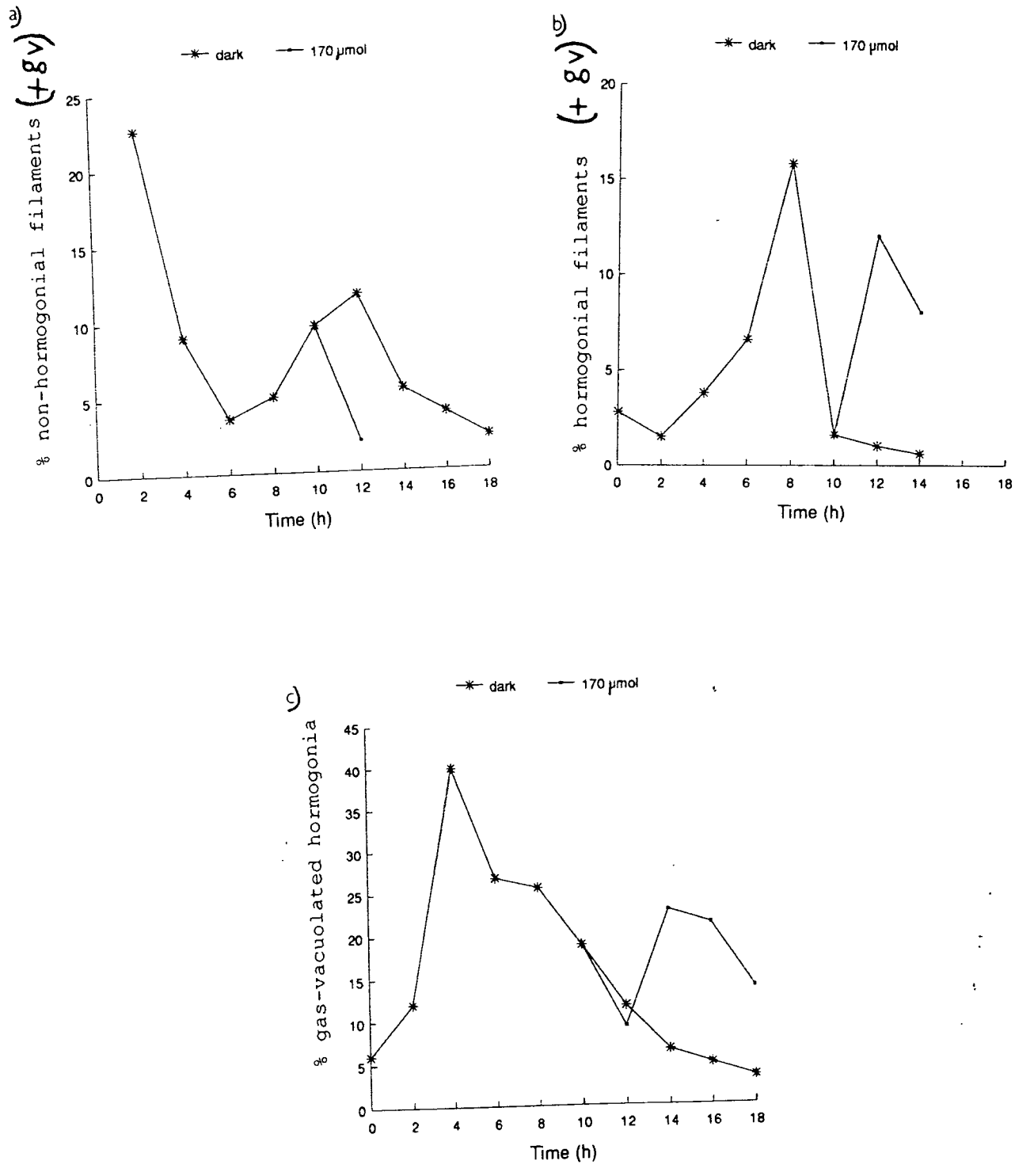


Fig. 8.5 Influence of shift of light on gas vacuole formation in Calothrix D794. The alga was grown in standard medium at 170 µmol photon m^{-2} s^{-1} for 5 d, sub-cultured and incubated in dark. The culture was shifted back to 170 µmol photon m^{-2} s^{-1} after an initial period of 4 h incubation at 10 µmol photon m^{-2} s^{-1}

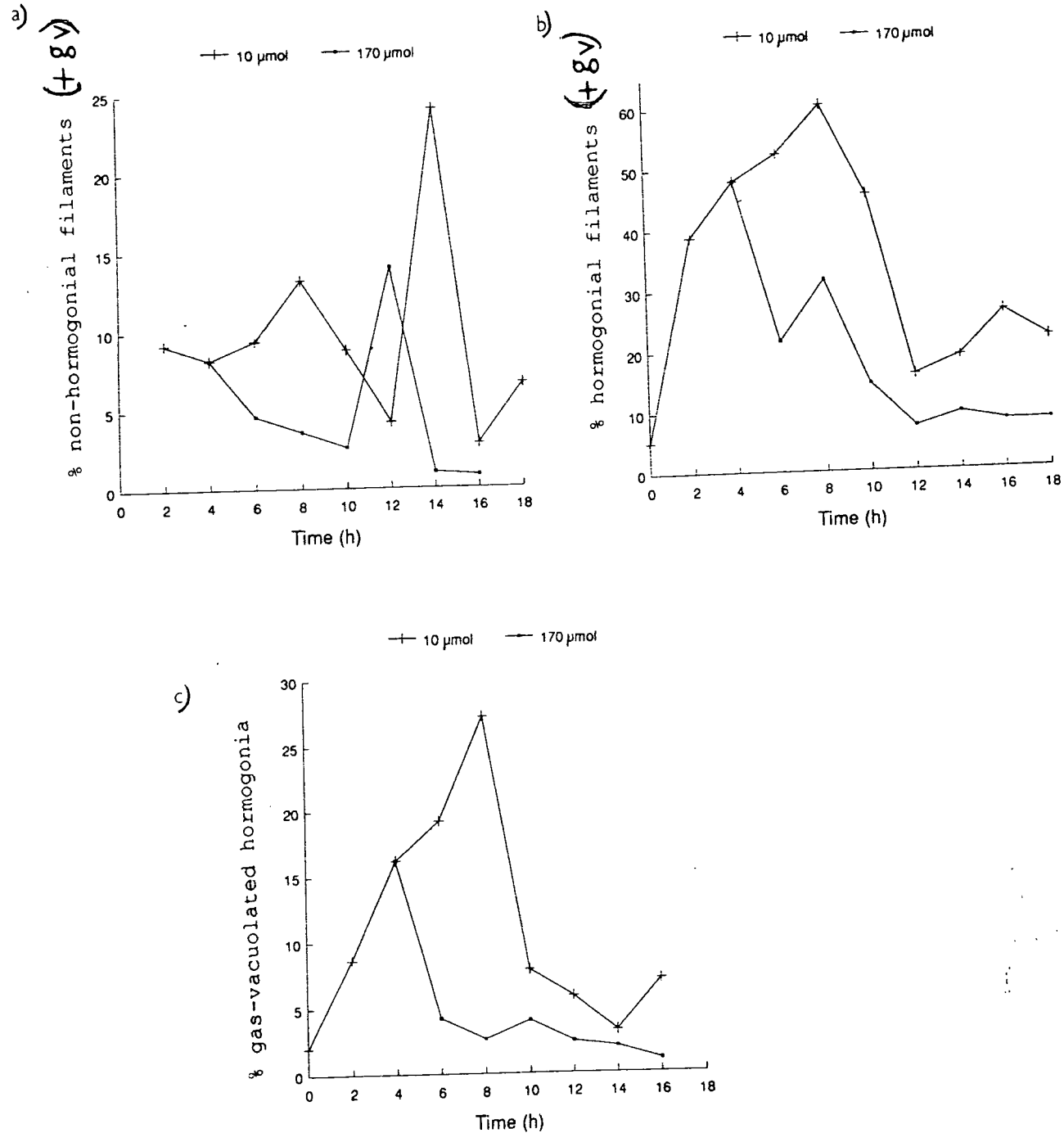
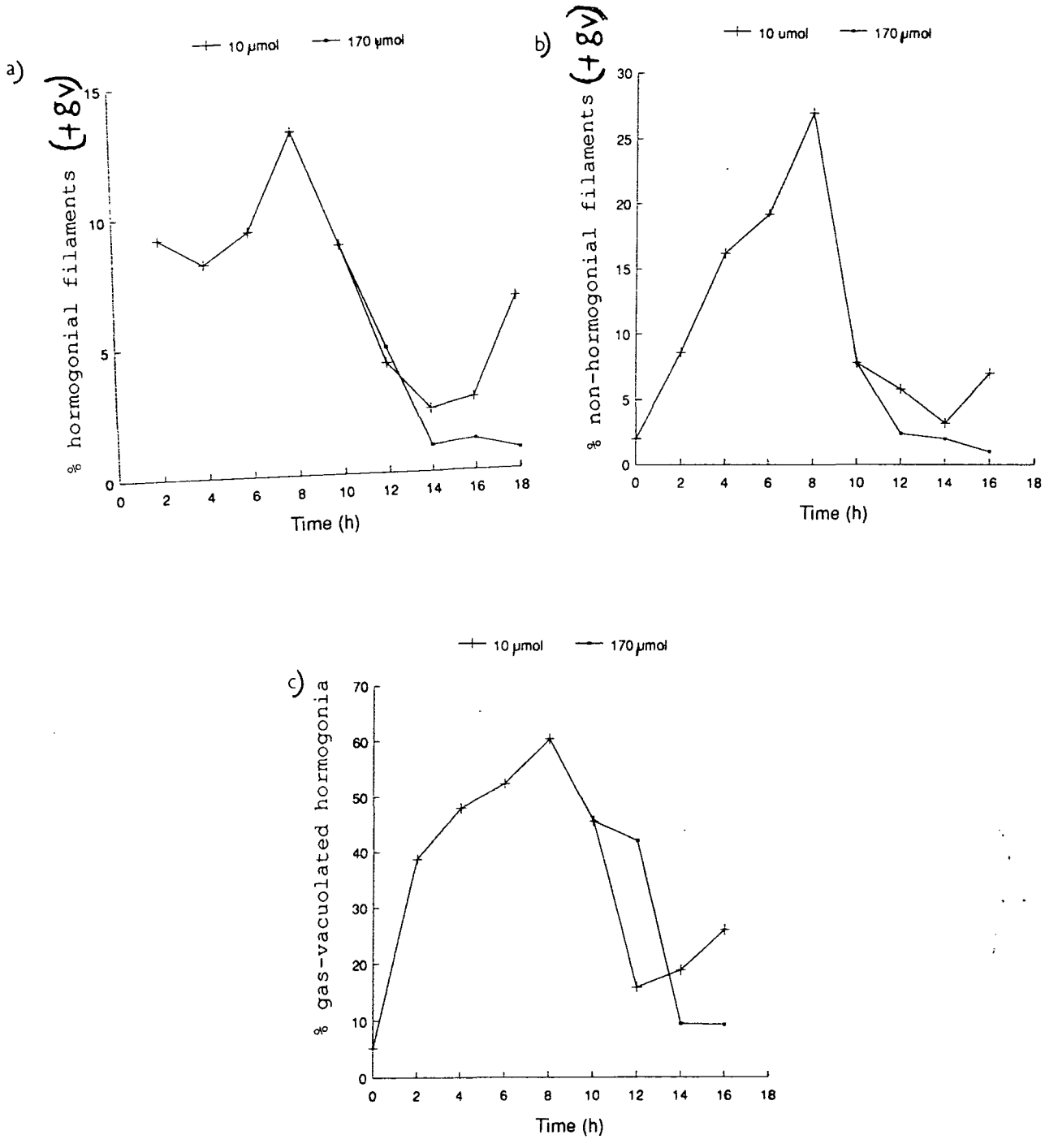


Fig. 8.6 Influence of shift of light on gas vacuole formation in Calothrix D794. The alga was grown in standard medium at 170 µmol photon m^{-2} s^{-1}, sub-cultured and incubated at 10 µmol photon m^{-2} s^{-1}. The culture was shifted back to 170 µmol photon m^{-2} s^{-1} after an initial period of 10 h incubation at 10 µmol photon m^{-2} s^{-1}



The results are summarized below:

At 170 µmol photon m^{-2} s^{-1}, gas vacuoles were just started to form at the tapered end of trichome in 2 h of incubation whilst in dark incubation, they had occupied 1/4th length of the filaments and there was already clear differentiation of gas-vacuolated hormogonia at 10 µmol photon m^{-2} s^{-1} (Table 8.1). In some filaments (less than 1%) gas vacuoles formed in all the cells except a few basal cells in 6 h dark incubation.

Gas vacuoles were small and indistinct initially in culture incubated in the dark, they subsequently increased in size and number to occupy much of the cell. By 4 h, hormogonia had clearly differentiated and been released in the dark and at 170 µmol photon m^{-2} s^{-1} whilst in 10 µmol photon m^{-2} s^{-1}, a long gas-vacuolated hormogonia released in 6 h.
2 percentage of gas-vacuolated hormogonia, both attached and free, were swollen after 12 h incubation in dark and at 10 µmol photon m^{-2} s^{-1}. Such features were also observed after the cultures were shifted to 170 µmol photon m^{-2} s^{-1} (Fig.8.4).

On transfer from dark to 170 µmol photon m^{-2} s^{-1}, gas vacuoles disappeared rapidly during the first 2 h and then continued to form and be released at a reduced rate. Gas vacuoles were confined to the periphery at the beginning, later they became restricted to cross walls and most of the gas vacuoles were wider at 170 µmol photon m^{-2} s^{-1}.

The percentage of the filaments with gas vacuole formation in the tapered end was lowered 4 times during first two hours of exposure and they continued to form at a lower rate up to 10 h (figs 8.2a and 8.3a) but it was stopped forming after 18h in (fig. 8.4a). The percentage of gas-vacuolated hormogonia differentiation was higher than in dark after initial reduction of 6.3 times during first 4 h of exposure in Fig. 8.2b, 2 h in Fig. 8.3b and no reduction was observed in Fig. 8.4b. The percentage of released gas-vacuolated

hormogonia was reduced at the beginning and then became higher than in dark in all cases.

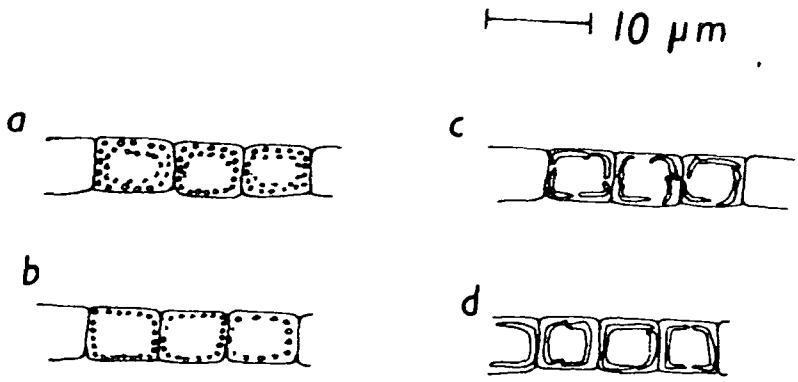
Similar results were observed in this strain on transfer from 10 to 170 μmol photon m^{-2} s^{-1}, however, the percentage of gas vacuoles were lower than in dark with the exception of higher percentage of gas-vacuolated hormogonia released on transfer to 170 μmol photon m^{-2} s^{-1} after an initial period of 10 h incubation in 10 μmol photon m^{-2} s^{-1} (Figs 8.5 and 8.6).

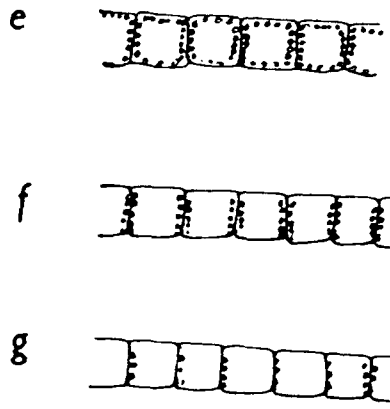
Fig. 8.7 Calothrix D794 showing locations of gas vacuoles in cells (under optical microscope) at low and high light and swelling of gas-vacuolated hormogonia when the culture was shifted to 170 μmol photon m^{-2} s^{-1} after 10 h incubation in dark.

a-d) at low light

e-g) at high light

h-i) swelling of gas-vacuolated hormogonium


10 µm
a
c
b
d


e
f
g

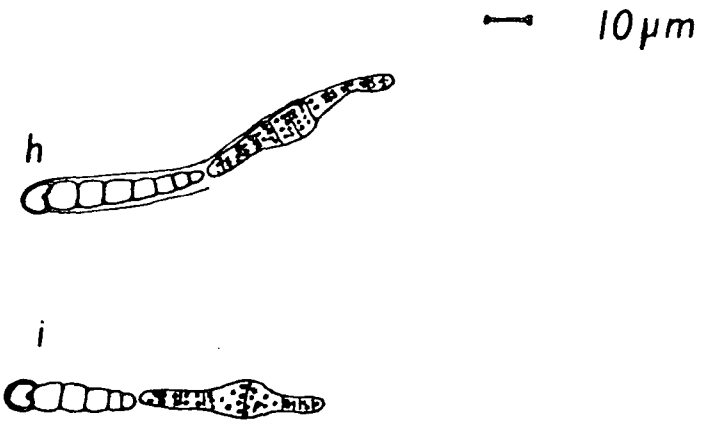
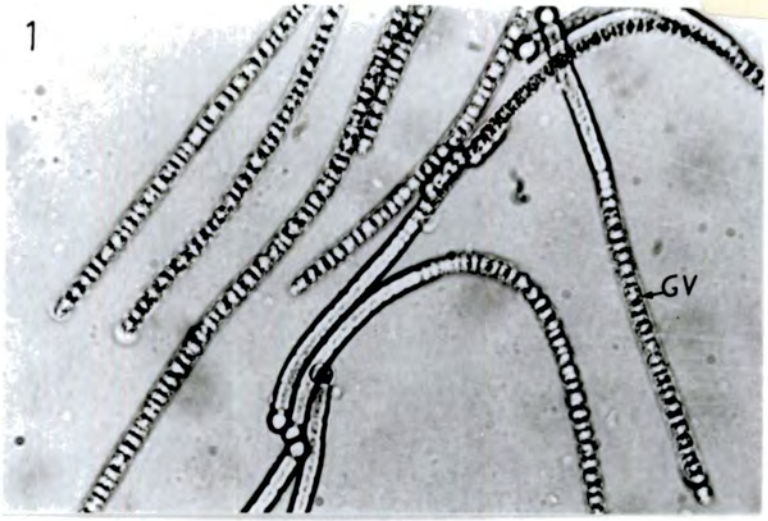
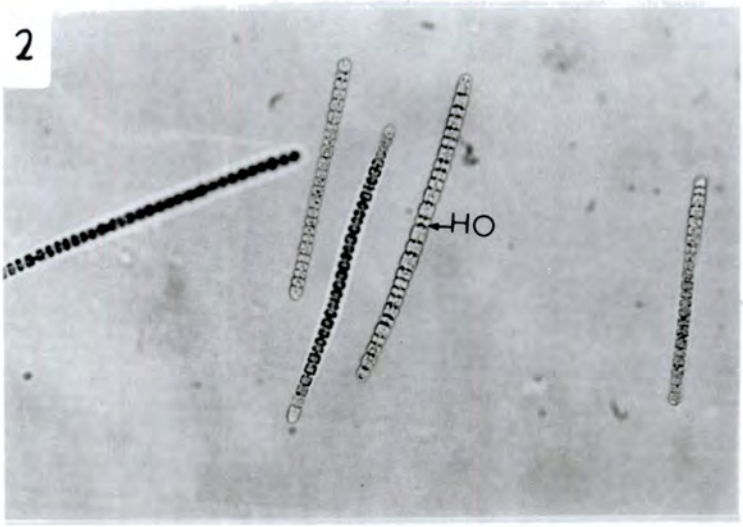
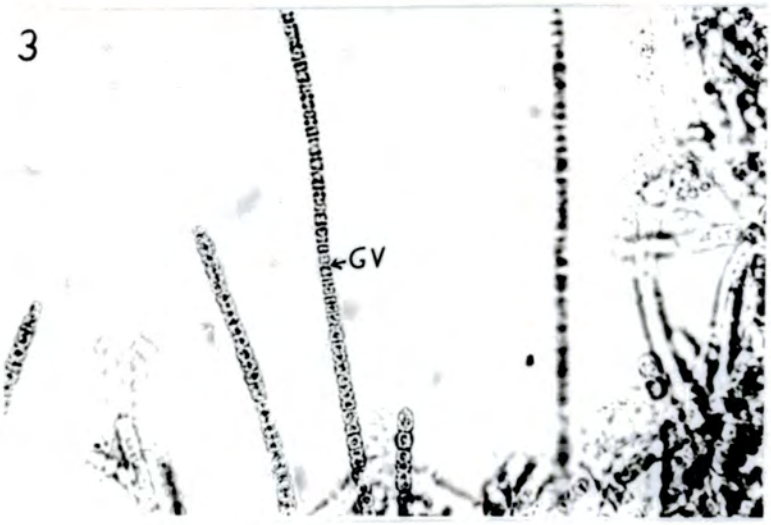

10µm
h
i
j

Fig. 8.8 Optical micrograph of Calothrix D794 showing gas vacuole formation in tapered part of trichomes and gas-vacuolated hormogonia
1) gas vacuole formation at 10 µmol photon m^{-2} s^{-1}
2) released gas-vacuolated hormogonia
3) gas vacuole formation in tapered part of trichomes in dark grown culture
4) releasing gas-vacuolated hormogonia

Scale bar = 10 µmol


1
GV


2
HO


3
GV

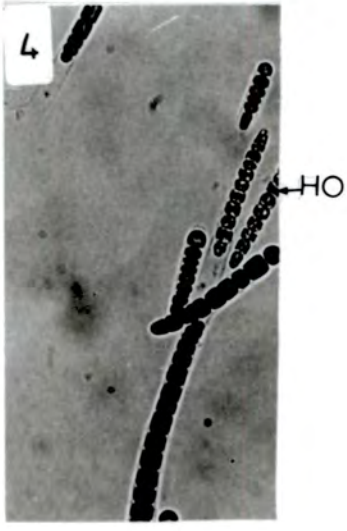
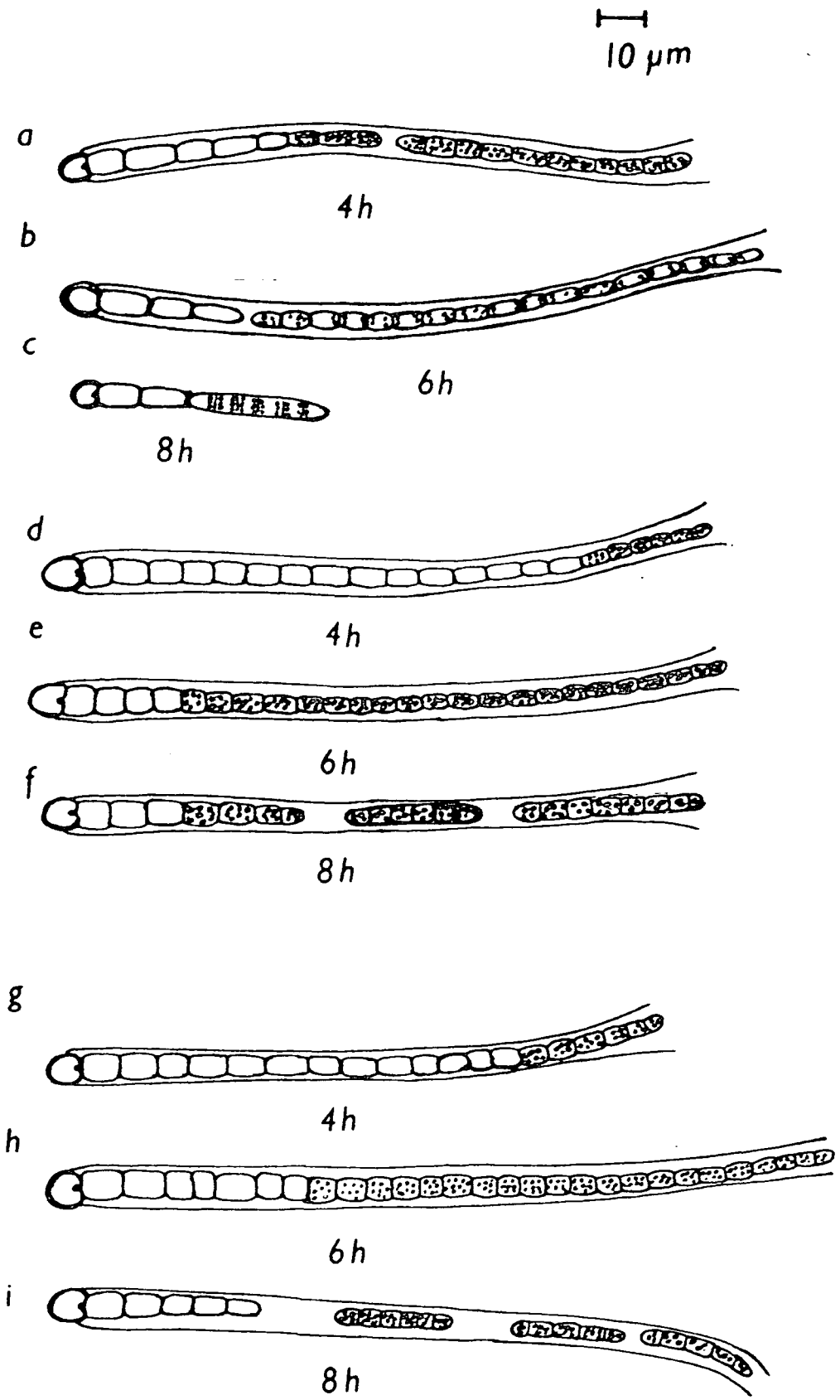

4
HO

Fig. 8.9 Calothrix D794 showing gas vacuole formation when the alga was grown at 170 µmol photon m^{-2} s^{-1} for 5 d, sub-cultured in fresh medium and incubated at 10, 170 µmol photon m^{-2} s^{-1} and in dark
a-c) 170-170 µmol photon m^{-2} s^{-1}
d-f) 170-10 µmol photon m^{-2} s^{-1}
g-i) 170-dark


10 μm
a
4h
b
c
6h
8h
d
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e
6h
f
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4h
h
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i
8h

9 DISCUSSION

9.1 ALGAL FLORA OF NEPAL

The algal flora at sites A and B was similar and dominated by blue-green algae. It is possible that similar soil conditions (pH 5-6 and medium to high available phosphorus and potassium content) overcame the striking differences in altitude and zone. The presence of blue-green algae in such conditions has been reported from rice-fields of India and Kerala (1.5). However, there were obvious floristic differences between the sites e.g. Aulosira was the predominant genus at site A whilst Anabaena dominated at site B. The algal flora at site C differed from the other two sites as it was dominated by green algae. Azolla pinnata and Spirogyra covered the sampling field where water was accumulated indicating the importance of nitrogen fixation in rice-fields situated at high mountain (2064 m). Azolla pinnata was reported only from Tarai and mid-hills (Maskey and Bhattarai, 1984) but not from high mountain. Though no visually obvious growth blue-green algae were found in the field, Anabaena, Calothrix and Tolypothrix were found in samples. Presence of blue-green algae in terraced rice-fields of Nepal has not been reported before. A higher abundance of blue-green algae has been reported in paddy soils than other cultivated soils (Watanabe and Yamamoto, 1971).

Blue-green algae, in this study, constituted 44% of the total algal flora (taxa). The proportion is similar to that reported by various authors under different climatic conditions in India, Japan, Ukraine and Bangladesh (1.3). These results are within the limits as sites were sampled only once with the exception of site B where three visits were made.

9.2 MORPHOLOGY OF CALOTHRIX ISOLATES

Each Calothrix isolate exhibited its own characteristic morphological features even when grown in identical culture medium. Characteristic features showed by all three strains were the presence of some blue heterocysts and lack of hair cells under P-deficiency but they differed from each other in many different respects (5.2, 5.3 and 5.4).

All three strains exhibited morphological variation in laboratory cultures depending on the developmental stage. A wide morphological range in species of Rivulariaceae in culture has been reported as a result of their complex life cycle (Singh and Tiwari, 1970; Maxwell, 1974 and Chang, 1983). Formation of false-branches, thick sheath in all three strains and twisting of filament (Calothrix D796, 12 wk old stock culture) was observed in old cultures as reported by (Jeeji-Bai, 1977; Chang, 1983).

9.3 INFLUENCE OF PHOSPHORUS ON MORPHOLOGY OF THREE STRAINS

Four stages of growth of Calothrix D794, D795 and D796 have been described, during batch culture. These strains differ from those described for Calothrix parietina by Livingstone and Whitton (1983) as none of the strains studied formed hairs under P-deficiency (0.1 mg l^{-1} P).

Gas-vacuolate hormogonium formation in Calothrix D794 was affected by low phosphorus medium; no marked effect was observed in Calothrix D795 up to 24 h. The absence of gas-vacuolated hormogonium in Calothrix D796 may be due to the absence of gvp genes or if these genes were present and gas vacuoles were formed, these were possibly not observed under the light microscope.

The flexibility of hormogonia in Calothrix D794, highly gas-vacuolated hormogonia in Calothrix D795 and highly motile Hormogonia in Calothrix D796, may have ecological importance in the field if they behave in the same way in the field as they did in the laboratory cultures. Calothrix D794 isolated from Parwanipur which lies in plain, is liable to flooding and has low night

and high day temperatures. The flexibility of non gas-vacuolated hormogonia in this strain may have ecological importance in migration during the flooded time.

The highly gas-vacuolated hormogonia in Calothrix D795 may have considerable ecological importance in aiding vertical migration in the rice-field water as water is maintained here most of the time. The firm sheath in this strain might have advantage when the organism is attached to the soil surface or aquatic plants. The inability of Calothrix D796 to form gas vacuoles may not be a disadvantage as the fields at Kakani tend to be dry most of the year. The highly motile hormogonia with an extended sheath in this strain may have an ecological importance in finding a suitable micro-habitat in the rice-fields.

Intra-thylakoidal vacuoles developed at the apical part of the trichomes in all strains. Though the formation of intra-thylakoidal vacuoles has been reported to be the first indication of hair formation in other strains of Calothrix (Livingstone and Whitton, 1983; Mahasneh, 1988), hairs were not formed in any of the strains used in this study. The lack of formation of hairs by Calothrix D794, D795 and D796 indicates that phosphorus is not responsible for hair formation in these strains.

Separation discs were formed in all strains, though they were formed earlier in Calothrix D796. The formation of separation discs in Calothrix D796 was much quicker in 0.1 mg l^{-1} P in comparison to standard medium. It seemed likely that separation discs were formed by active division of cells in cultures of all strains with 1.78 mg l^{-1} P whilst by lysis of the occasional cell in 0.1 mg l^{-1} P medium. Whatever the cause, the separation discs were followed by false-branches in all strains.

The method of replication in Calothrix D794 and D796 under P-limited conditions may possibly be by producing false-branches in which fragments of filaments remained immobile and grew in situ. The formation of intercalary

heterocysts in Calothrix D795 were frequent and the trichomes in this strain fragmented. Most of filaments aggregated in all strains in 0.1 mg l^{-1} P medium which was eventually followed by colony formation much earlier than in 1.7 mg l^{-1} P medium.

All strains accumulated polyphosphate granules within 10 min when phosphorus was added to P-limited cultures and released gas-vacuolated hormogonia in Calothrix D794 and D795 within 18 h of phosphorus addition, whilst no gas vacuoles were observed in Calothrix D796 though many hormogonia were released. The release of gas-vacuolated hormogonia after addition of phosphorus to P-limited culture in Calothrix parietina has been reported by Livingstone et al. (1983). The higher the percentage of polyphosphate granules accumulated, the higher the percentage of hormogonia were released as reported in other strains of Calothrix by Mahasneh (1988). This ability of accumulating polyphosphate granules by all three Calothrix strains may be of ecological importance to adopt under P-limited condition in the complex rice-field ecosystems. Blue-green algae assimilate more P than they require and store the excess as polyphosphate, which can be used under P-deficient conditions (Roger and Reynaud, 1979).

Thus phosphorus deficiency has a marked influence on morphology and cytology of three Calothrix D794, D795 and D796. These morphological features may provide a means of assessing nutrient status in the field. However, experimental results were based only on cultured material in laboratory conditions, hence, differences between field and cultured material are not known. Thus a study in fields on these organisms is essential.

9.4 EFFECT OF LIGHT FLUX AND NUTRIENT CONCENTRATION ON THREE STRAINS

Each Calothrix strain behaved differently at different light flux values. The ability of Calothrix D794 to grow at 170 µmol photon m^{-2} s^{-1} was not surprising as it was isolated from Tarai. The disappearance of gas vacuoles

and formation of intra-thylakoidal vacuoles in hormogonium of Calothrix D795 before release at 170 μmol photon m^{-2} s^{-1} could be possibly due to the inhibition effect of high light which may be related to its climate. The tolerance of Calothrix D796 to 170 μmol photon m^{-2} s^{-1} was interesting as it did not have gas-vacuolated hormogonium and was isolated from high mountain area.

Effect of low and high nutrient medium on gas vacuole formation in Calothrix strains was studied. Percentage of gas-vacuolated hormogonia formation in Calothrix D794 was much higher in 10 mg l^{-1} P medium at 10 μmol photon m^{-2} s^{-1}. Though the hormogonia in Calothrix D795 were always highly gas-vacuolated, the percentage of gas-vacuolated hormogonia was observed to be higher in 10 mg l^{-1} P medium at 10 μmol photon m^{-2} s^{-1} whilst no effect was observed in Calothrix D796.

9.5 EFFECT OF LIGHT ON GAS VACUOLE FORMATION ON CALOTHRIX D794

Calothrix D794 was grown in three environmental conditions: dark, 10 and 170 μmol photon m^{-2} s^{-1}. The percentage of gas-vacuolated hormogonia in this strain was highest at 10 μmol photon m^{-2} s^{-1} than in dark, and lowest at 170 μmol photon m^{-2} s^{-1}. In dark incubation, gas vacuoles were actively formed during first 4 h possibly due to the effect of energy carry over. Thereafter they decreased probably due to the high energy requirement for the production of hormogonia and formation of gas vacuole. After 14 h filaments stopped growing and separation discs were formed in between the vegetative cells.

Gas vacuoles always formed from the apical cells and gas vacuoles were obvious before clear differentiation of the hormogonium or before the hormogonium is detached from the parent trichome. In some filaments, gas vacuoles may be formed in 3/4th of the length of the trichome. During gas vacuole formation gas-vacuolated hormogonia release presumably takes place when the required amount of energy was achieved by the organism. Though

there was an equal supply of nutrients in the medium, some filaments continued to differentiate gas-vacuolated hormogonia one after another, whilst others delayed and released a single long hormogonium (200-300 μm). After release, they started to disappeared without any definite pattern, gas vacuoles may disappear from any cell in hormogonium. Thus gas vacuole formation and hormogonia differentiation are two linked phenomena which occur under the control of external factors during the developmental cycle (Damerval et al., 1987).

The effect of shift of light on gas vacuole formation was studied. Gas vacuole formation and release did not stop completely but at a lower rate, when the culture was shifted to 170 μmol photon m^{-2} s^{-1} after an initial period of 4 h incubation in dark. Release of gas-vacuolate hormogonia was higher in the culture exposed to high light after 8 h and 10 h incubation in dark. The cause of the high percentage of gas-vacuolated hormogonia on transfer from dark to 170 μmol photon m^{-2} s^{-1} may be due to genes which have switched on during the dark period but could not express due to limitation of light energy and these were expressed as soon as they were exposed to light which took place within four hours of exposure.

The release of gas-vacuolated hormogonia were found to be up and down (fluctuated) the cause of which might be due to release of unequal length of hormogonia.

Gas vacuoles in Calothrix D794 form in the specialized portion of the filaments. Once the gas vacuoles formed in the filament, the whole filament may become buoyant. However, not all of the filaments formed gas vacuoles in dark or in low light and few gas vacuolated hormogonia remained on exposure to when they were aggregated in high light (170 μmol photon m^{-2} s^{-1}). In dark incubation for 4 h, the ratio of filaments with gas vacuoles and filaments without gas vacuoles was 1:6.8 (preincubated at 170 μmol photon m^{-2} s^{-1}) whilst ratio

of released gas-vacuolated hormogonia and hormogonia without gas vacuoles 1:03. On transfer to 170 μmol photon m^{-2} s^{-1} ratio of filaments with gas vacuoles and filaments without gas vacuoles was 1:19 whilst ratio of gas-vacuolated hormogonia and hormogonia without gas vacuoles was 1:14 within 2 h of exposure.

Gas-vacuolated hormogonia once released started to float on the surface, so these were the first to expose in high light whilst the filaments with and without gas vacuoles may float below the surface after initial reduction in gas vacuole formation.

Gas vacuole formation in the tapered end of the trichome, gas-vacuolated hormogonia differentiation (to be detached from parent trichome), their release and disappearance are the complex sequences during the growth period. Each process is influenced by change in environmental condition such as condition of preincubation, period of incubation in dark/low light and duration of exposure. Calothrix D794, as Anabaena flos-aquae, may have an ability to regulate mechanisms of buoyancy by forming higher percentage of gas vacuoles in dark and lower percentage in high light.

Such responses to light intensity would enable this organism to migrate up and down during night and day which might have considerable ecological importance in absorbing nutrition through water and to occupy the preferred position in the rice-field water.

SUMMARY

1) Algal samples were collected from three different rice-fields in Nepal. 25 taxa (mostly genera) recorded in field samples and in laboratory cultures, 13 were blue-greens, 6 were greens and 6 were diatoms. The sites A and B were dominated by blue-green algae whilst at site C green-algae were the dominant phylum. Among blue-green algae, the heterocystous forms were (in decreasing order of abundance) Anabaena , Aulosira, Tolypothrix, Nostoc, Cylindrospermum, Calothrix and Gloeotrichia.

2) Each Calothrix isolate exhibited its own characteristic morphological features when grown in a standard medium. They differed from each other in filament structure, growth form and in presence or absence of gas vacuoles in hormogonia. They resembled each other in having some blue heterocysts under certain conditions and not forming hair cells. Formation of false-branches, thick sheath in all three strains, formation of intercalary heterocysts and breakage of filaments in Calothrix D795 and twisting of filaments in Calothrix D796 were observed in old cultures.

3) A study was made on the influence of phosphorus deficiency on the morphology of three isolates in batch culture using the standard medium (1.78 mg l^{-1} P) and a modified medium with 0.1 mg l^{-1} P. In 0.1 mg l^{-1} P medium, the concentration of Fe was increased to ensure that growth was limited by phosphorus.

The first response in P-limited cultures was observed in the behaviour of hormogonium of each strain. All three strains released hormogonia both in standard and in low P medium on the first 24 h of growth. The hormogonia in Calothrix D794 were gas-vacuolated (8% released hormogonia) in standard medium, hormogonia in Calothrix D795 were highly gas-vacuolated (80% hormogonia released) in Calothrix D795 whilst Calothrix D796 did not form gas-

vacuolated hormogonia in either standard or low P medium. As phosphorus deficiency increased, the trichomes increased in length and synthesis of hormogonia ceased in all strains with an obvious increase in cyanophycin granules and a decrease in polyphosphate granules.

In all strains, these morphological changes took place much earlier in 0.1 mg l^{-1} P. Intercalary heterocysts were formed in Calothrix D794 and D795 whilst in Calothrix D796, formation of separation discs was followed by that of intra-thylakoidal vacuoles but no hair was formed in any of the strains. The cultures were found to be P-limited (confirmed by staining) on day + 4 in 0.1 mg l^{-1} P medium and on day + 8 in standard medium.

Without addition of phosphorus at this stage, a reduction in cell dimensions of Calothrix D794 and D795 was observed, whereas in Calothrix D796, a few basal cells were swollen. The filaments in all strains clumped together. False-branches were formed in all strains but false-branches were repeatedly formed in Calothrix D794 and D796.

When phosphorus was added, there was a rapid increase in the number of polyphosphate granules, a decrease in the number of cyanophycin granules, a massive release of hormogonia within 18 h and formation of blue heterocysts.

4) The influence of light, low and high nutrient concentrations on morphology of the three strains was tested. Morphological changes were observed in high and low continuous light conditions. Calothrix D794 grew well at 170 µmol photon m^{-2} s^{-1} although a few basal cells elongated. However, growth was limited at very low light (4 µmol). Calothrix D795 did not tolerate 170 µmol photon m^{-2} s^{-1} after the second subculture as cells developed intra-thylakoidal vacuoles, lost pigments and died. Gas vacuoles in hormogonia were destroyed well before they were released from the filaments. At low light, this strain continued to grow although filaments were fragmented by

formation of intercalary heterocysts. Calothrix D796 can tolerate high and low light although the growth was slow for the first 2 d.

5) Formation of gas vacuoles in Calothrix D794 and D795 was stimulated by 10 μmol photon m^{-2} s^{-1} and 10 mg l^{-1} P medium. No effect was observed in D796. Gas vacuole formation in Calothrix D794 was suppressed in 170 μmol photon m^{-2} s^{-1}.

6) Calothrix D794 was used to investigate the influence of a shift in light on gas vacuole formation. Gas vacuoles continued to form at a lower rate when the alga was shifted back to 170 μmol photon m^{-2} s^{-1} after an initial period of 4, 8 and 10 h incubation in the dark. Formation of gas-vacuolated hormogonia, their release and the disappearance of gas vacuoles occurred within a few hours of exposure.

7) When the alga was grown at continuous 170 μmol photon m^{-2} s^{-1} for 5 d, 2.8% of the filaments were with a clear differentiation of gas-vacuolate hormogonia whilst no filaments were forming new gas vacuoles in the filaments. 6.0 % of released gas-vacuolated hormogonia were present.

When the culture in this stage was subcultured in to fresh medium and incubated in dark, 22% of the filaments formed gas vacuoles in tapered end, the % 0f hormogonial filament was lowered from 2.8-1.5 and % of released gas-vacuolate hormogonia was increased from 6.0-12.0 within 2 h 0f incubation.

8) When the culture was shifted back to 170 μmol photon after an initial period of 4 h incubation in dark, the % of non-hormogonial filaments with gas vacuoles in tapered end was again reduced from 9.0-2.6, % of gas-vacuolated

hormogonial filaments were reduced from 3.8-2.4 and % of released hormogonia from 40.4-6.4 within 2 h of exposure to 170 µmol photon m^{-2} s^{-1}.

9) After initial reduction in gas-vacuolated hormogonia, on transfer the culture from dark to high light, gas-vacuolated homogonia were observed to be reduced more than in the dark and a certain percentage remained for a long period when they were aggregated or entangled between the filaments.

Similar results were also observed in the cultures transfered to 170 µmol photon m^{-2} s^{-1} after 8 h and 10 h incubation in dark (Figs 8.3 and 8.4). Some of the gas-vacuolated hormogonia were swollen.

The gas vacuole formation at the specialized tapered part of the filament, a clear differentiation of gas-vacuolated hormogonia, their release and disappearance depend upon environmental factors such as dark, low and high light, duration of incubation and pre-incubation conditions.

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APPENDIX 1 LAND USE OF NEPAL (1977)

Land use	ha	%
Forest area	48,23,000	34.20
Cultivated area	23,26,000	16.0
Pasture	1,785,700	12.66
Water	400,000	2.83
Residential & road	30,000	0.21
Waste land	2,629,100	14.97

APPENDIX 2 RAIN FALL, AVERAGE MINIMUM AND MAXIMUM TEMPERATURE IN PARWANIPUR IN 1986.

Month	Rainfall (mm)	Av. min. tem.	Av. max. temp.
Jan.	6.0	8.0	23.25
Feb.	2.0	8.66	25.96
March	6.3	14.97	32.70
April	4.4	19.96	36.43
May	59.7	23.23	35.06
June	340.8	25.31	34.08
July	615.5	24.91	31.74
Aug.	453.8	25.72	33.10
Sept.	612.5	23.73	30.89
Dec.	117.8	20.43	30.66
Nov.	5.0	14.35	28.65
Dec.	36.6	11.35	24.75

APPENDIX 3 TABLES REPLACED BY FIGS IN TEXT

Table *a* Four different types of filaments (+ gv and - gv) expressed as percentage of total filament and released hormogonia (+ gv and - gv) as % of total released hormogonia in Calothrix D794. n = 500 (filamets)
+ gv = with gas vacuole, - gv = without gas vacuole, n = 250 (hormogonia)

Incubation time (h)	Filaments: non-hormogonia		hormogonia		Free hormogonia (released)	
	+ gv %	- gv %	+ gv %	- gv %	+ gv %	- gv %
0	none	65.4	2.8	31.8	6.0	94.0
2	3.4	61.0	2.0	28.0	15.2	84.8
4	9.0	50.0	15.0	26.0	30.4	69.6
6	1.8	72.8	4.0	21.4	15.6	84.4
8	2.6	74.0	2.0	21.4	10.0	90.0
10	2.4	75.4	1.6	20.6	8.0	92.0
12	3.2	75.8	2.2	19.2	7.2	92.8
14	2.6	79.8	1.2	16.4	3.6	96.4
16	1.6	82.2	0.8	15.4	2.8	97.6
18	1.8	76.4	1.2	20.6	10.0	90.0

Table *b* Filaments (+gv and -gv) expressed as percentage of total filaments and released hormogonia (+ gv and - gv) as percentage of total released hormogonia in Calothrix D794 at 10 µmol photon m^{-2} s^{-1} PAR (pre-incubated at 170 µmol photon)

0	none	77.2	2.0	20.8	5.2	94.8
2	9.2	63.0	8.6	19.2	38.8	61.2
4	8.2	58.6	16.2	17.0	48.0	52.0
6	9.4	55.0	19.2	16.4	52.4	47.6
8	13.2	51.0	27.0	8.8	60.4	39.6
10	8.8	70.8	7.8	12.6	45.6	54.4
12	4.2	69.2	5.8	20.8	15.8	68.4
14	2.4	70.4	3.2	24.0	18.8	86.0
16	2.8	74.4	7.0	15.8	26.0	74.0
18	6.6	77.0	4.6	4.6	21.6	78.4

Table *c* Four different types of hormogonia (+ gv and - gv) expressed as percentage of total filaments and gas-vacuolate hormogonia as % of total released hormogonia in Calothrix D794 incubated in dark (preincubated at 170 µmol photon)

0	none	65.4	2.8	31.8	6.0	94.0
2	22.0	53.6	1.5	24.4	12.0	88.0
4	9.0	61.9	3.8	25.2	40.4	41.6
6	3.6	52.0	6.6	36.6	26.8	73.2
8	5.0	53.4	15.8	25.8	25.6	74.4
10	9.6	58.8	1.6	30.0	18.8	81.2
12	11.6	45.0	1.0	42.4	11.6	88.4
14	5.4	69.8	0.6	24.2	6.4	93.6
16	3.8	69.2	none	27.0	4.8	95.2
18	2.2	65.6	none	32.2	3.2	96.8
20	1.6	69.6	none	28.8	2.0	98.0

Table d Four different types of filaments (+ gv and - gv) expressed as percentage of total filaments and released hormogonia (+ gv and - gv) as % of total released hormogonia in D794 when exposed to 170 µmol photon m^{-2} s^{-1} PAR after 4 h incubation in dark.

Incubation time (h)	Filaments				Released hormogonia	
	none-hormogonia		hormogonia			
	+ gv	- gv	+ gv	- gv	+ gv	- gv
4	9.2	61.9	none	29	40.4	41.6
6	2.6	62.8	2.4	32.2	6.4	93.6
8	2.0	71.8	1.0	25.2	9.6	90.4
10	1.0	71.0	1.8	26.2	14.0	86.0
12	none	82.0	1.6	16.4	23.2	76.8
14	none	81.0	none	19.0	16.0	84.0
16	none	78.2	none	21.8	6.4	93.6
18	none	78.4	none	21.6	4.0	96.0
20	none	83.8	none	16.2	6.8	93.2

Table e Four different types of filaments (+ gv and - gv) expressed as percentage of total filaments and released hormogonia (+ gv & - gv) as percentage of total released hormogonia in D794 when exposed to 170 µmol photon m^{-2} s^{-1} PAR after 8 h incubation in dark.

8	5.0	53.4	15.8	25.8	25.6	74.4
10	1.2	72.0	0.4	26.4	14.8	85.2
12	1.6	52.4	2.8	23.2	18.8	81.0
14	1.0	79.0	none	20.0	33.6	71.2
16	1.2	52.6	3.4	22.8	55.6	44.4
18	none	80.2	1.0	18.8	35.2	64.8
20	0.6	85.6	0.4	13.4	2.8	97.2

Table f Filaments (+ gv and - gv) expressed as % of total filaments and released hormogonia as % of total released hormogonia in D794 when exposed to 170 µmol photon m^{-2} s^{-1} PAR after 10 h incubation in dark.

12	2.0	75.6	12.0	21.2	9.2	90.8
14	none	80.0	none	20.0	22.8	77.2
16	none	83.4	none	16.6	21.2	78.8
18	none	79.4	none	20.6	13.6	10.0

Table g Filaments (+ gv and - gv) expressed as % of total filaments and released hormogonia as % of total hormogonia in D794 when exposed to 170 µmol photon $_m^{-2}$ s^{-1} PAR after 4 h incubation in 10 µmol photon m^{-2} s^{-1} PAR.

6	4.6	70.2	4.2	21.0	21.6	78.4
8	3.6	76.8	2.6	17.0	31.6	68.4
12	2.6	74.0	4.0	18.2	14.4	85.6
14	1.4	83.2	2.4	13.0	7.2	92.8
16	1.0	84.0	2.0	13.0	9.4	90.6

Table h Filaments (+ gv & - gv) expressed as % of total filaments and released hormogonia (+ gv and - gv) as % of total released hormogonia in D794 when exposed to 170 µmol photon m^{-2} s^{-1} PAR after incubated 10 h at 10 µmol photon m^{-2} s^{-1} PAR.

12	4.8	78.4	2.4	14.8	42.0	62.0
14	1.2	86.6	2.0	10.2	17.2	82.8
16	0.8	87.4	1.0	10.8	14.0	86.0
18	none	86.6	none	13.4	9.2	90.8

* Table i Filaments (+ gv and - gv) expressed as total filaments and released hormogonia (+ gv and - gv) as % of total hormogonia released in D794 grown in Chu 10D-N incubated in tank at 110 µmol photon m^{-2} s^{-1} PAR for 6 d and then sub-cultured in fresh medium and incubated exactly in the same condition without induction in low light / dark (control).

Incubation time (h)	Filaments: non -hormognial		hormogonial		Released hormogonia	
	+ gv	- gv	+ gv	- gv	+ gv	- gv
0	1.0	66.0	8.6	23.8	19.2	80.8
2	3.4	82.4	1.2	13.0	8.4	91.6
4	2.8	79.2	1.6	16.4	10.8	89.2
6	9.4	66.4	3.6	20.6	9.2	82.8
8	2.8	72.4	2.4	15.8	7.6	84.0
10	5.8	69.4	4.4	20.4	9.6	90.4
12	1.2	80.4	2.2	16.2	14.8	85.0
14	3.4	74.4	5.8	18.4	23.6	76.4
16	0.6	84.8	2.2	12.4	32.4	67.6
18	none	59.8	1.0	39.2	18.8	81.2

* Table j D794 incubated at 110 µmol photon m^{-2} s^{-1} PAR for 6 d, subcultured and incubated in dark.

0	1.0	66	8.6	80.8	19.2	80.8
2	5.8	51.2	5.2	20.0	30.4	69.6
4	6.8	58.6	5.8	28.8	32.8	67.2
6	11.2	36.4	18.0	34.4	40.0	60.0
8	16.2	48.4	11.0	24.4	35.2	64.8
10	8.6	68.0	3.0	20.4	20.0	80.0
12	5.4	67.0	1.8	25.8	12.0	88.0
14	1.6	68.8	0.6	29.0	6.8	93.2
16	none	78.8	none	21.2	none	100.0
18	none	80.5	none	19.5	none	100.0

* Table k D794 exposed to 110 µmol photon m^{-2} s^{-1} PAR after 4 h incubation in dark.

6	7.4	46.4	12.8	33.4	38.4	81.6
8	0.4	73.6	6.0	20.0	16.0	84.0
10	none	72.0	3.0	25.0	7.2	92.8
12	none	75.8	1.2	23.0	4.8	95.2
14	0.8	70.6	1.2	27.4	6.4	92.8
16	1.4	73.0	1.4	24.2	38.4	61.6
18	7.6	61.2	1.6	29.6	30.4	63.6

* Table *l* D794 expose to 110 μmol photon m^{-2} s^{-1} PAR after 8 h incubation in dark.

10	1.2	70.8	0.4	27.6	8.4	91.6
12	none	73.4	0.4	26.6	10.8	36.4
14	none	83.2	0.2	16.6	26.8	73.2
16	none	75.0	none	24.8	20.0	40.0
18	none	84.0	none	15.0	11.6	88.0

* Table *m* D794 exposed to 110 μmol photon m^{-2} s^{-1} PAR after 10 h incubation in dark.

12	1.4	78.0	0.4	20.2	9.6	90.4
14	0.8	80.0	1.2	18.0	13.6	86.4
16	none	82.6	1.4	16.0	22.0	78.0
18	none	83.4	none	16.6	10.0	90.0

* not replaced by fig.

APPENDIX 4 CULTURE COLLECTION RECORDS

D0655 ANABAENA SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on 03/02/83 by J.W.SIMON
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH 7.0 + HEPES
Notes: 4.5 um wide, soil sample from middle of rice field at Khumultar research station.

D0656 GLOEOTRICHIA SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on 03/02/83 by J.W.SIMON
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH 7.0 + HEPES
Notes: 4.5 um wide, gas-vacuolate hormogonia.
Khumultar research station.

D0657 NOSTOC SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on 03/02/83 by J.W.SIMON
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH 7.0 + HEPES
Notes: Culture: forms akinetes, motile hormogonia.

D0658 NOSTOC SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on 03/02/83 by J.W.SIMON
Growth: temp 32 light 100 medium CHU 10D -N pH 7.0 + HEPES
Notes: 2 um wide.

D0660 CALOTHRIX SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on not by
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH7.0 + HEPES
Notes: 4.5 um wide, narrow blue-green contaminant present. Forms gas-vacuolate hormogonia.

D0661 ANABAENA SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on not
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH 7.0 + HEPES
Notes: Forms akinetes, narrow blue-green contaminant present.

D0662 GLOEOTRICHIA SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on 03/02/83 by J.W.SIMON
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH 7.0 + HEPES
Notes: Possibly two forms present, narrow blue-green present.

D0663 GLOEOTRICHIA SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on not
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH 7.0 + HEPES
Notes: 10 um wide, narrow blue-green contaminant present.

D0666 NOSTOC SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 27/09/82 H.M.VAIDYA KHUMULTAR rice-field
Culture source: Date: Sender: Isolated by:
J.W.SIMON
Axenic on: not and clonal on 03/02/83 by J.W.SIMON
Growth: temp 32 light 100 medium CHU 10D Cl(17) -N pH 7.0 + HEPES
Notes:

D0794 CALOTHRIX SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 28/08/86 H.M.VAIDYA PARWANIPUR Rice
Res. Centre
Culture source: Date: Sender: Isolated by:
H.M.VAIDYA
Axenic on: 02/03/87 and clonal on 19/01/87 by H.M.VAIDYA
Growth: temp 32 light 100 medium CHU 10 -N + HEPES pH 7.0
Notes: Floating sample collected from edge of rice field.

D0795 CALOTHRIX SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 11/09/86 H.M.VAIDYA KHUMALTAR Rice
Res Centre
Culture source: Date: Sender: Isolated by:
H.M.VAIDYA
Axenic on: 02/03/87 and clonal on 19/01/87 by H.M.VAIDYA
Growth: temp 32 light 100 medium CHU IO-N + HEPES pH 7.0
Notes: Collected from rice field, floating mat attached to soil surface.

D0796 CALOTHRIX SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
NEPAL 21/09/87 H.M.VAIDYA KAKANI (2064 m)
Culture source: Date: Sender: Isolated by:
H.M.VAIDYA
Axenic on: 02/03/87 and clonal on 02/01/87 by H.M.VAIDYA
Growth: temp 32 light 100 medium CHU 10-N + HEPES pH7.0
Notes: Isolated from dried rice plant.

D0797 SYNECHOCOCCUS SP.

Strain:		Repeat isolates:	
Country found:	Date:	Finder:	Details of site:
NEPAL	21/09/86	H.M.VAIDYA	KAKANI rice-field
Culture source:	Date:	Sender:	Isolated by: J.W.SIMON

Axenic on: 01/06/87 and clonal on OO/10/86 by J.W.SIMON
Growth: temp 32 light 100 medium CHU IO-N + NH4-N (10)+HEPES pH 7.0
Notes: Kakani altitude 2064 m, raw water sample, no filtration.